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The Ecology and Behaviour of Insecticide Resistant Malaria Vectors and Implications for Control in Burkina Faso

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of Doctor of Philosophy

By

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"No man ever steps in the same river twice, for it's not the same river and he's not the same man." - Heraclitus (ca. 540 – ca. 480 BCE)

“No man's knowledge here can go beyond his experience” – John Locke (1632 - 1704)

“The greater the difficulty, the more glory in surmounting it” - Epicurus (341 - 270 B.C.)

“The only thing I know is that I know nothing” - Socrates (c. 469 - 399 B.C.)

“There is no law except the law that there is no law” - John Archibald Wheeler (1911 - 2008)

“Nothing in life is to be feared, it is only to be understood Now is the time to understand more, so that we may fear less” - Marie Curie (1867 - 1934)

“If I have seen further, it is by standing on the shoulders of Giants” - Sir Isaac Newton (1642 - 1727)

Abstract

Long-Lasting Insecticide-Treated Nets (LLINs) and Indoor Residual Spraying (IRS) are the most common and successful methods for malaria vector control in Africa. There is growing evidence of shifts in mosquito vector biting and resting behaviours in several African settings where high LLIN coverage has been achieved. These changes, combined with growing insecticide resistance, may reduce intervention success by decreasing the contact between vectors and insecticide-treated surfaces. While insecticide resistance in malaria vectors has been widely investigated, less is known about the implications of mosquito behavioural changes to malaria control. In recent years, LLIN programmes appear to have a reducing impact in a small number of high burden African countries including Burkina Faso. This reducing effectiveness is hypothesized to be the result of insecticide resistance, but the potential additional contribution of mosquito behavioural avoidance strategies has not yet been investigated in Burkina Faso. The aim of this PhD was to investigate the contribution of insecticide resistance and mosquito behaviours to the persistence of malaria transmission in southwestern Burkina Faso following a national LLIN-distribution campaign. Specific objectives were to (i) evaluate the performance of a new mosquito sampling method, the Mosquito Electrocuting Trap (MET) to measure spatial and temporal variation in human exposure to malaria vectors; and characterize the spatial, seasonal and longer-term trends in (ii) vector ecology and behaviours, (iii) insecticide resistance within *Anopheles gambiae sensu lato* (s.l.) and (iv) malaria vector survival and transmission potential in rural Burkina Faso.

A two-year programme of longitudinal mosquito vector surveillance was initiated within 12 villages of south-western Burkina Faso in 2016, shortly after completion of a mass LLIN distribution. Host seeking malaria vectors were sampled monthly using Human Landing Catches (HLC) and METs conducted inside houses and in the surrounding outdoor area (911 households in total). Resting bucket traps (RBTs) were used to sample indoor and outdoor resting vectors. In an initial study (Chapter 2), I evaluated the performance of the MET relative to the HLC for sampling host-seeking

malaria vectors over 15 months in 12 villages. Overall, the MET caught proportionately fewer *An. gambiae* s.l. than the HLC (mean estimated number of 0.78 *versus* 1.82 indoors, and 1.05 *versus* 2.04 outdoors). However provided a consistent representation of vector species composition, seasonal and spatial dynamics, biting behaviour (e.g. location and time) and malaria infection rates relative. The MET slightly underestimated the proportion of bites that could be prevented by LLINs relative to the HLC (5%). However, given the major advantage of the MET of reducing human infection risk during sampling, I conclude these limitations are acceptable and that the MET presents a promising and safer alternative for monitoring human exposure to malaria vectors in outdoor environments.

Vector sampling was extended (using HLCs and RBTs) to investigate longer-term temporal changes in vector ecology and behaviour (Chapter 3). Analysis of a subset (20%) of the *An. gambiae* s.l. (N= 7852) indicated that *An. coluzzii* (53.82%) and *An. gambiae* (45.9%) were the main vector species. There was substantial variation in vector abundance between sites and seasons, with a predicted ~23% reduction in *An. gambiae* s.l. biting density from start to end of study. A higher proportion of outdoor biting (~54%) was detected than expected from previous studies; but there was no evidence of spatial, seasonal or longer-term changes in exophagy. Species level analyses indicated that revealed moderate but statistically significant different in the exophagy and biting time between *An. coluzzii* and *An. gambiae*. Combining information on biting times and location (indoors versus outdoors), I estimated that ~85% of exposure could be prevented using good quality and effective LLINs during standard sleeping hours (10 pm – 5 am).

Bioassays were conducted on the *An. gambiae* s.l. population at 9 out of the original 12 study villages to estimate spatial, seasonal and longer-term variation in insecticide resistance (IR) over the study period. Overall, only 23% of *An. gambiae* s.l. exposed to a diagnostic dose of deltamethrin were killed within 24 hours; indicating that all surveyed populations are resistant. Furthermore, IR increased over the study period, with significant reduction in mortality after exposure to deltamethrin in bioassays. There was no evidence of variation in IR between *An. gambiae* and *An. coluzzii*.

Finally, the transmission potential of *An. gambiae* s.l. in this area was investigated through assessment of mosquito parity rates (a proxy of survival), malaria infection rates and estimation of annual Entomological Inoculation Rates (EIR; Chapter 5). The daily survival rate of malaria vectors in this area was > 90%), but with variation between villages and seasons. After controlling for this spatial and seasonal variation, there was evidence of a longer-term increase in vector survival over the study period. In contrast, both mosquito vector biting densities and their malaria infection rates declined over the study period. This resulted in a drop in the predicted EIR from 320 to 105 infective bites per person/year respectively in year 1 and 2. Considering the proportion of exposure estimated to be preventable by effective LLIN use (~85%, Chapter 2 &3), I estimated that residents in this area are still exposed to ~32 infective bites per person per year even when this intervention is used. This confirms that even with 100% coverage and usage of highly effective LLINs, high levels of transmission will persist in this setting.

Taking the case of Burkina Faso as an example, results obtained here confirm that both IR and outdoor biting by malaria vectors are contributing to the persistence of transmission in high burden African countries. Consequently, a successful vector control programme in this context need a clear insecticide resistance management plan and supplementary tools that target vectors feeding and resting outdoors.

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Author's Declaration

I hereby solemnly declare that the work presented in this thesis is entirely my own, except where otherwise stated. I honestly recognize the contribution of all volunteers, laboratory and field technicians, and the co-authorship of my supervisors Professor Heather M. Ferguson and Jason Matthioppoulos, colleagues and other co-authors, with intention for the works to be published. I further declare that no part of this work has been submitted as part of any other degree.

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Here is the specific contribution of each co-authors: AS designed the study, conducted the field data collection, did the molecular analysis, performed the data analysis, interpreted the results and drafted the manuscript. KHT contributed to the field data collection. WMG, NFS, and HMF contributed to the design of the study and the sampling protocol and provided comments upon the manuscript. HMF was a major contributor in writing this manuscript. SZ, PO and FC contributed to the molecular analysis in the labs. LN, JM, contributed to the data analysis. NM, designed, developed assisted the METs. All authors read and approved

List of Abbreviations

AIC: Akaike's Information Criterion
CDC-LT: Centre Disease Control Light Trap
DD: Discriminating Dose
EIR: Entomological Inoculation Rate
ELISA: Enzyme Linkage Immuno-Sorbent Assay
HDN: Human-baited Double Net
HDT: Host decoy Trap
HLC: Human Landing Catches
GAM: Generalized Additive Models
GAMM4: Generalized Additive Mixed-Models augmented with lme4
GLM: Generalized Linear Models
GLMM: Generalized Linear Mixed-Models
HLC: Human Landing Catches
ITT-C: Ifakara tent trap design C
IR: Insecticide resistance
LLINs: Long-Lasting Insecticide-Treated Nets
LRT: Likelihood Ratio Tests
MCMC: Markov Chain Monte Carlo
MET: Mosquito Electrocuting Trap
PCR: Polymerase Chain Reaction
PR: Parity rate
PVC: Polyvinyl Chloride
SSA: Sub-Saharan Africa
SR: *Plasmodium falciparum* sporozoite rate
WHO: World Health Organisation

Chapter 1: General Introduction

1.1. Malaria burden, pathology and trends

1.1.1 Malaria pathology

Malaria is a mosquito-borne disease caused by *Plasmodium* genus parasites transmitted to humans (host) by *Anopheles* mosquitoes (vector) [1]. The incubation period defined as the time between the inoculation of the parasites into a host and the appearance of the first symptoms. This period can last for six to ten days for *Plasmodium falciparum* malaria and approximately fifteen to sixteen days for the others parasites (non-*falciparum* malaria) [2]; and can vary with several factors such as host immunity, prophylaxis and previous anti-plasmodial treatment [3]. Several symptoms of ranging severity have been described [4]. In more than 60% of non-severe and uncomplicated malaria cases, patients report feeling cold and sweating, fever and headaches and it is also common to see patients with nausea, vomiting or stomach-ache [5]. In the absence of rapid and efficient care, the disease can be severe; leading to anaemia [6]and the development of neurological sequelae [7]. Disease severity varies with host age and immune status [8, 9]. In endemic and high transmission areas, malaria heavily impacts maternal and newborn infant health. For example, it has been shown that malaria can lead to low birthweight (birthweight < 2.5 kg) particularly in primigravidae mothers, maternal death, and to congenital malaria [9]. Furthermore, Dandorp and colleagues showed that severe anaemia and respiratory distress are most common in younger age groups [8].

Besides the threats of mortality and morbidity, malaria infection can also result in reduced intellectual capacity development and poor school performance in children [10]. Children with frequent malaria episodes at a critical moment of their brain development are likely to be subject to short term cognitive impairment, possibly resulting in poor results in school exams

[11]. In addition to that, long term (up to nine years) consequences following severe malaria may involve neurocognitive and cognitive impairment [12] which may extend malaria burden beyond the traditional mortality and morbidity.

1.1.2 Malaria in the world

Malaria is still one of the largest global killers. In 2017 the global risk at population was estimated at about 4 billion people [13]. In addition 2018, there were 228million (95%CI: 206 – 258 million) cases and a total of 435 000 (95%CI: 401 000 – 470 000) people lost their lives with 67% of these malaria victims being children under five-years old [14]. However, globally there has been a 28% reduction in mortality observed between 2010 to 2017 [13] and ~68% between 2000 and 2015 [15]. This was mainly due to improved diagnosis, treatment and upscaling of vector control. However, progress has stalled over the last few years (e.g. 2016 - 2018). This is the first time in 15 years that cases and deaths did not decrease [13, 14]. For example the number of cases increased from 219 million in 2017 to 228 million in 2018 [14]. Though, the biggest problem seems to be the continuation of transmission in small group of "high burden countries" (Burkina Faso, Cameroon, Democratic Republic of Congo, Ghana, Mali, Mozambique, Niger, Nigeria, Uganda, Republic of Tanzania and India; [13]). These countries accounted for 70% of the global malaria cases and related deaths. The reason for this slowdown is not clear, and whether this is just a temporary stalling or the start of a general reversal of progress. Despite averting > 60% of deaths since 2000, malaria still has a high burden in many African countries, particularly in West Africa.

1.1.3 Malaria in Africa

Malaria is one of the biggest public health problems in sub-Saharan Africa (SSA, Figure 1.1, [16, 17]). According to World Malaria Report, 93% of the children who died from malaria in 2017 were in the WHO African region [13]. In addition, 80% of the total cases registered in 2017 occurred in SSA. Over the last 20 years there has been a significant decrease in clinical malaria

prevalence and cases in Africa have fallen from 321 (253 – 427) ‰ in 2000 to 192 (135-265) ‰ globally in 2015 [15]. During the same period, the number of malaria deaths occurring in SSA dropped from 1,007,000 (CI: 666,000 – 1,736,000) to 631,000 (CI: 394,000 – 914,000) [18].

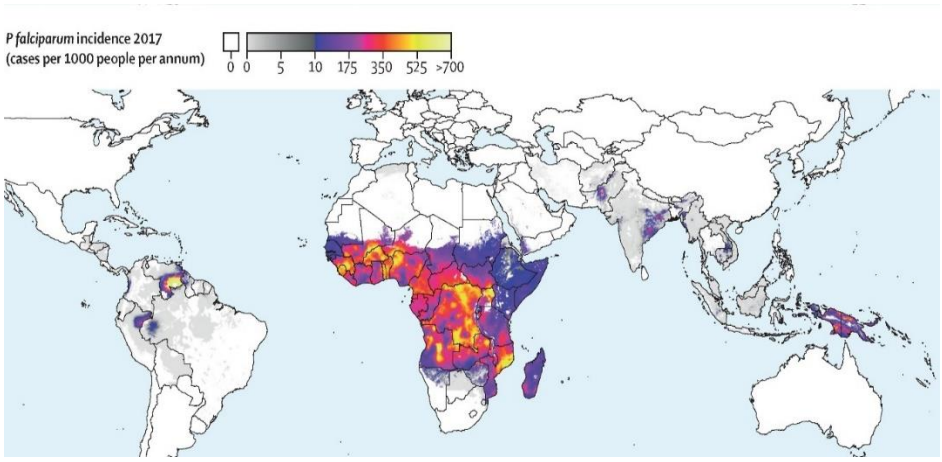


Figure 1.1 : Distribution of *Plasmodium falciparum* incidence in 2019 (source: use with permission from [16]).

1.2. Malaria parasite and life cycle

From approximately 100 parasite species in the *Plasmodium* genus, only five have been implicated in causing malaria in human. These are: *Plasmodium falciparum* Welch, 1897; *Plasmodium malariae* Laveran, 1881; *Plasmodium ovale Stephens, 1922; Plasmodium vivax* Grassi and Felleti, 1890 and *Plasmodium knowlesi* Sintonand Mulligan, 1932 [19]. Recently, *P. ovale Stephens* has been separated into two different species known as *P. ovale curtisi* and *P. ovale wallikeri* [20].

The malaria parasite life cycle is characterized by a sexual stage that occurs in Anopheles mosquito vectors (definitive host) and an asexual stage occurring in vertebrate hosts [19]. Mature male and female gametocytes are drawn up by the female mosquito while taking a blood meal. These gametocytes fuse to form a motile zygote in the blood meal which penetrates the mosquito midgut to form an oocyst on the outer midgut wall (~4 - 5 days following blood meal, [1]). Within each oocyst, several thousand sporozoite-stage parasites develop [1]. These sporozoites burst out into mosquito haemolymph and migrate from the haemolymph into the salivary glands

where they will be injected into the vertebrate host during the next blood feed [21]. The time required for parasites to develop from gametes to infection-stage sporozoites is the extrinsic incubation period (EIP). The length of the EIP varies with temperature, *Plasmodium* species, and is influenced by aspects of parasite-mosquito interactions [21]. For example, the EIP of *P. falciparum* ranges between 12 days for a daily temperature 27°C [22].

Once inside the vertebrate host, the parasite goes through several different stages of development starting with an exoerythrocytic schizogony inside liver cells [23-25]. During the liver stage, merozoites are produced and released into the blood stream in about five to eight days. These merozoites then penetrate red blood cells and undergo development to form trophozoites then schizonts, and merozoites known in a cyclical process known as erythrocytic schizogony [19]. Transmission stage gametocytes form from a small proportion of these asexual parasites, although the mechanism that governs this differentiation is unknown [26]. All *plasmodium* species go through the same life cycle but during the exoerythrocytic stages, some *P. vivax* and *P. ovale* parasites onto a dormant stage named hypnozoites that can persist in the liver for years before they re-emerging and causing infection [27, 28]. Figure 1.2 describes the life cycle of Plasmodium one inoculated into the vertebrate host.

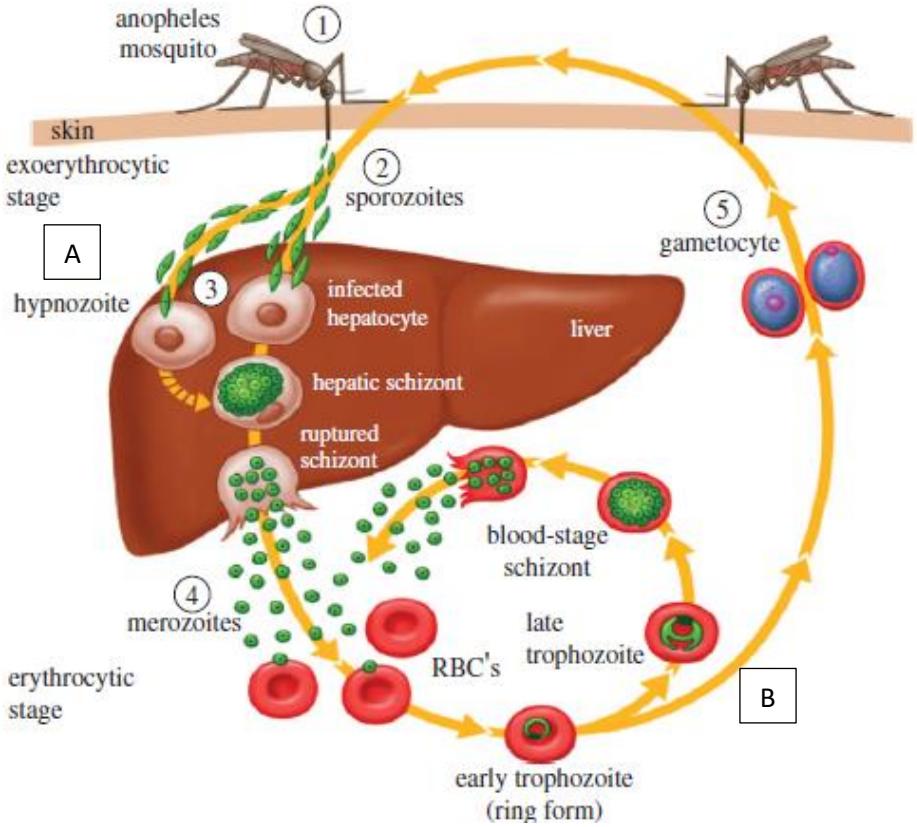


Figure 1.2: *Plasmodium sp* life cycle (Source: modified picture from [29]): Anopheles mosquito inoculating sporozoite to human host, A: exoerythrocytic stage 2) sporozoite inoculated, 3) Liver stages; B: erythrocytic stage 4) merozoites issue from schizonts rupture, 5) gametocyte stage and they ingestion by anopheles mosquito during blood feeding. RBC stand for red blood cells.

1.3. Malaria vectors

There are more than 400 mosquito species within the *Anopheles* genus, of which only about 30 are involved in malaria transmission [30]. Specifically, in sub-Saharan Africa less than twenty of the 140 known anopheline species are known to transmit malaria [31] from which five taxa are responsible for the majority of transmission: *An. gambiae sensu stricto* (ss), *An. funestus* s.l. Giles, *An. arabiensis* Patton, *An. nili* Theobald and *An. moucheti* Evans [32]. Historically, *An. gambiae* s.s. was thought to be composed of two distinct chromosomal forms, the "M" and "S" which can exist sympatrically in west Africa [33, 34]. In 2002, [35] raised the possibility that these molecular forms may be distinct species. This hypothesis has been subsequently confirmed by more detailed genetic analysis, with the M and S form now being recognized as the species *An. coluzzii and An. gambiae*

respectively [36]. *Anopheles gambiae*, *An. coluzzii* and *An. arabiensis* Patton with five other species (*An. melas* Theobald, 1903; *An. merus* Doenitz, 1902; *An. quadriannulatus* A Theobald, 1911; *An. bwambae White*, 1985 and *An. quadriannulatus* B Hunt, 1998) are part of the *An. gambiae* s.l. species complex. Members of this group are morphologically undistinguishable [37, 38]. *Anopheles funestus* s.l. is a species group, made up of nine different species (*An. funestus ss, An. rivulorum Leeson, An. Leesoni Evans, An. parensis Gillies, An. vaneedeni Gillies & Coetzee, An. confusus Evans & Leeson, An. aruni Sobti, An. brucei Service* and *An. fuscivenosus Leeson*; [39-42].

1.3.1 Malaria vectors life cycle

Mosquitoes have different life stages starting with an initial aquatic stage (from eggs, larvae and pupae) followed by a terrestrial adult phase (Figure 1.3). Adult females lay about 50 to 200 eggs in water bodies which hatch into first instar larvae within 2 to 3 days [1]. These undergo 4 moults to pass through 5 larval instar stages, during which they feed on debris and microorganisms [1]. Mosquito development from larvae into adults, and adult development/maturation is temperature dependant and varies between species. On average, *An. gambiae* s.l. larval development last approximately 10.92 days to 12.35 days respectively at 23°C and 31°C [43], after which larvae transform into pupae (2 - 3 days) before emerging as terrestrial adults [1].

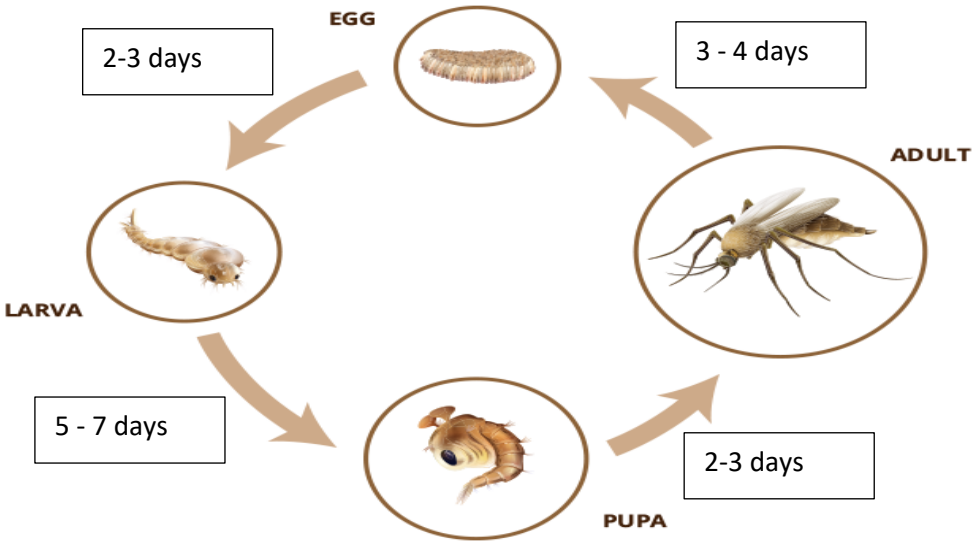


Figure 1.3: Mosquito life cycle and ranges of time between each stage as considered for *Anopheles gambiae* s.l. http://www.mosquitoes.org/education/, [Accessed on 09-07-2016]).

Adults of both sexes are thought to initially feed on plant nectar after emergence [1], with females going on to feed on blood. Males and females mating within two days after emergence [44]; which triggers the start of the gonotrophic cycle in females. The gonotrophic cycle describes a process beginning with blood feeding, followed by egg development, and ending with the oviposition of eggs into aquatic habitats [45, 46]. In African malaria vectors, this period is thought to be repeated every ~2-4 days [46].

1.3.2 Malaria vector bio-ecology and behaviour

The ecology and behaviour of malaria vectors varies between species. The type of larval habitats used by each species may depend on factors such as the presence of predators and physico-chemical aspects of the water [47]. For example, *Anopheles arabiensis* is mostly found in dry savannah or open woodland [48], and often in the vicinity of livestock [49]. *Anopheles coluzzii* larvae can breed in aquatic habitats flooded rice fields and larger, permanent water bodies with floating vegetation [50-52]. In contrast *An. gambiae* and *An. arabiensis* ae more likely to be found in manmade water bodies and small, sunny temporary pools close to houses [49], or rice field

without much floating vegetation [50]. *Anopheles funestus* is found in savannah-like habitats where its larvae breed in large ponds, shaded semi-permanent or permanent fresh water with vegetation as well as lake and rivers edges [49, 53, 54].

There is also notable variation in adult behaviour between vector species. For example, *An. funestus*, *An. gambiae* and *An. coluzzii* preferentially feed on humans [55] late at night [56]; while *An. arabiensis* is known to be more zoophilic; feeding both on livestock and human, and bites earlier in the evening or in the morning [53]. In 1998, an experimental study in Burkina Faso comparing the host preference of major malaria vectors showed that only 8% of *An. gambiae* s.l. trying to feed on non-human animals were *An. gambiae* and *An. coluzzii,* with the remaining 92% being *An. arabiensis* [57]. African vector species also vary in their choice of resting habitats. "Resting" refers to the 1-3 days period after females take a blood meal, while they refrain from seeking further blood meals as eggs develop. In terms of resting behaviour, *An. funestus* , *An. gambiae* and *An. coluzzii* were described resting primarily indoors (e.g. "endophilic"; [58, 59]); while *An. arabiensis* is found resting either outdoors or indoors [53].

Variation in biting and resting behaviours has also been described within Africa vector species [60-63]. This variation may be attributed to both genetic and environmental factors. For example, variation in feeding and resting behaviours within *An. funestus* in West African has been associated with chromosomal polymorphisms [61]. Inversion of one of these chromosomal forms, 2Ra, has been associated with host choice in indoor collection [62]. Variation in host choice within vector populations has also been associated with environmental factors such as livestock availably [64] and use of bed nets [65]. Although previous studies have provided a good understanding of vector ecology and behaviour, some estimates of key traits such as host preference and resting behaviour suffer biases due to a lack of standardization in sampling methods and assumptions [66]. However, it is important to acknowledge that vector behaviours may not be fixed either

within or between vector species, and there is need to update and measure within specific context

1.3.3 Malaria vectors in Burkina Faso

The distribution of major malaria vectors throughout sub-Saharan Africa varies in association with ecological factors such as rainfall, vegetation density and type of breeding sites (permanent or temporal, [67]. Investigation on the distribution of malaria vectors in south-western Burkina Faso showed that *An. gambiae* and *An. coluzzii* represented more than 95% of samples collected in areas of rice cultivation; with *An. funestus* constituting the remaining 5% [54]. In contrast, *An. funestus* was the dominant (65%) malaria vector species in a cotton-growing area of southwestern Burkina Faso [54]. Other vector species such as *An. nili* have also been detected in Burkina Faso, at low prevalence (e.g.< 1% to 9% of malaria vector community; [54]). As elsewhere in west Africa, malaria vector species vary seasonally and spatially in Burkina Faso. Seasonal variation in vector abundance and species composition follows the annual cycle of rainfall consisting of a single dry season between January-June, followed by a wet season between July-December where most malaria transmission occurs. In "Vallée du Kou" (VK, a village in the south west of Burkina Faso), *An. coluzzii* dominated at the beginning of the wet season, before being replaced by *An. gambiae* in the second half of the season with both species having similar proportion in the vector community towards the end [68]. In another study conducted 2013 – 2014; *An. coluzzii* was also found to dominate at the start of the rainy season; with the density of *An. gambiae* and *An. arabiensis* peaking near the end [69]. These spatial and temporal differences in the vector community may be related to differences in the ecological requirements of *An. coluzzii*, *An. arabiensis* and *An. gambiae* as discussed earlier.

1.4. Parameters involved in malaria transmission

Malaria transmission is classically described in terms of interactions between malaria parasites, mosquito vectors and vertebrate hosts [19, 70]. To be able to transmit malaria, the vector must feed on a human [70], be physiologically competent [71], and also live long enough for the *Plasmodium* parasites to complete their EIP [70]. Ronald Ross was the first to elucidate the role of mosquito vectors in malaria transmission [72], and highlighted the potential of larval source management to reduced malaria in some localities during his inaugural lecture [13]. Based on these assumptions, a mathematical model of malaria was developed [73] and improved through time [74] to describe transmission in terms of the basic reproductive number "R_o" as expressed in equation (1.1). Here R_o is defined as the number of secondary infections expected to be generated from a single infected human host within a susceptible population [75].

Equation 1.1: $$Ro = \frac{ma^2bcp^n}{-rln(p)}$$

Here, ***m*** indicates the ratio of mosquito to human density; ***a*** represents the average number of mosquito bites a person receives daily; **b** is the probability that a malaria-infected mosquito will transmit infection to a person upon a bite, **c** is the probability that a mosquito will pick up infection from a malaria-infected person (per bite); ***n*** is the length of the gonotrophic cycle; ***p*** is the vector daily survival rate and ***r*** the is the proportion of people that recover from the disease [74].

To best describe the relative potential for a mosquito population to transmit malaria, the concept of "vectorial capacity" **(*VC*;** Equation 1.2**)** was developed [76] and described in Chapter 5. The VC equation differs from *Ro* in considering only the transmission components dependent on mosquitoes The vectorial capacity equation is used to describe the ability of a vector population to drive transmission from one initial infective case [77] as follows:

Equation 1.2: $VC = \frac{ma^2p^n}{-ln(p)}$

Vectorial capacity is sometimes used to estimate the expected impact of vector control tools such as Indoor Residual spraying (IRS), Insecticide Treated Nets (ITNs) and larval source management on malaria transmission [77, 78]. Control methods that affect any of these components should lead to a reduction in malaria incidence [79]; with the greatest impact coming from reductions in vector survival [75]. The vectorial capacity and its component parameters are thus useful predictors of malaria transmission intensity and the impact of interventions.

1.5. Malaria vector control

Malaria vector control aims to reduce human-vector contact and protect individuals from infection, as well as reducing transmission at the community level [80]. This can be achieved through different methods including housing improvements [81], use of repellents [82], larval source management [83], large scale use of indoor residual spraying (IRS) [84] and LLINs [85]. Of these, LLINs and the IRS are the core interventions recommended by the WHO [80], and most commonly deployed in Africa. The vector control tools can be grouped into insecticide and non-insecticide-based methods as followed:

1.5.1 Insecticide based control

1.5.1.1 Insecticide Residual Spraying

Indoor Residual Spraying consists of coating the inner surface of house, but also structures such as animal sheds, with insecticides that repel kill mosquitoes that rests indoors (endophilic) [86, 87]. Since the beginning of malaria control, IRS has played an important role with early programmes using dichlorophenyl-dichloroethane (DDT) in Europe, Russia, Asia and Latin America during the 1955-1969 global malaria eradication campaign [84, 88]. Many insecticidal products such as Carbamates, Organophosphates, Pyrethroids, and DDT (only if alternatives are unavailable) and recently the Neonicotinoid (Clothianidin) have been recommended by the World Health

Organisation Prequalification Team for use of IRS [89]. This intervention offers the possibility for rotating different classes of insecticides in a setting, but due to resistance to DDT and pyrethroid, only carbamates and organophosphates remain viable options [90]. However, mechanisms conferring resistance to Carbamates and Organophosphates are also increasing in malaria vectors [91-93].

The success of IRS rests on its ability to target indoor resting mosquitoes [87], with any change in indoor resting behaviour being of concern as it could lead to its failure. Recently reductions in malaria transmission were observed in areas of several African countries [90, 94] where IRS was conducted using carbamates, DDT and pyrethroids. According to the WHO, about 116 million in 2017 compared to 93 million people at risk in 2018 were protected by IRS globally [13, 14]; From this, only 64 million people of this IRS protected population were found in Sub-Saharan Africa in 2018 [13]. Although effective, IRS has several limitations, including its need for regular implementation (every 2 – 12 months depending on insecticide, [95]), high implementation cost [96], logistical issues [97] and insecticide resistance [91, 92, 98]. Thus the number of countries using IRS has declined, leading to a reduction in coverage of total population at risk worldwide from 5% in 2010 to 3% in 2017 [13] and further to 2% in 2018 [14]. However, this proportion increased from 5.4% in 2016 to 6.6% in 2017 in Africa [13].

Burkina Faso, alongside many other West African countries, carries out IRS with the support of the President's Malaria Initiative [99]. This control method was mainly restricted to the South-west of the country, in the health district of Diebougou, where it has been carried out each year from 2010 to 2012. This program used Bendiocarb (a type of insecticide from the carbamate class) and aimed to protect more than 25 000 children under five years old and about 2000 pregnant women, with a coverage touching 99% each year [99]. However, this programme was stopped because of lack of sustainability and the short period of efficacy of the insecticide used. Due to increased availability of a new class of non-pyrethroid insecticide (Pirimephos-methyl, [100]), IRS was resumed in 3 regions of Burkina Faso in 2018 [101]. Reports suggest this programme had led to a significant reduction

of some entomological determinants of malaria transmission including sporozoite rates (the proportion of mosquitoes found with sporozoites of malaria parasite in there salivary glands) and entomological inoculation rate (EIR defined as the number of infectives a person could receive in a given area annually; [101]).

1.5.1.2 Long lasting Insecticide treated Nets (LLINs)

Long-Lasting Insecticide-Treated Nets are bed nets made of woven polyester or polyethylene fibres that are impregnated with insecticides. The insecticidal effect of these nets is designed to last for three to four years [102] depending on the fibres types. LLINs are fitted over a bed and operate as a physical barrier protecting the user from mosquito biting, as well as acting as a repellent and killer of mosquitoes attempting to feed on the protected users [103]; with the killing effect generating a community impact that protects non-users [104, 105]. LLINs thus primarily target anthrophilic vector species that feed on people mainly indoors and late at night [106-108]. LLINs use impacts vectorial capacity by reducing vector density, human biting rate and adult vector survival [78, 109]. The reduction in mosquito lifespan associated with LLINs is predicted to significantly reduce in malaria transmission [110]. Several studies showed significant reductions in malaria transmission and disease burden in many African countries [111, 112] following the large-scale introduction of LLINs and their predecessor ITNs (e.g. “insect treated nets”, with shorter-term efficacy; [113]).

The number of LLINs delivered and in use in malaria endemic African regions has increased drastically over the last decade [13]. The proportion of people sleeping under an ITN or LLIN in Africa has increased from an estimated 29% in 2010 to 50% in 2017 [13]. It has been estimated that ~578 million LLINs have been distributed globally between 2016 and 2018, with 87% of these in sub-Saharan Africa [14]. As an example, the proportion of households owning LLINs in Tanzania increased from a level approaching zero in 1999 to a range from 61.7 to 65.2% in 2010 [114].

As in other malaria endemic countries, vector control in Burkina Faso relies on distributing LLINs through mass campaigns [13, 115-117]. The estimated ownership of LLINs in the country is ~75% at household level in 2018 [118]. Despite the massive scale up of LLINs distribution in SSA, in 2018 it was estimated that about 40% of the African population still did not own a single insecticide treated net [14]. The protection provided by LLINs is influenced by mosquito biting behaviour [119-121], human behaviour including perception of use [122, 123] and the physical integrity of nets [124]. In addition, the protection obtained from LLINs may be reduced by insecticide resistance that is now widely reported in malaria vectors across Africa [125-128], as will be discussed further.

1.5.2 Non insecticide-based methods

1.5.2.1 Housing Improvement

House design and structure is a significant determinant of the vector abundance inside houses, which in turn predicts human malaria rise [81]. For instance, in South-East Tanzania, it has been reported that the abundance of *An. gambiae* and *An. funestus* indoors are significantly reduced by housing improvement such as screened eaves and windows using insecticide treated material [129] or without insecticides [130, 131]. Other housing factors that are positively associated with vector density include having thatched roofing, non-plastered interior walls and house size [132, 133] [133]. In 2015, the Roll Back Malaria programme highlighted housing improvement (door, window and roof protection and as well as wall plastering [134]) as strategies for reducing mosquito entry into houses. A study analysing cross-sectional data from Demographic and health Survey in addition to Malaria Indicators Survey found that in 2010 malaria prevalence was higher in children living in traditional houses (~71%) than that in modern houses (~40%; [135]). Implementation of housing improvement may be affordable and easy in some settings, and so could thus be a supplementary tool against malaria transmission.

1.5.2.2 Larval control

Larval source management (LSM) is a complex method that can be used to target the aquatic phases of malaria vectors to prevent their development into adults and thus reduce malaria transmission [136-139]. Several methods can be used for this LSM. Firstly, this can be done through physical manipulation and modification of the environment to reduce larval habitats through canal drainage, land levelling or filling pools [140]. In addition, LSM can be achieved through biological control (e.g release of predators, microbiological organisms) or chemical insecticide. Biological control includes agents such as larvivorus fish [141, 142], and microbial agents such as *Bacillus sphaericus,* and *Bacillus thuringensis* [143]. There is also the use of insect growth factors (IGF) [143] that prevent larvae from developing into adults [144]. Finally, LSM can be conducted by spreading oil to reduce the water surface tension which prevents larvae, pupae and newly emerged adults from staying at the surface [143]. An advantage of this approach is that it may work in the presence of insecticide resistance [145]. However, only few trials have demonstrated an epidemiological impact of LSM implementation [83]. In Kenya, LSM implementation led to a reduction of about 89% and 91% in *Anopheles* late instar larvae density by using canal drainage and *Bacillus thuringensis* (*Bti*) respectively [146]. Furthermore, the use of *Bti* in a large flood plain in the Gambia led to a significant decrease in the proportion of water bodies colonized by malaria vectors, but with no accompanying reduction in malaria incidence, vector density or sporozoite rates [147]. Larval control methods will be most effective in areas where breeding sites are well defined and transmission is low to moderate [145]; conditions that may not apply in many high burden African countries [148]. As LSM is difficult and costly to implement in some African settings [148] [145], it is not used on a wide scale.

1.5.2.3 Odour baited control methods

Vector control methods based on the use of baits include attractive sugar baits (ASB; [149, 150]) and plants odour baits [151]. Toxic sugar baits are solutions made from compounds toxic to mosquitoes, such boric acid mixed

with the juice of fruits that contain attractive volatiles which can be sprayed on plants, rice, and grass [149] or deposited in traps [150]. Another possibility that is being tested in the laboratory is the mixed use of sugar and toxic microorganism such as *Bti* [152] and fungi [153]. Pilot studies indicate that ASB have potential to significantly reduce adult malaria vector densities [154], but the epidemiological impact of this intervention has not yet been measured. Similarly, several odour- baited traps based on plant [155] or animal or human derived odours [156] have been proposed for vector surveillance and control [151]. As with ASB, the epidemiological impact of such approaches has not yet been demonstrated. These are relatively new approaches that are not yet in wide use for malaria vectors, and still in early stages of testing and evaluation [157].

1.5.2.4 Repellents

Repellents may also be used to protect Individuals (personal) and/or larger areas (e.g. spatial repellents [82]) from mosquito bites. Several repellents have been developed either synthetically or extracted from plants [82, 158, 159]. DEET (N,N-diethyl-1,3 methylbenzamide) is probably the most widely used synthetic insecticide; and has been shown to significantly reduce the number of malaria vector bites received by users for up to 8 hours [82]. An advantage of repellents is there potential to protect people at times and places where they are not using LLINs.

1.5.2.5 Genetically modified mosquitoes

Genetically modified mosquitoes (GMM) can be used for vector control by either suppressing populations, or replacing the population which one that cannot transmit malaria [160]. In GMM strategies aiming to suppress vector populations, the proposed strategy involves releasing modified males that will mate with wild females and cause them to produce unviable progeny [161]. Alternatively, the population replacement strategy aims to release vectors that have been modified vector to possess a trait that makes vectors refractory to malaria infection [162, 163]. These methods are still in early

stages of development, with as yet no field release trials being conducted in Africa [162].

1.6. Insecticide resistance

1.6.1 Definition and generality

Insecticides used for controlling pests both in agriculture and public health are grouped into four classes including pyrethroids, organochlorides, carbamates and organophosphates. They act according to two main modes of action; either by targeting the nervous system voltage gate sodium channel (VGSC) (Organochlorate and Pyrethroid; [164]) or acetylcholinesterase (carbamate and organophosphate, [165]). The only classes of insecticides recommended for use on LLINs are the pyrethroids and Pyrroles [89], based on their lower mammalian toxicity. Carbamates, Neonicotinoid and Organophosphates are additionally recommended for IRS [89, 116]. The widespread use of these chemicals in both agriculture [166, 167] and public health [168, 169] has generated widespread insecticide resistance (IR) in Africa [170-172].
Insecticide resistance is defined as the capability of a target insect population to survive a known dose of a given toxin [170, 173]. Based on the WHO definition, a malaria vector population is defined as phenotypically resistant if less than 90% are killed in the 24-hour period following exposure to a discriminating dose [174, 175].

Since DDT resistance was first reported in 1949 in California, [176]; insecticide resistance has been an increasing concern for malaria vector control [177]. Data retrieved from the IR mapper database in 2015 show there a huge decrease in the 24-hour mortality of *An. gambiae* after exposure to a decimating dose (DD) of pyrethroids in many African settings since 1995 [177]. Data indicate that there has been a rapid spread of IR throughout SSA in the last 10-20 years (e.g (http://irmapperjavascriptwcfservice.cloudapp.net/ , Figure 1.4).

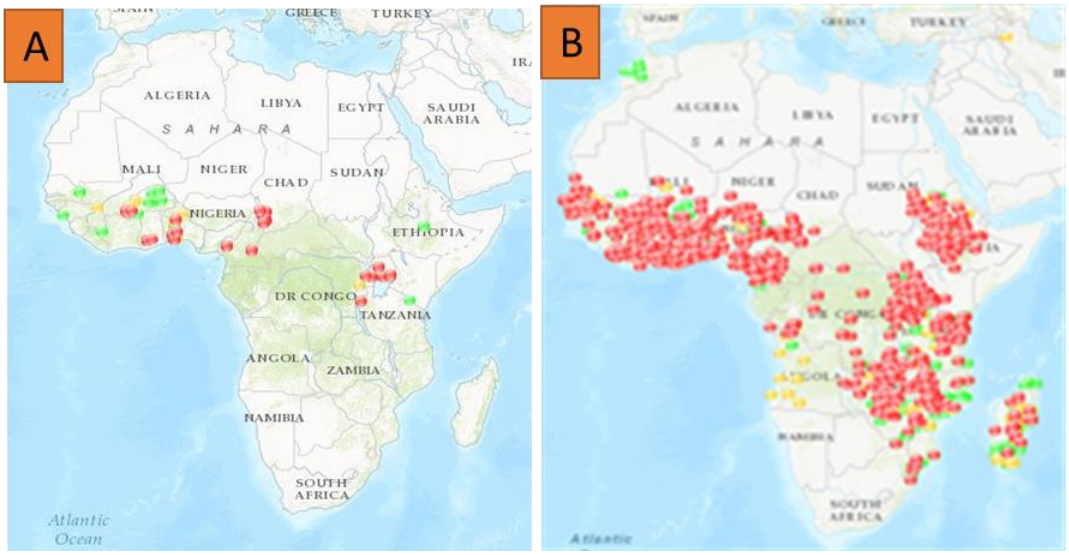


Figure 1.4: Pyrethroid resistance in *Anopheles sp* in **A)** 2005 - 2006 and **B)** 2018 – 2019. Red dots show resistant population with mortality < 90%, yellow dots show mortality between 90% and 98% (for these population there is need of confirmation) and green dots show susceptible population (mortality >98%). Reproduced from IR Mapper.

As will be discussed in Chapter 4, IR is hypothesized to be jeopardizing the effectiveness of malaria vector control based on LLINs and IRS. However, the epidemiological impact of IR in terms of its abillity to reduce the impact of interventions has yet to be established (further discussed in Chapter 4).

1.6.2 Mechanisms of insecticide resistance

1.6.2.1 Metabolic resistance

Metabolic resistance manifests as an increase in insecticide metabolization or degradation, leading to a decrease in the amount of insecticide available before its toxicity is expressed [170, 173]. Modification in coding sequences, gene overexpression and amplification are markers of this resistance mechanism [92, 126, 128]. There are a number of enzyme families involved in this mechanism, including carboxylesterases, cytochrome P450 monooxygenases (cytochrome P450s), and glutathione transferases (GSTs) [178, 179]. In 2015, it was demonstrated that detoxification genes are overexpressed within insecticide resistance populations of Anopheles in Burkina Faso [69]. There is some evidence that the ability of vector populations to mount a metabolic resistance response can be reversed

through pre-exposure to chemical synergists such as piperonyl butoxide (PBO), which inhibits the production of esterases involved in detoxification [126]. Trials in Cote d'Ivoire indicate that pre-exposing a highly resistant population of *An. coluzzii* led to an increase in their mortality following later exposure to bendiocarb (from 12.4 to 80%) [126]. Subsequently, LLINs have been developed that are impregnated with bothy pyrethroids and PBOs, with early studies indicating these combination nets have increased efficacy in areas of high IR [180, 181]. Although this has yet to be demonstrated in the field [181], WHO has prequalified next generation nets including pyrethroid-PBO and interceptor G2 nets [89] for use in areas of high IR.

1.6.2.2 Target site mutation

Another method through which malaria vectors become resistant to insecticides is target site mutation. Insecticides such pyrethroid and DDT act by blocking the insect sodium channel from closing, leading to the insect death [173]. Target site mutations are defined as non-silent modifications occurring in a given insecticide target site amino acid sequence [182] that prevent insecticide molecules from binding to such target sites, thus blocking their function [182]. Some described mutations in arthropods have indirect impacts on the kinetics of the voltage gate sodium channel (VGSC) [183] including a substitution of leucine in codon 1014 by either Phenylalanine [184] named L1014F or serine [185] called L1014S.

Target site mutations have been described in malaria vector populations in many African countries (e.g. [186-189]. The L1014F and L1014S mutations also known respectively as "knock down resistance" markers (west (Kdr-w; [184] and east Kdr-e [185]) are widespread in *An. gambiae* complex throughout Burkina Faso [190]. Mosquitoes with mutations are the most resistant to insecticides [186, 187].

1.6.2.3 Cuticular resistance

Cuticular resistance is defined as a reduction or delay in insecticide uptake [191], which reduces the toxic impacts on insect metabolism. This type of

resistance occurs through an alteration in their cuticle structure and composition [173, 192, 193]. Overexpression of genes encoding for putative cuticular proteins have been associated with decreased insecticide uptake in resistant bed bugs [194]. Moreover, microarray analysis of *An. gambiae* and *An. arabiensis* in West Africa indicated that putative genes encoding for both cuticular proteins and fatty acids are overexpressed in resistant mosquitoes [195, 196]. DDT- resistant strains of *Drosophila melanogaster* have a high concentration of some cuticular hydrocarbons which are associated with reduced insecticide penetration [192]; with a similar effect also being confirmed in resistant *An. gambiae* strains [197, 198]. At present, the frequency of this type of resistance in wild vector populations and its impact on control measures is unknown [199].

1.6.2.4 Behavioural resistance

Behaviour resistance is defined as ability of mosquitoes to avoid contact with insecticide treated surfaces or environments through their behaviour [173, 200]. At present, most insecticides for use in malaria vector control are based on application indoors (either on a bed net or walls of a house), targeting mosquitoes that prefer to feed on humans indoors, during sleeping hours (e.g. LLIN; [107, 201]). Thus, the key mosquito behaviours that underpin successful vector control are anthropophily (preference for humans), indoor feeding and resting, and biting during night time hours [107, 201]. Changes in any one of these behaviours would be expected to reduce vector contact with common control methods such as LLINs or IRS [202]. As will be discussed in Chapter 4, there is debate about whether mosquito vectors are shifting their behaviour in response to these type of control methods [203, 204] and whether any changes are due to evolutionary selection or phenotypic plasticity. There is evidence that mosquito behaviours such as host choice have some genetic basis [205]. Additionally, behaviours such as the location of biting and resting by malaria vectors have been associated with environmental factors such as the presence of cattle, use of insecticides and other climatic factors [64, 206, 207]. Behavioural

avoidance strategies could be either through evolutionary processes or phenotypic plasticity [208-210] as described in Chapter 3.

1.7. Rationale of the study Area

Burkina Faso is a landlocked country located in West Africa with an estimated population size of 19,034,397 inhabitants in 2016 [211]. It has three climatic zones: the Sahel, the Sudan-Sahel and the Sudan-Guinea zone (Figure 1. 5). The country is characterised by two main seasons: a rainy/wet season, generally lasting four to five months (from June to October), and a dry season [212, 213]. The rainfall in the Sudan-Guinea zone located in the southern part of the country (Figure 1.5) reaches 900mm a year, whereas it is less than 600mm in the Sahel zone situated in the northern part [214]. Furthermore, the district of Banfora (where the study is taking place) is part of the Cascades region (CR) and is in the South West in the humid climatic zone, about 450 km from the capital Ouagadougou (Figure 1.5 & 1.7). The population size of the region was estimated to 739,497 inhabitants in 2015 [211].

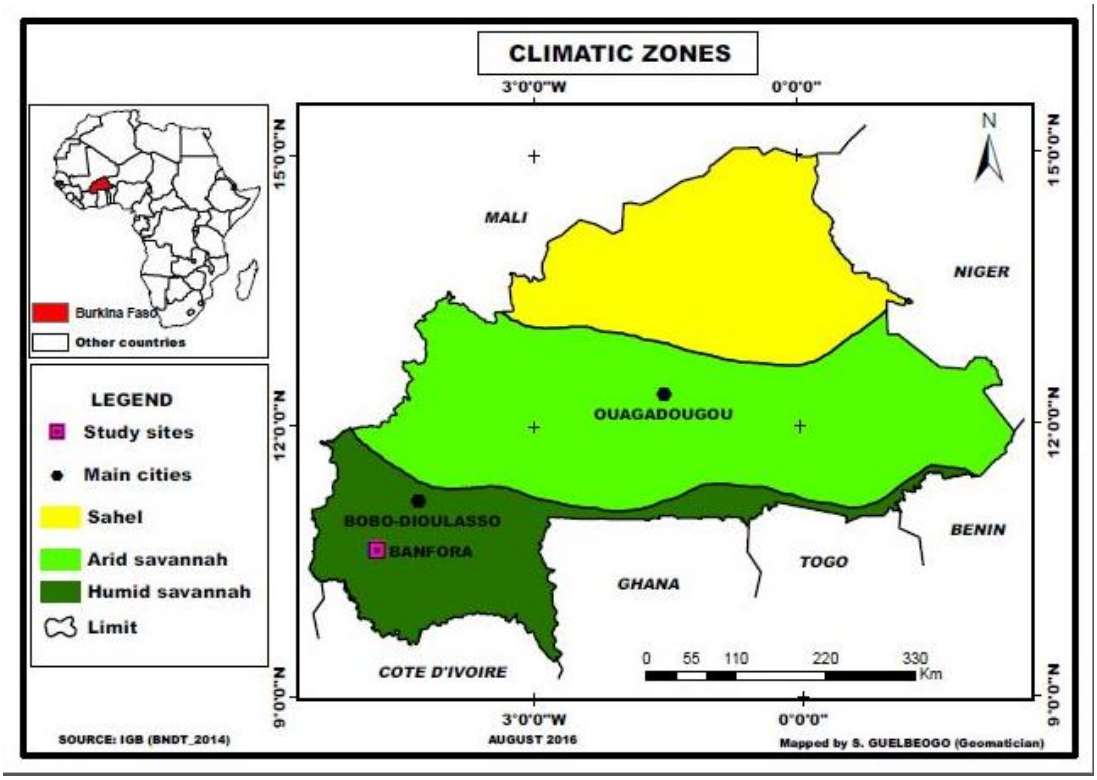


Figure 1.5: Map showing the three climatic zones of Burkina Faso, the main cities and the study site Banfora (Source: produced by Guelbéogo on my request).

As a malaria endemic country, transmission is seasonal, and the incidence varies mainly according to climatic zones and the seasons. Also, transmission is hyperendemic, varying from low in the dry season (October to May) to high in the rainy season (June to September).

Overall the number of malaria cases in 2018 was ~12 million with ~50% of the cases occurring in children under 5 years old, and 4 292 deaths according to the National Malaria Control Programme (Figure 1. 6; [115, 211, 215]). Despite the scale up in LLINs distribution, malaria control has little impact on the malaria incidence [212]. Compared to other SSA countries, Burkina Faso has one of the highest malaria prevalence rates [214], and remains one of the countries with high malaria burden [13, 117, 216]. Unlike some neighbouring countries, malaria cases do not appear to be decreasing and may be increasing in Burkina Faso despites several campaigns of LLINs distribution (Figure 1.6).

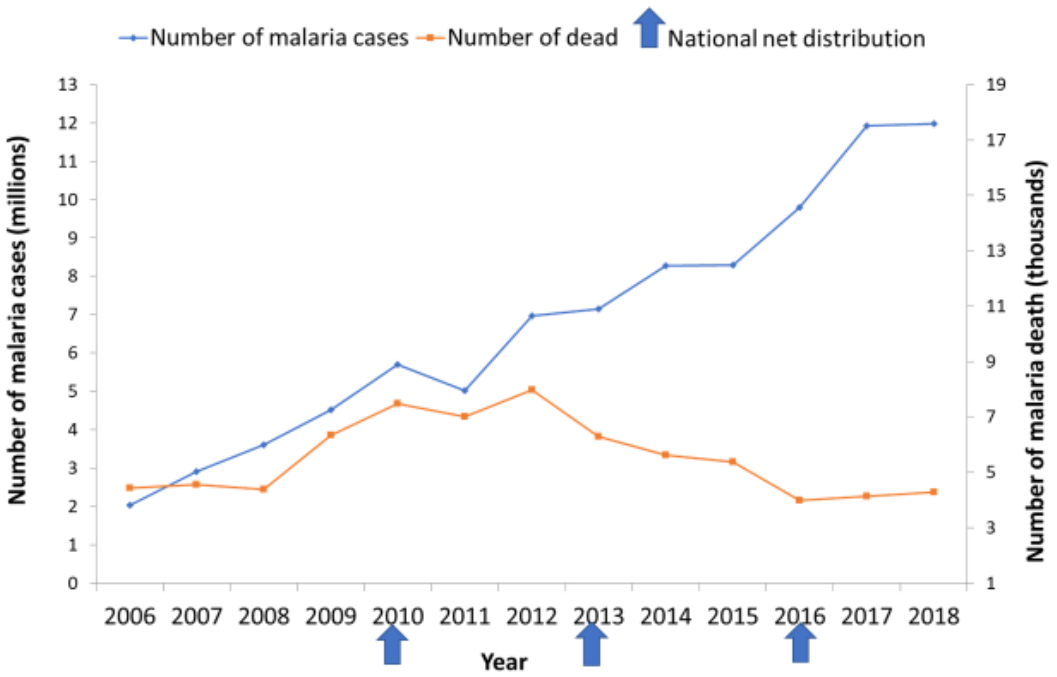


Figure 1.6: Reported number of malaria cases (blue line) and related deaths (orange line) between 2006 and 2018 in Burkina Faso (National Malaria Control Programme, unpublished data)

Although Burkina Faso is considered a high burden country, there is substantial variation in malaria cases within the country. The District of Banfora where this study was carried out, has a high numbers of reported cases [217, 218]. Banfora District is within the Cascades region of southwestern Burka Faso.

Insecticide resistance is hypothesized as the leading cause of the ineffectiveness of LLINs in Burkina Faso. Recent estimates suggest insecticide resistance is widespread [219, 220]; with levels of IR in southwestern Burkina Faso being exceptionally high (>80% surviving 24 hours after exposure to pyrethroids and DDT; [221]). It is also possible that the effectiveness of LLINs in Burkina Faso is being hampered by behavioural resilience and avoidance in vector populations. So far, there has been little investigation of behavioural resilience and avoidance in malaria vectors in Burkina Faso [222-224]. Although IR has been widely investigated in Burkina Faso [69, 219, 220, 225-227], the few studies that have investigated vector behaviour are patchy in time and space [61, 222, 223, 228-230]. Therefore, there is a need to undertake a comprehensive study of behaviour and ecology

of vector populations in Burkina Faso to assess the relative contribution of IR and behavioural resilience/avoidance to the decreasing effectiveness of LLINs and other control approaches.

However, to understand and measure behavioural variation in vector population, there is a need for reliable tools to characterise these behaviours. The only reliable approach for measuring human exposure to mosquito bites indoors and outdoors is the Human Landing Catches (HLC) [231]. For the collection using HLC, a human volunteer acting as bait catch host seeking mosquitoes using an aspirator and flash torch [232]. Given the growing recognition of the importance of outdoor biting to malaria transmission [233, 234] [234, 235] and the exposure of volunteers to vector-borne diseases [235] many attempts have been made to develop alternative "exposure-free" methods to HLC [236, 237]. Recently, a "Mosquito Electrocuting Trap" (MET) has been developed [238] and tested in Tanzania with the aim of providing an exposure-free method sampling of mosquito biting activity [238-240]. This trap has not yet been evaluated in West Africa, but if similarly, successful, would significantly enhance ability and offer more options to safely measure behaviour of IR vectors.

1.8. Aims and objectives

1.8.1 Aim

My PhD research study was part of an interdisciplinary project entitled "Improving the efficacy of malaria prevention in an insecticide-resistant Africa (MIRA)". The overall goal was to characterize the ecology, behaviour, insecticide resistance and transmission potential of malaria vectors in southwestern Burkina Faso; and assess the relative contribution of IR and behavioural avoidance to control failure. Specific aims were to investigate spatial and temporal heterogeneity in resistance and vector behaviours at twelve sentinel villages (Figure 1.7) in the Cascades regions of south-western Burkina Faso over a 2-year period following the 2016 mass LLIN distribution. Further details of the study area are provided in Chapter 2.

1.8.2 Objectives

Specific objectives were to:

1) Evaluate a new mosquito sampling method, the Mosquito Electrocuting Trap, as a safer alternative to the gold standard "Human Landing Catch" approach for measuring human exposure to malaria vectors (Chapter 2)

2) Test for spatial, seasonal and longer-term changes in the vector host seeking and resting behaviour following LLIN distribution (Chapter 3)

3) Assess spatial and temporal changes in insecticide resistance following LLIN distribution (Chapter 4)

4) Assess spatial and temporal changes in malaria vector survival and transmission intensity following LLIN distribution (Chapter 5)

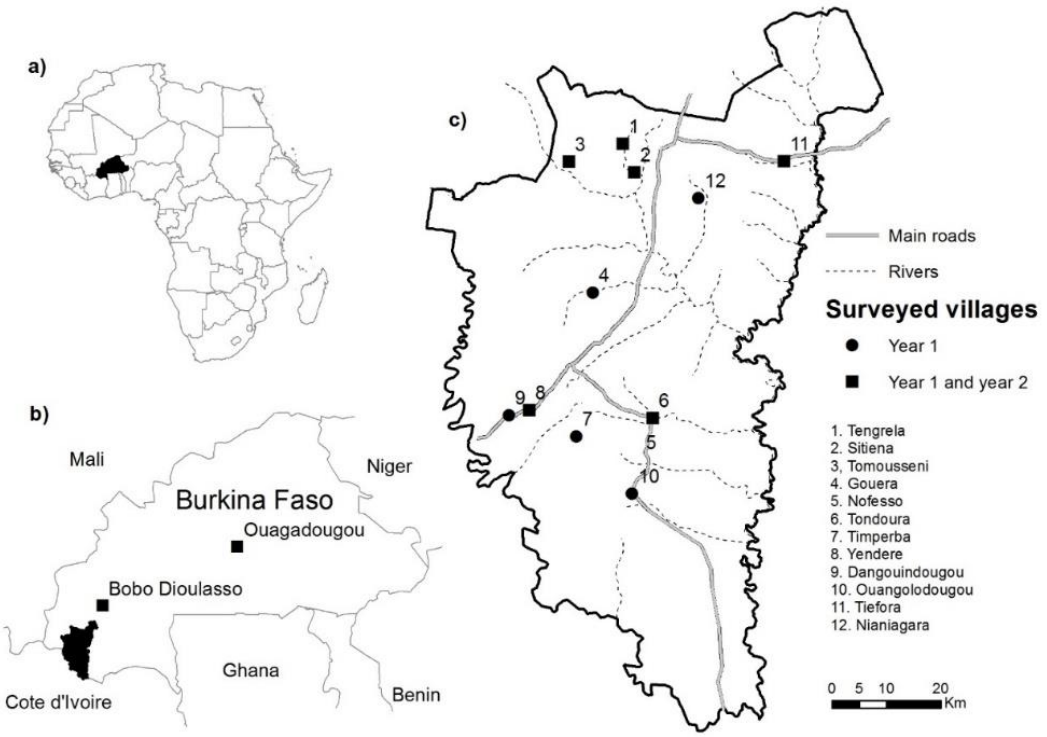


Figure 1.7: Map of the 12 study villages. a) location of Burkina Faso within Africa, b) study area in the Cascades Region in Burkina Faso, and c) villages where mosquito collection took place. Circles represent the villages sampled for 18 months and squares represent the villages that were part of the longer-term study site and where sampling was extended to 26 months.

1.9. Ethical approval and consent to participate

This study involved several procedures involving human subjects including the collection of mosquitoes using Human Landing Catches and Mosquito Electrocuting Traps and administering of questionnaires. To this extent, ethical clearance was obtained from the Ethical Committee for research in Health of the Ministry of Health of Burkina Faso (EC V3.0_CERS N°2016-09-097) and the Institutional Bioethical Committee of the local research institution (National Malaria Research and Training Centre, CNRFP) under EC V3.0_ N°2016-026/MS/SG/CNRFP/CIB) and the Liverpool School of Tropical Medicine (Certificate 16-038). Prior to starting the research, the project aims, and objectives were explained to community leaders in each village. Signed informed consent was also obtained from all household owners where mosquitoes were collected, and from volunteers who took part in mosquito collections by HLC and MET.

Chapter 2: Evaluation of mosquito electrocuting traps as a safe alternative to the human landing catch for measuring human exposure to malaria vectors in Burkina Faso.

NOTE: This chapter formed the basis for a paper published in Malaria Journal (Appendix 3)

Abstract

Background

Measuring human exposure to mosquito bites is a crucial component of vector-borne disease surveillance. For malaria vectors, the Human Landing Catch (HLC) remains the gold standard for direct estimation of exposure. This method, however, is controversial since participants risk exposure to potentially infected mosquito bites. Recently an exposure-free Mosquito Electrocuting Trap (MET) was developed to provide a safer alternative to the HLC. Early prototypes of the MET performed well in Tanzania but have yet to be tested in West Africa, where malaria vector species composition, ecology and behaviour are different. Here the performance of the MET was evaluated relative to HLC for characterizing mosquito vector population dynamics and biting behaviour in Burkina Faso.

Methods

A longitudinal study was initiated within 12 villages in Burkina Faso in October 2016. Host-seeking mosquitoes were sampled monthly using HLC and MET collections over 14 months. Collections were made at four households on each night, with METs deployed inside and outside at two houses, and HLC inside and outside at another two. Malaria vector abundance, species composition, sporozoite rate and location of biting (indoor *versus* outdoor) were recorded.

Results

In total, 41,800 mosquitoes were collected over 324 sampling nights, with the major malaria vector being *Anopheles gambiae sensu lato* (s.l.). Overall the MET caught fewer *An. gambiae* s.l. than the HLC (mean predicted number of *An. gambiae* s.l. per trap and per night: 0.78 *versus* 1.82 indoors,

and 1.05 *versus* 2.04 outdoors). However, MET collections gave a consistent representation of seasonal dynamics in vector populations, species composition, biting behaviour (location and time) and malaria infection rates relative to HLC. As the relative performance of the MET was somewhat higher in outdoor *versus* indoor settings, this trapping method slightly underestimated the proportion of bites preventable by LLINs compared to the HLC (MET = 82.08%; HLC = 87.19%).

Conclusions

The MET collected proportionately fewer mosquitoes than the HLC. However, estimates of *An. gambiae* s.l. density in METs were highly correlated with HLC. Thus, although less sensitive, the MET is a safer alternative than the HLC. Its use is recommended particularly for sampling vectors in outdoor environments where there are few validated alternatives to the HLC.

2.1. Background

Measurement of malaria transmission and evaluation of vector control requires estimation of human exposure to malaria-infected mosquitoes [241]. This exposure is often estimated in terms of the Entomological Inoculation Rate (EIR [242]) defined as the mean number of malaria-infected mosquito bites a person would be expected to receive in a given time period [241, 243]. Accurate estimation of exposure to mosquito bites is crucial for evaluating interventions, thus there is an urgent need for reliable and robust methods to give unbiased estimates of exposure in a range of settings [243]. Several methods have been used to measure mosquito host-seeking behaviour and human exposure to mosquitoes. Historically, the Human Landing Catch (HLC) has been the most commonly used method for African malaria vectors, and is considered a gold standard approach for direct measurement of human-mosquito contact in both indoors and outdoors settings [232]. In this method, human volunteers expose part of their body, usually the lower legs, to lure host-seeking mosquitoes that are then collected upon landing [232].

Although the HLC provides a direct measurement of human exposure to bites, its estimates can be biased due to variation in the skill of mosquito collectors and their attractiveness to mosquitoes [231, 244-246]. The HLC also raise ethical concerns as collectors are exposed to potentially infectious mosquito bites [247]. While this risk can be minimized by providing malaria prophylaxis to collectors, protection cannot be guaranteed in areas of drug resistance or where mosquitoes are carrying other pathogens, such as arboviruses [235, 237]. One African study indicated that HLC participants had no increased risk of malaria [248], but there remains a concern about disease exposure in areas where other mosquito-borne pathogens are circulating.

Due to these limitations of the HLC, a range of alternative “exposure-free” methods have been developed. Most common is the CDC light trap [232, 249-251], a trap that can be placed next to a person sleeping under a bed-net

and used to collect mosquitoes that would have otherwise have fed on them [250]. Although effective and easy to use in indoor environments [66], this method is harder to implement outdoors and may not accurately reflect human exposure in this setting [66, 239, 252]. Furthermore, CDC light catches can be affected by variation in light intensity [253, 254] and colour [66]. Other "exposure-free" methods include the human-baited double net trap (HDN) [252], Suna Trap [255], Host Decoy Trap (HDT; [256]), Ifakara tent trap design C (ITT-C) [236]and the Mbita trap [237]. Of these, the last two have the same limitation as the CDC light trap of not being suitable or representative for measuring exposure in outdoor environments. For example, the tent trap only samples mosquitoes that are capable of entering a small enclosed structure, therefore, disproportionately catch indoor biting mosquito species [257]. The HDN was as efficient as the HLC in collecting outdoor anthropophilic mosquitoes. However, like the Tent Trap, it may also be selectively biased towards indoor biting mosquitoes, or sample vectors that enter the net to rest instead of biting [252, 258]. Similarly the Mbita trap had poor performance relative to the HLC in a setting where most vectors were exophilic and zoophilic [259]. Both the SUNA and Host Decoy Trap have shown promise for sampling outdoor biting malaria vectors [255, 256]; although may under [260] or overestimate [256] human exposure relative to the HLC. Given the growing recognition of outdoor biting as a major source of residual transmission in Africa [233, 234, 261] there is a clear need for improved methods that can reliably and safely measure exposure outside of homes.

The Mosquito Electrocuting Trap (MET) has been developed as a safer alternative method to the HLC for measuring human exposure to mosquito vectors both indoors and outdoors [238-240]. As previously described [238], the MET builds on previous work using electrified nets and grids to trap flies [262, 263] and mosquitoes [264-268] attracted to hosts or their odours. This trap consists of four panels that can be assembled into a box around the lower legs of seated human [238, 239] (Appendix 1), or an entire host (human or cow) [240]. Each panel consists of an electrified surface that allows free air movement and is safe to use in close proximity to a human volunteer,

and intercepts and kills mosquitoes just before they land on hosts. An advantage of this method is that in addition to protecting participants from mosquito bites, it can be used in a standardized way in both indoor and outdoor environments. This method has shown promise as alternative to the HLC for sampling malaria vectors in Tanzania [238-240]. For instance, the first prototype achieved a sampling efficiency of ~60% relative to the HLC for sampling *Anopheles arabiensis* outdoors in rural Tanzania, falling to 20% when used indoors [238]. Further study on an improved prototype carried out in an urban area indicated the MET had a similar performance to the HLC [239]. A recent study evaluated a further prototype of the MET in which the electrified trapping panels were expanded to encompass the whole body of a human volunteer or calf [240], with the performance of the MET exceeding that of the HLC. The MET has not been tested outside of Tanzania yet, thus its effectiveness in different ecological settings is unknown. There is a need to evaluate the MET in west African settings where vector species composition, ecology and biting behaviour is often markedly different from East Africa and evaluate how its performance varies between sites and seasons.

This study aimed to evaluate the performance of the MET relative to the HLC in a longitudinal study in south-western Burkina Faso. Sampling was conducted over a 14-month period in 12 villages, where malaria vector abundance and species composition are known to vary considerably between seasons and sites (Chapter 3). Aims were to test the performance of the MET relative to the HLC for estimating vector abundance, and location of biting (indoor *vs* outdoor) i) over the study period, ii) over the course of the night, and iii) in relation to mosquito density. Additional aims were to compare estimates of mosquito vector species composition and infection rates between HLC and MET collections and assess if they produce comparable estimates of exposure to *Anopheles gambiae sensu lato* (s.l.) based on human behaviour.

2.2. Methods

2.2.1 Study site

This study took place in 12 villages within the Cascades Region of south-western Burkina Faso (Figure 1. 7), where mosquito sampling was conducted over 14 months between October 2016 and December 2017. Residents of these villages live within compounds consisting of one or more households. Most residents are subsistence farmers whose primary crops are cereals, vegetables, rice and cotton. Domestic animals including dogs, cattle, sheep, goats, pigs, donkey and poultry are usually kept within compounds. The area has two distinct seasons: a rainy season (May to October) and a dry season (from November to April) [212, 214]. Annual rainfall in the area ranges from 600-900 mm, with a mean temperature of 26.78 °C (range: 15.7 °C - 38.84 °C) and mean humidity of 61.89 % (range: 15.11 - 99.95%) during the study period. *Anopheles gambiae s.l.* is the most abundant malaria (> 90%) vector in this area [217, 269].

2.2.2. Trapping methods

Mosquitoes were collected using HLCs [270] and METs [238]. The MET used was an improved prototype of the version used previously [238, 239]. In brief, it consists of four 50 cm x 50 cm grid panels that can be assembled into a square with the bottom and top open. Panels are made from polyvinyl chloride (PVC) frames. Stainless steel wires (1.2 mm thick) were embedded to run from the top to bottom of each frame at a spacing of 5 mm (Appendix 1 and 2). Adjacent wires were differentially charged as negative or positive, such that an insect would be shocked on contact with both. The assembled grid panels were connected to a power supply sourced by two 12-volt batteries in series (Appendix 2). A protective shield made from PVC was fitted into the interior side of each panel to prevent any accidental contact between users and the electrified surface.

2.2.3. Experimental design

Across the study period (Oct 2016 - Dec 2017), adult mosquitoes were collected twice a month in each of the 12 villages with the occasional breaks for holidays and team training. Additionally, only one night of sampling was conducted in each village during the first month. This resulted in mosquitoes being sampled from 4 households at each village for approximately 14 months. The same group of four households was sampled on 2 nights each month; with a different group of households being selected the following month to maximize the spatial coverage of sampling within villages. There was a minimum distance of 30 m between houses sampled on the same night. This culminated in a total of 672 households being sampled over 14 months. Collections were made both inside houses and, in the peri-domestic area (within 8-10 m of the house). Indoor collections were usually conducted in the sitting rooms of houses or in single-room houses.

2.2.4. Mosquito collection

On each night, host-seeking mosquitoes were collected using the HLC and MET. On the first night of sampling during each 2-day period, two houses were randomly allocated for collections with HLC and two others with METs. On the second night, these methods were rotated between households in a cross-over design. Participants involved in mosquito collections also rotated between indoor and outdoor trapping stations each hour to avoid confounding location with individual differences in attractiveness to mosquitoes.

When collecting mosquitoes by HLC, the volunteers sat on a chair with their legs exposed up to the knees. Mosquitoes landing on their legs were sucked into pre-labelled papers cups using a mouth aspirator and a torch (Figure 2.1A). For MET sampling, volunteers sat on a chair with their legs up to their knees placed inside the trap (Figure 2.1B-C), while the remaining part of their body was protected from mosquito bites using protective clothing (first 6 months, Fig. 2.1B) or a netting screen (from April 2017, Figure 2..1C). The METs were placed on top of a plastic mat, which was covered with a white

cloth to make it easier to see electrocuted mosquitoes that fell off the trap and onto the ground.

Each night, the HLC and MET collections were run from 7 pm to 6 am, with participants conducting trapping for 45 minutes of each hour followed by a 15-minute rest break. During the break period, the MET was switched off and technicians collected mosquitoes trapped on the outer surface and those that had fallen on the white cloth using tweezers. All mosquitoes collected using METs were stored in pre-labelled Petri dishes while those collected by HLC were transferred into paper cups labelled to identify the household and trapping location (indoors or outside, trap type and collection hour).

Overall mosquitoes were sampled on 324 nights in the 14 months of data collection, culminating in a total of 1296 HLC collections. According to the experimental design, a similar number of HLC and MET collections should have been performed. However, due to problems with the functioning of METs and heavy rainfall on some nights which caused battery problems and short circuiting; only 1080 MET collections (outdoor = 531, indoor = 549) were conducted.

Figure 2.1: A) A volunteer collecting mosquitoes landed on his leg using the human landing catch (HLC) method. **B, C)** Volunteers using mosquito electrocuting traps (METs).

2.2.5. Mosquito processing

Cups containing mosquitoes collected by HLC were placed into a cool box. Cotton pads soaked in a 10% sugar solution were placed on top of collection cups to feed any survivors and transferred to the laboratory. Once in the laboratory, mosquitoes were killed by putting them in a freezer, then sorted to species complex level using morphological keys [271] and stored in labelled 1.5 mL Eppendorf tubes containing silica gel. A subsample of 3199

females (36.3% of total) morphologically identified as *An. gambiae* s.l., were selected to provide a representative sample from each month, village, trapping location (indoor *vs* outdoor) and method (HLC, MET). The subsampling strategy was guided by consideration of the minimum sample size likely to be required to detect malaria infection in one unique mosquito collection (e.g permutation of night, trapping method and location). Based on baseline data collected in nearby areas [217], the average *Plasmodium falciparum* sporozoite infection rate in *An. gambiae* s.l. was estimated as ~5% [217]. Assuming rates are similar in our study area, we would need to test a minimum of 40 females from each group of interest to have a chance of detecting two infected individuals. On this basis, we proposed to subsample ~40 female *An. gambiae* s.l. from each trapping method (HLC and MET) and location (indoors and outdoors) on each night of sampling both for testing for *P. falciparum*. By aiming to analyze a roughly similar number of individuals, the relative precision with which this proportion could be estimated was standardized across collections. It was possible to achieve this sample size in the rainy season of 2016 (October) and 2017 (June to October) when mosquito densities were high, but not always during the dry season (November 2016 to May 2017) when densities were much lower. Consequently, all samples were subjected to molecular analysis when this number was lower than forty (the mean number analyzed per collection in these dry months was ~13). Legs from individual mosquitoes from this subsample were analysed by PCR analysis to confirm their species following [272]. Likewise the head and thorax of the same specimens were tested for *Plasmodium falciparum* sporozoite infection using the Enzyme-Linked Immuno-Sorbent Assay (ELISA, [273]).

2.2.6. Environmental and human behaviour data collection

During the mosquito collection, temperature (°C) and humidity (%) were recorded using Tiny Tag data loggers (Tiny Tag application Explorer 4.9) at each trapping location. Additionally, the time at which residents from the houses where the sampling was taking place went into of their houses at night and came in the morning was also recorded alongside the mosquito

collection. These data were used to calculate P_{fl} = proportion of mosquito bites occurring when most people are inside their dwellings and likely asleep and π_i = the proportion of exposure to malaria transmission that occurs indoors and could be prevented using LLINs. These exposure metrics are calculated on the assumption that the time spent indoors at night reflects the time spent sleeping where you can be protected from mosquito bites by a LLIN. The research team were present over all hours of the night at the 672 households where mosquitoes were collected. Each hour, the team recorded whether residents were outdoors (= 0) or inside. The values given here represent the raw proportion of residents observed to be inside their house pooling across all households (672 households representing 12 villages, from October 2016 to December 2018). Questionnaires data indicated that there were ~6186 residents in total at the 672 households surveyed for mosquito collections. From these data approximately 50% (including adults and kids) were indoors by 10 pm, with 50% or more leaving their homes in the morning after 5 am (Figure 2.2). The solid black line indicates the proportion of people observed to be inside their house at different times of the night. The red dashed line indicates when 50% of the surveyed population were observed to be indoors and thus had the potential to be protected by LLINs.

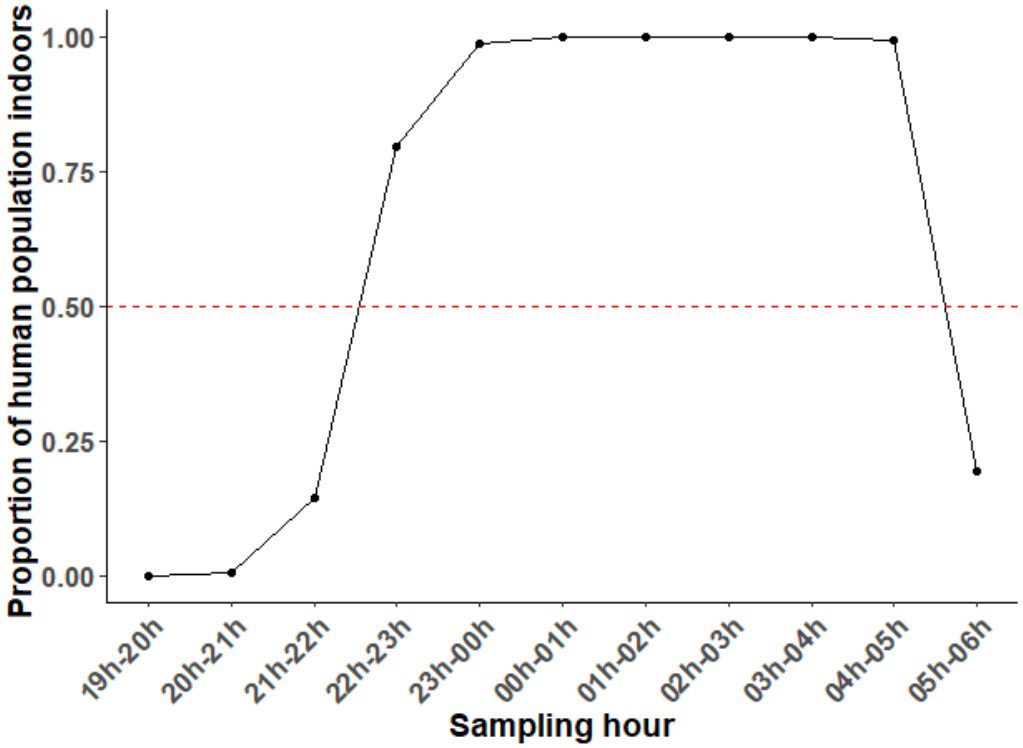


Figure 2.2*:* Graphs indicating the proportion of residents (at households where mosquitoes were being collected) that were observed to be inside their houses during different hours of the night.

2.2.7. Statistical analysis

Analysis was conducted to test for (i) variation in mosquito abundance between traps (per night, per hour and across the study period), (ii) density dependence in the performance of the MET relative to the HLC (iii) variation in malaria vector species composition between trapping methods (defined by the proportion of *Anopheles coluzzii* within the *An. gambiae* complex), and (iv) variation in *An. gambiae* s.l. sporozoite infection rate between traps. Additionally,(v) estimates of hourly and location-dependent (indoor *vs* out) vector densities were used to calculate and compare three key metrics of human exposure to bites as described below [274-276]. Generalised Linear Mixed Effect Models (GLMMs) were constructed within the R statistical software version 3.5.0 (2018-04-23) [277] augmented with the lme4 packages for analysis [278], with the exception of the analysis for density dependence across the study period as described below.

The relative efficiency of the MET compared to the HLC was assessed in terms of the number of *An. gambiae* s.l. caught per night. Mosquito abundance data were highly over-dispersed so they were modelled using a negative binomial distribution [279]. Initially, trapping method and its interaction with village and trap location were included in the maximum model of *An. gambiae s.l.* abundance along with other covariates (Model 2.1, Table 2.1) to allow testing of whether trap performance varied between sites and trap location. Additionally, variation between trapping methods was also assessed in relation to mean temperature and humidity; by including interactions between method and these environmental variables (Model 2.1, Table 2.1).

Variation in the relative efficiency of MET compared to the HLC was assessed separately for outdoor and indoor collections using Generalized Additive Models (GAM) with a negative binomial distribution [280]. This package allowed estimation of a nonparametric function to capture non-linear seasonal dynamics by using a smoothing spline on week which assigned all sampling weeks to an annual scale running from "1" (first week in January) – "52" (last week in December). In the full model, the response variable was the number of *An. gambiae* s.l. caught per night whilst the explanatory fixed effect variables were trapping method and its interaction with the temporal smoothing spline. To assess whether the interaction was significant in each location (indoor and outdoor), the model with interactions was compared to the basic model without interaction using the Akaike Information Criteria (AIC). Here, no random effect was included as the primary aim was to assess seasonally dependent trap performance.

In addition, to test whether the relative performance of the MET compared to HLC changed over the course of night, a model was constructed with the response variable of the proportion of *An. gambiae* s.l. caught in METs in each hour of sampling out of the total in MET and HLC combined (Model 2.2, Table 2.1). Here sampling "nHour" was defined as a continuous variable where "1" corresponded to the first hour of collection (7 pm to 8 pm) and "11" the last hour (5 am to 6 am).

Density dependence in MET performance was assessed by testing for linearity between *An. gambiae* s.l. catches in the MET and HLC following the method described in [239] using Markov Chain Monte Carlo (MCMC) in the programme Jags [281, 282]. Here the response variable was the number of *An. gambiae s.l.* collected using the MET and the explanatory variable the number collected using HLC.

To investigate variation in species composition and malaria infection rates as estimated by different trapping methods, analysis was conducted on the subset of *An. gambiae* s.l. (n = 3199) that were individually identified to species level. In the analysis related to species composition the response variable was the proportion of *An. coluzzii* in the *An. gambiae* s.l. catch per collection, with explanatory variables for trapping method, location, temperature and humidity (Model 2.3, Table 2.1). A similar model was constructed to analyse variation in the sporozoite rate of *An. gambiae* s.l. with the explanatory variables being mosquito species, trapping method, the interaction between species and location, village, temperature and humidity (Model 2.4, Table 2.1). It was not possible to include analysis of seasonality in these models because of sample sizes of mosquitoes in the dry season at some of the villages were too low. Both data on % *An. coluzzii* and infection rate were modelled using a binomial distribution.

Finally, data on the time and location of biting (indoors *vs* outside houses) were used to estimate three standard epidemiological parameters of relevance for estimating human exposure to mosquito bites and the impact of LLINs [276, 283]. These are defined as the (i) proportion of *An. gambiae* s.l. host-seeking indoors (P_i), (ii) proportion of mosquito bites occurring when most people are inside (time spent inside estimated based on observations, Figure 2.2) their dwellings and likely asleep (P_{fl}) and (iii) proportion of human exposure to *An. gambiae s.l.* bites occurring indoors (π_i). The π_i metric estimates the proportion of exposure to malaria transmission that occurs indoors and could be prevented using LLINs [276, 283]. These proportions were used as response variables in analyses that tested whether these exposure estimates varied between trapping methods

and in response to season, temperature and humidity (Model 2.5 – 2.7, Table 2.1).

In all the analysis, random effects were incorporated at the intercept to capture the baseline variability by day (Date), compound, household and village except for Model 2.1 (Table 2.1). For each variable of interest, model selection was conducted through a process of backward elimination starting from a maximal model (Table 2.1) in which Likelihood Ratio Tests (LRTs) were used to evaluate the significance of individual terms. Mean values and 95% confidence intervals for all statistically-significant effects in the minimum model ("best model") were obtained from the GLMMs using the effects package [284].

Table 2.1: Maximal models used for the modelling including the primary response variable, explanatory variables and statistical distribution used. ":" indicates an "interaction" Methods are MET: Mosquito Electrocuting trap and HLC: Human Landing Catch and location indicates indoors versus outdoors. The average temperature and relative humidity were obtained by averaging the records over the course of the collection night. Here locations are the collection points inside houses or outdoor while seasons are dry or wet seasons. nHour represents here hours as discrete variables from "1" being the first hour of collection (7 pm - 8 pm) to the last hour of collection of the night being "11" (5 am – 6 am). The season was defined here as categorical variable dry (November to April) or wet (May to October). P_i was calculated as the number of *An. gambiae* sl. caught indoors (I) divided by the total caught indoors (I) and outdoors (O) over a sampling night (7 pm- 6 am): $I_{7pm \rightarrow 6am}$ / ($I_{7pm \rightarrow 6am}$ + $O_{7pm \rightarrow 6am}$) (Govella et al., 2010, Russell et al., 2011). P_{fl} is the number of *An. gambiae* s.l. collected during hours when more than 50% of people are indoors and could be protected by LLINs, divided by the total caught over the entire night of sampling (in and out). This P_{fl} was calculated by dividing the total *An. gambiae* s.l. collected between 10 pm and 5 am indoors and outdoors ($I_{10pm->5am}$ + $O_{10pm->5am}$) by the total collected between 7pm and 6am ($I_{7pm->6am}$ + $O_{7pm->6am}$) (Govella et al., 2010, Russell et al., 2011). Values of π_i were computed as the proportion of total *An. gambiae* s.l. collected indoors during hours when people could be protected by an LLIN ($I_{10pm \rightarrow 5am}$) over itself and the total *An. gambiae* s.l. collected outside during non-sleeping hours ($I_{10pm \rightarrow 5am}$ + $O_{7pm \rightarrow 10pm, 5am \rightarrow 6am}$) (Govella et al., 2010). Here, "subset of *An. gambiae* s.l. "refers to subset that were individually identified to species levels and individually tested for sporozoite infection.

Model	Tests	Response variables	Fixed Effect variables	Random effect variables	Type of data	Distribu-tion
2.1	Trap efficiency	Abundance	Village + Method + Location + Season + Temperature + Humidity + Method: Village + Method: Location + Method: Season + Method: Temperature + Method: Humidity,	Date + Compound + Household	Nightly count *of An. gambiae* s.l.	Negative binomial

Model	Tests	Response variables	Fixed Effect variables	Random effect variables	Type of data	Distribu-tion
2.2	Proportion of MET collection across night	MET_ total/ (MET_ total + HLC_ Total)	nHour + Location + nHour: Location,	Date + Village	Hourly count *of An. gambiae* s.l.	Binomial
2.3	Proportion of *An. coluzzii*	(An. coluzzii/An. coluzzii + An. gambiae)	Village + Method + Location + Method: Village + Method: Location + Temperature + Humidity,	Date + Compound + Household	Subset of *An. gambiae* s.l. data	Binomial
2.4	Sporozoite infection rate	Positive/ (Positive + Negative)	Village + Method + Location + Method: Village + Method: Location + Species + Temperature+ Humidity,	Date + Compound + Household	Subset of *An. gambiae* s.l. data	Binomial
2.5	Proportion of indoor biting (P_i)	$I_{7pm \text{ -> } 6am}$ / ($I_{7pm \text{ -> } 6am}$ + $O_{7pm \text{ -> } 6am}$)	Method + Season + Method: Season + Temperature + Humidity,	Village + Date + Compound + Household	*An. gambiae* s.l. data	Binomial
2.6	Proportion of mosquito when people are indoor (P_{fl})	($I_{10pm->5am}$ + $O_{10pm->5am}$) / ($I_{7pm->6am}$ + $O_{7pm->6am}$)	Method + Season + Method: Season + Temperature + Humidity,	Village + Date + Compound + Household	*An. gambiae* s.l. data	Binomial

Model	Tests	Response variables	Fixed Effect variables	Random effect variables	Type of data	Distribu-tion
2.7	Human exposure to mosquito bite indoor (π_i)	$I_{10pm \text{ -> } 5am}$ / ($I_{10pm \text{ -> } 5am}$ + $O_{7pm \text{ -> } 10pm,\ 5am \text{ -> } 6am}$)	Method + Season + Method: Season + Temperature + Humidity,	Village + Date + Compound + Household	*An. gambiae* s.l. data	Binomial

2.3. Results

2.3.1. General results

A total of 41,800 mosquitoes were collected over 324 trapping nights, of which 41,395 were females (Table 2.2). Most of the female mosquitoes were anophelines (86.4%), with the remainder being culicines (Table 2.2). *Anopheles gambiae* s.l. represented 97.7% of all anophelines, (Table 2.2). Within the subset of *An. gambiae* s.l. individually analyzed to species level (n=3199, 36.3% of total), *An. gambiae* constituted 41.58%, *An. coluzzii* 58.17% and *An. arabiensis* 0.25%. No molecular identification of species within the *Anopheles funestus* group was performed because the small number collected indicated this is not a major vector in the area (n = 35).

Table 2.2: Number of mosquitoes collected pooled over the collection methods (Human Landing Catch and Mosquito Electrocuting Trap) and displayed by species and per village over 15 months (October 2016 to December 2017). Totals include both female and male mosquitoes.

Village	*Culex sp*	*Mansonia sp*	*Aedes sp*	*Anopheles sp*	*An. gambiae* s.l.
Dangouindougou	1118	1494	21	2411	2359
Gouera	277	80	18	2131	2111
Nianiagara	10	30	20	1252	1231
Nofesso	9	8	3	1188	1187
Ouangolodougou	16	9	11	833	830
Sitiena	53	186	8	4015	3777
Tengrela	260	1239	7	9540	9291
Tiefora	52	147	7	7185	6964
Timperba	105	35	43	1449	1436
Tondoura	2	19	17	1491	1483
Toumousseni	106	179	11	2566	2509
Yendere	96	237	6	1800	1766
Total	2104	3663	172	35861	34944

2.3.2. Trap sampling efficiency

Overall, there were notable differences in *An. gambiae s.l.* abundance between villages, trapping methods and locations (Table 2.3). In addition, *An. gambiae s.l.* abundance also varied notably across the collection period, with peaks during the rainy season (May –Oct) followed by decline in the dry season (Nov-April, Figure 2.3).

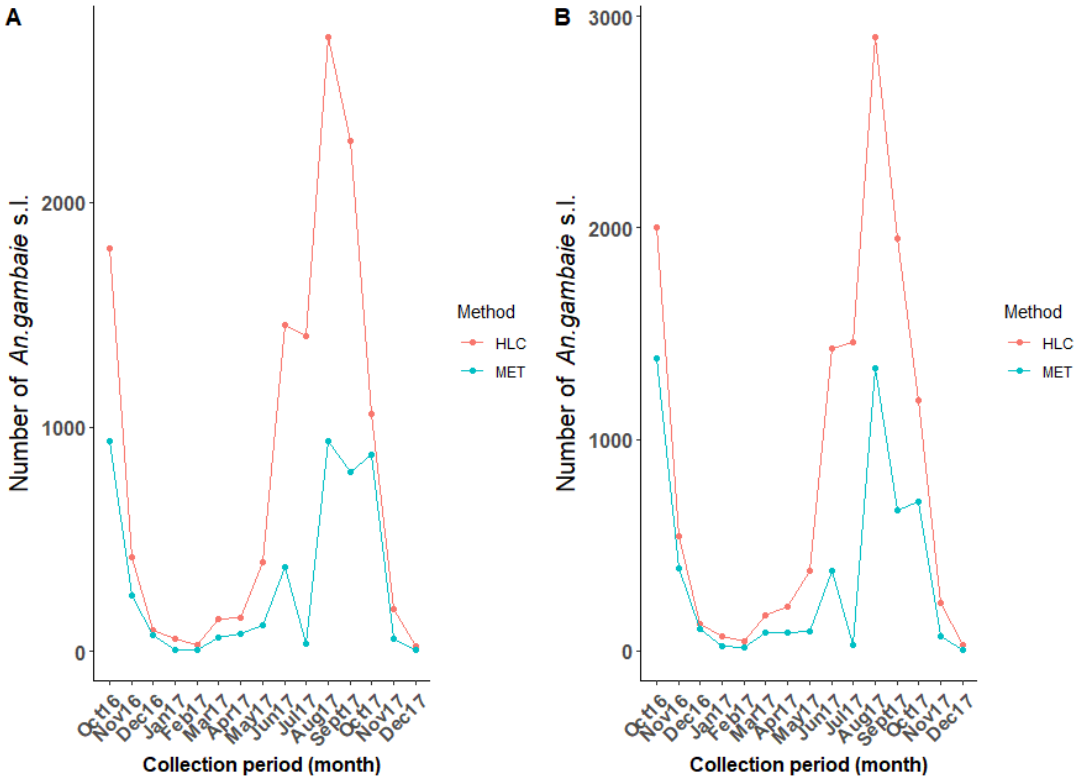


Figure 2.3: Number (raw data) of *An. gambiae* s.l. collected per month from (October 2016 to December 2017 by trapping methods A) indoor and B) outdoor using Mosquito Electrocuting Trap (MET) and Human Landing Catch (HLC).

Table 2.3: Number of *An. gambiae* s.l. females collected using different trapping methods, and at different locations (indoor versus outdoor) across the 12 study villages between October 2016 and December 2017. HLC = Human Landing Catch and MET = Mosquito Electrocuting Trap.

Village	HLC			MET		
	Indoor	Outdoor	HLC Total	Indoor	Outdoor	MET Total
Dangouindougou	787	784	1571	334	454	788
Gouera	762	866	1628	113	370	483
Nianiagara	477	480	957	125	149	274
Nofesso	338	540	878	103	206	309
Ouangolodougou	268	407	675	73	82	155
Sitiena	1588	1609	3197	313	267	580
Tengrela	3407	3104	6511	1457	1323	2780
Tiefora	2276	2389	4665	1174	1125	2299
Timperba	444	414	858	225	353	578
Tondoura	550	575	1125	197	161	358
Toumousseni	787	893	1680	309	520	829
Yendere	546	676	1222	185	359	544
Total	12230	12737	24967	4608	5369	9977

Table 2.4: Significance of terms included in the full Models 2.1; 2.3 and 2.4. Here, df is the degree of freedom and LRT (x^2) represents the values of Likelihood Ratio Test. n/a indicate that the given variable was not included in the model or its interaction with other variable is significant.

	Model 2.1: Trap efficiency			Model 2.3: Proportion of An. coluzzii			Model 2.4: Proportion of sporozoite		
Variables	LRT	df	p-value	LRT	df	p-value	LRT	df	p-value
Humidity	9.795	1	0.0017*	20.323	1	<0.0001*	0.083	1	0.773
Location	n/a	n/a	n/a	0.12	1	0.72	1.474	1	0.224
Method	n/a	n/a	n/a	0.027	1	0.87	0.783	1	0.376
Village	n/a	n/a	n/a	95.4	1	<0.0001*	27.631	11	0.003*
Season	244.427	1	<0.0001*	n/a	n/a	n/a	n/a	n/a	n/a
Species	n/a	n/a	n/a	n/a	n/a	n/a	1.1575	1	0.282
Temperature	0.587	1	0.443	2.84	1	0.09	0.019	1	0.889
Hour: Location	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
Village: Method	59.936	11	<0.0001*	10.324	11	0.501	5.654	11	0.895
Method: Location	4.205	1	0.038*	0.571	1	0.449	0.126	1	0.721
Species: Location	n/a	n/a	n/a	n/a	n/a	n/a	6.15	1	0.013
Method: Season	0.022	1	0.88	n/a	n/a	n/a	n/a	n/a	n/a
Method: Humidity	0.471	1	0.49	n/a	n/a	n/a	n/a	n/a	n/a
Method: Temperature	0.532	1	0.465	n/a	n/a	n/a	n/a	n/a	n/a

* Indicates significant term retained in the final model with $p<0.05$

The mean nightly abundance of *An. gambiae s.l.* was best explained in a final model that included the interaction between trapping method and village (df = 11, x^2 = 59.936, p < 0.0001), trapping method and location (df = 1, x^2 = 4.20, p = 0.04), season (as dry or wet season, (df = 1, x^2 = 244.42, p < 0.0001)) and humidity (df = 1, x^2 = 9.795, p = 0.002; Table 2.4 & 2.5). The significance of these interactions indicates that there is spatial variability in trap performance (Table 2.3 & 2.5, Figure 2.4) as well as between outdoor and indoor locations (Table 2.3 & 2.5, Figure 2.5). Overall the relative performance of the MET compared to the HLC was 46.88% (95% CI: 46.20 – 47.42%), but there was considerable variation between villages from a low of ~17% relative sensitivity in Sitiena to a high of ~100% in Toumousseni (Figure 2.4). Similarly, there was variation in trap performance between indoor and outdoor settings. However, regardless of location (in or outside), the number of *An. gambiae s.l.* collected using METs was less than the HLC (indoor: z = -5.93, p < 0.0001; outdoor: z = -5.42, p < 0.0001) with the performance of the MET relative to HLC being slightly higher in outdoor (Figure 2.5, 51.47%; 95% CI: 50.89 – 52.22%) than indoor settings (Figure 2.5, 42.86%; 95% CI: 42.0 - 43.44%). In general, mean nightly temperatures were higher and humidity lower inside of houses than outdoors (Table 2.6). None of the interactions between the trapping method and the temperature or humidity was significantly associated with the variation in the number of *An. gambiae* s.l. collected (Table 2.4). However, accounting for other significant variables in the model, *An. gambiae s.l.* abundance was positively associated with humidity taken (z = 3.162, p = 0.002; Table 2.4 & 2.5, Figure 2. 6), and significantly higher in the wet than dry season (df = 1, x^2 = 244.42, p < 0.0001; Table 2.4 & 2.5, Figure 2.7), irrespective of trapping method.

Table 2.5: Summary of the estimate (ß), standard errors, z values and p-value for each explanatory variable included in the final Model 2.1 used for assessing the variation in trapping efficiency. Here, df is the degree of freedom and Chi-sq (x^2) represents the values of Likelihood Ratio Test. The temperature and relative humidity were obtained by averaging the records over the course of the collection night. MET= Mosquito electrocuting trap. * indicates $p < 0.05$.

	ß	Std. Error	z value	Pr(>|z|)
Intercept	-1.799	0.299	-6.020	0.000*
MET	-0.826	0.146	-5.674	0.000*
Outdoor	0.092	0.064	1.443	0.149
Gouera	-0.941	0.416	-2.261	0.024
Nianiagara	-1.861	0.428	-4.345	0.000*
Nofesso	-2.208	0.418	-5.287	0.000*
Ouangolodougou	-1.560	0.422	-3.698	0.000*
Sitiena	-1.653	0.434	-3.810	0.000*
Tengrela	1.116	0.410	2.718	0.007*
Tiefora	0.126	0.411	0.306	0.760
Timperba	-1.674	0.411	-4.071	0.000*
Tondoura	-2.355	0.424	-5.554	0.000*
Toumousseni	-1.662	0.430	-3.863	0.000*
Yendere	-0.752	0.409	-1.837	0.066
Wet season	3.294	0.191	17.212	0.000*
Temperature	-0.037	0.052	-0.716	0.474
Humidity	0.158	0.050	3.162	0.002*
MET: Outdoor	0.184	0.090	2.050	0.040*
MET: Gouera	-0.222	0.227	-0.979	0.327
MET: Nianiagara	0.009	0.237	0.038	0.970
MET: Nofesso	-0.237	0.245	-0.967	0.334
MET: Ouangolodougou	0.209	0.235	0.888	0.375
MET: Sitiena	-1.038	0.230	-4.517	0.000*
MET: Tengrela	0.039	0.191	0.206	0.837
MET: Tiefora	-0.082	0.200	-0.408	0.683
MET: Timperba	0.033	0.219	0.151	0.880
MET: Tondoura	0.311	0.229	1.362	0.173
MET: Toumousseni	0.752	0.221	3.405	0.001*
MET: Yendere	-0.076	0.205	-0.371	0.711

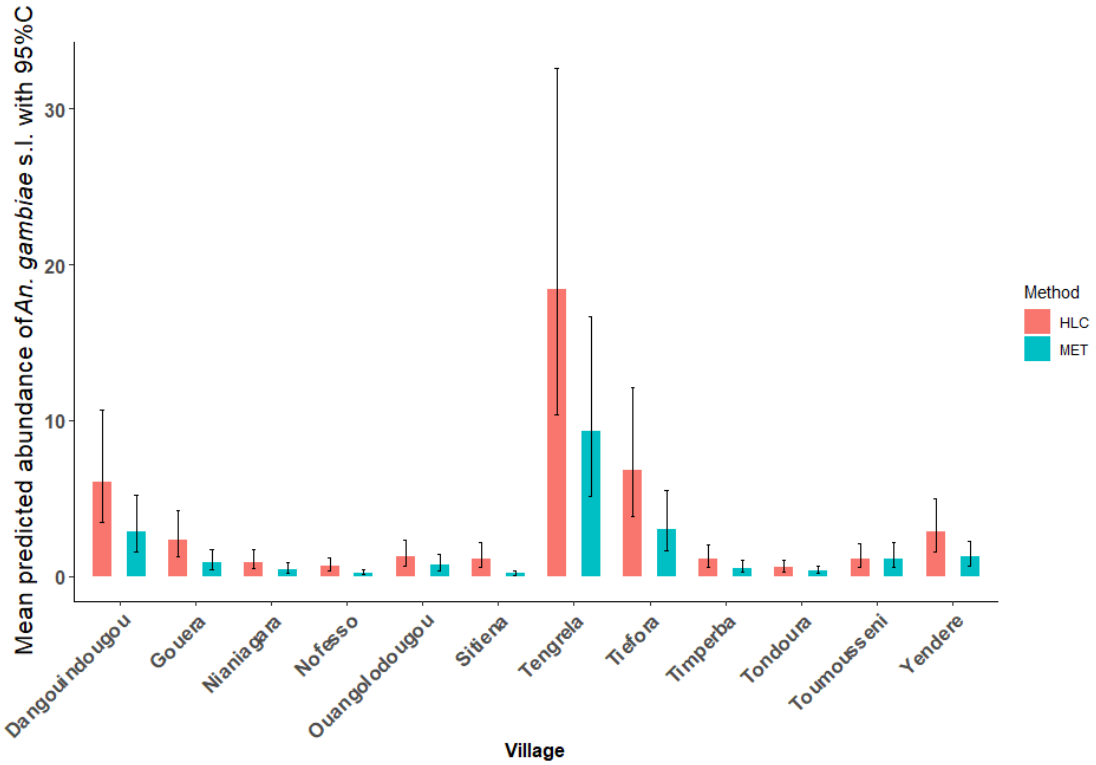


Figure 2.4: Mean predicted abundance of *An. gambiae s.l.* caught per night using different trapping methods in 12 villages in southwestern Burkina Faso. Data are pooled across trapping location (inside houses or outdoors) and the study period (October 2016 to December 2017). Error bars are with 95% confidence intervals. Here pink bars indicate HLC collection, and blue bars MET collections

Table 2.6: Range of average temperature (˚C) and relative humidity (%) recorded at the mosquito collection point using data logger.

	Temperature (C)	Relative humidity (%)
Indoor	27.6 (17 - 37.24)	58.62 (15.11 - 99.9)
Outdoor	25.16 (15.7 - 3884)	64.06 (11.73 - 99.95)

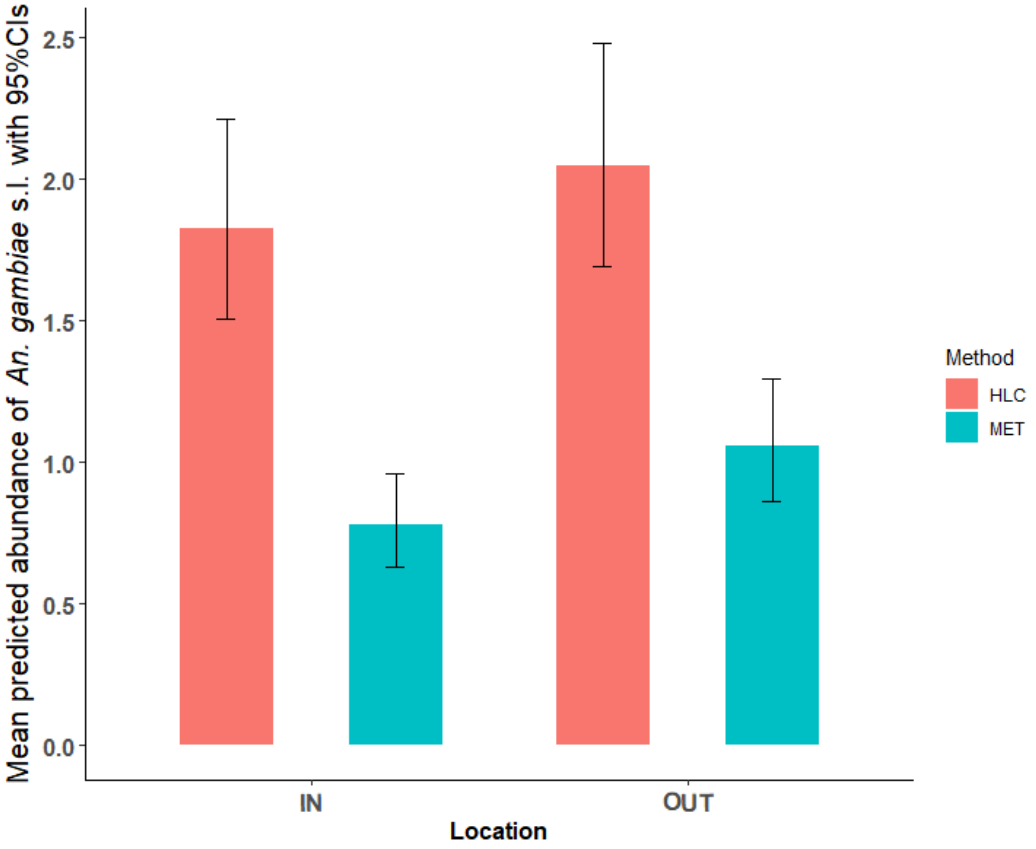


Figure 2.5: Mean predicted abundance of *An. gambiae* s.l. per night made at different trapping locations (IN = inside houses, OUT = peri-domestic area outside of houses) using two different trapping methods (pink bars = HLC; blue bars = MET) between October 2016 and December 2017. Errors bars are 95% confidence intervals.

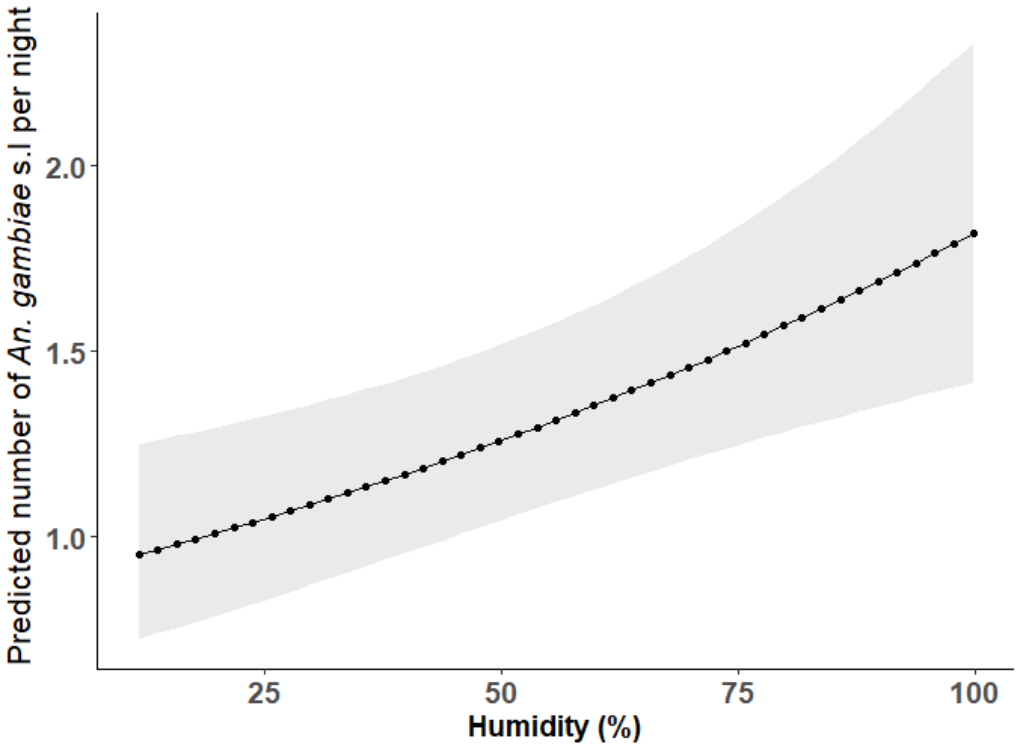


Figure 2.6: Predicted effect of humidity on the mean predicted number of *An. gambiae* s.l. collected per night over all trapping methods, locations and villages. The solid black line indicates the regression line based on the model and grey-shaded area indicates the 95% CIs. Humidity data were only available for part of the sampling period (e.g. mostly during the dry season months [Nov 2016-April 2017, and Nov -Dec 2017], and a few months in wet season [October 2016 and May - October 2017]. The predicted relationship between relative humidity and vector abundance is thus based on months in which matched data were available.

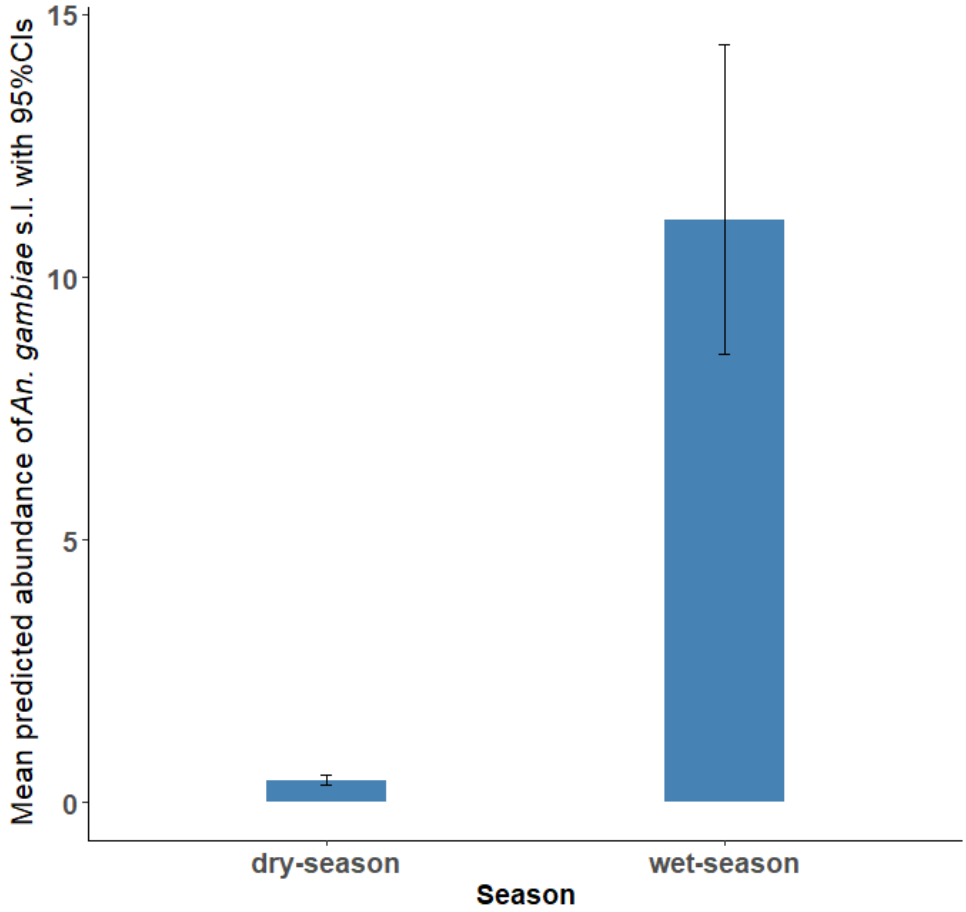


Figure 2.7: Mean predicted number of *An. gambiae s.l.* collected per night and season over the trapping methods, location and village with 95% CIs. Dry season indicates *An. gambiae* s.l. collected from November to April whilst wet season corresponds to period between May and October.

2.3.3. Relative performance of trapping methods across seasons

Analysis by GAMs indicated there was significant seasonal variation in *An. gambiae s.l.* abundance indoors (edf = 6.697, x^2 = 700.3, p < 0.0001) and outdoors (edf = 6.346, x^2 = 624.3, p < 0.0001). However, seasonal trends in *An. gambiae s.l.* abundance were indistinguishable as predicted from MET and HLC collections. The simple model, with no interaction between trapping method and season had the lowest AIC compared to the models with interactions (difference in AIC between simple and interaction model of 0.55 indoors, and 5.66 outdoors); indicating both methods predict similar trends (Figure 2.8).

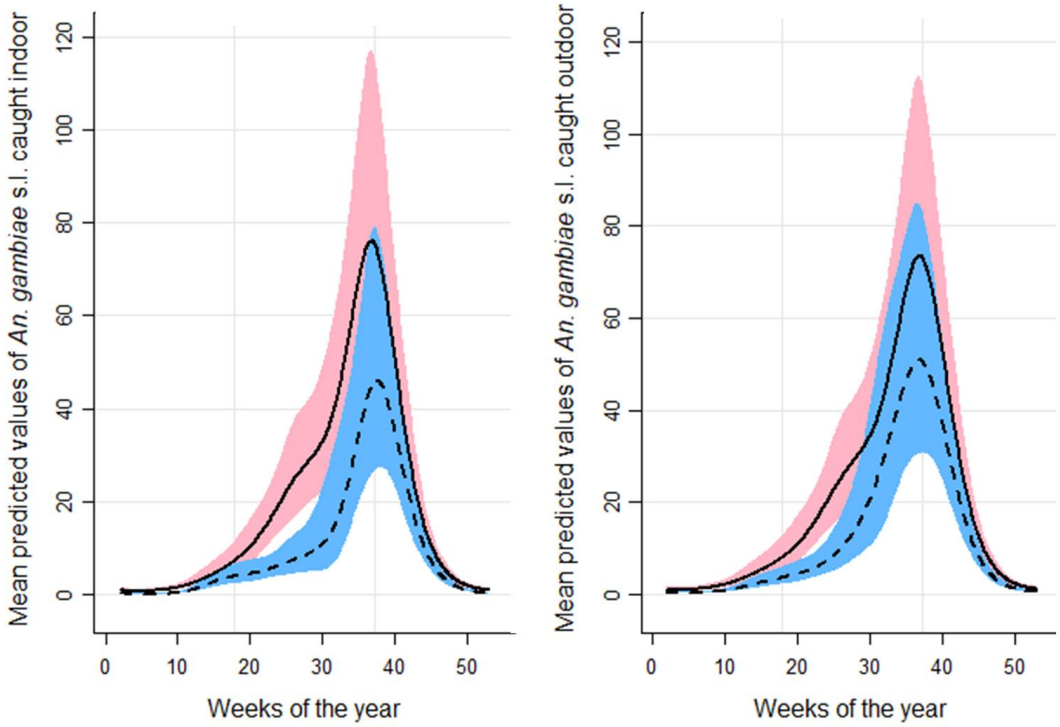


Figure 2.8: Mean predicted values of *An. gambiae* s.l. from a generalized additive model (GAM) with a negative binomial distribution. The full and open dots indicate respectively the observed number of *An. gambiae* s.l. in mosquito electrocuting trap and human landing catch through the course year indoors (left panel) and outdoors (right panel). The pink and blue areas are 95% confidence bands for the splines. The solid line and the pink indicate the data from HLC whilst the dashed-line and the blue represents the MET. Week "1" represents the first week of January, with weeks running consecutively up to week "52" (last week of December).

2.3.4. Relative performance of trapping methods across the night

The proportion of *An. gambiae* s.l. caught in METs relative to HLC was significantly influenced by the interaction between the sampling hour and trapping location (df = 1, x^2 = 10.83, p < 0.001). In indoor environments, the performance of the MET relative to the HLC stayed constant over all hours of the night (df = 1, x^2 = 0.13, p = 0.71). However, MET relative performance significantly declined (df = 1, x^2 = 27.63, p < 0.0001) between the first to the last hour of collection in outdoor settings (Figure 2.9).

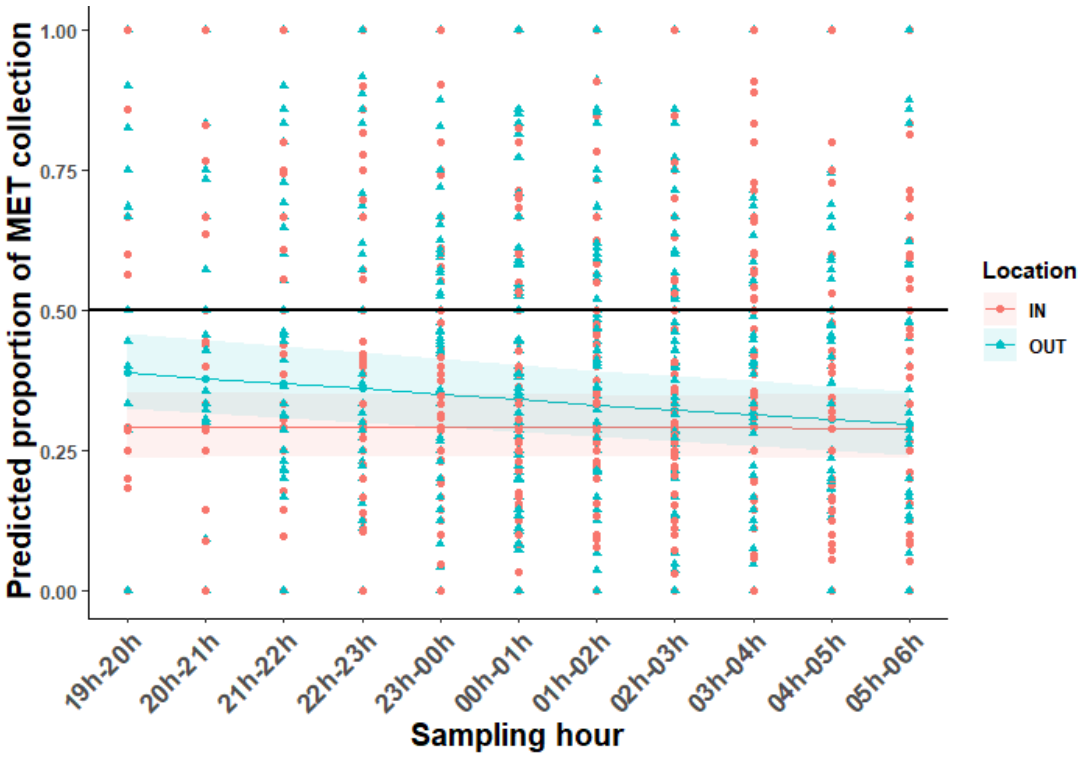


Figure 2.9: Mean predicted proportion of *An. gambiae* s.l. caught in mosquito electrocuting trap (MET) collections relative to the human landing catch (HLC) over the course of the night (7 p.m.–6 a.m.). The red dots and blue triangles indicate the ratio MET/ (MET + HLC) from the actual raw data respectively collected at indoor and outdoor sampling points. The black solid line indicates the scenario in which MET and HLC catch rates were equivalent. The red and blue lines represent the predicted regression line from models fit on data collected inside houses (IN) and outdoors (OUT). The shaded areas around the predicted lines represent 95% confidence intervals.

2.3.5. Density dependence in MET performance

The number of mosquitoes collected using HLC ranged from 0 - 575 indoors, and 0 - 672 outdoors, compared to 0 - 385 indoors and 0 - 542 outdoors for the MET. The degree of dependence (β) between HLC and MET collections across this range was estimated to be 0.92 (CI: 0.79 – 1.06) indoors and 1.00 outdoors (CI: 0.68 – 1.14). These values indicate there was no density-dependence as the credible intervals of estimates include 1 at each location (Figure 2.10). There was also a strong linear correlation between the number of *An. gambiae s.l.* caught in MET and HLC collections both indoors (r = 0.84 (CI: 0.79 – 0.89)) and outdoors (r = 0.86 (CI: 0.81 – 91).

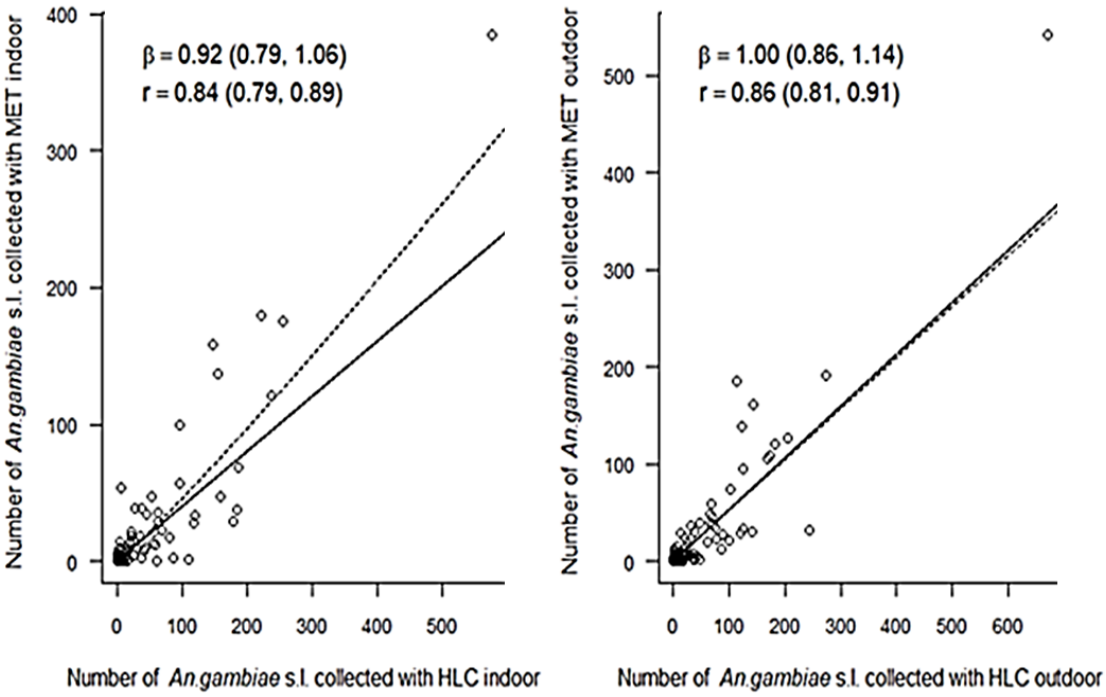


Figure 2.10: Observed values (open dots) and predicted relationships between the density of *An. gambiae* s.l. caught in mosquito electrocuting trap (MET) collections and human landing catches (HLC) at indoor (left panel) and outdoor (right panel) locations. In each graph, the dashed-lines indicate the model-predicted relationship between the traps and the black solid lines show the density independent relationship between MET and HLC collections. *B* and *r* indicate respectively the degree of dependence and linear correlation between HLC and MET in the number of *An. gambiae s.l.* collected.

2.3.6. Proportion of *Anopheles coluzzii* in host seeking collections

The composition of *An. gambiae* s.l. varied substantially across villages (df = 1, x^2 = 95.4, p < 0.0001; Table 2.4 & 2.7), with *An. coluzzii* representing more than 75% of the complex at four villages, *An. gambiae* dominating at six, and a roughly equal composition of *An. coluzzii* and *An. gambiae* at the remaining two sites (Figure 2.11). However, the variation in the proportion of *An. coluzzii* was not associated with the interaction between method and location, method and village (df = 11, x^2 = 10.324, p = 0.5), or trapping methods (df = 1, x^2 = 0.027, p = 0.87), location (df = 1, x^2 = 0.12, p = 0.72) or in relation to the mean temperature (df = 1, x^2 = 2.84, p = 0.09; Table 2.4) taken individually. Additionally, the proportion of *An. coluzzii* in collections was negatively associated with humidity (z = -4.67, p < 0.0001;

Table 2.4 & 2.7; Figure 2.12) with *An. gambiae* being more prevalent as humidity rose.

Table 2.7: Summary of the estimate (ß), standard errors, z values and p-value for each explanatory variable included in the final Models 2.3 and 2.4 used respectively for assessing the variation in proportion of *An. coluzzii* and the proportion of sporozoite. The relative humidity was obtained by averaging the records over the course of the collection night. n/a indicate that the given variable was not included in the model.

	Model 2.3: Proportion of An. coluzzii				Model 2.4: Proportion of sporozoite			
	ß	Std. Error	z value	Pr(>|z|)	ß	Std. Error	z value	Pr(>|z|)
Intercept	1.526	0.378	4.034	0.000*	-2.846	0.219	-12.98	0.000*
Gouera	-2.479	0.596	-4.160	0.000*	0.730	0.323	2.263	0.024*
Nianiagara	-3.006	0.716	-4.199	0.000*	-0.650	0.750	-0.866	0.386
Nofesso	-4.378	1.258	-3.481	0.000*	0.544	0.568	0.957	0.339
Ouangolodougou	-2.698	0.722	-3.737	0.000*	0.464	0.480	0.967	0.334
Sitiena	1.983	0.975	2.033	0.042*	0.308	0.410	0.750	0.453
Tengrela	2.927	0.642	4.559	0.000*	-1.262	0.347	-3.633	0.000*
Tiefora	-0.394	0.547	-0.719	0.472	-1.026	0.356	-2.884	0.004*
Timperba	-3.701	0.676	-5.476	0.000*	0.519	0.342	1.520	0.129
Tondoura	-3.893	0.699	-5.572	0.000*	0.563	0.298	1.887	0.059
Toumousseni	-1.441	0.627	-2.299	0.021*	0.346	0.340	1.017	0.309
Yendere	-1.490	0.567	-2.630	0.009*	0.291	0.428	0.681	0.496
Humidity	-0.590	0.126	-4.666	0.000*	n/a	n/a	n/a	n/a

* Indicates $p < 0.05$

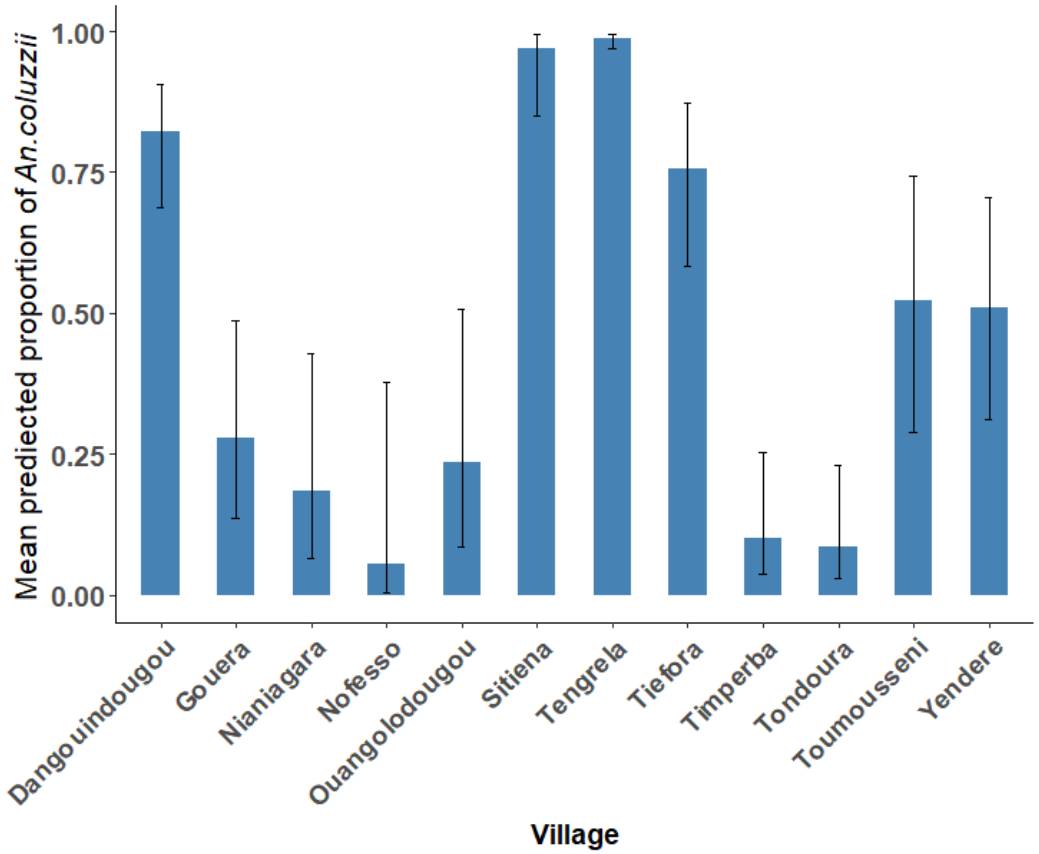


Figure 2.11: Mean predicted proportion of *An. coluzzii* relative to *An. gambiae* collected per village from October 2016 to December 2017, pooled over the trapping location and methods, with 95% CIs.

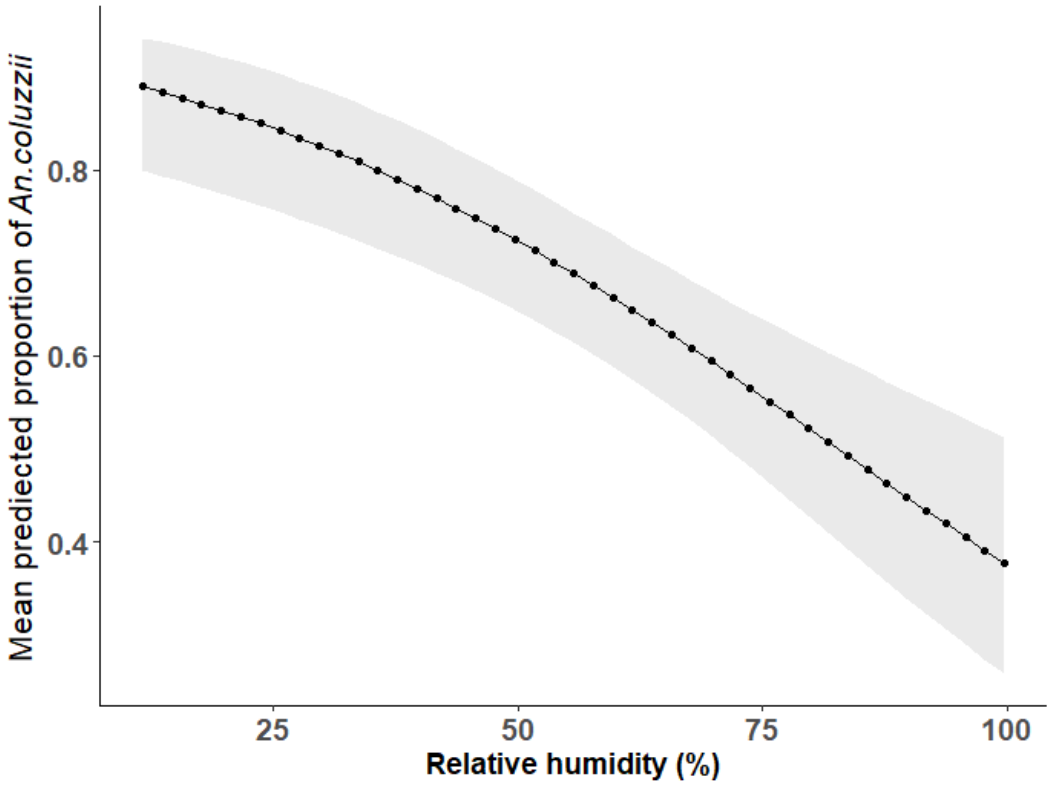


Figure 2.12: Effect of the mean relative humidity on the estimation of the proportion of *An. coluzzii* in the *An. gambiae* s.l. population. The solid black line is the regression line of the predicted proportions and the grey-shaded area indicate 95% CIs.

2.3.7. Malaria infection

A total of 157 out of 3199 *An. gambiae s.l.* tested were positive for *P. falciparum* sporozoite infection (4.9% infection rate). Sporozoite rates varied significantly between villages (df = 11, x^2 = 27.631, p = 0.003; Table 2.4 & 2.7; Figure 2.13). However, the variation in the sporozoite rate was not associated with the interaction between method and village (df = 11, x^2 = 5.654, p = 0.895), the interaction between method and location (df = 1, x^2 = 0.126, p = 0.72) but with the vector species and trapping location (df = 1, x^2 = 6.15, p = 0.013; Table 2.4). The *P. falciparum* sporozoite infection rate in *An. gambiae* was similar at indoor (5.16%; 95% CI: 3.64 – 7.26%) and outdoor trapping locations (5.67%; 95 % CI: 4.17 – 7.66%), whereas sporozoite rates were higher in *An. coluzzii* caught indoors (5.91%; 95 % CI: 4.2 – 8.28 %) than outside (2.8%; 95% CI: 1.78 – 4.39%; Figure 2.14). However, sporozoite rates in the overall *An. gambiae* s.l. sample did not vary between trapping

methods (df = 1, x^2 = 0.78, p = 0.38; Table 2.4), temperature (df = 1, x^2 = 0.02, p= 0.88) or humidity (df = 1, x^2 = 0.08, p = 0.77; Table 2.4).

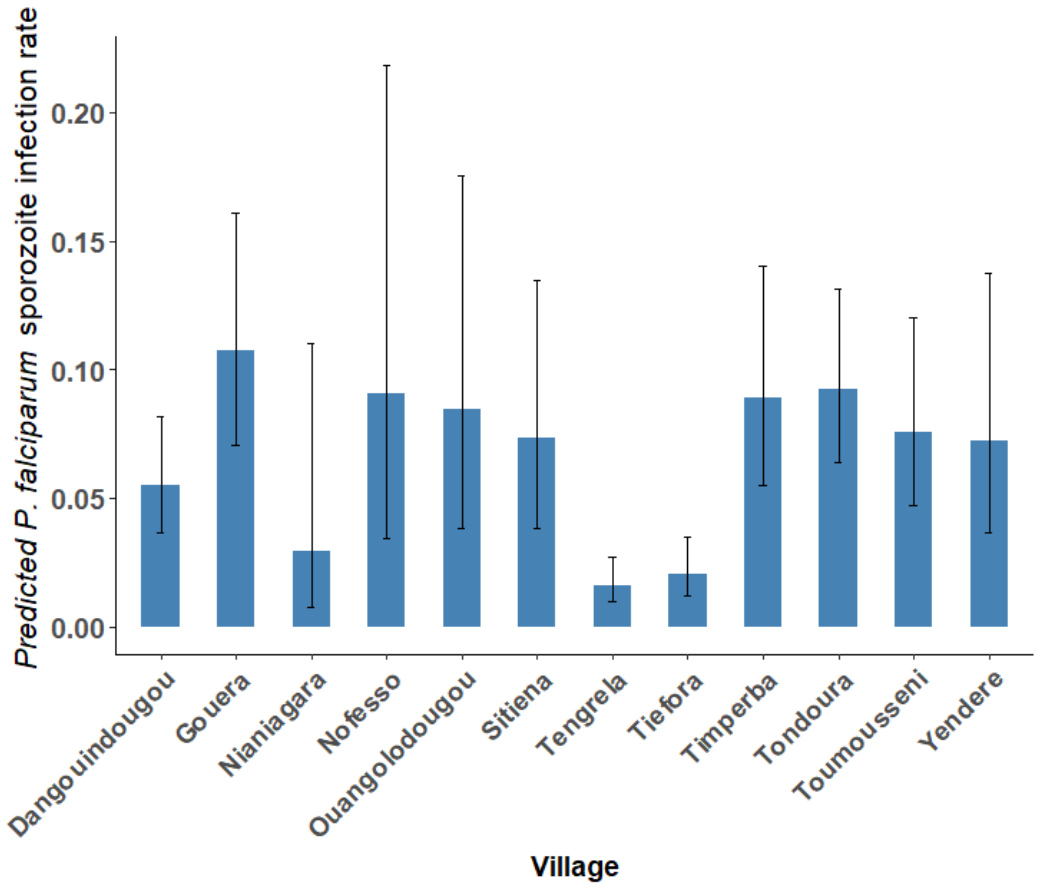


Figure 2.13: Mean predicted *Plasmodium falciparum* infection rate in *An. gambiae* s.l. collected per village from October 2016 to December 2017, pooled over the trapping location and methods, with 95% CIs.

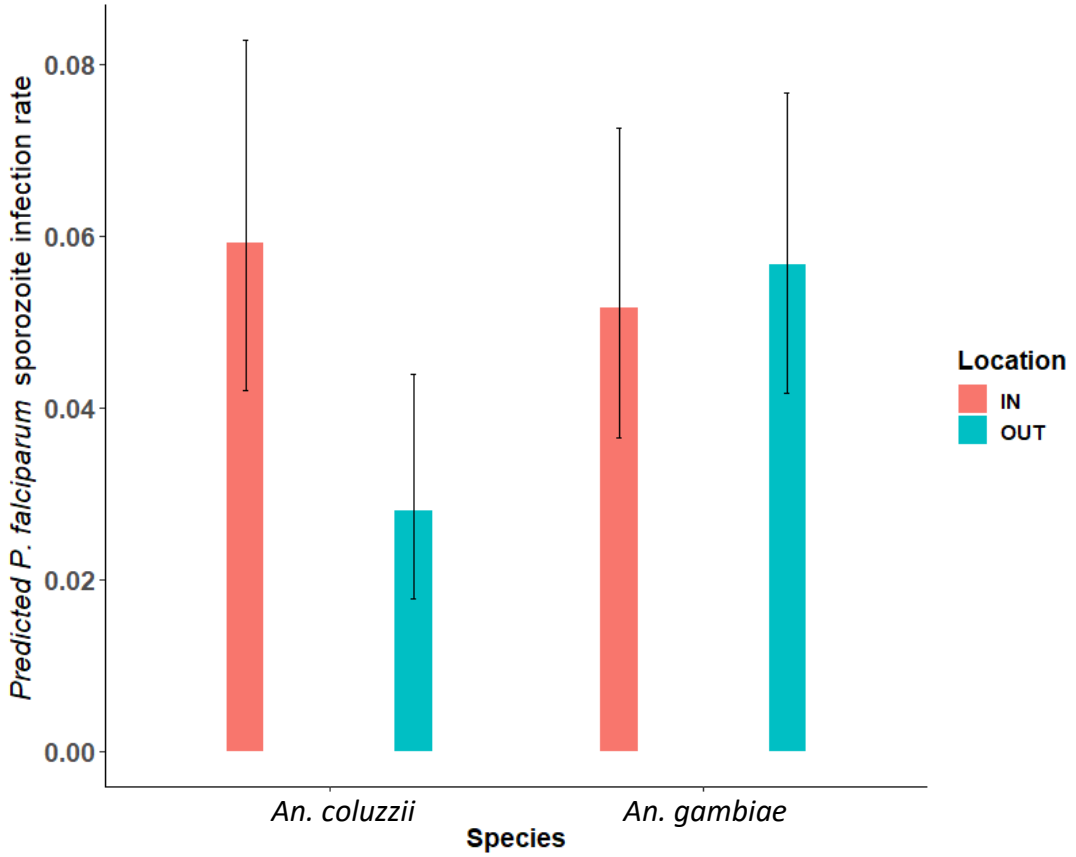


Figure 2.14: Mean predicted *Plasmodium falciparum* infection rate by *An. gambiae* s.l. complex species and location (IN= indoor, OUT = outdoor) collected from October 2016 to December 2017, pooled over the methods and villages. Error bars indicate95% CIs.

2.3.8. Vector behaviour and human exposure

The *An. gambiae* s.l. population in the study area was relatively exophilic, with numbers host-seeking outdoors being similar or slightly higher than those indoors (Figure 2.15A). However, estimates of the proportion of indoor biting (P_i) varied somewhat between trapping methods (df = 1, x^2 = 4.25, p = 0.039; Table 2.8 & 2.9); with the HLC predicting a slightly higher degree of outdoor biting (45.73%; 95 % CI: 43.2 – 48.27%) compared to the MET (43.42%; 95% CI: 40.47 – 46.4 %), Figure 2.15B). Similarly, estimates of the proportion of *An. gambiae* s.l. caught during times when most people are indoors ($P_{fl,}$ x^2 = 11.28, p < 0.001; Table 2.8), and the proportion of human exposure to *An. gambiae* s.l. estimated to occur indoors (πi, x^2 = 21.03, p < 0.0001; Table 2.8) were slightly but significantly higher in HLC than MET collections (Figure 2.15C). There was no significant additional effect of

temperature, humidity or season on these human exposure traits (P_i, P_{fl}, and πi; Table 2.8).

Table 2.8: Significance of terms included in the full Models 2.5; 2.6 and 2.7. Here LRT represents Likelihood Ratio Test and df is the degree of freedom equal to "1" for all the terms.

	Method		Season: Method		Humidity		Temperature	
	LRT	p-value	LRT	p-value	LRT	p-value	LRT	p-value
Model 2.5: Proportion of indoor biting (P_i)	4.25	0.039*	0.31	0.57	0.21	0.64	0.02	0.87
Model 2.6: Proportion caught when most people are indoors (P_{fl})	11.28	0.000*	0.28	0.6	0.33	0.56	0.75	0.38
Model 2.7: Proportion of human exposure occurring indoors (π_i)	21.03	0.000*	0.02	0.3	0.2	0.65	0.03	0.84

* Indicates $p < 0.05$

Table 2.9: Summary of the estimate (β), standard errors, z values and p-value for each explanatory variable included in the final Model 2.5, 2.6 and 2.7 used for assessing the variation in human exposure and mosquito behaviour.

	β	Std. Error	z value	Pr(>|z|)
Model 2.5: Proportion of indoor biting (Pi)				
Intercept	-0.171	0.052	-3.289	0.001*
Method MET	-0.094	0.045	-2.062	0.039*
Model 2.6: Proportion caught when most people are indoors (Pfl)				
Intercept	1.957	0.082	23.778	0.000*
Method MET	-0.217	0.058	-3.756	0.000*
Model 2.7: Proportion of human exposure occurring indoors (πi)				
Intercept	1.918	0.111	17.201	0.000*
Method MET	-0.396	0.080	-4.960	0.000*

* Indicates $p < 0.05$

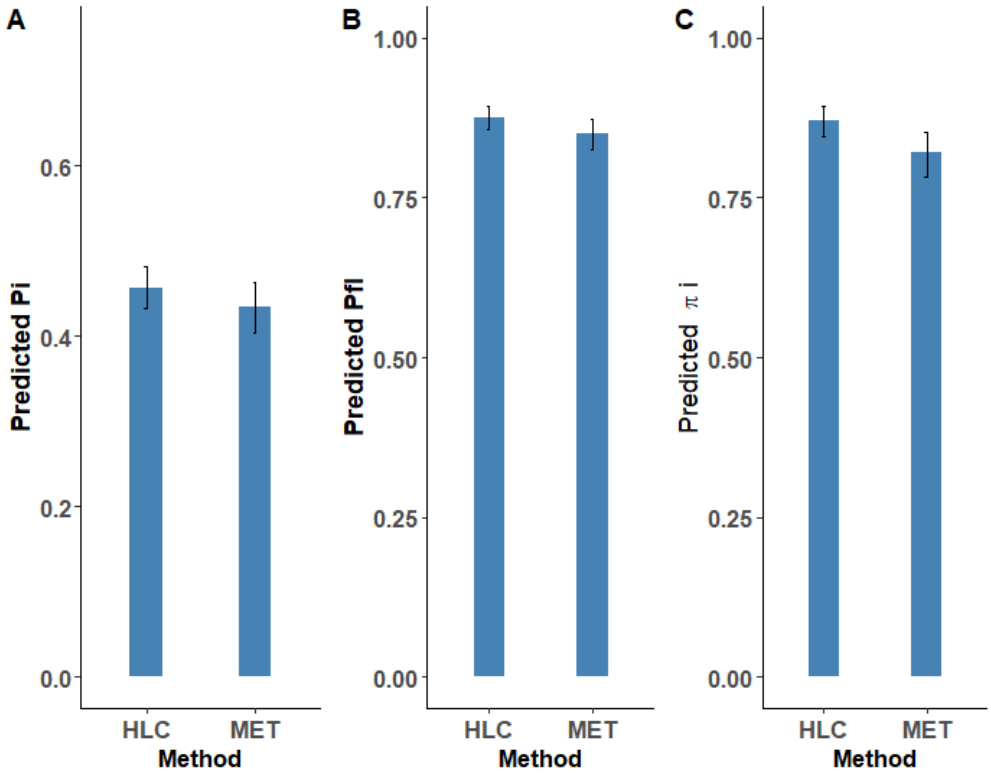


***Figure 2.15**: Estimated proportion of* An. gambiae *s.l.* ***A) Pi** = caught indoors, **B) Pfl** = bites occurring when most people are inside their dwellings and likely asleep and **C)** π_i = the proportion of human exposure to* An. gambiae *s.l. bites occurring indoors from human landing catch (HLC) and mosquito electrocuting trap (MET).* Error bars indicate 95% CIs.

2.4. Discussion

Here the performance of METs was evaluated as an alternative to the gold standard "HLC" for estimating human exposure to malaria vectors. This was the first time that METs were evaluated outside Tanzania, and in a West African setting. In general, the MET caught fewer *An. gambiae s.l.* than HLC with relative performance being higher in outdoor (52%) than indoor environments (43%). The overall efficiency (combining in and outdoors) of the MET (~46%) was similar to that described for first prototype trialled in rural Tanzania by [238], but below the near 100% relative performance reported with further prototypes tested in Tanzania [239, 240]. However, estimates of vector species composition, seasonal dynamics, biting behaviour (indoor *vs* outdoor) and malaria infections rates were generally similar between MET and HLC collections. This strengthens evidence that METs can provide a safe alternative to the HLC for characterizing attributes of malaria vector populations; even though they may require location-specific calibration for prediction of vector density.

It is unclear why MET performance was relatively lower in this study than reported in Tanzania. However, several factors may account for this. One possibility is that the current study incorporated more intra-site variability. Previous work in Tanzania has involved evaluation at a limited number of fixed sampling points in a 1 - 2 sites. Here the METs were tested at multiple households across 12 different villages, with considerable variation in MET relative performance being noted between sites (17– 100%). Thus, local characteristics of the study site may have a significant impact on trap performance. The relatively lower sampling efficiency of the MET here compared to Tanzania could also be due to operational problems that arose after the first batch of METs had been in continuous use for several months, exaggerated by wear and tear during the regular transport between villages (up to 100 km apart, on poor roads). These operational problems included short-circuits, and power supplier failure in addition to dipping in current/voltage, some of which may not have been noticed until traps failed.

Although only data from days in which both MET and HLC collections were conducted was used for analysis, these faults indicate that the MET prototype may need further improvement for stable use over long periods of time. Additionally, there were small differences in trap design between the prototype used here and in Tanzania, which may have contributed to the reduced performance. For example in contrast to previous studies in Tanzania [238, 239], the MET prototype here used white untreated net to protect the part of participant's bodies that were not in the trap. It has been shown that *An. gambiae s.l.* are more attracted to traps with high visual contrast [256], and the use of white netting to protect participants here may have diminished the contrast between the trap and host bait compared to previous versions. Another factor that could make a difference is vector ecology and species composition. The major vectors in areas where the MET has been used in Tanzania is *An. arabiensis* [238, 239] whereas *An. gambiae* and *An. coluzzii* dominated in our study area in Burkina Faso [217, 269]. Cuticular hydrocarbon composition (CHC) varies between Anopheles species [285-287]. Furthermore, the electrical conductivity of insects can vary with their CHC, water content and body size [288]. Therefore, the variation in the MET performance between the current study and those carried out in Tanzania could also be due to local variation in vector species composition that influenced the susceptibility of vectors to electrocution.

The results from the present study suggested METs performed better in outdoor ~52% relative sensitivity compared to the HLC) than indoor (~43%) settings. Earlier trials in Tanzania also found MET performance to be higher outdoors than inside houses [238]. It is unclear why MET sampling efficiency tends to be higher outdoors, with further work required to address this bias. Given the growing recognition of the importance of outdoor biting in maintaining residual malaria transmission [233, 234, 261] and current lack of satisfactory alternatives to the HLC for measuring this, the MET can serve a useful purpose even if only suitable for use outdoors. The relatively good performance of the MET relative to the HLC for sampling malaria vectors outdoors reported here and elsewhere [239, 240] indicate that it may be suitable for monitoring exophagic and zoophilic vector [240] populations.

Both MET and HLC sampling methods confirmed strong seasonal variability in vector abundance as has been widely documented in Burkina Faso and other parts of West Africa [289, 290]. The current results indicate that the relative performance of the MET compared to the HLC stays constant across seasons, and that both methods predict similar seasonal trends in vector abundance. Additionally, there was no evidence of density dependence in the sampling efficiency of METs over a wide range of *An. gambiae* s.l. density. This contrasts with results from an earlier prototype where MET performance showed signs of density dependence indoors but not outside [239], but no density dependence was found in another relatively short (21 days) study period [238]. Based on the current and previous studies, it can be concluded that the MET can provide relatively accurate estimates of vector population dynamics that are unbiased by season or underlying density.

Consistent with previous studies [238, 239], there was no detection of any difference in MET sampling efficiency throughout the night when it was used indoors. However, there was a reduction in relative MET performance throughout the night when used outdoors. Such a decrease in MET sampling efficiency outdoors was reported with an early MET prototype in Tanzania [238], but not in a follow up with a new version [239]. It is unclear why MET sampling efficiency falls during the night in outdoor but not indoor settings. One possibility is variation in microclimatic conditions like humidity, which is generally higher outdoors than indoors. Humidity can trigger more rapid discharge of batteries [291]. To maintain consistent MET performance when used outdoors, batteries could be changed during the sampling night.

The malaria vector species composition in this study area varied notably compared to that of previous MET trials in Tanzania. Specifically, *An. coluzzii* and *An. gambiae* were the dominant vector species here compared to *An. arabiensis* and *An. funestus* in Tanzania [203, 239, 240, 292]. Previous work in Tanzania indicated MET capture efficiency varied between malaria vector species (e.g. *An. arabiensis* and *An. funestus* [238]). However, vector species composition was similar in collections made by HLC and MET here; indicating no differential sampling performance between *An. coluzzii* and

An. gambiae. Further calibration may be required to ensure the MET gives unbiased estimates of composition of malaria vector species in new settings. Similar to previous studies [239, 240], we found no difference in malaria sporozoite rates between vectors in HLC and MET collections. Thus, the MET also appears to yield unbiased estimates of *An. gambiae s.l.* infection rates.

Finally, the three-key human-mosquito exposure metrics were evaluated to assess whether they were reliably predicted by the MET. In general, estimates of these three exposure-metrics were similar between HLC and MET collections. However, the MET tended to slightly underestimate all three metrics likely because of its slightly lower sampling performance in indoor *versus* outdoor settings. Even this with these biases, estimates of exposure as calculated by the different trapping methods were generally within a few percentage points of one another. For operational use, estimates of exposure derived from MET collections could be adjusted to compensate for this bias.

The multi-site nature of this study allowed assessment of wider aspects of MET feasibility for programmatic sampling. In contrast to previous trials in Tanzania where the MET was used in fixed, single locations [238, 239]; here was carried out in 12 villages requiring the MET to be moved every few days and sometimes as far as 100 km. The integrity of electrified surfaces on the METS were checked before and after transport in the field. The output voltage was also regularly checked during collections to ensure it was meeting the necessary target. On occasions where voltage output was suboptimal (~ 0.4% of days), MET operation was stopped and the problem reported to technical support team. Overall, MET collections were performed on ~17% fewer sampling hours than the HLC. However, this does not represent the proportion of times that the MET failed. Most of these MET hours (~9%) were lost while waiting for a replacement unit to be made and delivered (~ 4-week period). The most frequent problem encountered with MET use was power failure due to short-circuiting (~ 6% of time) with occasional sparking on the frame. Therefore, further improvements in MET design are needed to resolve this issue. In addition, it was noted that short-

circuiting was more likely to occur when there was high level of moisture in the environment (e.g. rainy season, times of high humidity). This was probably due to small water droplets condensing on the frame and occasionally running down the wires. Regular wiping of the MET surface (e.g. during 15 minutes break periods from sampling) could help avoid a build-up moisture of trap surface. Alternately, redesigning the trap with wires running horizontally instead of vertically will prevent droplets from running down into the frame. METs were subjected to heavy use in this study, under challenging field conditions. It is perhaps not surprising that traps exhibited some degree of physical damage and breakage under these intense circumstances. These issues could be resolved by making future prototypes more robust, and /or keeping METs in fixed locations rather than in constant transport. In addition, on some other nights, MET sampling was intentionally stopped (~1% of the sampling hours) due to high wind and rainfall that was anticipated to drive water onto the MET surface and cause short-circuiting. Even with these difficulties, the METs still performed relatively well and consistently with the HLC in this study. To increase the protection of volunteers from bites of very small biting insects (those with wingspan less than 5mm) that may be present at some study sites, we recommend fitting fine-mesh insecticide-free netting on the inner panel of MET surfaces with very small holes.

An additional consideration is the relative expense of doing collections with METs *versus* HLC. Currently, MET are individually built to order by a small team; with the combined cost for all components and manufacture of ~£ 650-700 per unit. This cost is prohibitively high for large-scale surveillance (e.g by comparison, a standard CDC light trap costs ~$ 100 USD per unit). However, it is anticipated that the production cost would significantly decrease if produced at scale. While costs of MET collections may always be more expensive than a simple HLC where no equipment is required, we believe this additional expenditure is justified in terms of the improved safety to human subjects that it can provide.

2.5. Conclusions

This is the first-time that the MET was evaluated outside of East Africa. Overall, the MET collected proportionately fewer malaria vectors than the HLC, and slightly overestimated the proportion of outdoor biting. However, the performance of METs relative to the HLC was consistent over time, and provided similar estimates of seasonal dynamics, biting behaviour, species composition and infection rates in malaria vector populations. Thus, despite some technical problems arising after prolonged MET usage under field conditions, we conclude it presents a promising and safer alternative for monitoring human exposure to malaria vectors in outdoor environments.

Chapter 3: Spatial and temporal variation in the abundance and behaviour malaria vectors following a scaling up in LLINs in rural Burkina Faso

Abstract

Background: Long-Lasting Insecticide-Treated Nets (LLINs) and Indoor Residual Spraying (IRS) are the most common and successful methods for malaria vector control in Africa. There is growing evidence of shifts in vector biting and resting behaviours in several African settings where high LLIN coverage has been achieved. These changes, combined with insecticide resistance, may reduce intervention success by decreasing the contact between vectors and insecticide-treated surfaces. The implications of such mosquito behavioural changes to malaria control are not yet known but may contribute to the limited impact of LLIN programmes in some African countries. The existence and magnitude of behavioural avoidance strategies in vector populations in Burkina Faso has not been yet documented. This study aimed to characterize the spatial and temporal distribution of the abundance, biting and resting behaviour of the major malaria vectors in southern Burkina Faso following a national LLIN-distribution campaign.

Methods: A two-year programme of longitudinal mosquito vector surveillance (October 2016 – December 2018) was initiated within 12 villages of south-western Burkina Faso in 2016, shortly after completion of a mass LLIN distribution. Malaria vectors were sampled monthly using Human Landing Catches conducted inside houses and in the surrounding outdoor area (total = 911 houses). Additionally, resting bucket traps were used to sample indoor and outdoor resting vectors. GAMMs were performed to test whether vector abundance, the proportion of outdoor biting and resting, and the median time of biting changed between sites, seasons and over the 2-year period of the study (October 2016 – December 2018).

Results: A total of 49 482 mosquitoes were collected during the study, with most being from the major malaria vector group *Anopheles gambiae* s.l. (96.74%). The abundance of *An. gambiae* s.l. varied significantly between villages and seasons (wet vs dry). Controlling for these spatial and seasonal effects, there was evidence of significant decline in vector density over the course of the study. Overall, ~54% of *An. gambiae* s.l. were collected host-seeking outdoors; with the proportion of outdoor biting being higher in *An. gambiae* (~55%) than *An. coluzzii* (~51%). Most

mosquito biting took place between 00 h and 02 h, with evidence of variation in median biting times between species, villages and seasons. Based on these biting times and locations, it is estimated that ~85% of exposure to vector bites could be prevented through use of LLINs during typical sleeping hours (22 h – 5 am). Approximately 47% of *An. gambiae* s.l. females rested outdoors, but this varied between seasons.

Discussion: This study revealed substantial spatial and seasonal variation in malaria vector abundance in the study area, and evidence of longer-term decline across the 2-years following mass LLIN distribution. A higher degree of outdoor biting and resting by malaria vectors was detected than has previously been reported in Burkina Faso. Most outdoor biting occurred during typical sleeping hours when people are indoors; indicating that ~85% of human exposure could be prevented by LLINs. Although this suggests LLINs can prevent the bulk of exposure, this degree of protection is unlikely to be sufficient for control given the generally high abundance and outdoor biting of vectors in the study area.

3.1. Background

Long lasting Insecticide-Treated nets (LLINs) and Indoor Residual Spraying (IRS) are the main malaria vector controls tools [111, 293] recommended by the World Health Organisation [80]. As reviewed in Chapter 1, these interventions have had a significant impact on malaria control in Africa [15, 294, 295]. The success of these interventions rests on their ability to exploit key aspects of malaria vector behaviour; e.g., the tendency to feed mainly on humans (anthropophagic), inside houses (endophagic), during sleeping hours, and to rest inside houses (endophilic) after feeding [106, 107]. Consequently, LLINs and IRS are expected to work best against anthropophilic, endophilic and endophagic vectors that have high susceptibility to insecticides [121, 296, 297]. Accordingly these tools were shown to be more effective against anthropophilic than zoophilic species in Africa [298, 299] and British Guiana [300]. Historically, most of the major vectors responsible for malaria transmission in Africa (members of the *An. gambiae* s.l. complex) are described as feeding primarily on humans [57, 301-306] and having endophilic behaviour [40] characterized by late night-biting [40, 56, 307]. This combination of behaviours is thought to account for the early success of current vector control approaches in Africa [308, 309].

However, the suite of mosquito behaviours that predispose them to LLINs or IRS may not be fixed within vector populations or species. There is growing evidence that widespread use of LLINs and IRS may be selecting for behavioural and ecological changes in mosquito vector communities that allow them to reduce their contact rates with domestic insecticides (e.g. Chapter 1, [310]). Such changes have been observed to arise through two different mechanisms: ecological and evolutionary. Ecologically-driven shifts or behavioural resilience (defined as natural and invariable behaviour or pre-existing behaviours) in vector behaviour arise from shifts in vector species composition [311, 312], with the relative proportion of endophagic and endophilic species being reduced relative to those that are most likely to feed early in the evening or morning [65], outdoors and/or on animals as well as humans [311, 312]. For example, following an IRS intervention in Zambia the relative abundance of the endophagic *An. quadriannulatus* decreased compared to the more exophagic and zoophagic *An. arabiensis* [313]. Similarly, *An. gambiae* was replaced as the main source of transmission by its more exophilic sibling species *An. arabiensis* after the scaling

up of LLINs in Kenya [314] and Tanzania [315]. Furthermore, following the introduction of IRS, the anthropophagic *An. funestus* [40] was replaced by more zoophilic and exophagic species (*An. parensis and An. rivulorum*) in Kenya in 1960s [316]. Evolutionary-based shifts or behavioural resistance refer to changes in mosquito behavioural traits that occur within a vector species, reflective of an adaptation to avoid the intervention. For example after introduction and high coverage with LLINs, *An funestus* appears to have switched its biting time from late night to early morning in Benin [317]. Furthermore, this species was found biting in broad daylight in Senegal [318], which was speculated to be due a response to growing LLIN coverage. Such ecological and adaptive changes have been documented in several settings [204, 319, 320]. However, despite the widespread reporting of such mosquito behavioural shifts [65, 204, 230, 317], their implications for malaria control including LLIN performance remain to be quantified. For example, a study in Kenya found that *An gambiae* and *An. funestus* were more likely to bite outdoors and/or early in the evening after LLIN scale up; however this had little epidemiological impact because this intervention could still prevent > 90% of human exposure [321]. Therefore, there is a need to not only measure shifts in malaria vector behaviour in response to interventions, but also to estimate its impact on human exposure. Such understanding is required to assess the relative contribution of behavioural resistance to residual malaria transmission.

Assessment of the impact of mosquito behavioural resilience and resistance on malaria control is becoming increasingly important, especially considering the growing evidence that LLIN programmes are having little impact in a small but growing group of African countries. As reviewed in Chapter 1, Burkina Faso is one of 10 African countries with the highest burden of malaria[13]. The prevalence and number of malaria cases in these high burden countries is either not falling, or increasing [13]. In Burkina Faso, this stagnation in progress has arisen concurrently several rounds of mass LLIN distribution [322-324]. Mass LLIN distribution in Burkina Faso began in 2010, with ~ 90% of households owning at least one LLIN by 2014 [323, 325]. This is of great concern because it is not yet clear whether Burkina Faso and other high malaria burden countries are just exceptions to the general trend of decline across most of Africa, or early warnings

of the start of malaria control failure that may soon spread throughout the continent.

The reasons for the declining LLIN impact in these high burden African countries is uncertain, but the leading hypothesis is insecticide resistance ([171, 326], Chapter 1 & 4). As previously discussed (Chapter 1 & 4), insecticide resistance is now widespread in malaria vectors across Africa [125, 177, 219, 327-329], but no clear association with control failure has been demonstrated [112]. Shifts in mosquito species composition and/or within-species adaptations that allow vectors to evade LLINs may also be undermining LLIN performance; however, this hypothesis has not been as thoroughly investigated as insecticide resistance. One reason for the limited investigation of mosquito behavioural shifts is the lack of high resolution/quality surveillance data on mosquito behaviours through time, of sufficient spatial and temporal resolution to discern longer-term trends from background environmental variation.

It is generally assumed that malaria vector populations in Burkina Faso are behaviourally susceptible to LLINs because they have been reported to be highly endophagic [54, 222], endophilic [223] and anthropophilic [57]. In addition, early work in Burkina Faso before mass LLIN distribution [56] indicated that *An. gambiae* s.l. were generally active late in the night from 01 h – 06 h. As reviewed in Chapter 1, the major malaria vectors in Burkina Faso are two species within the *An. gambiae* species complex: *An. gambiae* and *An. coluzzii* [54, 219, 223, 330]. As described in Chapter 1 knowledge of the biting and resting behaviours of vectors within Burkina Faso is quite patchy. Other studies conducted in plateau central region in the late 1990s [224] and early 2000s [222] showed that > 50% of *An. gambiae* s.l. bite indoors. Thus, even within the limited data available, there is evidence of variation in behaviours between malaria vector species, populations and time periods. Much of the knowledge of malaria vector behaviours in Burkina Faso comes from before the period of mass LLIN distribution ([54, 222-224, 331], and thus may not reflect any ecological or evolutionary changes triggered by these interventions. It is therefore important to update knowledge of mosquito vector behaviour and assess spatial and temporal variation to anticipate whether behavioural resistance is limiting the impact of LLINs for malaria control in Burkina Faso.

The aim of this study was to i) update information on the feeding and resting behaviours of major vector species in southwestern Burkina Faso, ii) quantify spatial and seasonal variation in malaria vector ecology, biting and resting behaviours and iii) test for evidence of long-term shifts in mosquito behavioural traits following a mass LLIN distribution campaign. Particular focus was placed on mosquito behavioural traits that could reduce the impact of LLINs including outdoor biting, earlier biting, outdoor resting.

3.2 Methods

3.2.1 Study sites and study design

An entomological surveillance programme was conducted within 12 villages of south-western Burkina Faso between October 2016 and February 2018, as described in Chapter 2 (Figure 1.7). The key behaviours of interest were time and location of biting (indoors versus outside) and location of resting (indoors versus outside).

The study was instigated a couple of months after a mass LLIN-distribution campaign took place in the study area. Monthly mosquito collection was conducted at all 12 village sites for the first 16 months of the study. To address the aim of testing for long-term shifts in vector ecology and behaviour following mass LLIN distribution, sampling was continued for an additional 10 months (February 2018 to December 2018) at a subset of 6 villages from the original group of 12 (Figure 1.7). This group of "long-term" sites was selected to reflect a range of variation in vector ecology (abundance, species composition), behaviours (outdoor biting), malaria infectivity rates (presented in Chapter 5) and insecticide resistance levels (presented in Chapter 4). Additionally, the long-term study villages were selected to achieve a relatively broad spatial distribution (Figure 1.7). In total, monthly sampling at these long-term sites was conducted over 26 months, in comparison to 16 months at all study sites.

3.2.2 Mosquito collection

Overall, twenty-six rounds of monthly mosquito collections were made (sixteen in the 12 original villages plus a further ten at the longer-term study villages). Over this period, host-seeking malaria vectors were sampled using Human Landing Catches (HLC) as described in the Chapter 2. In the current Chapter, only the data

collected using the HLC were used as it is the "gold standard method" recommended for measuring malaria vector host-seeking behaviour [232]. Mosquito were collected as described in the method section in Chapter 2. Data from HLCs were used to estimate mean vector abundance (number per night), hourly biting rates (number per hour) and the proportion of outdoor biting and resting.

In addition, malaria vector resting behaviour was investigated using Resting Box Traps (RBTs) placed in and outside of homes [332]. The RBTs were made using 20 L plastic buckets purchased from a local market, with their inner surface covered with moistened black cotton cloth to create a high contrast and humid environment. RBTs were placed in the same households where host-seeking mosquito collections took place but set at a different house within the compound. Inside houses, RBTs were placed on the floor in a relatively shaded corner of the sitting room. RBTs were set up in the peri-domestic area around houses (~5 metres radius of the house) to capture outdoor resting mosquitoes. On each night of collections, two RBTs were placed inside and outside at each household. RBTs were set up at approximately 7 pm each night and emptied the following morning (~5 am) using electrical aspirators (Figure 3.1).

Figure 3.1: Photo of a field technician collecting resting mosquitoes from an RBT using a prokopack aspirator in outdoor environment earlier (5h30) in the morning.

3.2.3 Mosquito processing

All mosquitoes collected were stored in labelled collection cups and processed as described in the methods section of Chapter 2. In brief, female mosquitoes were morphologically identified and those belonging to the *An. gambiae* s.l. species group or *An. funestus* were retained for molecular analysis. In addition, female *Anopheles* collected from the RBTs were further classified based on their abdominal (physiological) status into categories of blood-fed, unfed, gravid, or half gravid [333].

3.2.4 Molecular analysis

A subsample of 7852 females (~20 % of total collected in HLCs) morphologically identified as *An. gambiae* s.l. were selected for further identification to species level by PCR [272] to provide a representative sample from each month, village, and trapping location (indoor vs outdoor) as described in Chapter 2. In addition, ~77% (n = 449) of the 584 *An. gambiae* s.l. females collected in RBTs were retained for molecular analysis to confirm their species using polymerase chain reaction as described in Chapter 2.

3.2.5 Statistical analysis

Statistical analysis was conducted with the aims of testing for spatial (between villages) and temporal (seasonal and "long-term") variation in five key metrics of malaria vector ecology and behaviour: i) abundance, (ii) species composition (proportion of *An. coluzzii* in *An. gambiae s.l.*), (iii) proportion of outdoor biting, (iv) median biting time, and (v) proportion of outdoor resting. Here, "abundance" was defined as the mean number of *An. gambiae* s.l. caught per person per night in HLC. This measure can also be interpreted as the human biting rate (HBR; as these data are described in Chapter 5); a measure of human exposure. Additionally, data on the time and location of biting were used to estimate two metrics of human exposure to malaria vector bites: (1) the proportion of *An. gambiae* s.l. caught during hours when most people were indoors and likely to be in bed (P_{fl}), and (2) the proportion of human exposure to bites that occur indoors (π_i) [204, 276, 283] calculated as described in Table 2.1 in Chapter 2.

Analysis of spatial variation for all these mosquito ecological, behavioural and human-exposure metrics was based on the first 16 months of data from all 12 sites. The temporal analysis focused on investigating seasonal and longer-term (linear) trends in the variables of interest. To assess seasonal variation, each day of the year was classified on a scale running from "1" (set as January 1st) to "365" (December 31st) hereafter called cDate. This means that collections made on the same day but in different years got the same value. To assess longer term trends occurring over the full 26 months of the study, an additional temporal variable was created by assigning a continuous value to each day of collections from the first day (October 1st, 2016) until the last of collections (December 4th,2018), hereafter called nDate. Environmental covariates of mean nightly temperature and relative humidity at households (from Tiny Tag application Explorer 4.9) were used as additional explanatory variables in the models. This allowed assessment of whether mean temperature and humidity had additional independent effects on vectors trait after accounting for seasonal variation. However, these environmental covariates were not recorded at resting traps and were thus not included in analysis of resting behaviours (Table 3.1).

Variation in biting time between mosquito species was initially considered in terms of clock hour (Model 3.5 and 3.7, Table 3.1) and other environmental covariates. However it is recognized that biting activity is circadian and likely modulated by light levels [334]; with the onset of biting in *An. gambiae* s.l. occurring after sunset [307].There is some seasonal variation in the timing of sunset of this area of Burkina Faso, from an earliest time of 17 h 53 in November to latest time of 18h47 in july. Thus, in addition to clock hour, biting activity was also investigated in term of the time after sunset (retrieved from R using the function "getSunlightTimes" from the "suncalc" packages). Later this was used to estimate the time after sunset that was used as response variable in Model 3.6 and 3.8 (Table 3.1). Here, the time after sunset (in hour) was calculated by doing the subtraction: "the collection time minus exact the sunset time" and then used to estimate the median biting time after sunset at each night of collection. For example, if the sunset time was 6:12 pm and the collection was at 7 pm the considered time after sunset will be 0.78 h (~47 minutes). Therefore, time after sunset was 0.78 h despite that this could have had any length of time between 0.78 and 1.55 h (~47 min to 1 h 33 min) as collection could have been at exactly

7 : 00 pm or later at 7:45 pm (time at which collection stopped for 7 to 8 pm). Though, this median biting time after sunset (in hour) was later used as response variable.

Generalised Additive Mixed Effect Models (GAMMs) within the 'mgcv package' [335] augmented with the lme4 package [278] named GAMM4 were used to test for associations between all vector ecological and behavioural metrics and explanatory variables of interest. This approach was implemented using the R statistical software version 3.5.0 (2018 – 04 - 23) [277]. GAMM4 was used because of its flexibility, that enables incorporation of "splines" to model non-linear temporal effects (e.g. seasonal variation) as well as unidirectional changes (long-term trends). In addition, it allowed both random and fixed effects to be fitted. For each response variable, a maximal model was created which included all fixed and random effects of interest as listed in Table 3.1. Whilst almost all analyses were conducted using GAMM4s, Generalised Linear Models (GLMs) were used to estimate the average number of bites per hour as required to reconstruct the hourly biting pattern (Table 3.1). In all cases, model selection was conducted by systematically removing terms from the maximal model (Table 3.1) using the 'anova.gam' function from the 'mgcv package' [336] from GAMM4s. During model selection, Likelihood Ratio Tests (LRTs) were used to evaluate the significance of individual terms. Fitted values and 95% confidence intervals for all statistically-significant effects in the minimum model ("best model") were obtained using the effects package [284] for the GLMs and the 'predict.gam function' for GAMMs [337]. Mosquito abundance data (number per night) were found to be highly over-dispersed based on the overdispersion parameter calculation as described in [338], and thus modelled using a negative binomial distribution [339]. Proportion data (% *An. coluzzii* in *An. gambiae* s.l.; outdoor biting and resting, the P_{fl}, and π_i) were modelled using a binomial distribution whilst the median biting time after sunset was modelled using a loglinear distribution with a gamma likelihood.

In all relevant analyses, the longer-term trend was assessed by including nDate as a discrete independent variable. Seasonality was modelled through fitting a non-linear smoothing function to cData (spline named as t2(cDate, bs = cc) in the models; (Table 3.1)). A non-linear term is appropriate for describing the typical pattern for seasonal variation in mosquito abundance that is characterized by a

strong peak in the wet season and significant decline in the dry season. In general, random effects such as compound and household were incorporated into models to account for baseline variability.

Although, analyses were conducted to test for spatial and temporal variation in most entomological variables of interest, there was an exception. For the resting behaviour, the sample sizes were too low for robust assessment of spatial variation. Consequently, in this analysis "village" was fit as a random effect. As most female *An. gambiae* s.l. caught in resting collections (77%) were individually identified to species level by PCR, it was possible to do a further sub-analysis to investigate vector species-specific differences in resting behaviour (*An. gambiae* and *An. coluzzii*). Further details of the modelling approach for each variable of interest including choice of random and fixed effects, and distribution are given in Table 3.1.

Table 3.1: Maximal models used for modelling seasonality including the primary response variable, explanatory variables and statistical distribution used. The average temperature and relative humidity were obtained by averaging the records over the course of the collection night. Hyphens (-) indicates no random effect used. Physiology means the abdominal status indicating if fed, unfed, gravid or partially fed mosquitoes. nDate indicates the longer-term trend over the collection periods fitted as a linear term. The seasonality term was fitted using a non-linear smoothing function (spline t2(cDate, bs=cc)) on days as a period of 365 days (cDate). nHour and the I(nHour^2) respectively represent here hours as discrete variables from 1 being the first hour of collection (7pm-8pm) to the last hour of collection of the night being 11 (5am – 6am) and its quadratic term. Here, "subset of An. gambiae *s.l.* "refers to subset that were individually identified to species levels and tested for sporozoite infection.

Group-Model	Trait	Response variables	Fixed Effect variables	Random effects	Type of data	Distribution
i-3.1	Abundance	Abundance	Village + Location+ Temperature+ Humidity +Village : Location +nDate + t2(cDate, bs=cc),	Compound + Household	Host-seeking nightly *An. gambiae* s.l	Negative binomial
ii-3.2	Proportion of *An. coluzzii*	(*An. coluzzii*/*An. coluzzii* + *An. gambiae*)	Village+ Location+ Temperature+ Humidity +Village : Location + nDate + t2(cDate)	Compound+ Household	Subset: *An. gambiae* s.l. lab-processed data	Binomial
iii-3.3	Outdoor biting proportion	(Outdoor/ Outdoor +Indoor)	Village+ Temperature+ Humidity + nDate+ t2(cDate, bs=cc),	Compound+ Household	Host-seeking nightly *An. gambiae* s.l.	Binomial
iv-3.4	Mean number of bite/person/hours	Hourly count	nHour + I(nHour^2)	-	Host-seeking hourly *An. gambiae* s.l.	Negative binomial
iv-3.5	Median biting time	Median of hours	Village+ Location+ nDate+ Temperature + Humidity + Village : Location + t2(date, bs=cc)	Compound+ Household	Host-seeking hourly *An. gambiae* s.l.	Poisson
iv-3.6	Median biting time after sunset	Median time after sunset	Village+ Location+ nDate+ Temperature + Humidity + Village : Location + t2(date, bs=cc)	Compound+ Household	Host-seeking hourly *An. gambiae* s.l.	Poisson

Model	Trait	Response variables	Fixed Effect variables	Random effects	Type of data	Distribution
iv-3.7	Median biting time by species	Median of hours	Species + Village + Location + nDate + Temperature + Humidity + t2(date, bs = cc)	Compound + Household	Subset of *An. gambiae* s.l. data	Poisson
iv-3.8	Median biting time by species after sunset	Median time after sunset	Species + Village + Location + nDate + Temperature + Humidity + t2(date, bs = cc)	Compound + Household	Subset of *An. gambiae* s.l. data	Poisson
v-3.9	Outdoor resting proportion (male)	(Outdoor/ Outdoor +Indoor)	nDate + t2(date, bs=cc)	Village + Household	Field collected resting *An. gambiae* s.l.	Binomial
3.10	Outdoor resting proportion (female)	(Outdoor/ Outdoor +Indoor)	nDate + Species + Physiology + Species: Physiology + t2(date, bs = cc)	Village + Compound + Household	Subset: *An. gambiae* s.l. resting lab-processed data	Binomial
3.11	Proportion of mosquito when people are indoor (P_{fi})	($I_{10pm->5am}$ + $O_{10pm->5am}$) / ($I_{7pm->6am}$ + $O_{7pm->6am}$)	Village + Temperature + Humidity + nDate+ t2(cDate, bs = cc),	Compound + Household	Host-seeking nightly *An. gambiae* s.l.	Binomial
3.12	Human exposure to mosquito bite indoor (π_i)	$I_{10pm -> 5am}$ / ($I_{10pm -> 5am}$ + $O_{7pm -> 10pm, 5am -> 6am}$)	Village + Temperature + Humidity + nDate + t2(cDate, bs = cc),	Compound + Household	Host-seeking nightly *An. gambiae* s.l.	Binomial in
3.13	Outdoor biting by species	(Indoor, Outdoor)	Species + nDate + t2(cDate, bs = cc),	Compound + Household + Village	Subset of *An. gambiae* s.l. data	Binomial

Results

3.3.1 General results

A total of 49 482 mosquitoes comprising 4 genera were collected in HLC collections during the study (Table 3.2). Here Anopheles was the most abundant genera (84.2%), followed in decreasing order by *Mansonia sp* (7.69%), *Culex sp* (4.24%) and *Aedes sp* (0.33%; (Table 3. 2).

Table 3.2: Total number of female mosquitoes caught using Human Landing Catches in southwestern Burkina Faso from October 2016 to December 2018 (pooled across villages and trapping locations).

Village	*Anopheles sp*	*Culex sp*	*Mansonia sp*	*Aedes sp*
Dangouindougou	1611	801	1178	5
Gouera	1642	214	49	15
Nianiagara	984	7	26	12
Nofesso	881	1	7	4
Ouangolodougou	675	9	8	12
Sitiena	5722	80	213	9
Tengrela	9621	410	1504	12
Tiefora	8889	122	168	12
Timperba	861	70	15	22
Tondoura	2227	14	22	26
Toumousseni	5800	166	168	12
Yendere	2742	156	226	16
Grand Total	41662	2096	3807	161

Within HLC collections, 96.54% of *Anophelines* were morphologically identified as belonging to the *An. gambiae* s.l. group (N = 40 220), followed by *An. coustani, An. funestus, An. nili*, *An. obscursus*, *An. pharoensis and An. rufipes* (Table 3.3). Within the subset of *An. gambiae* s.l. on which PCR analysis for species identification was performed (~20 % of total) three species were identified: *An. coluzzii* (53.82%), *An. gambiae* (45.9%) and *An. arabiensis* (0.28%). A total of 927 mosquitoes were collected from the RBTs in which *Anopheline* females represented ~61% (Table 3.4). Most female Anophelines in RBTS were *An. gambiae* s.l. (95.65%), followed by *An. rufipes* (2.76%), *An. funestus sp* (1.62%), *An. nili* (0.49%)., *An. pharoensis* (0.32%) and *An. coustani* (0.32%). Approximately similar

numbers of *An. gambiae* s.l. females were found in indoor and outdoor RBTs (Table 3.5). Of the 449 resting female *An. gambiae* s.l. identified by PCR, 61.25% were *An. coluzzii* and 38.08% were *An. gambiae* (Table 3.6). Two *An. arabiensis* (0.45%) and one hybrid between *An. coluzzii* and *An. gambiae* (0.22) were also found (Table 3.6).

Table 3.3: Total number of females Anophelines caught in Human Landing Catches in southwestern Burkina Faso October 2016 to December 2018 (pooled between villages and over trapping locations).

Village	*An. coustani*	*An. funestus*	*An. gambiae* s.l.	*An. nili*	*An. obscursus*	*An. pharoensis*	*An. rufipes*	*An. zeimani*
Dangouindougou	4	6	1574	1	0	25	1	0
Gouera	0	5	1629	3	0	5	0	0
Nianiagara	0	1	973	4	0	6	0	0
Nofesso	0	1	880	0	0	0	0	0
Ouangolodougou	0	0	674	0	0	1	0	0
Sitiena	43	2	5138	466	13	59	0	1
Tengrela	137	7	9177	72	1	215	7	5
Tiefora	3	6	8671	38	0	171	0	0
Timperba	1	1	858	1	0	0	0	0
Tondoura	0	1	2217	7	0	2	0	0
Toumousseni	2	4	5726	25	0	43	0	0
Yendere	3	4	2703	14	0	18	0	0
Total	193	38	40220	631	14	552	8	6
Proportion (%)	0.46	0.09	96.48	1.51	0.03	1.32	0.02	0.01

Table 3.4: Total number of mosquito males and females caught using Resting Bucket Traps 12 villages of southwestern Burkina Faso, from October 2016 to December 2018. Results are displayed by trapping location (IN = indoor and OUT = outdoor) and village.

Village	Total mosquitoes		*Anopheline sp*		*Culex sp*		*Mansonia sp*		*Aedes sp*	
	IN	OUT	IN	OUT	IN	OUT	IN	OUT	IN	OUT
Dangouindougou	10	21	2	15	8	3	0	3	0	0
Gouera	23	66	16	64	5	2	0	0	2	0
Nianiagara	8	6	8	6	0	0	0	0	0	0
Nofesso	2	11	2	10	0	1	0	0	0	0
Ouangolodougou	4	2	4	2	0	0	0	0	0	0
Sitiena	52	28	50	28	0	0	2	0	0	0
Tengrela	223	133	210	111	6	15	7	7	0	0
Tiefora	64	98	64	96	0	1	0	1	0	0
Timperba	11	1	11	1	0	0	0	0	0	0
Tondoura	27	26	24	25	3	0	0	0	0	1
Toumousseni	56	46	46	42	8	3	2	0	0	1
Yendere	7	2	6	2	1	0	0	0	0	0
Total	487	440	443	402	31	25	11	11	2	2

Table 3.5: Total number of female Anophelines caught using Resting Bucket Traps in 12 villages of southwestern Burkina Faso, from October 2016 to December 2018. Results are displayed by trapping location (IN = indoor and OUT = outdoor) and village.

	An. coustani		*An. funestus*		*An. gambiae* s.l.		*An. nili*		*An. pharoensis*		*An. rufipes*		
	IN	OUT	IN	OUT	IN	OUT	IN	OUT	IN	OUT	IN	OUT	Total
Dangouindougou	0	0	0	1	2	4	0	0	0	0	0	1	8
Gouera	0	0	0	4	11	25	0	0	0	0	0	11	51
Nianiagara	0	0	0	0	8	6	0	0	0	0	0	0	14
Nofesso	0	0	0	0	1	2	0	0	0	0	0	0	3
Ouangolodougou	0	0	0	0	4	0	0	0	0	0	0	0	4
Sitiena	0	0	0	0	22	19	0	0	0	0	0	0	41
Tengrela	0	2	2	1	142	101	0	2	1	1	5	0	256
Tiefora	0	0	0	2	58	73	0	1	0	0	0	0	134
Timperba	0	0	0	0	2	1	0	0	0	0	0	0	3
Tondoura	0	0	0	0	12	11	0	0	0	0	0	0	23
Toumousseni	0	0	0	0	32	40	0	0	0	0	0	0	72
Yendere	0	0	0	0	6	2	0	0	0	0	0	0	8
Total	0	2	2	8	300	284	0	3	1	1	5	12	618

Table 3.6: Total numbers of female *An. gambiae* s.l. caught using Resting Bucket Trap in 12 villages in southwestern Burkina Faso, from October 2016 to December 2018 (RBT) and display by species pool over villages, trapping location according to their physiology status.

		Abdominal status				
Species	Location	Unfed	Fed	Half-gravid	Gravid	Total
An. arabiensis	Indoor	0	1	0	0	1
	Outdoor	0	1	0	0	1
An. coluzzii	Indoor	59	79	3	18	159
	Outdoor	87	22	1	6	116
An. gambiae	Indoor	37	35	0	11	83
	Outdoor	51	25	0	12	88
An. gambiae-coluzzii	Indoor	0	1	0	0	1
	Outdoor	0	0	0	0	0

3.3.2 Mosquito abundance and species distribution

The total number of *An. gambiae* s.l. collected varied between villages; with the highest numbers obtained at Tengrela and Tiefora (Table 3.3). There was a clear seasonal trend in *An. gambiae* s.l. abundance across the year (pooling across villages), with numbers peaking during the rainy season (June to October) and crashing during the dry season (December to May; Figure 3.2). Variation in the mean abundance of *An. gambiae* s.l. was best explained in a model that accounted for village (df = 11, x^2 = 230.54, p < 0.0001; Table 3.4, 3.7 & 3.8), trapping location (indoors versus outside, df =1, x^2 = 21.28, p < 0.0001; Table 3.7 & 3.8), seasonal variation (x^2 = 1165, edf = 6.84, p = 0.0007; Table 3.7 & 3.8) and a long-term trend (df = 1, x^2 = 6.63, p = 0.01; Table 3.7 & 3.8). Accounting for these effects, there was no additional impact of mean temperature (df = 1, x^2 = 0.15, p = 0.70), humidity (df = 1, x^2 = 0.004, p = 0.95), or the interaction between village and location (df = 11, x^2 = 14.93, p = 0.18; Table 3.7). Spatial variation in *An. gambiae* s.l. abundance was considerable; with mean densities varying from less than 1 per night in Nofesso to > 93 in Tengrela (Figure 3.3). The mean nightly biting rate was ~18 outdoors compared to ~16 bites per person per

night indoors (Figure 3.4). As expected, there was a significant seasonal variation in *An. gambiae* s.l. abundance with numbers being ~ 70 times higher at the height of the wet season (month of September with predicted mean = 72, 95% CI: 36 - 108) than dry season (December to April, estimated mean = 0.3, 95% CI: 0.15 – 0.5; Figure 3.5). After controlling for the variation due to village and season, there was also evidence of slight longer-term trend decline in *An. gambiae* s.l. abundance across the study period (Table 3.4).

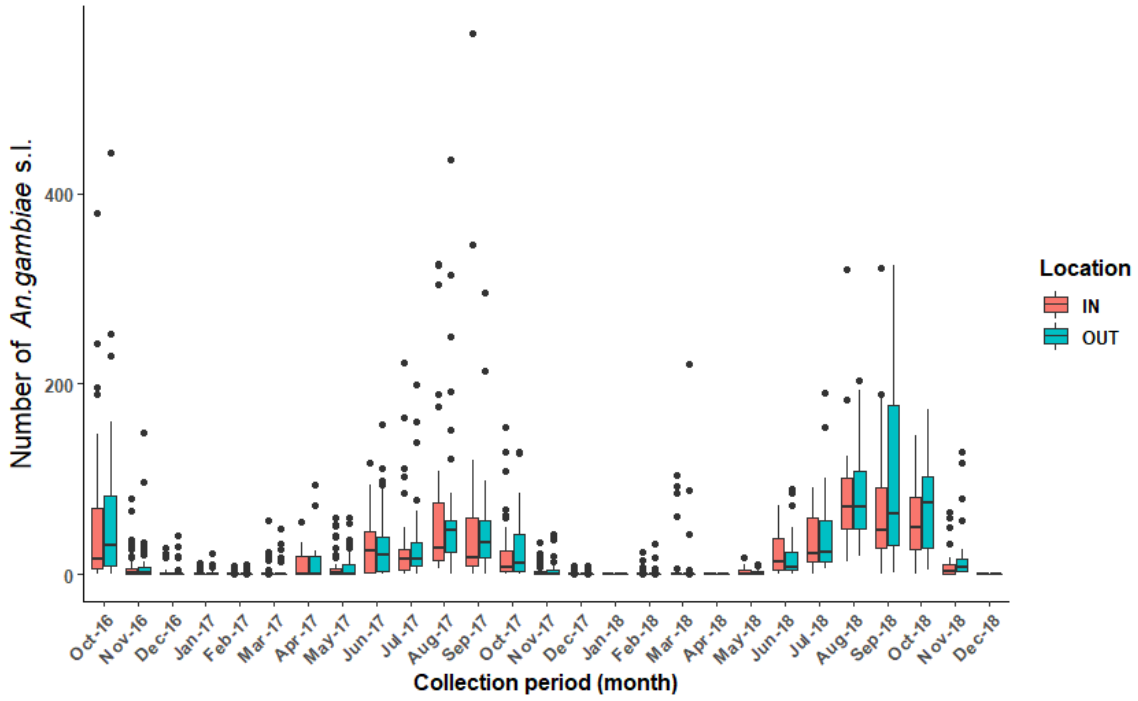


Figure 3.2: Box plot showing the number of *An. gambiae* s.l. (from raw data) and pooled per month and trapping location (inside houses or outdoors) collected from October 2016 to December 2017 using Human Landing Catch in southwestern Burkina Faso.

Table 3.7: Significance of terms included in the full Models 3.1, 3.2 and 3.3. Here Chi.sq (x^2) represents Likelihood Ratio Test and df is the degree of freedom equal to 1 for all the terms. n/a indicate that the given variable was not included in the model.

	Model 3.1: Abundance			Model 3.2: Proportion of *An. coluzzii*			Model 3.3: proportion of outdoor biting		
Variables	Chi.sq	df	p-value	Chi.sq	df	p-value	Chi.sq	df	p-value
Humidity	0.004	1	0.95	0.04	1	0.84	2.76	1	0.09
Location	21.28	1	<0.0001*	n/a	n/a	n/a	n/a	n/a	n/a
Village	230.54	11	<0.0001*	n/a	n/a	n/a	13.7	11	0.25
cDate	1165	6.84[a]	0.0007*	68.35	5.22[a]	0.04*	3.06	1.55[a]	0.19
nDate	6.63	1	0.01*	32.78	1	<0.0001*	1.31	1	0.25
Temperature	0.15	1	0.7	1.53	1	0.22	0.49	1	0.48
Village: Location	14.93	11	0.185	24.78	11	0.01*	n/a	n/a	n/a
Location: Species	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a

* indicates significant term retained in the final model with $p < 0.05$ and

[a] indicate the given df represent the estimate degree of freedom (edf = degree of wigginess) from smoother term.

Table 3.8: Summary of the estimate (β), standard errors, z values and p-value of explanatory variables in the final Model 3.1 using the total female *An. gambiae* s.l. collected nightly (as response) at each village over location (indoor and outdoor) in southwestern Burkina Faso variable. nDate is a discrete variable representing the longer-term starting from the first day in October 2016 to the last day at the end December 2018. The reference village is Dangouindougou and the adjusted R^2 is 0.20.

	β	Std. Error	z value	Pr(>|z|)
Intercept	2.161	0.223	9.674	< 0.0001*
Gouera	-1.323	0.313	-4.235	< 0.0001*
Nianiagara	-2.102	0.344	-6.105	< 0.0001*
Nofesso	-2.628	0.339	-7.741	< 0.0001*
Ouangolodougou	-1.923	0.320	-6.004	< 0.0001*
Sitiena	-1.700	0.339	-5.012	< 0.0001*
Tengrela	0.665	0.300	2.219	0.026*
Tiefora	0.266	0.311	0.853	0.393
Timperba	-1.708	0.315	-5.425	< 0.0001*
Tondoura	-1.971	0.304	-6.487	< 0.0001*
Toumousseni	-1.367	0.331	-4.131	< 0.0001*
Yendere	-0.850	0.275	-3.091	0.002*
Location	0.138	0.0298	4.613	< 0.0001*
nDate	-0.0007	0.0003	-2.576	0.01*

* indicates $p < 0.05$.

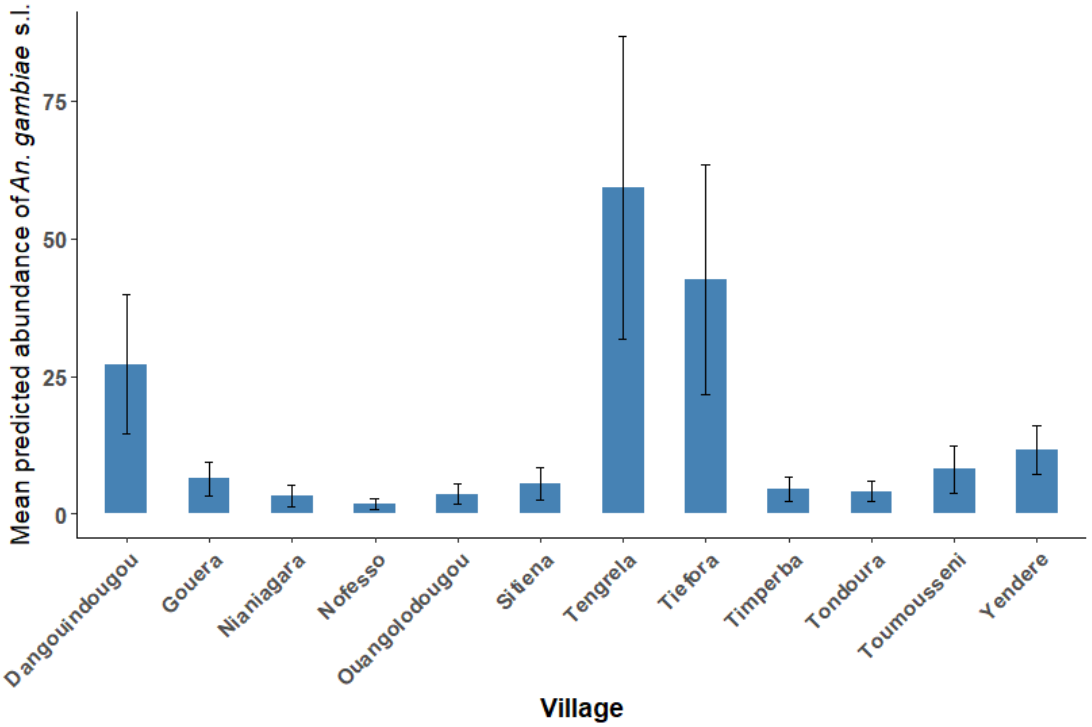


Figure 3.3: Mean predicted number of *An. gambiae* s.l. caught per night using Human Landing Catch in 12 villages in southwestern Burkina Faso. Data are pooled across trapping location (inside or outdoors houses) and the study period (October 2016 to December 2018). Error bars indicate 95% confidence intervals.

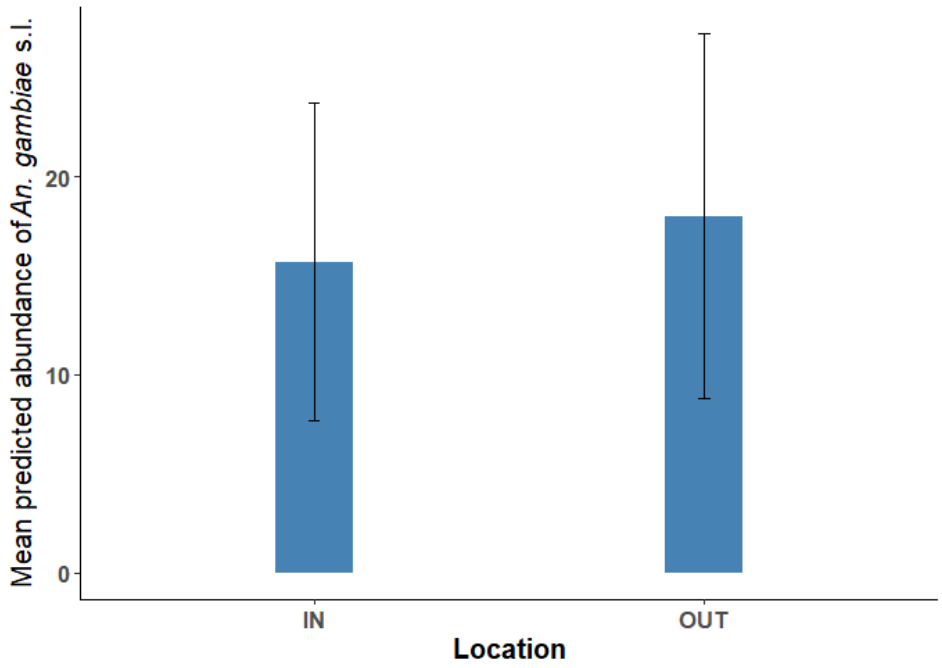


Figure 3.4: Mean predicted number of *An. gambiae* s.l. caught per night at each location (IN= inside houses or OUT= outdoors) as estimated from the final model using data from Human Landing Catches from 12 villages in southwestern Burkina Faso. Data are pooled across trapping location (inside or outdoors houses) and the study period (October 2016 to December 2018). Error bars indicate 95% confidence intervals.

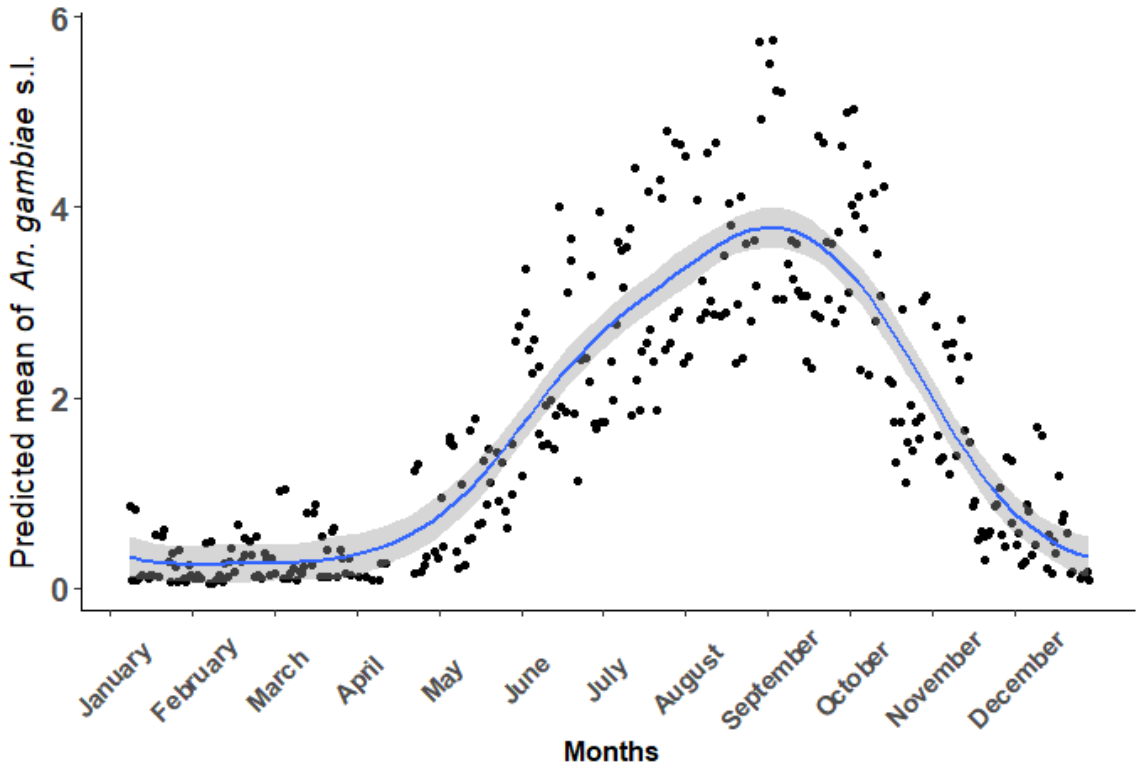


Figure 3.5: Estimated seasonal variation of *An. gambiae s.l.* abundance from human landing catches. The dots represent the predicted number of mosquitoes collected per month and sampling event. The blue line corresponds to the mean fitted regression line from the model with corresponding 95% confidence intervals as grey-shaded area. Data are pooled over 12 villages in southwestern Burkina Faso and trapping location (inside houses or outdoors).

3.3.3 *Anopheles gambiae* s.l. complex species: spatial and temporal distribution

Anopheles coluzzii and *An. gambiae* were the most abundant species within the subsample of *An. gambiae* s.l. identified by PCR (4222 *An. coluzzii*, 3600 *An. gambiae* and 22 *An. arabiensis* and 7 hybrids from *An. coluzzii* and *An. gambiae*). Due to their small numbers, *An. arabiensis* and the hybrids were excluded from statistical analysis. The proportion of *An. coluzzii* within *An. gambiae* s.l. sample was best explained in a model that accounted for an interaction between villages and trapping location (df = 11, x^2 = 24.78, p = 0.01), seasonality (edf = 5.22, x2 = 68.35, p = 0.04), and a long-term trend over the study period (df = 1, x^2 = 32.78, p < 0.0001; Table 3.7 & 3.9). A village and trapping location interaction were required because the difference between the proportion of *An. coluzzii* collected indoor and outdoor varied drastically between villages. However, these

differences were relatively minor in comparison to the much larger variation in species composition occurring between villages (Figure 3.6). For example, in several villages, the proportion of *An. coluzzii* was roughly similar in indoor and outdoor HLCs (e.g. Nianiagara, Sitiena, Tengrela), whereas in others there was higher proportion of *An. coluzzii* in indoor collections (e.g. Gouera) or outdoor collections (e.g. Nofesso). Both vector species were present throughout the year, but their relative composition varied considerably across the season. Specifically, *An. coluzzii* was much more abundant in the dry season, while *An. gambiae* dominated in the rainy season (Figure 3.7A). In addition, there was evidence of a long-term decline in the proportion of *An. coluzzii* ($z = -5.65$, $p < 0.0001$; Table 3.7 & 3.9) across the study period (Figure 3.7B). Accounting for these effects, there was no additional impact of mean temperature ($df = 1$, $x^2 = 1.53$, $p = 0.22$, Table 3.7) or humidity ($df = 1$, $x^2 = 0.04$, $p = 0.84$, Table3.7) on the proportion of *An. coluzzii* in the *An. gambiae* s.l. population.

Table 3.9: Summary of the estimate (β), standard errors, z values and p-value of explanatory variables in the final Model 3.2 using the proportion of female *An. coluzzii* relative to *An. gambiae* collected nightly (as response) at each village over location (indoor and outdoor) in southwestern Burkina Faso variable. nDate is a discrete variable representing the longer-term starting from the first day in October 2016 to the last day at the end December 2018. OUT= Outdoor location. The reference for village is Dangouindougou, for Location =indoor. The adjusted R^2 is 0.59.

	β	Std. Error	z value	Pr(>|z|)
Intercept	0.643	0.236	2.724	0.006*
Gouera	-0.765	0.352	-2.176	0.030*
Nianiagara	-0.968	0.441	-2.195	0.028*
Nofesso	-3.354	0.669	-5.015	0.000*
Ouangolodougou	-2.414	0.506	-4.775	0.000*
Sitiena	2.359	0.371	6.363	0.000*
Tengrela	4.706	0.406	11.582	0.000*
Tiefora	1.192	0.306	3.897	0.000*
Timperba	-1.607	0.425	-3.784	0.000*
Tondoura	-2.611	0.451	-5.788	0.000*
Toumousseni	0.856	0.332	2.575	0.010*
Yendere	-0.256	0.316	-0.808	0.419
OUT	0.206	0.232	0.889	0.374
nDate	-0.002	0.000	-5.645	0.000*
Gouera: OUT	-0.635	0.343	-1.853	0.064
Nianiagara: OUT	-0.499	0.475	-1.050	0.294
Nofesso: OUT	1.027	0.673	1.527	0.127
Ouangolodougou: OUT	0.032	0.603	0.053	0.958
Sitiena: OUT	-0.247	0.311	-0.795	0.427
Tengrela: OUT	-0.471	0.387	-1.215	0.224
Tiefora: OUT	-0.554	0.253	-2.184	0.029*
Timperba: OUT	-0.610	0.481	-1.269	0.205
Tondoura: OUT	-0.423	0.545	-0.776	0.438
Toumousseni: OUT	0.172	0.279	0.615	0.539
Yendere: OUT	-0.537	0.318	-1.692	0.091

* indicates $p < 0.05$

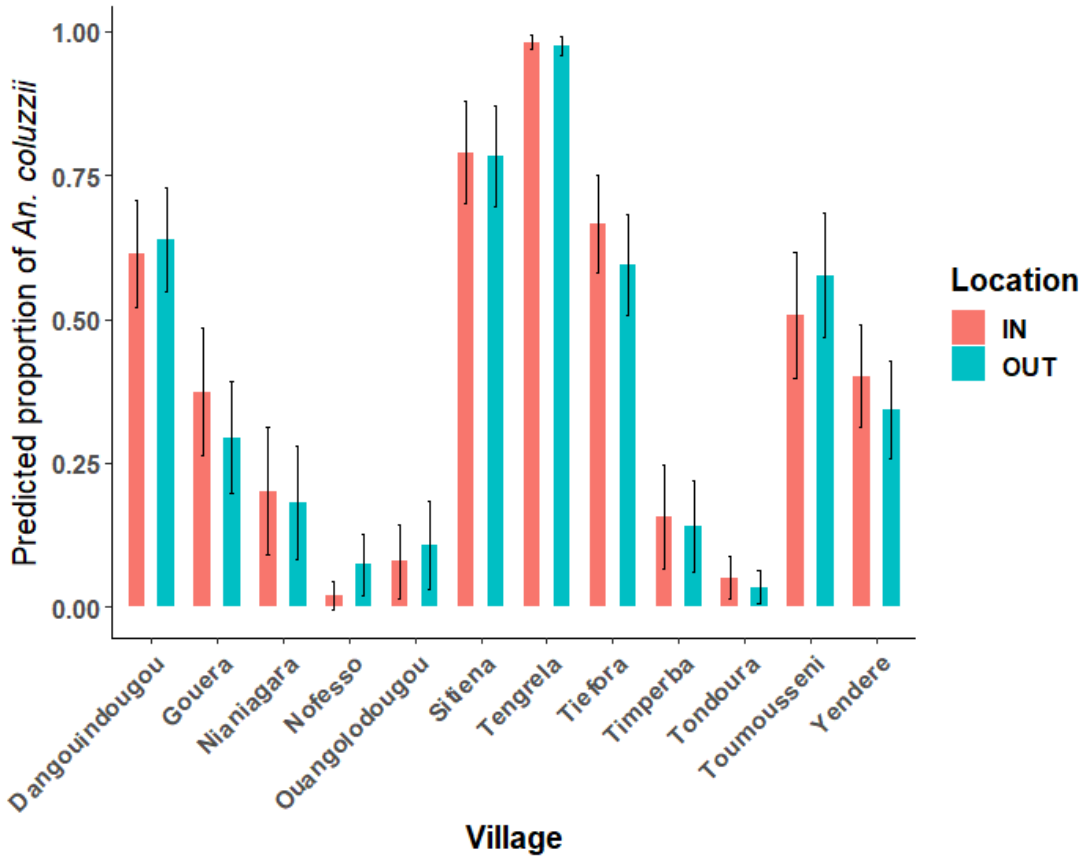


Figure 3.6: Predicted proportion of *An. coluzzii* in *An. gambiae* s.l. caught using Human Landing Catches in 12 villages in southwestern Burkina Faso, pooled over the collection period (October 2016 to December 2018) at each village by trapping location (IN= inside houses or OUT= outdoors). The error bars indicate 95% confidence intervals.

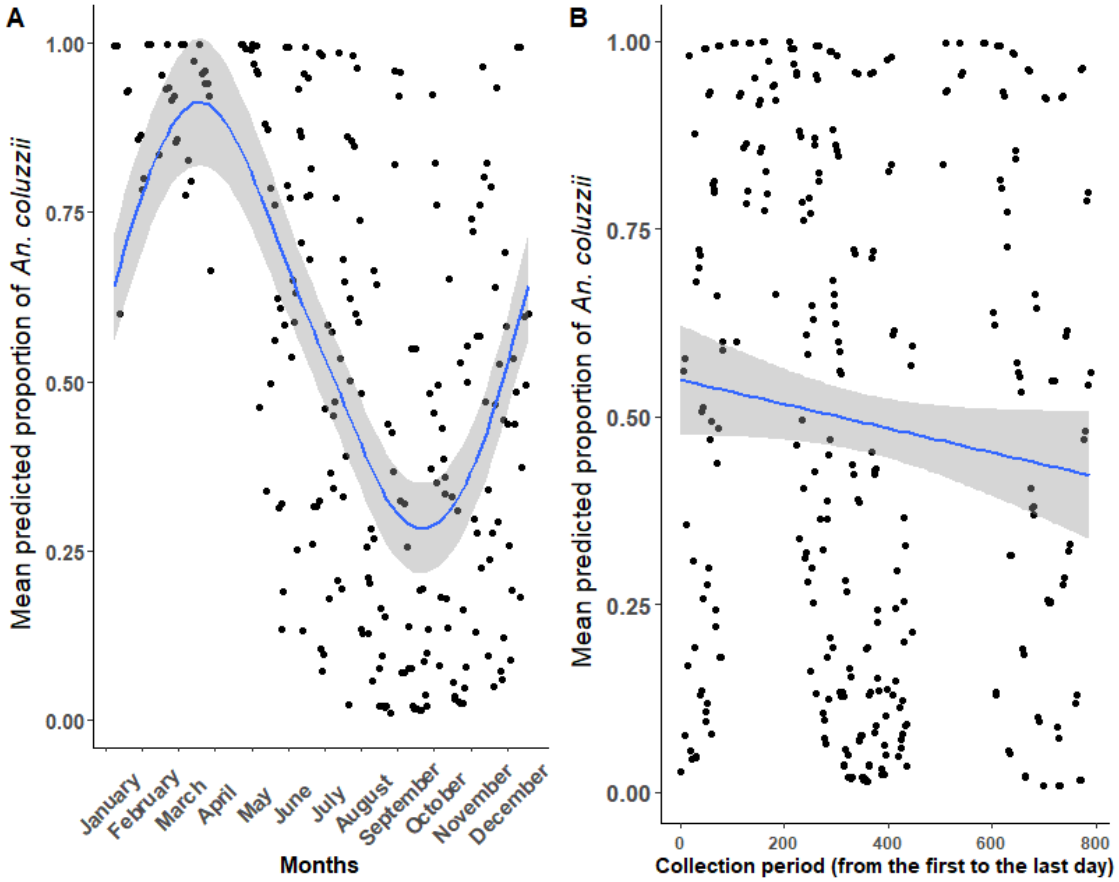


Figure 3.7: Predicted seasonal **(A)** and long-term trends **(B)** in malaria vector species composition as estimated from Human Landing Catches in 12 villages in southwestern Burkina Faso. Species composition was modelled in terms of the proportion of *An. coluzzii* within the *An. gambiae* s.l. complex. The blue curve and line represent the predicted regressions from models accounting for additional variation due to village and trapping location (inside and outdoors houses), with the grey-shaded area around them indicating the 95% confidence intervals.

3.3.4 *Anopheles gambiae* s.l. biting location

Anopheles gambiae s.l. were collected host seeking both indoors and outdoors in most villages (Tables 3.10); enabling the proportion of outdoor biting to be calculated with precision. Overall, ~54% (95% CI: ~51 – 57%) of *An. gambiae* s.l. host-seeking occurred outdoors, with no evidence of variation between villages (df = 11, x^2 = 13.70, p = 0.25), seasons (edf = 1.55, x^2 = 3.06, p = 0.19) or of a long-term change across the study period (df =1, x^2 = 1.31, p = 0.25; Table 3.7). The proportion of outdoor biting was also not significantly associated with mean

nightly temperature (df = 1, x^2 = 0.49, p = 0.48) or humidity (df = 1, x^2 = 2.76, p= 0.09; Table 3.7).

Table 3.10: Total number of host-seeking *An. gambiae* s.l. female collected using Human Landing Catches in southwestern Burkina Faso for each of the 12 village by trapping method from October 2016 to December 2018.

Village	Indoor	Outdoor	Total
Dangouindougou	788	786	1574
Gouera	762	867	1629
Nianiagara	451	522	973
Nofesso	340	540	880
Ouangolodougou	265	409	674
Sitiena	2549	2589	5138
Tengrela	4579	4598	9177
Tiefora	3917	4754	8671
Timperba	445	413	858
Tondoura	1121	1096	2217
Toumousseni	2670	3056	5726
Yendere	1272	1431	2703
Total	19159	21061	40220

Further analysis was conducted on the subset of *An. gambiae* s.l. that were individually identified to species level by PCR. Within this data, there was some evidence that the proportion of outdoor biting varied between *An. gambiae* and *An. coluzzii* (df = 1, x^2 = 6.82, p = 0.009). Both vector species were slightly more abundant in outdoor versus indoor HLCs, however *An. gambiae* was predicted to be somewhat more exophagic (Figure 3.8, proportion of outdoor biting: 54.73%, 95% CI: 52.35 - 57.12) compared to *An. coluzzii* (51.4%, 95% CI: 48.9 – 53.9%).

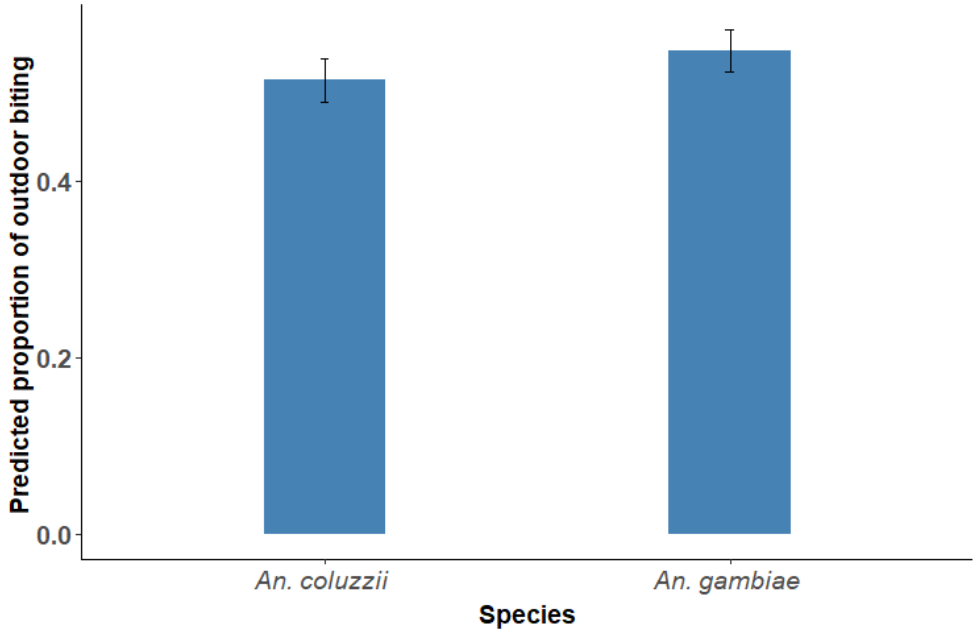


Figure 3.8: Predicted mean of the proportion of outdoor biting from *An. coluzzii* and *An. gambiae* (as assessed from Human Landing Catches) in 12 villages in southwestern Burkina Faso. Prediction after controlling for the variation between village, season over the study period using data collected from October 2016 to December 2018. Error bars indicate 95% confidence intervals.

3.3.5 Biting time in *Anopheles gambiae* s.l. population

The biting activity of *An. gambiae* s.l. exhibited a typical pattern of steady increase from low numbers in the early evening, up to a peak between 00 h and 04 h, with median biting time occurring at 01 h – 02 h. Similar patterns of biting activity were observed in indoor and outdoor collections (Figure 3.9). Although most biting took place during the middle of the night, some *An. gambiae* s.l. were caught biting during the final hour of collections (5am - 6am, Figure 3.9). Both analyses based on the clock time and time since sunset generated the same conclusions (Table 3.11) when considering the biting time of *An. gambiae* s.l. The median time of biting varied between villages (df = 11, F = 2.25, $p < 0.002$; Table 3.11, Figure 3.10) and was one hour earlier in Tengrela and Sitiena where *An. coluzzii* dominated compared to Nofesso and Tondoura (Figure 3.10) and *An. gambiae* was most dominant. Furthermore, median biting time showed a seasonal trend (edf = 2.26, F = 326.5, $p < 0.002$; Figure 3.9; Table 3.11) with biting occurring earlier between December – April (dry season) compared to July - September (wet season, Figure 3.11) where *An. gambiae* was again the most

prevalent species (Figure 3.6). No other explanatory variables had a significant association with median biting time (Table 3.11). Further analysis of the subsample of *An. gambiae* s.l. identified to species level indicated that the median biting time differs according to species after considering the effects of inter villages and seasonal variations (Table 3.11). Here, it was estimated that *An. coluzzii* was biting one hour earlier than *An. gambiae* ($df = 1$, $F = 23.49$, $p < 0.0001$, Figure 3.12). However, no seasonality was found in the median biting time when considering the clock time from the subsample identified to species level ($edf = 1.82$, $F = 0.58$, $p < 0.08$; Table 3.11). Considering the similarity in conclusion when using the full data, the contrast in the seasonality between the median time based on the "clock time" and "since sunset" may be due to the sample selection for the molecular works. This selection for species ideitification was not homogenously across the night. However, given the relatively small variation in sunset time in the area (≤ 1 hour) and difference in units of time measurement for sunset (hour: minute: seconde) and the biting time (aggregated by hour:00) graphs included here were based on clock time.

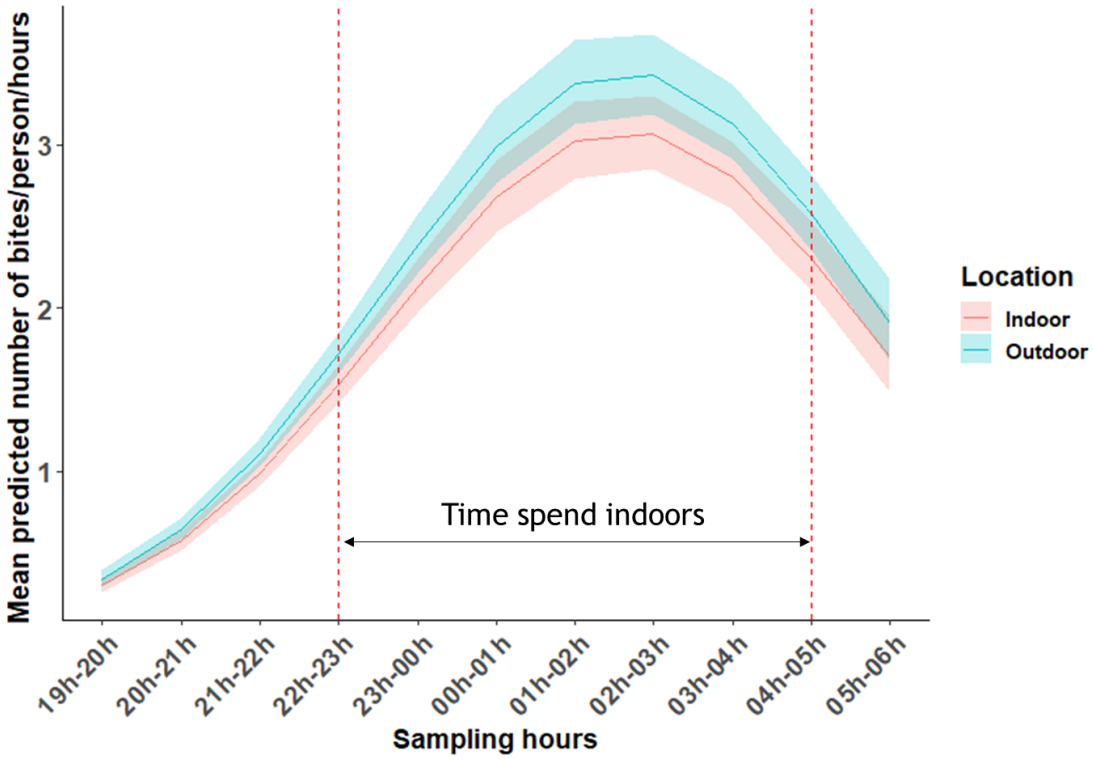


Figure 3.9: Mean number of *An. gambiae* s.l. biting per hour (as assessed from Human Landing Catches) in 12 villages in southwestern Burkina Faso, from October 2016 to December 2018. Data are pooled over village and collection period. The period between the red vertical dashed lines indicate period-time coinciding when most people are inside their dwellings as defined in Chapter 2 (Figure 2.2). The blue and red full lines indicate the predicted number biting in outdoor and indoor settings, respectively; with the shaded areas around the lines indicating the 95% confidence intervals.

Table 3.11: Significance of terms from the full model of median biting times by *An. gambiae* s.l. nDate indicates the long-term trend. n/a indicate that the given variable was not included in the model. F indicates the F-Statistic.

	Model 3.5: Median biting			Model 3.6: Median biting after sunset			Model 3.7: species median biting			Model 3.8: species median biting after sunset		
Variables	F	df	p-value	F	df	p-value	F	df	p-value	F	df	p-value
Humidity	0.08	1	0.776	0.21	1	0,647	0.004	1	0.95	0.079	1	0.778
Location	0.075	1	0.783	0.872	1	0.351	1.5	1	0.22	1.316	1	0.251
Village	2.246	11	0.01	2.294	11	0.008	3.437	11	0.000*	3.695	11	0.000*
cDate	326.5	2.26[a]	0.002*	104	1.87[a]	0.001*	20.58	1.819[a]	0.088	25.38	1.715[a]	0.0148*
nDate	0.202	1	0.653	0.082	1	0.77	3.092	1	0.079	2.801	1	0.094
Species	n/a	n/a	n/a	n/a	n/a	n/a	23.489	1	0.000*	24.321	1	0.000*
Temperature	0.932	1	0.334	0.324	1	0.569	1.318	1	0.251	1.281	1	0.257
Village: Location	0.613	11	0.819	0.60	11	0.83	n/a	n/a	n/a	n/a	n/a	n/a

* indicates significant term retained in the final model with $p < 0.05$ and

[a] indicate the given df represent the estimate degree of freedom (edf = degree of wigginess) from smoother term.

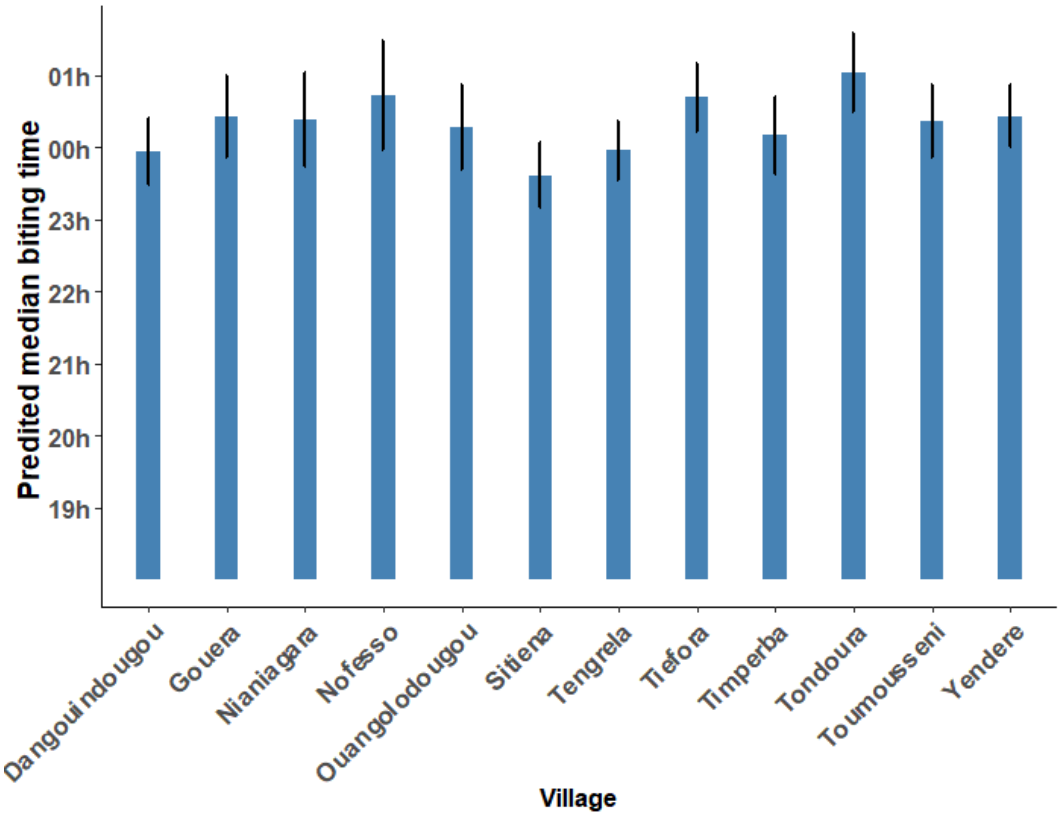


Figure 3.10: Corresponding predicted median biting times (hour) of *An. gambiae* s.l. as estimated from Human Landing Catches conducted in southwestern Burkina Faso from October 2016 to December 2018. Data are pooled over trapping location (inside or outdoor houses). The bars indicate the predicted medians and error bars indicate 95% confidence intervals.

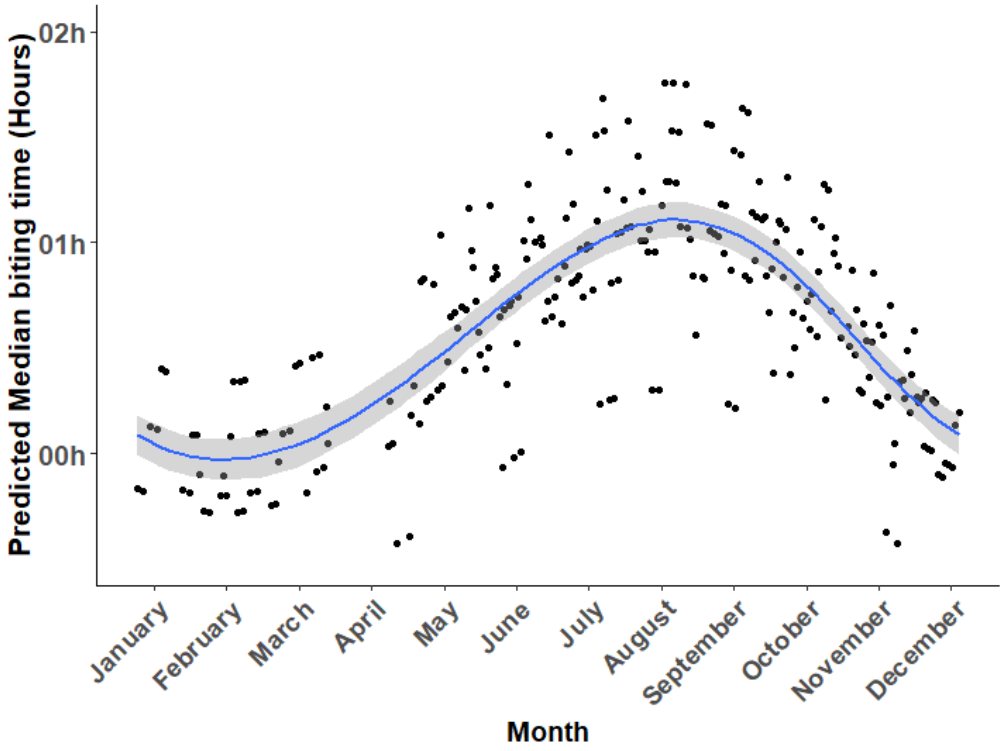


Figure 3.11: The corresponding predicted median biting time (hour) of *An. gambiae* s.l. across the year in southwestern Burkina Faso. Data are pooled over village, trapping location (inside or outdoor houses). The curve line indicates the regression line of the median biting time (dots) and grey-shaded area 95% confidence interval.

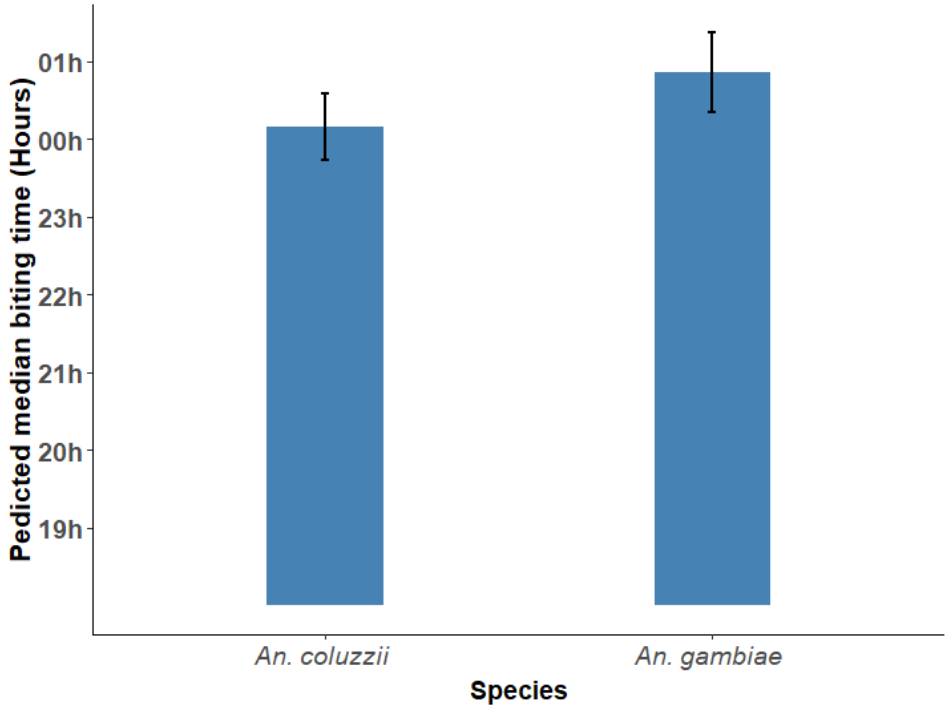


Figure 3.12: The corresponding predicted median biting time (hour) of *An. coluzzii* and *An. gambiae* as predicted from the final model using data from Human Landing Catches conducted in southwestern Burkina Faso from October 2016 to December 2018. The bars indicate the predicted medians and error bars indicate the 95% confidence intervals.

3.3.6 Resting behaviour in *Anopheles gambiae* s.l.

Separate analyses were conducted for female and male *An. gambiae* s.l. Further analysis was carried out on the subset of female *An. gambiae* s.l. (77% of total) that were individually identified to species level after exclusion of *An. arabiensis* and hybrids. Both sexes of *An. gambiae* s.l. were found resting inside and outside houses, with females slightly less likely to be found outdoors (46.97%, 95% CI: 23.53 – 70.41%) than males (54.14%, 95% CI: 18.47 – 89.8%). The resting behaviour of female *An. gambiae* s.l. varied seasonally (edf = 2.58, x^2 = 10.01, p = 0.007; Table 3.12; Figure 3.13), with more indoor resting in the wet than dry season. In contrast, there was no seasonal variation in male resting behaviour (edf = 0.33, x^2 = 0.268, p = 0.36; Table 3.12). There was no evidence of a longer-term shift in resting behaviour over the study period in females (df = 1, x^2 = 1.12, p = 0.29; Table 3.12) or males (df =1, x2 = 0.216, p = 0.64; Table 3.12).

Table 3.12: Significance of terms from the full model 3.9 and 3.10 of outdoor resting by *An. gambiae* s.l. nDate indicates the long-term trend. Here Chi.sq (x^2) represents Likelihood Ratio Test. n/a indicate that the given variable was not included in the model.

	Model 3.9: Male outdoor resting			Model 3.10: female outdoor resting		
Variables	Chi.sq	df	p-value	Chi.sq	df	p-value
cDate	0.268	0.329[a]	0.358	10.01	2.584[a]	0.059
nDate	0.216	1	0.642	1.116	1	0.29
Species	n/a	n/a	n/a	3.07	1	0.084
Sporozoite	n/a	n/a	n/a	16.49	2	0.0003*
Species: Sporozoite	n/a	n/a	n/a	2.595	2	0.273

* indicates significant term retained in the final model with p < 0.05 and
[a] indicate the given df represent the estimate degree of freedom (edf = degree of wigginess) from smoother term

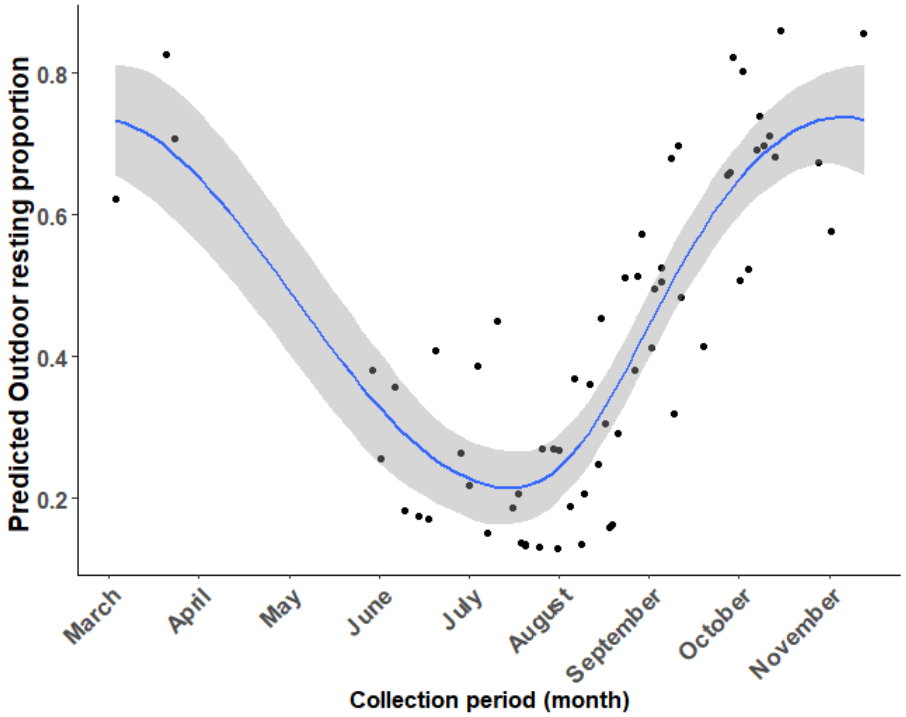


Figure 3.13: The predicted proportion of outdoor resting (dots) of the female *An. gambiae* s.l. from October to September in southwestern Burkina Faso. The blue curve indicates the regression line describing the trend in seasonality from a model after controlling for variation between villages. The grey-shaded area indicates 95% confidence interval.

Within the 449 *An. gambiae* s.l. females collected from the RBTs, 52.12% were unfed, 36.53% blood fed, 10.47% gravid and 0.9% half-gravid (Table 3.6). The half gravid" individuals were not included in the statistical analysis because there was only a few (N = 4). Analysis of all other *An. gambiae* s.l. females indicated that resting location varied significantly with physiological status (df = 2, x^2 = 16.49, p = 0.0003; Table 3.12), but not with species (df =1, x^2 = 3.07, p = 0.08; Table 3.12). Only ~30% (95% CI: 7.4 - 49.64%) of blood fed *An. gambiae* s.l. were found resting outdoors compared to ~65% (95% CI: 41.71 – 87.55%) of unfed and 44% of gravid females (95% CI: 3.12-75.16%, Table 3.6).

3.3.7 Predicted human exposure

As described in the method section of Chapter 2, estimates of the degree of exposure that can be prevented by LLINs assuming that most people were indoors and under nets between 10 pm – 5 am, and otherwise outdoors and unprotected between 7 – 10 pm, and 5 – 6 am when mosquito collections stopped. Information on the timing and location (in vs out) of *An. gambiae* s.l. biting activity described above was combined with observational-derived data on human behaviour (Figure 2.2) to estimate predicted exposure to mosquito bites. Overall, the proportion of *An. gambiae* s.l. biting occurring during hours when most people are indoors (10 pm – 5 am, Pfl) was 86.81% (95% CI: 83.6 – 90.02%), with 85.45% (95% CI: 80.64 – 90.26%) of human exposure occurring when people are indoors (πi). However, these estimates varied somewhat between villages (Table 3.13, Figure 3.14). Values of P_{fl} and πi varied from a low of ~81% and ~79% in Tengrela up to ~91% to ~92% respectively in a group of villages including Nianiagara, Ouangolodougou, Timperba and Tondoura (Figure 3.14). There was no evidence of seasonal variation in these exposure metrics (P_{fl}: x^2 = 0.001, edf = 0.002, p = 0.39; πi: x^2= 1.42, edf = 0.86, p = 0.11; Table 3.13), or an additional impact of temperature and humidity (Table 3.10). However, there was evidence of a reduction in the proportion of host seeking occurring during times when people are expected to be indoors (P_{fl}: z = -3.14, p = 0.002, Figure 3.15A, ~7% decline), and in the proportion of exposure predicted to be preventable using LLINs (πi: z = -3.72, p = 0.0002, Figure 3.15B, ~10%) over the study period.

Table 3.13: Significance of explanatory variables in the model 3.11 and 3.12. Chi.sq (x^2) represents the value of the Likelihood Ratio Test. cDate indicates the seasonality term fitted using a non-linear smoothing function (spline t2(cDate, bs = cc)) on days as a period of 365 days. nDate corresponds to the longer-term trend of the proportions modelled as a discrete variable (1 to 798 days). The temperature and relative humidity were obtained by averaging the records over the course of the collection night.

	Model 3.11: Proportion caught when most people are indoors (Pfl)			Model 3.12: Proportion of human exposure occurring indoors (πi)		
Variables	Chi.sq	df	p-value	Chi.sq	df	p-value
cDate	0.01	0.002	0.39	1.42	0.86	0.11
Humidity	0.34	1	0.56	0.29	1	0.59
nDate	9.86	1	0.002	13.81	1	0.0002
Village	50.16	11	0	41.51	11	0
Temperature	1.28	1	0.126	3.58	1	0.06

* indicates significant term retained in the final model with $p < 0.05$ and

[a] indicate the given df represent the estimate degree of freedom (edf = degree of wigginess) from smoother term.

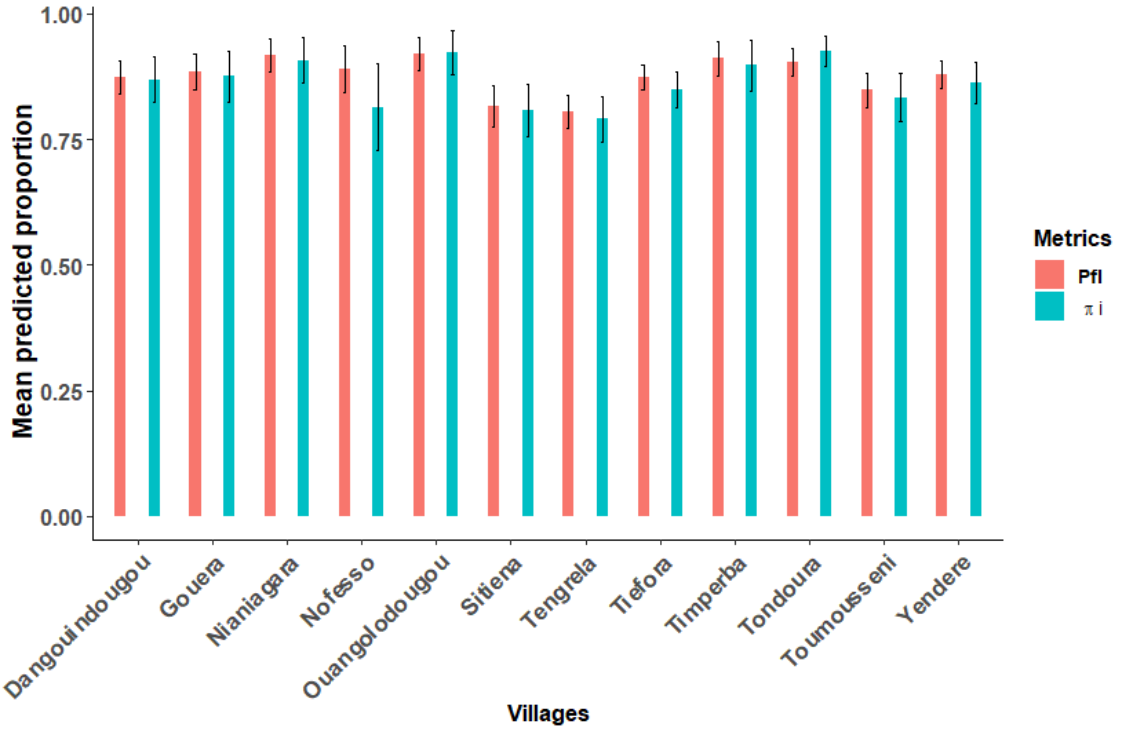


Figure 3.14: Estimated predicted mean of the proportions of *An. gambiae* s.l. caught during hours when most people are inside their dwellings and likely asleep (e.g. between 10 pm – 5 am; **Pfl**, red bars) and total human exposure to *An. gambiae* s.l. bites occurring indoors (**πi**, blue bars) based on Human Landing Catch data in the 12 villages in southwestern Burkina Faso. The error bars indicate the 95% confidence intervals.

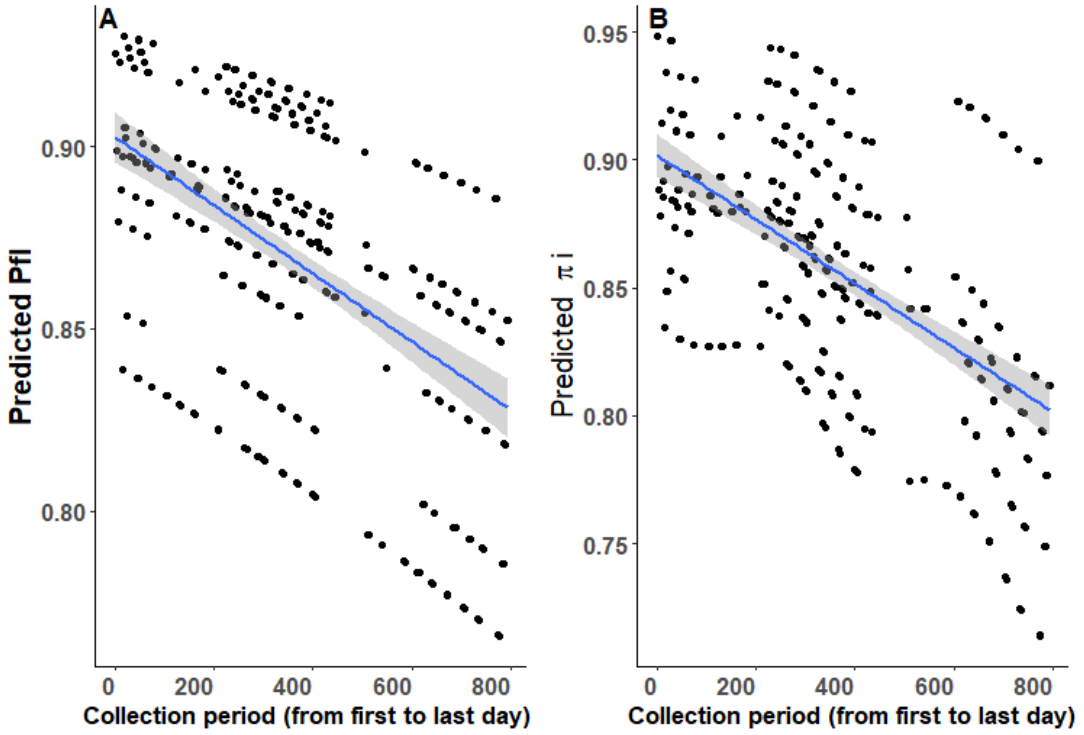


Figure 3.15: Estimated mean of **A) Pfl** = the proportion of *An. gambiae* s.l. bites occurring when most people are inside their dwellings and likely asleep (e.g. between 10 pm - 5 am; Pfl), **B) πi** = total human exposure to *An. gambiae* s.l. bites occurring indoors (πi) based on Human Landing Catch from 6 villages over 2 years (Oct 1st, 2016 to Dec 4th, 2018) in southwestern Burkina Faso. Dots represents the predicted values (Pfl and πi) at each sampling night. The blue line represents the regression line from the model and the grey-shaded area the 95% confidence intervals.

3.3 Discussion

As anticipated, here malaria vector abundance was shown to vary considerably between sites and seasons. Taking this heterogeneity into account, there was also evidence of a longer-term reduction in *An. gambiae* s.l. abundance (from ~25 bites per person per night in year 1 versus 17 bites per person per night in year 2, Chapter 5) over the course of the study. This coincided with a shift in vector species composition, with the proportion of *An. coluzzii* relative to *An. gambiae* decreasing by ~23% over the study period. The proportion of outdoor biting and resting by *An. gambiae* s.l. in the study area was higher than expected ($\geq$ 50%). However, neither the proportion of outdoor biting, median biting time or proportion of outdoor resting by malaria vectors changed during the study period. Combining data on the timing and location of mosquito biting, it was

estimated that ~85% of human exposure to *An. gambiae* s.l. bites could be prevented by use of effective LLINs during typical sleeping hours (10 pm-5 am). However, there was evidence of a gradual reduction in the degree of protection expected from LLINs over the course of the study. I speculate this apparent reduction in expected protection from LLINs may be due to a longer-term change in malaria vector species composition, with the slightly more exophagic *An. gambiae* increasing in proportion relative to *An. coluzzii* through time. Despite this potential change in the proportion of exposure that could be prevented by LLINs, the overall reduction in vector density suggests that transmission was falling during the study period. Further investigation of the vectorial capacity of these populations (Chapter 5) and their susceptibility to insecticides (Chapter 4) is needed to confirm how LLIN effectiveness and associated human exposure to malaria changed over the study period.

In Burkina Faso and in many other African countries, there has been relatively little investigation of *An. gambiae* s.l. behaviour in comparison to their insecticide resistance status [68, 69, 220, 225, 340]. Information presented here addresses this gap and provides updated estimates of vector behaviour within the current context of mass LLIN distribution. Previous studies from the Central, Plateau Central and West regions (2001-2015) of Burkina Faso indicated that the proportion of indoor feeding either exceeded 50% [222, 341], or was split evenly with outdoor biting [52]. Analysis of data presented in an earlier study on *An. gambiae* s.s. in Ghana (West Africa), before the taxonomic split into *An. gambiae* and *An. coluzzii* [36] indicated that this vector is endophagic; with 78% of bites taken indoors [342]. More recently, a systematic review of malaria vector biting behaviour from a range of African countries indicates that generally > 80% of vector bites occur indoors [343]. This follows historical data from human landing catches carried out in Burkina Faso from 1985 to 1988 [224], and 2002 to 2004 [222], that estimated the proportion of indoor biting by *An. gambiae* s.l. to be ~57% and ~59% respectively still somewhat higher than the 45% estimated here. However, it could be argued that there may be heterogeneities in *An. gambiae* s.l. biting behaviour. For example, these findings slightly contrast with results of a study from Benin where the majority of *An. gambiae s*.l. (~55%) still occurred indoors two years after LLIN distribution (e.g LLIN distribution in 2014,

study in 2016; [344]). However, it is unknown whether the slightly higher outdoor biting rates in this setting reflects natural between-population variability in comparison to other sites (from previous studies in Burkina Faso and elsehwhere (e.g. Benin); or are the result of selection against indoor biting in response to the mass introduction of LLINs. As elsewhere, previously endophagic malaria vectors have been observed to increase outdoor biting following introduction of LLINs and IRS [345, 346]. For example, a study in south-eastern part of Benin showed a significant reduction in the proportion of indoor biting from ~67% to 43% before and after an IRS campaigns, compared to change from 71% to 57% in control arms [345]. However, many of these studies are based on short-term observation of mosquito behaviour in the few months before and after the intervention, making it difficult to discern a longer-term trend from seasonal or other sources of variability. The more systematic monitoring of vector behaviour over two years following an intervention helps elucidate this.

Malaria vectors exhibited relatively similar patterns of biting activity in indoors and outdoors. In both cases, there was a single peak late in at night between 00 h to 04 h. This is consistent with early work on *An. gambiae* s.l. before mass ITN use, in which peak biting rates occurred between 00 h [56, 307, 347, 348]. A study in western Kenya showed that *An. gambiae* s.l. and *An. funestus* continued to biting late in the night even in presence of LLINs [321], as observed here. Further, results here are also consistent with that from a study in Senegal where the biting pattern of An. gambiae s.l. was similar in indoor and outdoor settings prior to mass LLIN distribution [349]. However, heterogeneity in *An. gambiae* s.l. biting time between and within countries has been documented [229, 350, 351]. Results presented here contrast with recent reports from north-western Burkina Faso where *An. gambiae* s.l. biting activity reached an earlier maximum of 8 pm [229, 351]. It is unclear whether this is be due to variation in the *An. gambiae* complex species composition (no species-level identification was performed in the previous study) or within-species variation. However, the activity of vectors in our study area suggests that most biting occurs during times when most people are indoors and could thus be protected by LLINs.

The major malaria vectors species in the current study area are *An. coluzzii* (53.82%) and *An. gambiae* (45.8%); with a minor proportion of other known vectors (*An. arabiensis* = 0.28%, *An. funestus* = 0.09%). This is consistent with previous studies in Burkina where *An. coluzzii* and *An. gambiae* together represented >80% of the *An. gambiae* s.l. complex [52, 223, 229, 352-354]. Analysis of the subset of *An. gambiae* s.l. which were individually identified to species level indicates some ecological and behavioural differences between species. First, the relative abundance of *An. coluzzii* was higher in the dry season (> 50% of *An. gambiae* s.l.), whereas *An. gambiae* dominated in the wet season (> 50%). This variation in the seasonal dynamics of *An. coluzzii* and *An. gambiae* has been previously described in Burkina Faso [50, 68, 223, 225, 355] and other parts of west Africa [356]. Second, the proportion of outdoor biting was slightly higher in *An. gambiae* (55%) than *An. coluzzii* (51%). These between-species differences in biting time differ from those reported elsewhere in west Africa (e.g Benin [356] and Cote d'Ivoire [357]). For example, the proportion of outdoor biting was higher in *An. coluzzii* (~57%) than *An. gambiae* (43%) in Benin [356]. Thus, the differences reported here may not reflect fixed species-specific differences, but characteristics of the local populations. Finally, the median biting time of *An. coluzzii* was slightly earlier (by ~1 hour) than *An. gambiae*. This may explain why, median biting times were 1 hour earlier in villages and dry season dominated by *An. coluzzii*, compared to those (villages) and wet season dominated by *An. gambiae*. To my knowledge, this is the first time that differences in biting time between these species have been investigated. However, no difference in resting behaviour between vector species was detected here, in contrast to a previous study in Burkina Faso where *An. gambiae* females were more exophilic (> 60%) than *An. coluzzii* (<50%; [330, 340]. Further evidence is required to confirm whether observations reported here reflect innate differences between these species or simply phenotypic plasticity between populations. However, these results highlight that both vector species are commonly found biting and resting outdoors. This could pose a challenge to current indoor-based vector control interventions.

Here, the densities of *An. gambiae* s.l. varied up to 32-fold between villages; with abundance being highest at the three villages with permanent water sources

(e.g. lakes, damps; Dangouindougou, Tengrela and Tiefora) compared to those with semi-permanent (e.g. small rivers and streams; Gouera, Nianiagara, Nofesso, Ouangolodougou, Sitiena, Timperba, and Toumousseni) and temporary ones (e.g. water-filled tyre-prints, footprints, natural and manmade pools and ditches; Tondoura and Yendere). Lakes and rivers are often exploited for agricultural practices in this region, creating aquatic microhabitats for larvae [358, 359] that are associated with *Anopheles* abundance and species composition [224, 360-363]. For example, malaria vector abundance was found to be higher in irrigated versus non-irrigated areas in Mali [362]. Here, *An. coluzzii* was the dominant vector in three villages (Sitiena, Tengrela and Tiefora) that had substantial rice and vegetable cultivation on flooded parcels of arable land. Such permanent habitats are more associated with *An. coluzzii* larvae elsewhere in Burkina Faso and other African countries [362, 364-369] in contrast to the temporary man-made breeding sites and water-filled tyre-prints favoured by *An. gambiae*. Consequently, the predominance of *An. gambiae* at the six sites (Nianiagara, Nofesso, Ouangolodougou, Timperba Tondoura and Yendere) without permanent water bodies here is in line with expectation. Here, two aspects of malaria vector behaviour were shown to vary seasonally; the location of resting (indoors versus outdoors) and median biting time (earlier in dry than wet season). As described above, seasonality in the *An. gambiae* s.l. species composition may account for at least some of this behavioural variation. To my knowledge no study has reported seasonality in *An. gambiae* resting behaviours in Burkina Faso. In east Africa, the relative difference in temperature and humidity between indoor and outdoor can vary seasonally, and has been linked to variation in outdoor biting by malaria vectors [207]. It is similarly possible that the seasonal variation in resting behaviour described here could be due to changes in microclimatic conditions between indoor and outdoor habitats. This seasonal variation in resting behaviour could have implications for the effectiveness of IRS, with a greater proportion of the vector population being found outdoors during the dry than wet season. In contrast, the lack of seasonality in the location (in vs out) of vector biting suggests LLIN effectiveness may be relatively stable across the year. After controlling for seasonal and spatial sources of variation, there was an evidence of long-term changes *An. gambiae*

s.l. ecology over the 2-year period of this study. First, there was a moderate decline in *An. gambiae* s.l. abundance, and the relative proportion of *An. coluzzii* within *An. gambiae* s.l. complex. Consistent reductions in vector densities have been reported 1-2 years following LLIN and IRS introduction in west Africa [370] and other settings across and beyond Africa (Papua New Guinea, [371]). However, there are also accounts of vectors abundance rebounding in the second year following an intervention [346, 372], which was not observed here. This reduction in *An. gambiae* s.l. density coincided with a gradual shift in species composition characterized by a moderate (~23%) reduction in the proportion of *An. coluzzii* (more endophilic and anthropophagic) relative to *An. gambiae*. Shifts in vector species composition from more to less endophagic species following the introduction of LLINs and IRS have been documented in other African countries [312-314, 373]. For example, substantial declines in *An. gambiae* relative to that more zoophilic and exophilic *An. arabiensis* have been widely documented in East Africa as LLIN coverage increased [204, 314]. Similarly, *An. arabiensis* become predominant in areas of Senegal following LLINs distribution decreases in *An. gambiae* and *An. coluzzii* [311]. In the absence of historical data from before the most recent LLIN distribution or earlier, we cannot confirm if this change in species composition is due to the intervention. However, the direction of change is consistent with the hypothesis that LLINs impose greater selection toward exophagic species.

This study has some notable limitations that require consideration. First, our ability to detect long-term shifts in malaria vector behaviours in response to LLINs was restricted by the lack of baseline data from before the 2016 LLIN campaign, or before any LLIN distribution (held in 2010 and 2013). Additionally, whilst the 2-years of monthly follow up at several sites is considerably more intensive in most previous studies of mosquito behaviour, it is still a relatively short-period of observation for detection of evolutionary changes. Longer-term data sets may be required to conclusively identify the relative contribution of interventions and concurrent environmental change with shifts in malaria vector ecology and transmission. While several other additional environmental factors such as temperature and humidity were included as covariates in this analysis, other important covariates such as rainfall and housing structure, LLIN use were

not. Thus, we cannot exclude the possibility that some of the long-term trends in vector ecology reported here were influenced by changes in these variables. Finally, this study likely overestimated the proportion of malaria exposure that can be prevented by LLINs because of the assumption of universal LLIN coverage and consistent sleeping patterns in the community.

Based on the timing and location of mosquito biting measured here, I estimated that ~85% of human exposure to malaria vectors could be prevented by appropriate use of good quality LLINs. This reinforces the general conclusion that LLINs can still effectively prevent the bulk of malaria exposure in this and other African settings (>90%, [65, 222, 275], however the predicted degree of protection estimated here (85%) is somewhat lower than previous estimates from Burkina Faso (90%, in 2002-2004), and other parts of Africa (95 – 99%; [374]). Additionally, both the proportion of host-seeking predicted to occur during times when people were indoors (P_{fl}), and proportion of exposure preventable by using LLINs (πi) appeared to fall over the study period. Similarly, a decrease (from 63% to 45%) in the in the proportion of that is preventable using LLINs (πi) in Senegal was observed following several rounds of LLIN mass distribution [349]. Even if the proportion of human exposure that can be prevented by LLINs remains at current levels, high levels of residual transmission are likely to persist because of the relatively high abundance of malaria vectors (Chapter 5).

The proportion of exposure that can be prevented by LLINs in this study area should be interpreted as a "best case scenario" based on the assumption of 100% population of coverage with good quality of LLINs. The true estimate of protection gained from the LLINs may considerably drop when considering that only ~ 90% of households own at least a LLIN, and only ~67% of people may effectively sleep under them [323]. Both these parameters of coverage and use may vary between communities, socio-economic and demographic groups and between seasons [123, 323, 325, 375]. The protective value of LLINs also declines with physical deterioration or damage to the nets through time as seen elsewhere in east [124, 376] and west Africa [377]. Additionally, these calculations were made based on the assumption that all community members are in bed and protected by LLINs for the same fixed period (10pm-5am). A recent

anthropological investigation in the study area highlighted significant heterogeneity in the sleeping hours and night-time activities of community members [378], indicating individual differences in human behaviour may further reduce LLIN-derived exposure. The combination of all these factors and widespread insecticide resistance (Chapter 4) in vector populations could help explain why LLIN-based strategies on their own are not making much impact in Burkina Faso (discussed in Chapter 6).

3.4 Conclusions

Here I show that malaria vector populations underwent significant ecological changes in the two years following a mass LLIN distribution in southwestern Burkina Faso. Most notably, there was evidence of a long-term decline in *An. gambiae* s.l. abundance, and a shift in species composition with *An. coluzzii* declining relative to *An. gambiae*. Rates of outdoor biting higher than anticipated from previous reports but showed little change over the study period. It was estimated that the bulk of human exposure to malaria vectors (85%) should be preventable by use of LLINs during sleeping hours, however this proportion of protection was predicted to decline over the study period. Although, most human exposure to malaria vectors in the study area likely happens indoors, outdoor biting constitutes an important source of residual transmission.

Chapter 4: Spatial and temporal variation in insecticide resistance within *Anopheles gambiae sensu lato* (s.l.) populations following a mass LLIN distribution in rural Burkina Faso

Abstract

Background

Burkina Faso has one of the highest rates of malaria transmission in Africa. In 2016, the government of Burkina Faso distributed more than 10 million Long-Lasting Insecticide-Treated Nets (LLINs) throughout the country as its primary malaria control strategy. However, the impact of this intervention may be impeded by increases in insecticide resistance (IR) in mosquito vector populations. This study investigated spatial, seasonal and longer-term changes in IR in the 2 years following a mass LLIN distribution, with the aim of measuring changes in resistance associated with this intervention.

Methods

Larvae of *Anopheles gambiae* s.l. mosquitoes were collected from nine villages in southwestern Burkina Faso and reared to adulthood in insectary conditions for use in insecticide resistance bioassays. Standard WHO Tube bioassays were performed on cohorts of larvae from different sites and time points after LLIN distribution to test for spatial, seasonal, and longer-term variation in the prevalence of IR. Additionally, bioassays were conducted using different doses of deltamethrin to quantify the intensity of IR in some populations. A subset of *An. gambiae* s.l. used in bioassays were identified to species-level, with results used to test for vector species-specific variation in IR.

Results

A total of 10,464 females adult *An. gambiae* s.l. from 9 villages were assayed for IR. Overall, the mortality rate of *An. gambiae* s.l. 24 hours after exposure to a discriminating dose of deltamethrin (0.05%) was 26.39% (95% CI: 23.5 – 29. 28%). The 24-hour mortality of all vector populations was below the 90%;

meeting the WHO criteria for high resistance. However, the post-exposure mortality rate varied between villages and seasons (< 21% in dry season versus ~30% in wet season), reduced considerably over course of the study (~38% at start to ~20% at the end). Furthermore, < 90% of *An. gambiae* s.l. were killed in the 24-hours following exposure to 5, 10 and 15 X the discriminating dose; confirming an extremely high level of IR in these populations. There was no evidence of a consistent difference in IR between *An. coluzzii* and *An. gambiae*.

Discussion

Insecticide resistance in the study site was very high and increased over the course of the study. This confirms that these vector populations have capacity for rapid and increasingly intense IR; which has potential to erode the impact of LLINs. There is a need for continual monitoring of IR within these malaria vector populations to quantify its impact on current control programmes and identify which alternative complementary strategies are most likely to overcome it.

4.1. Background

As in many other African countries, the primary malaria control strategy carried out by the government of Burkina Faso is the mass distribution of LLINs on a 3-year cycle (Chapter 1, [115]). A mass LLIN distribution in Burkina Faso occurred in 2016, within which more than 10 million LLINs were distributed throughout the country by the national malaria control programme. In the 2016 LLIN programme, the Permanet® 2 was distributed throughout Burkina, a product that contains the pyrethroid type Deltamethrin. However, there is concern that the impact of such interventions is being eroded by the widespread emergence and increase in insecticide resistance (IR) occurring in Burkina Faso and other African countries [69, 177, 379].

Widespread resistance has been previously documented in malaria vectors throughout Burkina Faso, where it has been attributed to wide-scale use of pyrethroids in agriculture [225, 380] and public health [168, 169, 188]. Pyrethroid resistance in malaria vectors has also now been documented in almost all African countries [125, 128, 227, 367, 381-383], with rates in Burkina Faso being amongst the highest. The co-occurrence of such high IR in Burkina Faso with a very high malaria burden suggests that this resistance is jeopardising the effectiveness of LLINs [326, 384]. As reviewed in chapter 1 and other studies [91, 220, 385], pyrethroid resistance is a complex phenotype arising through several different mechanisms including target site mutations [92, 127, 173, 185, 386], and the elevated expression of detoxification enzymes [173, 387]. These two mechanisms have been documented within *An. gambiae* s.l. populations in Southwestern Burkina Faso [220, 326, 340]. A third mechanism “cuticular resistance”, consisting of reduced in insecticide uptake, has also been observed in these vector population [197, 198]. The existence of multifaceted IR in addition to behavioural avoidance strategies (e.g. Chapter 3) in these vector populations is of concern for malaria control in Burkina Faso and other African countries.

As introduced earlier in Chapter 1, despite clear evidence of the existence and intensification of IR, there is still controversy surrounding its epidemiological impact. Experimental hut trials [329, 388], modelling [389], laboratory-based [326] and field [390-392] studies indicate IR can compromise LLINs efficacy. However, so far there is limited evidence of association between IR and epidemiological outcomes such as malaria incidence and prevalence in people [112, 172]. One reason for the lack of clear evidence linking insecticide resistance to malaria control failure could be that the transmission potential of resistant mosquitoes is impaired in other ways. For example, insecticide resistance is typically defined based on the ability of mosquitoes to survive the first 24-hours after insecticide exposure [175]. However, malaria transmission is dependent on the long-term survival of vectors; as the parasite requires at least 10 - 12 days development within mosquitoes living at 26 °C to 28 °C before it becomes infectious [22]. A recent study found that even highly resistant malaria vectors experience delayed mortality following exposure to insecticides, which reduces their transmission potential by 50% [393]. There are two ways LLINs impact on vector population: first, the personal protection impact that comes from preventing mosquito bites to users, and second the additional community impact arising from the insecticide where by mass killing impact reduces vector populations [108], protecting even non users [104, 105]. Insecticide resistance may erode the latter effect, but not the personal protection one. Thus, even if the killing impact of the LLINs is removed, nets still offer personal protection which may reduce malaria transmission [394].

The appearance and evolution of IR within a vector population depends on the strength of selection; which may vary in response to the source of insecticide, concentration and frequency of application. In malaria vectors, as stated earlier, IR has been associated both with insecticide use in vector control (LLINs and IRS, [168, 169, 188]) and agriculture [166, 225, 379, 395]. Whereas mass LLIN distribution campaigns aim to achieve high and universal population coverage within countries, there may be considerable subnational spatial and temporal variation in the use of agricultural insecticides in response to local ecology and farm practices. Heterogeneity

in the use of insecticides between villages and seasons in agriculture [166, 379, 395] could generate spatial and temporal variation in IR intensity in malaria vectors, with knock on effects for the efficacy of insecticide-based interventions. In addition, the spatial and temporal variation in A*n. gambiae* s.l. complex (e.g. Chapter 3) could result in differences between vector species in their exposure to insecticide and resultant IR. Though there is need of a finer-scale spatial, seasonal and mosquito-species specific variation in IR for understanding heterogeneity in intervention effectiveness.

This study aimed to quantify IR in malaria vector populations in southwestern Burkina Faso in the 2-year period following a mass LLIN distribution campaign. Objectives were to update information on the magnitude IR, and test for variation between i) villages, ii) seasons and iii) vector species. Additionally, bioassay data collected across the 2-year study period was used to assess longer-term trends in IR following mass LLIN deployment.

4.2. Methods

4.2.1 Study site

As part of a comprehensive longitudinal study of malaria vector demography, behavior (Chapter 3) and transmission potential (Chapter 5), insecticide resistance in malaria vector populations in southwestern Burkina Faso was monitored in the 2 years following a mass LLIN distribution. Originally, IR monitoring was planned to occur in all 12 original study sites (Figure 1.7, Chapter 1); with assays conducted at least once during the wet (July - October) and dry season (November - April) of each year. However, due to limitations in availability of breeding sites in some villages and months, especially during the dry season, IR monitoring was only possible in 9 villages (Figure 4.1, Table 4.1).

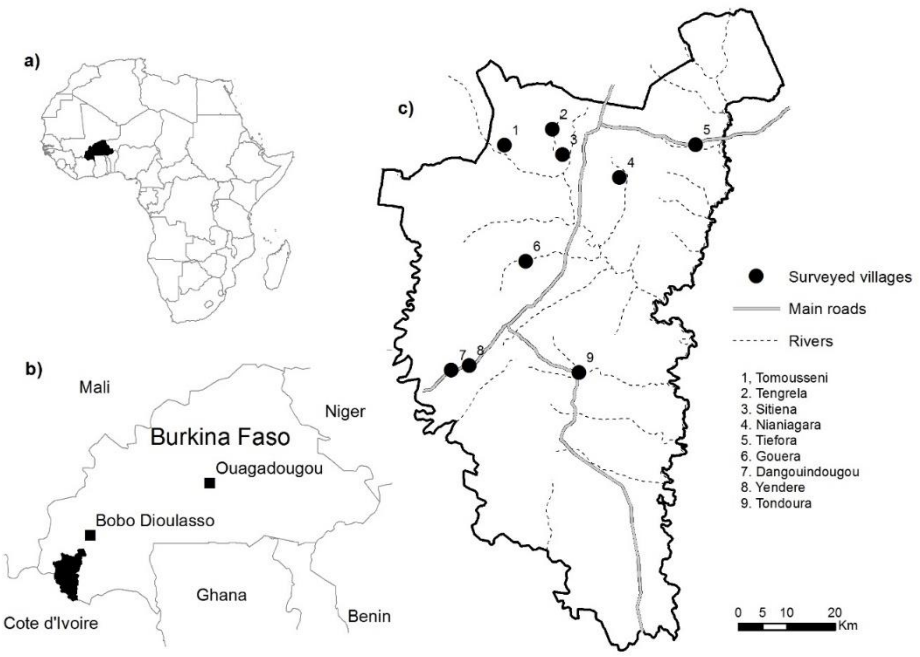


Figure 4.1: Map of the study sites showing the a) location of Burkina Faso within Africa, b) study area in the Cascades Region, c) the surveyed villages for insecticide resistance monitoring.

4.2.2 Experimental design:

Larvae were collected from different breeding sites using ladles (Figures 4.2), in each surveyed village then brought to an insectary located in the regional health center of the Cascades region and reared into adults. Larval sites were identified during random walks through villages to look for muddy footprints, puddles, ponds, irrigated rice fields, and streams. Once in the insectary, larvae were transferred into trays containing water from a drilling well and reared at a standard condition on a diet of fish food (Tetramin®) until pupal stage. Temperature and humidity in the insectary were maintained respectively at 27 +/-2°C and 70 +/- 10%, under a twelve-hours day/night photoperiod [396]. Pupae were collected from rearing trays and transferred into cages to emerge into adults. In cages, adults had access to moistened cotton using 10% glucose solution before being used in bioassays. Overall, wet season bioassays were conducted at 5 sites in 2016, 9 in 2017 and 7 in 2018 (Table 4.1). Dry season bioassays were only possible at 2 and 4 villages in 2017 and 2018 respectively (Table 4.1).

Table 4.1: Number of females Anopheles gambiae s.l. exposed at different seasons to deltamethrin and untreated paper (control) in bioassays between September 2016 and December 2018, summed across replicates. Numbers inside brackets are the total number of bioassays replicates conducted. "n/a" indicates no bioassays done at this level du to larvae not being available.

	Seasons					
Village	wet-16	dry-17	wet-17	dry-18	wet-18	Total
Dangouindougou	n/a	n/a	98(4)	n/a	332(13)	430
Gouera	n/a	n/a	549(22)	n/a	n/a	549
Nianiagara	n/a	n/a	91(3)	n/a	n/a	91
Sitiena	232(9)	n/a	465(17)	168(7)	239(10)	1104
Tengrela	307(12)	447(18)	663(27)	173(7)	1004(40)	2594
Tiefora	116(5)	426(17)	527(21)	469(19)	635(25)	2173
Tondoura	n/a	n/a	419(17)	n/a	673(27)	1092
Toumousseni	464(17)	n/a	371(15)	n/a	734(29)	1569
Yendere	249(10)	n/a	77(3)	286(11)	270(11)	857
Total	1368	873	3260	1096	3887	10484

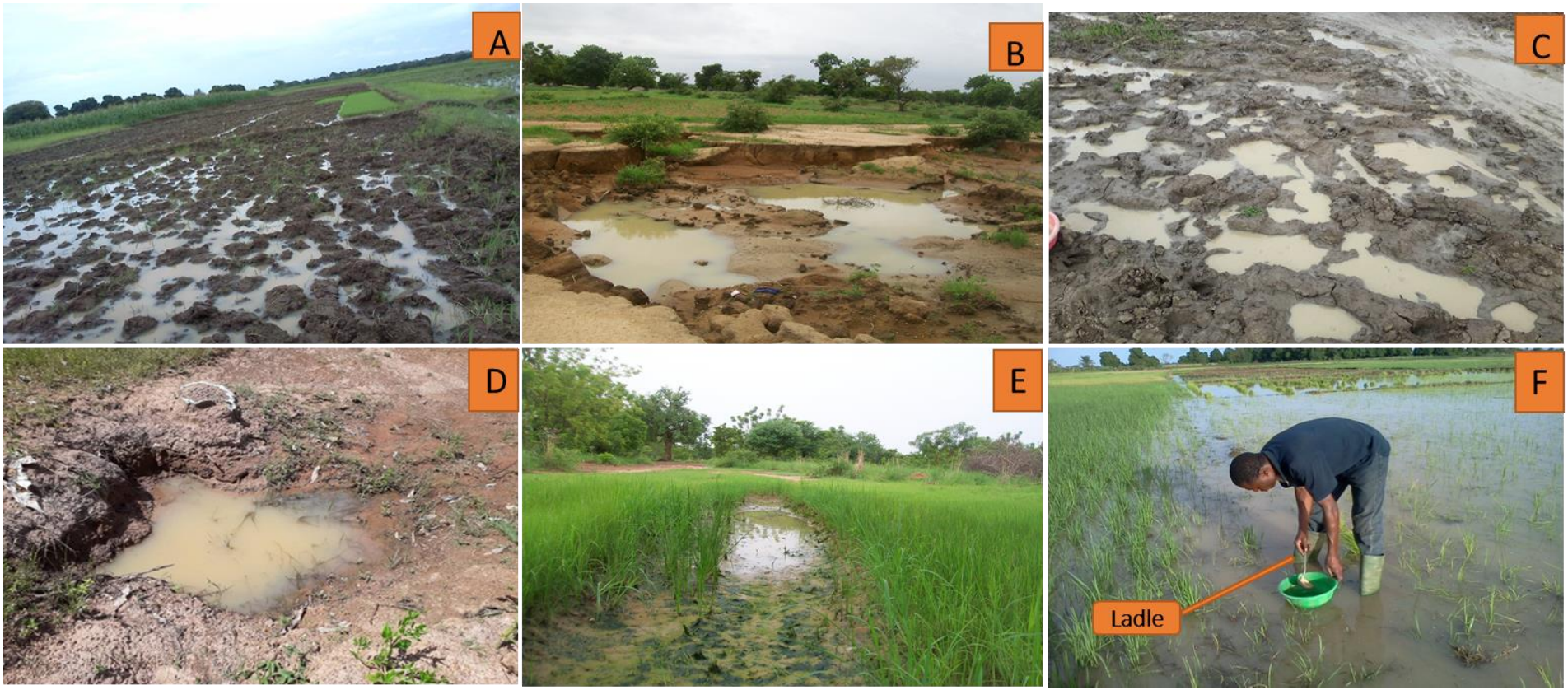


Figure 4.2: Different type of aquatic habitats that were surveyed for *An. gambiae* s.l. larvae **A)** irrigated field in Tengrela, **B)** edge of river and **C)** muddy footprints in Tondoura, **D)** a small ditch along a road in Tiefora, **E)** a puddle at the edge of a road in Tondoura and **F)** man collecting larvae from rice fields in Tengrela.

4.2.3 Insecticide susceptibility tests

Adult female *Anopheles gambiae* s.l. from field-collected larvae were used in a series of bioassays to measure IR following WHO guidelines for Tube bioassays [175]. In brief, cohorts of similarly aged female mosquitoes (3 - 5 days) were exposed to insecticide treated papers (provided by WHO/Vector Control Research Unit, Universiti Sains Malaysia) within a standardized plastic tube (125 mm length and 44 mm in diameter) for a sixty-minute period. The survival of mosquitoes in the 24-hour period following insecticide exposure was recorded [175]. These bioassays had two aims, first to test for the presence of resistance to deltamethrin (yes/no) based on the standard WHO definition following exposure to a discriminating dose (DD, also known as diagnostic dose). A DD is a concentration of insecticide, here 0.05% of deltamethrin, that can kill 100% of an exposed susceptible population thus allowing discrimination between susceptible and resistant population [175]. Under this definition, a mosquito population with lower than 90% mortality in the 24 hours following one hour of exposure to the DD is classified as resistant. The second aim was to quantify the intensity of resistance by estimating vector mortality after exposure to a range of increasing deltamethrin concentrations [175]. Here, in addition to exposing vectors to 5 and 10 times the DD (0.25% and 0.5%) as recommended by [175], they were also exposed to 15 times (0.75%) the DD. This additional concentration was implemented of account on the known high level of IR within these populations [69]. Bioassays using a range of concentrations were performed on larvae from all villages except Nianiagara (Table 4.1 & 4.2).

Following standard protocol [175], control groups were also established in which groups of mosquitoes were exposed to untreated test papers at the same time and duration as those exposed to insecticides. The aim of these control assays was to estimate the background mortality in non-insecticide exposed mosquito batches. Between 20 - 27 adult female mosquitoes were used in each bioassay, with the aim of conducting a minimum of four and maximum of six replicate bioassays per population per season each year, paired with at least two control replicates. Due to low larval abundance in

some villages (e.g. Nianiagara), there were fewer than 4 replicates for some insecticide concentrations (Table 4.1 & 4.2).

Table 4.2*:* Number of females *Anopheles gambiae* s.l. exposed to different concentrations of deltamethrin and untreated paper (control) in bioassays between September 2016, December 2018 and summed across replicates. Numbers inside brackets are the total number of bioassay replicates conducted. "n/a" indicates no bioassays done at this level.

	Concentrations of deltamethrin used (%)					
Village	0	0.05	0.25	0.5	0.75	Total
Dangouindougou	80 (3)	200(8)	50(2)	51(2)	49(2)	430
Gouera	150 (6)	54(2)	148(6)	125(5)	72(3)	549
Nianiagara	n/a	71(3)	n/a	n/a	n/a	91
Sitiena	315(13)	382(15)	252(10)	128(5)	27(1)	1104
Tengrela	757(31)	411(16)	502(20)	503(20)	421(17)	2594
Tiefora	528(21)	492(20)	488(20)	434(17)	231 (9)	2173
Tondoura	282(11)	300(12)	240(10)	147(6)	123(5)	1092
Toumousseni	356(14)	356(14)	315(13)	292(12)	250(10)	1569
Yendere	205(8)	253(10)	167(7)	186(7)	71(3)	857
Total	2693	2519	2162	1866	1244	10484

4.2.4 Molecular analysis of exposed mosquitoes

Initially, insecticide bioassays were performed only on groups of *An. gambiae* s.l. that were unidentified to species level (Table 4.1 & 4.2). The major malaria vector species within the *An. gambiae* s.l. group in the study area are *An. coluzzii* and *An. gambiae*, with previous work showing that the species composition varies between sites and seasons (Chapter 3). All the *An. gambiae* s.l. sample used in the diagnostic dose bioassays (Table 4.1) including those recorded both as dead or alive (but not the controls) were retrospectively identified to individual species level using PCR. This allowed assessment of whether there was a difference in mortality rate (dead over the total tested) between different species. Species identification was

performed using the Polymerase Chain Reaction (PCR; [272]) as described in Chapters 2 and 3.

4.2.5 Statistical analysis

In all analyses, the primary response variable was the proportion of mosquitoes that died (mortality rate) within the 24-hours following exposure to either the control (Model 4.1; Table 4.3) or different deltamethrin doses (Model 4.2 – 4.5; Table 4.3). According to WHO guidelines [175], mortality in the control groups should be < 5% for the tests to be validated. Any mortality ≥ 5 and < 20% should be corrected using the equation 4.1 described by Abbot [175]. In cases where the mortality rate in controls is ≥ 20%, the results were discarded. Here, the mortality rates in the control groups were averaged between the tests (on the same day). Then, mortality rates were assessed on a daily basis within the control group by including collection date as explanatory variable (Model 4.1, Table 4.3).

Equation 4.1:

$$\text{Corrected mortality} = \frac{Obverved\ mortlaity(\%)\ - Control\ mortlaity(\%)}{100 - Control\ mortality(\%)}$$

Further, explanatory variables of village and season were included in all models, along with a temporal spline to test for longer-term temporal variation between the start and end of study. Here, the longer-term trend was assessed by including a discrete independent variable named nMonth (that started as "1" on the first month of collection and counted upwards until the last month, month "25"). This term allowed the test for unidirectional temporal changes across the study period (consistent rise or fall; Table 4.3). Seasonality was modelled through fitting a non-linear smoothing function (spline named as t2(cMonth, bs = cc) in the models) which assigned all sampling month to an annual scale running from 1 – 12 (Table 4.3). The smoothing term (seasonal effect) described the annual non-linear trend, that can be repeated between years. Full models were created to assess *An. gambiae* s.l. mortality rates following exposure to each concentration using a series of models described in Table 4.3. Random

effects were incorporated into models to account for baseline variability between replicates. These analyses were carried out using Generalised Additive Mixed Effect Models (GAMMs) within the 'mgcv package' [335] augmented with the lme4 package [278] named GAMM4 [397] in the R statistical software as described in Chapter 3.

Secondary analysis was conducted on the subset of bioassays in which *An. gambiae* s.l. were identified to species level. Here a binary logistic regression was used to test how mortality varied between vector species (Model 6, Table 4.3). In all analyses, model selection was conducted through a process of backward elimination by sequentially removing terms, and assessing their significance using the 'anova.gam' function in the 'mgcv package' [336]. After model selection, mean values and 95% confidence intervals for statistically-significant terms were estimated as described in Chapter 3 using the 'predict.gam function' [337] from the 'mgcv package' [335]. The significance of the terms in the analysis was set at 0.05 level.

Table 4.3*:* Maximal models used for modelling seasonality in insecticide resistance including the primary response variable, explanatory variables and statistical distribution used. nMonth indicates the longer-term trend over the collection periods (starting from the first month considered as 1 to the last month of collection and counted upwards until the last month of sampling "25") fitted as a linear term. The seasonality term was fitted using a non-linear smoothing function (spline t2(cMonth, bs = cc)) on months as a period of 12 months (cMonth). PCR indicates the Polymerase Chain Reaction. Replicate indicate each tube of exposure.

Model	Tests	Response variables	Fixed Effect variables	Random effect variables	Type of data	Distribu-tion
4.1	Mortality rate	(Dead/ (Dead +Alive))	Date + Village + nMonth+ t2(cMonth, bs = cc),	Replicate	*Subset of An. gambiae* s.l. exposed as control	binomial
4.2	Mortality rate	(Dead/ (Dead +Alive))	Village + nMonth + t2(cMonth, bs = cc),	Replicate	*Subset of An. gambiae* s.l. exposed to 0.05% of deltamethrin	binomial
4.3	Mortality rate	(Dead/ (Dead +Alive))	Village + nMonth + t2(cMonth, bs = cc),	Replicate	*Subset of An. gambiae* s.l. exposed to 0.25% of deltamethrin	binomial
4.4	Mortality rate	(Dead/ (Dead +Alive))	Village + nMonth + t2(cMonth, bs=cc),	Replicate	*Subset of An. gambiae* s.l. exposed to 0.5% of deltamethrin	binomial
4.5	Mortality rate	(Dead/ (Dead +Alive))	Village + nMonth + t2(cMonth, bs = cc),	Replicate	*Subset of An. gambiae* s.l. exposed to 0.75% of deltamethrin	binomial
4.6	Mortality rate	(Dead/ (Dead +Alive))	Village + Species + nMonth + Village: Species + t2(cMonth, bs = cc),	Replicate	Subset of *An. gambiae* s.l. identified to species level after exposure to 0.05% of deltamethrin	binomial

4.3. Results

4.3.1 Mortality rate after exposure to different doses of deltamethrin

In total 10,464 female *An. gambiae* s.l. from 9 different sites were used in bioassays to measure IR (Table 4.1 & 4.2). The overall mortality rate in the control group was 1.55% (95%CI: 0.48 – 2.63%). However, at three occasions (tests) the mortality rate exceeded 5% (Figure 4.3) which did not meet the WHO criteria for validation of minimal background mortality (< 5%) in the control group. These tests were excluded from the analysis when assessing the mortality after exposure to different dose of deltamethrin instead of attempting mortality adjustment. Overall, the mortality rates of *An. gambiae* s.l. in the 24 hours following exposure to the DD was 23.33% (95%CI: 14.63 – 32.05%), indicating that all populations meet the WHO criteria of resistance. Further, mortality varied between villages (df = 8, x^2 = 29.61, p = 0.0002; Figure 4.4; Table 4.4 & 4.5); ranging from a low of ~ 10% in Sitiena to ~ 60% in Nianiagara. There was also evidence of seasonal variation in resistance after exposure to the DD (edf = 3.95, x^2 = 15.1, p = 0.003; Figure 4.5; Table 4.4 & 4.5). This is reflected by lower mortality following insecticide exposure in the late rainy season (~14%) compared to the dry (March – April; ~ 33%) and early wet season (~33% in June - August). However, vector mortality following insecticide exposure was predicted to be highest in November (late rainy season; Figure 4.5) where data are from only one village. Over the study period, vector mortality following exposure to a DD was deltamethrin was predicted to dropped from ~38% at the beginning (October 2016) to ~17% toward the end (December 2018; df = 1, x^2 = 20.91, p < 0.001; Figure 4.6; Table 4.4 & 4.5).

Table 4.4: Significance of explanatory variables included in the model for assessing variation in *An. gambiae* s.l. mortality rates from Model 4.2; 4.3; 4.4 and 4.5. Here, df is the degree of freedom, Chi.sq (x^2) represents the values of Likelihood Ratio Test. and p indicates the p-values associated with each term. nMonth is a discrete variable representing the longer-term starting from the first date of test in October 2016 to the date of last test toward December 2018. cMonth is the seasonality term fitted using a non-linear smoothing function on months as a period of 12 months.

	0.05% deltamethrin (Model 4.2)			0.25% deltamethrin (Model 4.3)			0.5% deltamethrin (Model 4.4)			0.75% deltamethrin (Model 4.5)		
Explanatory variable	Chi.sq	df	p	Chi.sq	df	p	Chi.sq	df	p	LRT	df	p
cMonth	15.1	3.95[a]	0.003 *	1.54	0.7[a]	0.12	0.001	0.01[a]	0.31	0.001	0.01[a]	0.13
nMonth	20.91	1	< 0.001 *	3.726	1	0.05	0.11	1	0.74	11.25	1	0.001 *
Village	29.61	8	0.0002 *	17.44	8	0.00 9 *	12.72	7	0.008 *	16.81	7	0.019 *

* indicates significant term retained in the final model with $p < 0.05$ and

[a] indicate the given df represent the estimate degree of freedom (edf = degree of wigginess) from smoother term.

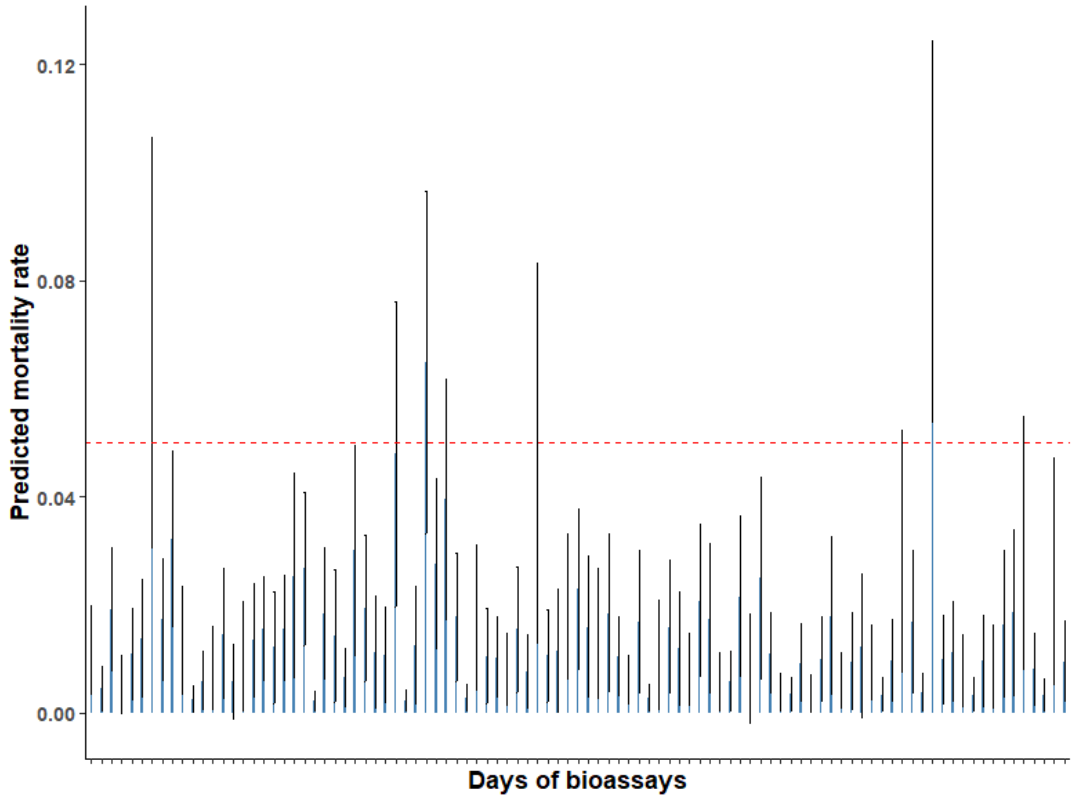


Figure 4.3: Mean predicted mortality rates in *An. gambiae* s.l. in the control group, after exposure to untreated paper based on prediction from the final model. The error bars indicate the 95% confidence intervals. The dashed line represents the 5% mortality rate below which the test is considered valid.

Table 4.5: Summary of the estimate (ß), standard errors, z values and p-value of explanatory variables in the final Model 4.2 and 4.3 using the mortality rate (as response) at each village in southwestern Burkina Faso variable. nMonth is a discrete variable representing the longer-term starting from the first date of test in October 2016 to the date of last test toward December 2018. cMonth is the seasonality term fitted using a non-linear smoothing function on months as a period of 12 months. The reference village is Dangouindougou and the adjusted R^2 are respectively 0.57 and 0.24. n/a indicate that the given variable is not part of the final model.

	Model 4.2: Mortality rate to DD				Model 4.3: Mortality rate to 5 X the DD			
	ß	Std. Error	z value	Pr(>|z|)	ß	Std. Error	z value	Pr(>|z|)
Intercept	-0.844	0.231	-3.644	0.000*	0.568	0.322	1.766	0.077
Gouera	1.012	0.397	2.550	0.011*	2.413	0.430	5.617	0.000*
Nianiagara	3.539	0.383	9.245	0.000*	n/a	n/a	n/a	n/a
Sitiena	0.908	0.253	3.596	0.000*	0.792	0.311	2.551	0.011*
Tengrela	0.927	0.277	3.345	0.001*	0.447	0.306	1.461	0.144
Tiefora	0.207	0.260	0.794	0.427	0.204	0.307	0.664	0.507
Tondoura	1.928	0.246	7.844	0.000*	1.215	0.321	3.790	0.000*
Toumousseni	1.212	0.264	4.586	0.000*	-0.040	0.313	-0.127	0.899
Yendere	2.514	0.272	9.232	0.000*	1.119	0.330	3.388	0.001*
nMonth	-0.144	0.011	-13.504	0.000*	-0.038	0.007	-5.454	0.000*
cDate	-0.797	0.091	-8.765	0.000*	n/a	n/a	n/a	n/a

* indicates significant term retained in the final model with $p < 0.05$ and

[a] indicate the given df represent the estimate degree of freedom (edf = degree of wigginess) from smoother term.

Table 4.6: Summary of the estimate (ß), standard errors, z values and p-value of explanatory variables in the final Model 4.4 and 4.5 respectively using the mortality rate (as response) at each village in southwestern Burkina Faso variable. nMonth indicates the longer-term trend over the collection periods (starting from the first month considered as "1" to the last month of collection and counted upwards until the last month of sampling "25") fitted as a linear term. The reference village is Dangouindougou and the adjusted R^2 are respectively 0.10 and 0.20. n/a indicate that the given variable is not part of the final model.

	Model 4.4: Mortality rate to 10 X the DD				Model 4.5: Mortality rate to 15 X the DD			
	ß	Std. Error	z value	Pr(>|z|)	ß	Std. Error	z value	Pr(>|z|)
Intercept	2.313	0.670	3.453	0.001*	2.587	0.484	5.348	0.000*
Gouera	0.575	1.054	0.545	0.586	3.205	0.860	3.729	0.000*
Sitiena	0.124	0.880	0.141	0.888	17.289	295.603	0.058	0.953
Tengrela	-0.795	0.726	-1.094	0.274	1.589	0.417	3.814	0.000*
Tiefora	-0.922	0.727	-1.269	0.204	-0.039	0.344	-0.112	0.911
Tondoura	0.033	0.826	0.040	0.968	1.651	0.410	4.030	0.000*
Toumousseni	0.071	0.755	0.095	0.925	1.411	0.372	3.791	0.000*
Yendere	-0.199	0.461	-0.433	0.665	-0.465	0.372	-1.249	0.212
nMonth	n/a	n/a	n/a	n/a	-0.115	0.019	-5.926	0.000*

* indicates significant term retained in the final model with $p < 0.05$ and

[a] indicate the given df represent the estimate degree of freedom (edf = degree of wigginess) from smoother term.

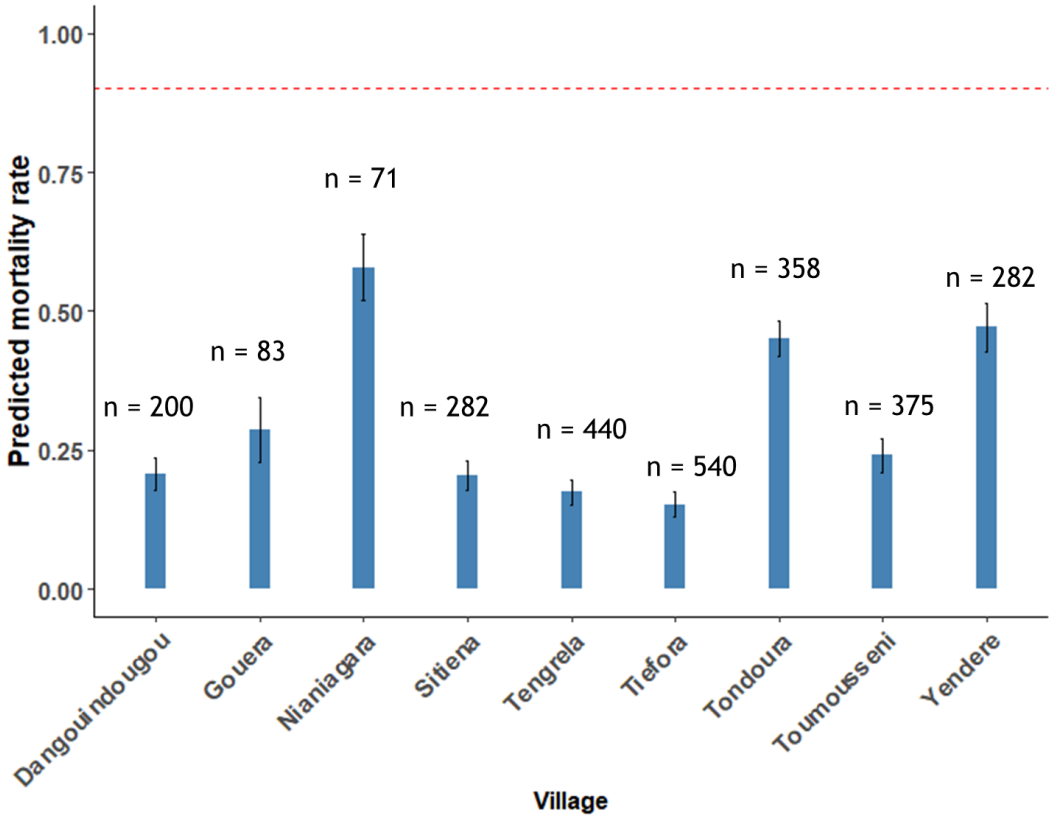


Figure 4.4: Mean predicted mortality rates in *An. gambiae* s.l. from 9 villages in southwestern Burkina Faso, after exposure to the discriminating dose (0.05%), of deltamethrin based on prediction from the final models. The error bars indicate the 95% confidence intervals. The dashed line represents the 90% mortality rate below which a vector population is considered resistant.

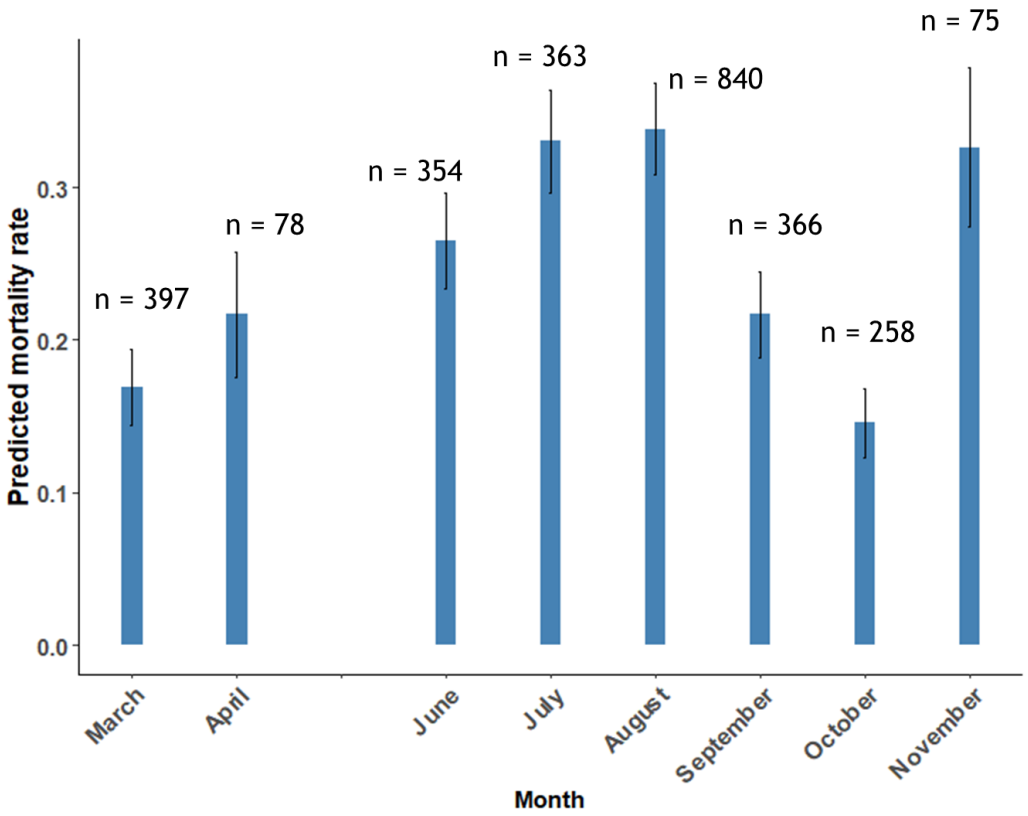


Figure 4.5: Mean predicted mortality rates in *An. gambiae* s.l., at each month of collection over 9 villages in southwestern Burkina Faso, after exposure to the discriminating dose (0.05%) of deltamethrin based on prediction from the final model. The error bars indicate 95% confidence intervals.

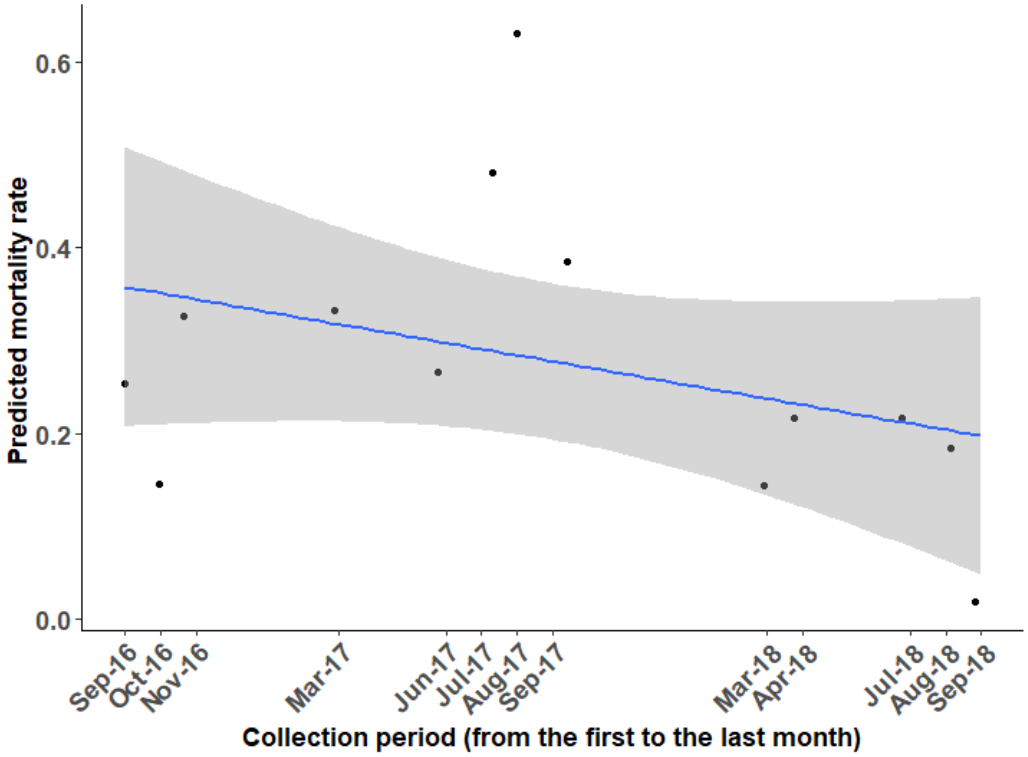


Figure 4.6: Predicted longer-term trend in the mortality of An. gambiae sl. 24 hours following exposure to a diagnostic dose of deltamethrin across the study period. Black dots indicate mean predicted mortality at each study month between September 2016 to September 2018 across 9 villages in southwestern Burkina Faso. The blue line indicates the predicted linear change in *An. gambiae* s.l. across the study period based on the final model, with the grey-shaded areas around them indicate 95% confidence intervals.

Furthermore, the mortality rate varied between villages after exposure to increase doses (Table 4.4; 4.5 & 4.6). Mortality following insecticide exposure increased to 64.14% (95% CI: 55.27 – 73.0); 86.03 (95% CI: 81.87 – 90.2%) and 87.48% (95% CI: 81.52 – 92.43%) when insecticide concentrations were increased to 5, 10 and 15 times the DD respectively (Figure 4.7; Table 4.5 & 4.6). As expected, the 24-hour mortality of vectors increased with the insecticide concentration, but even at the highest dose (15 X DD) only exceeded the target threshold for susceptibility of 98% in two out of nine villages (e.g Gouera and Sitiena; Figure 4.7). Evidence of longer-term variation in vector mortality remained in bioassays using 5 (df = 1, x^2 = 3.73, p = 0.05) and 15 times the DD (df = 1, x^2 = 11.25, p = 0.001), but not in assays conducted at 10 times the DD (df = 1, x^2 = 0.11, p = 0.74; Table 4.4, 4.5 & 4.6). There was some inconsistency in the pattern of spatial variation as

predicted from different concentrations (Figure 4.7). Additionally, there was no evidence of seasonal variation in IR in bioassays conducted at 5 (edf = 0.7, x^2 = 1.54, p = 0.12), 10 (edf = 0.01, x^2 = 0.001, p = 0.31) and 15 times the DD (edf = 0.01, x^2 = 0.001, p = 0.003; Table 4.4, 4.5 & 4.6).

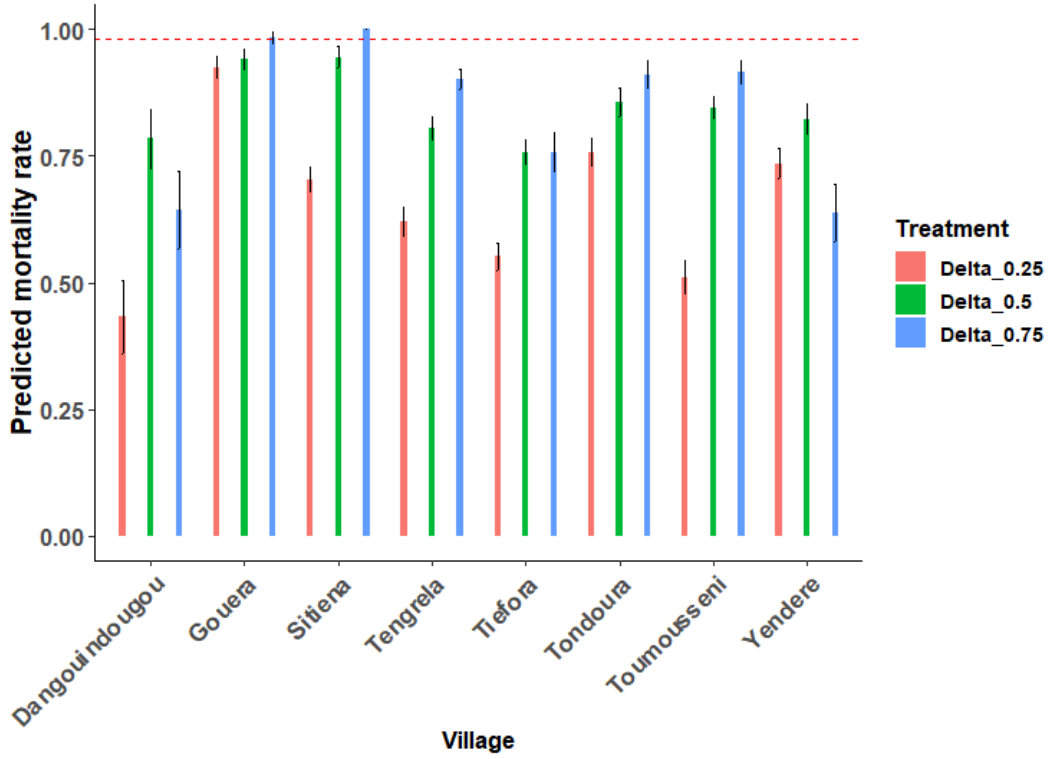


Figure 4.7: Mean predicted mortality in *An. gambiae s.l.*, from 8 villages in southwestern Burkina Faso, after exposure to 0.25%, 0.5% and 0.75% of deltamethrin based on prediction from the final models. The error bars indicate the 95% confidence intervals. The dashed line represents the 98% mortality rate below which a vector population is considered moderate to highly resistant if mortality < 90% at the DD.

4.3.2 Species-specific mortality rate analysis

Of the 2519 *An. gambiae* s.l. analysed in DD bioassays, ~84% (N = 2112; Table 4.7) were successfully identified to species level. In this subsample 53.6% of individuals were *An. gambiae*, 46.12% *An. coluzzii* and 0.28% *An. arabiensis*. *Anopheles arabiensis* were excluded from analysis as the sample size was too small for robust analysis. Within this subset of data; mortality after exposure to the DD was best explained in a model containing an interaction between village and species (df = 7, x^2 = 20.03, p = 0.005; Table 4.8 & 4.9). There was

no consistent difference in 24-hour mortality between *An. coluzzii* and *An. gambiae*, with the order and magnitude of difference in mortality between these species varying between villages (Figure 4.8). There was no evidence of seasonal variation in IR in this analysis (edf = 2.66, x^2 = 4.56, p = 0.11), but a longer-term increase in IR (e.g reducing post exposure mortality) was detected (df = 1, x^2 = 8.3, p = 0.004; Table 4.8 & 4.9), similar to that observed in the larger data set of unidentified *An. gambiae* s.l. (Table 4.4).

Table 4.7*:* Number of females from different species within the *An. gambiae* s.l. complex that were exposed to the discriminating dose (0.05%) of deltamethrin in bioassays, in each village. "Alive" indicates that total number that survived 24 hours after exposure, and "Dead" the number that died before 24 hours. "0" indicates that the given species was not detected in the sample.

Village	*An. arabiensis*		*An. coluzzii*		*An. gambiae*		Total
	Alive	Dead	Alive	Dead	Alive	Dead	
Dangouindougou	0	0	31	15	97	19	162
Gouera	0	0	17	30	38	31	116
Nianiagara	0	0	0	11	5	22	38
Sitiena	2	0	119	12	109	75	317
Tengrela	0	0	233	44	37	24	338
Tiefora	1	1	204	22	215	52	495
Tondoura	0	0	8	61	40	85	194
Toumousseni	1	0	21	16	101	20	159
Yendere	1	0	81	49	85	77	293
Total	5	1	714	260	727	405	2112

Table 4.8: Significance of explanatory variables included in the model 4.6 for assessing variation in *An. gambiae* s.l. mortality rates using the subset of data on sample molecularly analysed (Table 4.4). Here, df is the degree of freedom, Chi.sq (X^2) represents the values of Likelihood Ratio Test associated with each term. nMonth is a discrete variable representing the longer-term starting from the first date of test in October 2016 to the date of last test toward December 2018. cMonth is the seasonality term fitted using a non-linear smoothing function on months as a period of 12 months. The seasonality term was fitted using a non-linear smoothing function on months as a period of 12 months (cMonth).

	Model 4.6: Mortality rate in species		
Variables	Chi.sq	df	p-value
cMonth	4.56	2.66[a]	0.11
nMonth	8.3	1	0.004*
Village: species	20.03	7	0.005*

* indicates significant term with $p < 0.05$ and

[a] indicate the given df represent the estimate degree of freedom (edf = degree of wigginess) from smoother term.

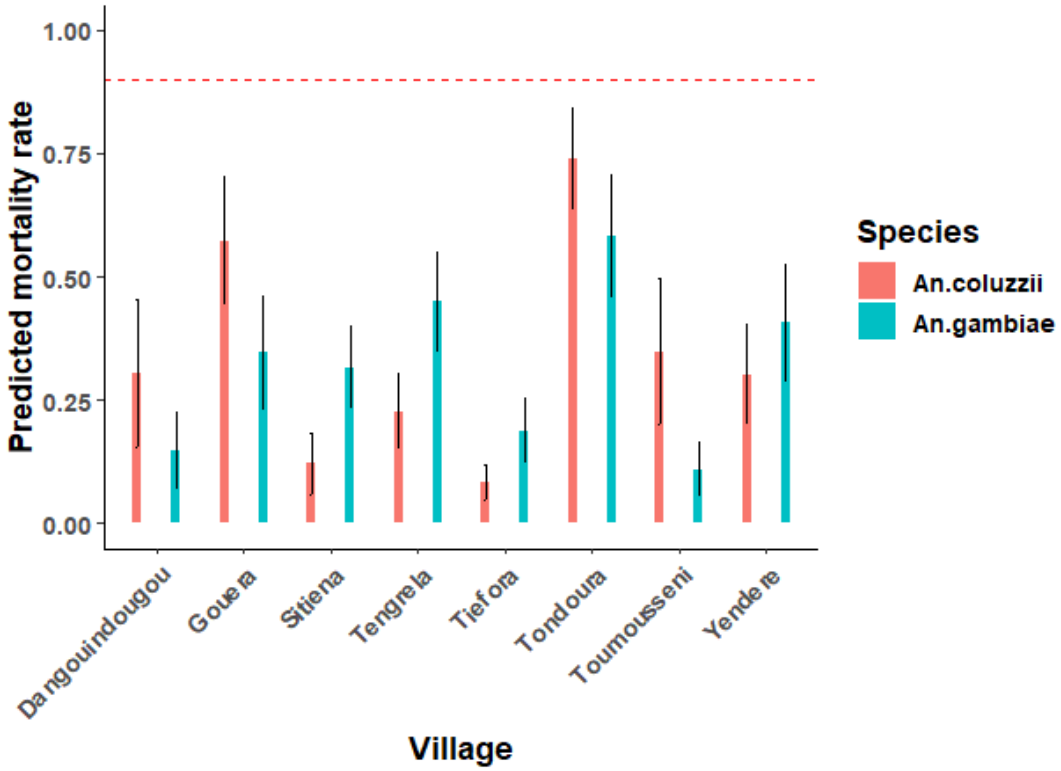


Figure 4.8: Mean predicted mortality rates after exposure to the discriminating dose (0.05%) of deltamethrin in *An. coluzzii* and *An. gambiae*., from 8 villages in southwestern Burkina Faso, based on prediction from the final model. The error bars indicate 95% confidence intervals. Here the dashed line represents the 98% mortality rate.

Table 4.9: Summary of the estimate (ß), standard errors, z values and p-value of explanatory variables in the final Model 4.6 using the mortality rate (as response) at each village in southwestern Burkina Faso variable from subsample molecularly analyzed (Table 4.4). nMonth is a discrete variable representing the longer-term starting from the first date of test in October 2016 to the date of last test toward December 2018. cMonth is the seasonality term fitted using a non-linear smoothing function on months as a period of 12 months. The references consist of Dangouindougou for village, *An. coluzzii* for species, and Dangouidougou: *An. coluzzii* for the interaction between village and species. The adjusted R^2 is 0.25.

	ß	Std. Error	z value	Pr(>|z|)
Intercept	-1.301	0.845	-1.541	0.123
Gouera	1.819	0.897	2.027	0.043*
Sitiena	0.344	0.877	0.392	0.695
Tengrela	0.462	0.817	0.565	0.572
Tiefora	-0.681	0.817	-0.833	0.405
Tondoura	2.318	0.887	2.614	0.009*
Toumousseni	0.091	0.984	0.092	0.927
Yendere	1.482	0.846	1.752	0.080
An. gambiae	-0.228	0.472	-0.483	0.629
nMonth	-0.085	0.030	-2.832	0.005*
Gouera: An. gambiae	-0.868	0.640	-1.356	0.175
Sitiena: An. gambiae	0.767	0.653	1.175	0.240
Tengrela: An. gambiae	0.982	0.591	1.661	0.097
Tiefora: An. gambiae	0.925	0.568	1.628	0.104
Tondoura: An. gambiae	-0.411	0.749	-0.549	0.583
Toumousseni: An. gambiae	-0.033	0.848	-0.039	0.969
Yendere: An. gambiae	0.843	0.573	1.470	0.141
cDate	-0.285	0.281	-1.014	0.311

* indicates $p < 0.05$ and

4.4. Discussion

This study confirmed a high prevalence of insecticide resistance in malaria vectors in southwestern Burkina Faso, with the overall mortality rate in *An. gambiae* s.l. in the 24 hours following exposure to a discriminating dose of (0.05%) deltamethrin being < 60%. This indicates that *An. gambiae* s.l. at all the surveyed villages met the WHO's definition of insecticide resistant (< 90% mortality in the 24 hours follow exposure). However, this mortality rate varied up to 4-fold between villages; suggesting the possibility of spatial variation in IR. Additionally, there was evidence of seasonal variation in IR with mosquito mortality following insecticide exposure being higher in the rainy than dry season. Most notably, there was evidence of a consistent increase in IR over the study period, with the mean mortality after exposure to the DD predicted to fall by ~47% between the start and end of the study after accounting for spatial and seasonal variation. Thus, insecticide resistance showed a consistent, rapid increase over the 2-year period following the recent mass LLIN distribution. Combined with other temporal changes in vector behaviour (Chapter 3) and net durability, this could lead to a considerable reduction in LLIN impact over time.

Here IR was quantified not only by changes in vector mortality after exposure to a diagnostic dose (DD) of deltamethrin, but also by their ability to survive 5, 10 and 15X the DD. This revealed a very high intensity of resistance, with ~36%, ~14% and ~12% of *An. gambiae* s.l. being able to survive exposure to 5X, 10X and 15X the DD respectively. Only two (Gouera and Sitiena) of the 9 populations tested met the WHO definition of susceptibility (e.g. ≥ 98% mortality in the 24 hours following exposure) when exposed to a concentration of 15X the DD (0.75%). According to [175] the intensity of resistance of a vector population can be classified as "moderate" or "high" if the mortality rate 24-hours after exposure is respectively ≤ 98% to 5 X the DD and < 98% to 10X the DD. By this definition, all the *An. gambiae* s.l. populations surveyed are "highly resistant". Furthermore, as post-exposure mortality rates were ≤ 98% at 15-times the DD, most of the *An. gambiae* s.l. vector populations may be classified as "super resistant".

Although IR was found throughout the study region, there was notable variation between the 9 vector populations surveyed. For example, mosquito mortality following exposure to the DD was considerably lower at some places (e.g. Sitiena, Tengrela, Tiefora and Toumousseni) than others (e.g. Dangouindougou, Nianiagara Tondoura and Yendere). The causes of the apparent spatial differences in IR are unclear, but may be linked to local variation in agricultural practices and associated chemical use ([167, 225]. Across the 9 villages assayed here, residents generally cultivate similar types of crops (cotton, maize, rice, and vegetables). However, pesticides may be used at different intensities across study areas. Mosquito larvae are more likely to come into contact with sub-lethal concentrations of insecticide if they are collected in rice field pools than if they were collected from puddles formed by footprints. For example, at Tengrela and Tiefora rice are grown twice a year (in dry and wet season), which likely requires increasing pesticide use compared to villages with one annual harvest. Substantial local variation in IR has also been reported in malaria vectors from other region of Burkina Faso, where *An. gambiae* s.l. mortality following DD exposure ranged from ~15% up to ~38% between sites [220]. Similar variation in IR at the sub-national level has been reported in other African countries (e.g. west [379, 398], east [399, 400] and south-east [401] Africa). Similarly, a recent study from the central Cote d'Ivoire showed substantial variation in the post-exposure mortality rate in *An. gambiae s*.l. ranging from ~18% to ~73% between sites [93]. This confirms IR can be spatially patchy, which could generate heterogeneity in the degree of protection provided by LLINs.

Local variation in IR could also arise due to interspecific variation in IR between vector species. I tested for vector species-specific variation in IR here, but found no consistent difference between *An. coluzzii* and *An. gambiae*. Instead, vector mortality was significantly associated with the interaction between vector species and site; with mortality being lower in *An. coluzzii* than *An. gambiae* in some villages (Sitiena, Tengrela, Tiefora and Yendere), whereas mortality lower in *An. gambiae* than *An. coluzzii* at the other sites. This interaction implies that IR is associated with vector species but only in a localized manner, with the species that is most resistant

varying between villages. Previous studies in Burkina Faso have suggested that IR differs between vector species [219, 402]. For example, the post insecticide-exposure survival of *An. gambiae* (formerly described as S form of *An. gambiae sensu stricto*) was higher (>55%) than in *An. coluzzii* (<40%; formerly described *An. gambiae s.s.* M-form [36]) after exposure to permethrin [219]. In central Africa, it has also been shown that the post insecticide-exposure survival was lower in *An. coluzzii* (~55%) than *An. gambiae* (~84%) [403]; suggesting the latter is more resistant. However, data from another region of Burkina Faso indicated IR was lower in sites dominated by *An. gambiae* compared to those with *An. coluzzii* [402]. In combination, these results indicate there is no consistent difference in IR between vector species in Burkina Faso.

A seasonal trend in IR was detected here, with *An. gambiae* s.l. being likely to die after insecticide exposure during and toward the end of the rainy than throughout the dry season. Other studies across Africa have indicated there may be seasonal variation in IR within malaria vectors, with susceptibility being higher in the wet season than dry season [381, 404]. Results here are consistent with a previous study from a cotton growing area of southwestern Burkina Faso [225] where the mortality of *An. gambiae s.*l. in insecticide bioassays fell by 37% between the start and towards the end of the rainy season; indicating an increase in IR across the season. Similarly, a study in Tanzania found that the mortality rate of *An. arabiensis* after insecticide exposure was lower in the dry (~45%) than wet season (~72%, [46]). In sub-Saharan Africa, most farmers use the same class of insecticides for agriculture as is used on LLINs (e.g. pyrethroids; [405, 406]). The emergence of IR in African malaria vectors has thus been linked to the use of agricultural pesticides [166, 380, 406]. The use of pesticides for agriculture varies seasonally in response to peak planting and harvest times. Consequently, selection pressure for insecticide resistance in malaria vectors changes over the course of a year. For example, the amount of insecticides in the environment may be relatively low at the start of the rainy season when farmers are just beginning to plant but rise consistently throughout the rainy season. If so, one might predict that malaria vectors sampled at the start of

the wet season (June – August) are under less selection for resistance than those sampled during the late wet (September – October) and the dry (March – April) seasons. Results obtained here suggest mosquitoes sampled at the beginning of the rainy season are more susceptible to insecticides than those sampled towards the end; where only individuals that were able to withstand high levels of exposure would remain [407]. This indicates that LLINs could be more effective in the rainy season when mosquitoes are more abundant (Chapter 3) and less resistant than in the dry season.

Insecticide resistance appeared to increase consistently over the study period, resulting in a ~47% decrease in the mortality of *An. gambiae* s.l. following exposure to the DD between the start and end of the study. Similar increases in IR have been documented over relatively short time periods in other African settings (e.g. in west [379] and central Africa [408, 409]). Similarly, a recent study from Mali found a ~10 - 25% decrease in the post-exposure mortality of *An gambiae* s.l. in WHO standard tube tests over two years [398]. Resistance in malaria vector populations (*An. funestus* and *An. gambiae* s.l.) to Dieldrin and DDT was first reported in Burkina Faso in the 1960s [410, 411]. In the 1970s the mortality rate of ~95% in *An. gambiae* s.l. population to deltamethrin was subsequently reported in the southwestern part of the country [412]. Similar mortality in the same *An. gambiae* s.l. population was also reported later in 2010s [413]. Over the past two-decades, IR has intensified in vector populations in Burkina Faso [69, 220, 352, 414]. Insecticide resistance was previously measured in Tiefora, one of the 9 study sites surveyed here, in 2014 [414]. At that time, the post exposure mortality of *An. gambiae* s.l. to the DD was ~39% compared to ~15% observed in the current study. Additionally, a previous study in Tengrela (another site in this study) found that vector mortality after DD exposure declined from ~92% to ~19% between 2011 and 2013 during rainy seasons [69]. These results illustrate the rapid intensification of IR throughout the study area since the early 2010s, coinciding with initiation of mass LLIN distribution programmes. Due to the high level of resistance within malaria vectors in the Cascades region described here, ideally Burkina Faso should

move to use a different insecticide class for vector control and complement LLIN programmes with alternative insecticide-free approaches.

The current study has some limitations. First, the susceptibility of the local vector population in the study area was based on the standard W.H.O tube tests, which are known to be sensitive to test conditions including larval rearing conditions, and the temperature and humidity of the testing room [175, 177]. Here, the temperature and humidity were only recorded in bioassays conducted in 2017 and 2018, making difficult to take these environmental factors into account when analysing the whole data set. In addition, the design of the tube assays can allow mosquitoes, to avoid contact with the insecticide-treated paper/surfaces; meaning they may not pick up the same quantity of insecticide as expected from resting or feeding through a LLIN. Further, data on IR was not available at all sites and all months; meaning there are spatial and temporal gaps in sampling that prevent full interpolation over the entire study period and area. In general, there is huge variability in IR within and between sites making difficult direct comparisons of resistance level and its possible impact on interventions. There is need for more realistic and efficient ways to measure the IR.

4.5. Conclusions

The current study confirms that IR is very high in malaria vector populations in the Cascades region of Burkina Faso, and amongst the highest described in Africa. Although IR was universally high there was evidence of spatial and seasonal variation in the killing effect of deltamethrin, a commonly used chemical in vector control. This indicates suggesting that there is fine-scale heterogeneity in selection pressure for resistance. Notably, there was an evidence of a substantial increase in IR over the course of this 2-year study. This highlights that urgent action needs to be taken to mitigate the potential impacts of IR on control failure.

Chapter 5: Spatial and seasonal variation in malaria vector survival and transmission following scaling up of LLINs in rural Burkina Faso.

Abstract

Background. Insecticide resistance and behavioural avoidance in mosquito vectors are hypothesized to be responsible for the stalling of progress in malaria control that has recently been observed in Africa. In the Cascades Region of Burkina Faso, high rates of outdoor biting (Chapter 3) and insecticide resistance (Chapter 4) have been described in local vector populations. Assessment of the potential epidemiological impact of such mosquito phenotypes requires estimation of their association with malaria transmission potential and human exposure. Here I assessed several aspects for malaria transmission potential (mosquito vector survival, infection rates, and human blood index) and corresponding entomological inoculation rates in 12 communities within this area over a 2-year period following a mass LLIN distribution programme. Aims were to quantify the amount of residual transmission that can be maintained in this area even with full LLIN coverage.

Methods: Host-seeking and resting malaria vectors were collected twice a month at 12 villages in the southwest Burkina Faso as described in Chapter 3. Mosquito samples were processed to estimate four key aspects of vectorial capacity and malaria transmission: (i) vector survival, (ii) sporozoite infection rates (SR), (iii) human blood index and (iv) entomological inoculation rates (EIR). These metrics were calculated for each village and year of study to characterize temporal and spatial variation in residual malaria transmission following LLIN distribution. Vector survival was estimated from the mean parity rates in *An. gambiae* s.l. over a full year.

Results: A total of 40,220 *An. gambiae* s.l. females were sampled in host seeking collections, of which 16,249 were dissected to assess parity. Across villages, parity rates (PR) in *An. gambiae* s.l. ranged from 71.4 - 83.8%; translating into a mean daily vector survival rate of 91% across the study

period. Parity rates varied between seasons (dry versus wet) and showed evidence of a longer-term increase over the study period (~12%; October 2016 - December 2018) indicating a gradual reduction in mosquito survival. The mean SR in *An. gambiae* s.l. was 3.4% (95% CI: 1.51 – 5.26%) but varied up to ~9-fold between villages. The long-term reduction in SR was detected, with infection rates in vectors falling by ~67% over the study period. The Human Blood Index of *An. gambiae s*.l. was 64.9%. Combining estimates of human biting rates (Chapter 3) with sporozoite infection rates, the EIR across the study area was estimated to be ~320 infective bites/person/year and 105 infective bites/person/year respectively in year 1 and 2.

Discussion: This study demonstrates a gradual reduction in malaria transmission intensity and human exposure across the 2-years following a mass LLIN distribution in Burkina Faso. However even with this reduction, the survival and sporozoite rates in these *An. gambiae* s.l. populations remained high; giving rise to expected annual Entomological Inoculation Rates of more than 100 infected bites per person per year. Even assuming the best-case scenario of full coverage of effective LLINs, only ~85% of these bites could be prevented by this intervention (πi, Chapter 3) with the remaining exposure being enough to maintain high transmission. Both the relatively high rates of outdoor biting (Chapter 3) and cattle feeding (here) in these vector populations indicate that supplementary methods targeting vector outside homes will be needed for control.

5.1. Background

As described previously (Chapter 1, 3-4), the continued success of primary vector control methods of LLINs and IRS is increasingly threatened by the emergence of insecticide resistance (IR, [400, 415]) and behavioural avoidance strategies in African malaria vector populations [311, 312]. Both phenotypes have been confirmed in the malaria vectors in the Cascades region of Burkina Faso (Chapter 3-4), where they may be responsible for the limit impact of recent LLIN programmes. However, the existence of behavioural resistance and IR does not necessarily imply that control methods are failing. Instead these phenomena may be "side effects" of very effective interventions that are successfully reducing vector densities and thus generating selection for resistance within the small number of remaining survivors, but not enough to counteract control. For instance, meta-analysis of a series of cohort studies in west Africa, east Africa and India [112] failed to demonstrate a link between insecticide resistance in local vector populations and malaria infection prevalence or clinical incidence. In this study, authors highlight that even in clusters where malaria vector mortality is considerably lower than the WHO threshold for classifying vectors as "susceptible" [175]), LLINs still appear to be effective. Another study in Kenya indicated that malaria transmission continued to decline even in the presence of a shift in vector biting behaviour that would reduce their contact with LLINs [321]. In contrast, malaria transmission in Burkina Faso appears to be stable and perhaps even increasing despite three rounds of mass LLIN distribution since 2010. It is thus crucial to identify the drivers on residual transmission in high burden settings like Burkina Faso and assess the relative contribution of insecticide and behavioural resistance.

The reproductive number "R_o" as expressed in equation (1.1 in Chapter 1) elucidates the role of mosquito vectors in malaria transmission [72], and has been used to illustrate the impact of vector control measure on malaria transmission (e.g. [72]). Although Ro provides a useful theoretical basis for understanding the conditions under which disease transmission may increase, it is difficult to directly measure in the field because of the

complex nature of the human, parasite and vector traits it encompasses. Further, its core assumption of predicting transmission within a completely susceptible host population is rarely relevant in field settings where most populations have some degree of immunity through repeated exposure. These limitations spurred the development of an entomological based measurement of transmission as an alternative, simplification to Ro equation. This metric, "Vectorial Capacity" ***(VC*****,** [76]*)*, is essentially a reformulation of the *Ro* equation that incorporates the mosquito components:

Equation 3. 1: $\boldsymbol{VC = \frac{ma^2p^n}{-ln(p)}}$

Where, ***m*** indicates the ratio of mosquito to human density; ***a*** represent the average number of mosquito bites a person receives daily; ***n*** is the length of the gonotrophic cycle; ***p*** is the vector daily survival rate and ***r*** the is the proportion of people that recover from the disease [76].

Malaria transmission intensity is crucially determined by the propensity of mosquito vector populations to feed on human hosts, their susceptibility to parasite infection, and survival through the parasite's extrinsic incubation period [70, 416]. Vectorial capacity and its constituent elements are regularly estimated as proxies for malaria transmission [75, 417], and to give insights into how vector control methods are working [418-421]. Of all the mosquito demographic and behavioural traits included in vectorial capacity, the one with the greatest effect is vector survival (**p**). This is because vector survival has an exponential relationship with VC, with a small change generating a large impact [79]. The exponential impact of vector survival arises because no transmission can occur unless vectors survive through the extrinsic incubation period of the parasite (9 to 11 days, at an average temperature of 29°C,[422]. Other crucial components of VC include the human biting rate (a); as only vectors feeding on humans have potential to become infected and transmit infection.

Although widely used to conceptualize malaria transmission, VC can be difficult to measure in the field and may not correlate well with estimates of infection in human populations. Instead, malaria transmission intensity is frequently measured in terms of the "Entomological Inoculation Rate" ("EIR", [423]). The Entomological Inoculation rate (EIR) is defined as the average number of infective bites a person would expect to receive from a given malaria vector in a given location per year, calculated as the product of human biting rate (***ma***) and vector sporozoite infection rates [423, 424]. EIR is considered to be one of the most direct estimates of human exposure to malaria and has a relatively good correlation with human epidemiological parameters such as malaria incidence [423, 425-427]. The EIR has been used widely in studies in Africa [428-430] and elsewhere [431] to assess the impact of insecticide treated materials on malaria transmission. Both parameters from VC (mosquito survival) and EIR are commonly estimated within routine entomological surveillance to assess the impact of control measures (e.g. LLINs and IRS, [432-435]).

Both the Ro and VC metrics require information on daily mosquito mortality ("***p***"). This is difficult to measure in the field but may be indirectly estimated from mosquito parity rates (parity = proportion of mosquitoes that have laid at least one egg batch). Based on the simplifying assumptions that the period between mosquito blood feeding and egg laying ("gonotrophic cycle" length) is fixed, that mosquitoes only blood feed once per cycle, and that the mosquito population has a stable age range over time period considered, the daily survival rate (S) of vectors can be approximated by [436]:

Equation 5.2: $\boldsymbol{S = \sqrt[n]{pr}}$

Here, n is the length of the gonotrophic cycle in days, and **"*pr*"** is the proportion of parous individuals. The mean life expectancy (LE) of vectors can be further estimated from their daily survival (S) as [436]:

Equation 5. 3: $\boldsymbol{LE = \frac{1}{-ln(S)}}$

Interventions measure that successfully target mosquito biting behaviours (e.g. LLINs, [437]) or resting behaviours (e.g. IRS [438, 439]) are expected to reduce mosquito survival rates and associated life expectancies. Consequently, mosquito parity rates are commonly measured to estimate the impact of interventions on vector populations (e.g. [76, 110, 440-443] .

Another mosquito trait commonly used to assess malaria transmission potential and intervention success is the human blood index (HBI). The HBI is calculated as the proportion of blood meals a vector population takes from humans relative to other host types in the environment [444], and reflects the degree of "anthrophagy" in the population. As the malaria parasite species of greatest significance in Africa (*Plasmodium falciparum*) can only be transmitted to and from humans, transmission intensity is positively and exponentially related to human biting rates. The epidemiological significance of HBI was first described by Garrett-Jones [444]. Globally, there is a positive relationship between the HBI of local vector populations and malaria transmission intensity [445]. Consequently, HBI is additional useful metric to assess the impact of interventions.

In Burkina Faso and much of west Africa, the major malaria vectors are *An. gambiae* and *An. coluzzii* [125, 217, 223]. These vector species are thought to be highly anthropophagic [57, 230, 446]. However, there is some evidence that malaria vectors may feed on livestock and other animals when humans are not readily accessible due to the presence of interventions (e.g. [230, 435, 447]). There is little up-to-date information on patterns of host choice in malaria vectors in the current context of mass LLIN distribution in Burkina Faso.

The aim of this study was to assess the transmission potential of malaria vectors in southwest Burkina Faso in the two years following a mass LLIN distribution. Several proxy measures of malaria transmission intensity and human exposure were measured and used to investigate spatially, seasonal and long-term temporal trends following a mass LLIN distribution. Specific measurement was made of: i) mosquito survival (as indexed by parity), ii)

malaria sporozoite rates, iii) human blood index and iv) the EIR within the study area. In combination, these results will help quantify the magnitude of residual transmission in this area, how effectively it is being tackled by existing interventions (LLINs) and suggest what additional control strategies may be most beneficial.

5.2 Methods

5.2.1. Study sites and mosquito processing

This current study was based on longitudinal surveillance of malaria vectors at 12 villages in southwestern Burkina Faso between October 2016 and December 2018 as described in Chapter 3. Here, host seeking malaria vectors were sampled from 911 households using Human Landing Catches (as described in Chapter 2). Resting mosquitoes were also collected using resting buckets (RBTs, [332]) as previously described (Chapter 3). Bi-monthly mosquito collections were conducted at all 12 villages for 15 months, with sampling continuing in a subset of 6 villages for a further 8 months (Figure 1.7). After collection mosquitoes were brought to the laboratory and identified to genera or species group level using morphological keys [271]. The primary of target of collection was the major malaria vector group *An. gambiae sensu lato* (s.l.). Additionally, female *An. gambiae s.l.* from the RBT collections were visually graded according to their repletion status (abdominal condition) into categories of blood fed (BF), unfed (UF), gravid (G), and half gravid (HG) [333].

5.2.2. Mosquito age-grading and survival

Overall, 49,482 mosquitoes comprising 4 genera were collected in HLCs across the study, of which 40,220 were identified as female *An. gambiae* s.l. (Table 3.2 in Chapter 3). A subset of these females were age-graded on the basis of their parity status [448]. Parity dissections are labour intensive and time consuming and can only be performed on "fresh" specimens that have been alive until just prior to dissection, thus only specimens from human landing collections were suitable for analysis. To provide a representative sample of parity in mosquitoes across study villages and time periods, a

random selection of up to five "fresh" *An. gambiae* s.l. (unfed or semi-gravid females) were dissected for each collection hour at each location (indoor or outdoor) and household on each night of HLC collection. Following this subsampling strategy, a total of 16,220 female *An. gambiae* s.l. were dissected (40.33% of total). In this method, the ovaries of female mosquitoes were dissected and inspected under a microscope (400X). Individuals were classified into categories of "nulliparous" (describing individuals that have not yet laid eggs; Figure 5.1A) or "parous" (those that have laid at least one egg batch; Figure 5.1B) based on the degree of tracheation in their ovaries [449].

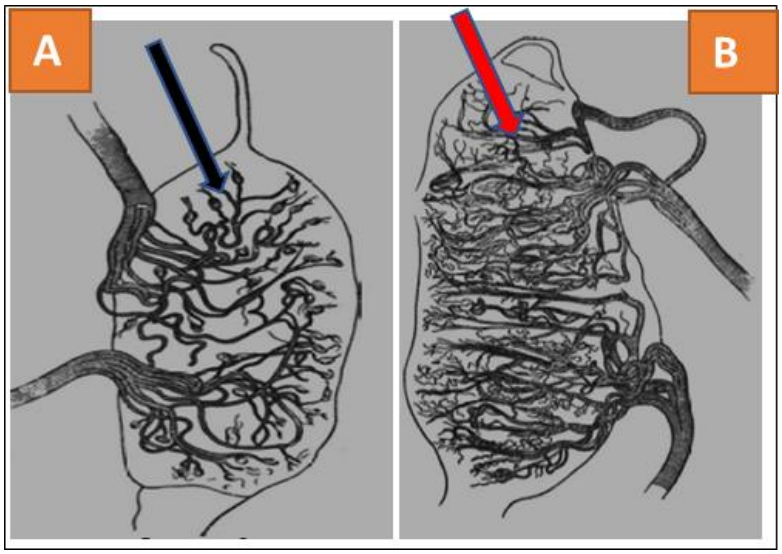


Figure 5.1: Ovary tracheations **A)** from nulliparous mosquitoes showing skein tracheoles indicated by the black arrows and **B)** parous mosquitoes showing unravelled tracheoles showed by red arrows (these Figures are from [449]).

5.2.3. Mosquito molecular analyses

Of 40,220 *An. gambiae s.l.* females collected, a subsample of 7,852 were selected to provide a representative sample from each month, village, trapping location (indoor *vs* outdoor). This subsampling strategy was guided by consideration of the minimum sample size likely to be required to detect malaria infection in one unique mosquito collection (e.g. permutation of night, trapping method and location) as described in method section in Chapter 2). Three types of molecular analyses were performed on this subsets of *An. gambiae* s.l. females: (i) Polymerase Chain Reaction (PCR) for

species identification (following [272]); (ii) malaria sporozoite detection [273] and (iii) blood meal identification ([450], to calculate the human blood index, HBI). Species identification and sporozoite detection were carried out on the same subset of *An. gambiae* s.l. females collected in HLCs (N = 7852). Additionally, species identification, blood meal identification and sporozoite detection were performed on all blood fed *An. gambiae* s.l. females collected in resting bucket traps (N=164). For analysis, each individual *An. gambiae* s.l. specimen was split so that legs and wings were used for PCR, the head and thorax for sporozoite detection, and abdomen (only if mosquitoes were collected from RBTs and were blood-fed) for blood meal analysis. Different tissues from the same mosquito sample were coded with a unique individual identifier so they could be traced and linked.

In ELISA tests (for sporozoite and blood meal source identification), two technical replicates of each sample were run in two different microplates at the same time and retested in cases where the first result was ambiguous. The absorbance of the solutions/reactions at the end of each ELISA was measured using microplate reader (Elx808; Bio-Tek) at 450 nm. To avoid any false positives (due to background noise), a sample was considered positive for an assay when its optical density (OD) was 2-fold higher than the average of the OD of both negative controls. Positive controls were also used in all ELISAs to ensure the procedure was working.

5.2.3.1 *Plasmodium falciparum* circumsporozoite protein detection

Malaria infection in mosquitoes was assessed by testing for the presence of *Plasmodium falciparum* circumsporozoite protein (CSP) in their head and thoraxes using a monoclonal sandwich Enzyme Linkage Immuno-Sorbent Assay (ELISA) developed by [273]. Presence of CSP in mosquito head and thorax samples indicates the presence of transmission stage sporozoites, as earlier parasite stages (e.g. oocysts) would only be found in the abdomen. Here, the head and thorax of individual females were placed in 1.5 mL Eppendorf tubes, and grinded in a solution of 250 µL of blocking-buffer (BB) with IGEPAL CA 630 (Sigma-Aldrich, cat. number I32021) using separate

Pellet Pestle® (Sigma-Aldrich cat. number Z359947) for each sample. Next, 50 µL of monoclonal antibody anti-CSP *P. falciparum* (mAb anti-CSP; from KPL, cat number 37-00-24-2, Gaithersburg, MD, USA) was dispensed into a 96-well microplate (Nunc, Roskilde, Denmark) and incubated overnight at Room Temperature (RT). The next morning (18 to 20 hours later), wells were emptied and filled with 200 µL of BB. This step allows the neutral proteins (albumin contained in the BB) to block unoccupied sites on the well surface to prevent non-specific binding of the antigen to the plate. After an hour of incubation at RT, wells were emptied again, washed to eliminate the unattached excess of antibodies using the washing solution (WS) and filled with 50 µL of antigen consisting of extracts of the ground mosquito solution; except for the 4-wells of the last column (12E to 12H, Figure 5.2) in each plate that were used for controls. Negative (e.g. 12G and 12H, Figure 5.2;) and positive (e.g. 12E and 12F, Figure 5.2) controls were used to determine the optical density cut-off values. For this, negative control samples were created by grinding the head-thorax of male *Anopheles gambiae* s.l. from field collections in a solution of 250 µL of blocking-buffer (BB) with IGEPAL CA 630 (Sigma Aldrich, cat. number I32021). A 50 µL aliquot of the negative control was added into wells 12G and 12H of each microplate. *Plasmodium falciparum* positive controls (BEI Resources®, cat. number MRA-890) at a concentration of 2pg/µL were created by diluting the acquired solution from the manufacture of 1µg/µL using the same buffer. The rest of the plate was filled by samples from individual *An. gambiae* s.l. females (one well per specimen). After 2-hours of incubation at RT, the plates were washed twice using WS made of Phosphate Buffer saline (PBS, Sigma-Aldrich cat. number D5773) 1X and 0.05% Tween 20 at pH 7.2) at 0.5% (volume/volume) to eliminate all unattached protein. Then, 50 µL of a solution made of peroxidase-conjugated mAb anti-CSP and (KPL, cat number 37-00-24-3, Gaithersburg, MD, USA) was added into each well and incubated for an hour at RT. Then the washing step was repeated four times using the WS. Next, 100 µL of O-phenylenediamine (OPD; SIGMA P5412-100TB) as substrate was added at each well and covered for thirty minutes of incubation. At the end

of the incubation, the reaction was stopped by adding 50 µL of phosphoric acid (concentration = 4N) followed by reading of absorbance of the product.

5.2.3.2 Blood meal source identification

Blood meal identification was carried out on the subset of *An. gambiae* s.l. that were identified as having recently blood fed and collected using the resting RBTs. A direct ELISA was used to identify if mosquito blood meals were from humans, cattle or both to allow estimation of the Human Blood Index (HBI) as described by [450]. The decision to only test for blood from human and cattle hosts since sera for use in positive controls was readily available for these hosts. Here, different microplates were prepared and processed according to the antibody (Ab) to be detected (human or bovine). Each sample was placed in a 1.5 mL Eppendorf and grinded in a solution of 150 µL of PBS (pH 7.4) Azide using a separate Pellet Pestle® (Sigma-Aldrich cat. number Z359947) for each sample. A 100 µL extract from the ground solution was diluted (1:3) in a PBS (pH 7.4) Azide 1X solution, then dispensed into individual wells of a 96-well microplate. Positive controls were made from 1/1000 diluted samples of human or bovine sera (diluted in PBS Azide). The bovine serum was obtained (after centrifugation at 5000 rotations per minute (rpm) for 5 minutes) from blood collected without anticoagulant from the national slaughterhouse. Similarly, human serum was obtained after centrifugation of blood collected from colleagues. The negative controls were made using reciprocal sera; each bovine serum was used as a negative control for human bloodmeal detection; and vice versa. For each test, wells on the last column of the microplate were used for negative and positive control respectively. Each plate was incubated overnight at RT, then emptied the following day. Then, 100 µL of conjugated Ab-anti-human (Sigma-Aldrich®, cat. number A0293-1ML) was added to each well of microplates used for human blood source identification and incubated for an hour. Similarly, the microplates for bovine blood source identification received 100 µL of Ab-anti-bovine (Sigma-Aldrich®, cat. number A5295-1ML). Next the substrate OPD was added to each well for 30 minutes. The reaction was then blocked by adding 50 µL of sulfuric acid 4N to each well follow by

reading the absorbance of the yellowish solution using microplate reader at 450 nm.

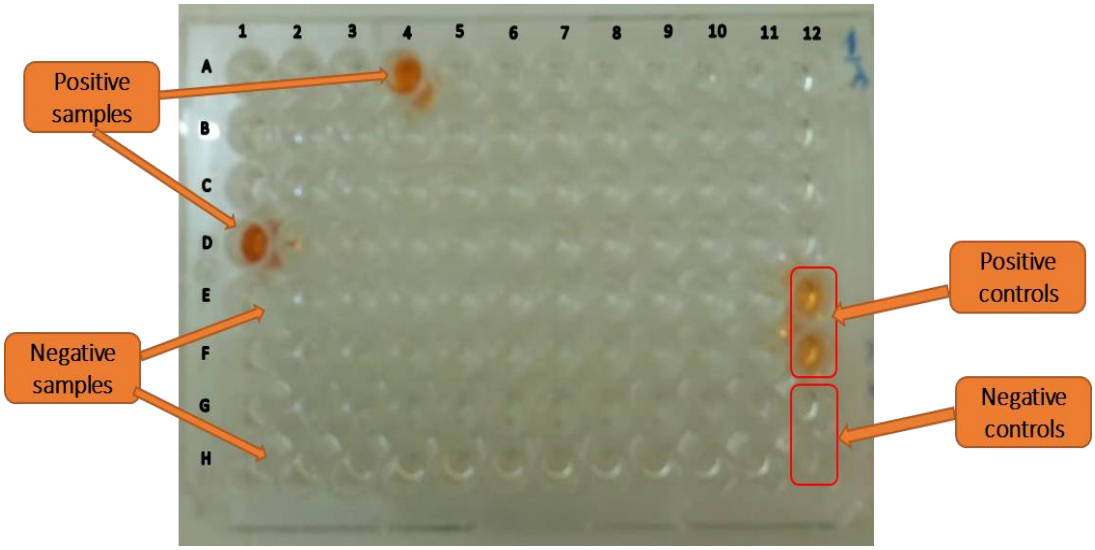


Figure 5.2: A microplate after running Enzyme-linked Immunosorbent Assay (ELISA) showing positive (pinkish solution) and negative (colourless solution) samples and controls.

5.2.4. Statistical analysis

Statistical analysis was carried out to test for spatial, seasonal and longer-term (over 26 months period) variation in parity (PR) and sporozoite infection rates (SR) in *An. gambiae* s.l. malaria vectors. Then, (iii) the human biting rate (HBR) and SR were used to estimate the EIR at different study sites and between the two study years. A series of generalized linear mixed models were created to test for spatial, seasonal and longer-term variation in these variables as described in Table 5.1. For all these three response variables, spatial variation was evaluated in models using data from all 12 villages surveyed in the first 18 months. Seasonal variation was assessed by coding each day of the year on a scale running from "1" (January 1st) to "365" (December 31st). This temporal variable was modelled as a non-linear smoothing function (spline named as t2(cDate, bs = cc) as described in Chapter 3. In addition, a secondary temporal variable was created to assess for longer-term trends within the subset of 6 villages that were sampled over 26 months. Here, a discrete independent variable named nDate was created that started as "1" on the first day of collection and counted upwards until

the last day of the study (e.g day "789") as described in Chapter 3. This was fit as a linear term in models test for a unidirectional rise or fall in the trait of interest across the study period. Both seasonal and longer-term temporal variation was modelled in analysis of parity rates (Table 5. 1, Model 5.1) and sporozoite rates (Table 5.1, Models 5.2). Secondary analyses were performed to estimate the mean SR and HBR of vectors between the first (Oct 2016 - Sept 2017) and second year (Oct 2017-Sept 2018) of study (Table 5.1, Model 5.3 and 5.4); with annual variation being modelled as a category (year 1 and 2) instead of a spline. As all *An. gambiae* s.l. in these data sets have been identified to species level by PCR, it was possible to include an explanatory variable for vector species (*An. coluzzii* or *An. gambiae*). An interaction term was fit between trapping location and vector species (Model 5.1 and 5.2; Table 5.1); to test if transmission traits varied between vectors host seeking in indoor versus outdoor locations. I also tested the influence of nightly mean temperature and humidity on parity rates, sporozoite rates and the human biting rate (Model 1, 2 and 4, Table 5.1) whilst accounting for seasonal variation. Thus, additional covariates of daily temperature and humidity at each household and location were included in models, along with random effects for compound and household (Table 5.1).

Due to the low sample sizes generated from mosquito resting collections, the data set for blood meal analysis was much smaller than that for parity or sporozoite rates. Here, the HBI was estimated as the proportion of *An. gambiae* s.l. that tested positive for human blood out of the total from which blood meals could be identified (as either human or bovine, N = 94) over the study period. Individuals whose blood meals could not be identified (N = 70) were excluded from analysis. Due to the small sample size, no statistical analysis was performed on these data set for HBI.

After testing the significance of key variables of interest for each trait as described above, secondary analyses were performed to estimate derived parameters from these models. First, mean parity rates ***(p)*** were estimated for *An. gambiae* s.l. for each village (Model 1) and used to calculate the mean daily survival rate (*S, Equation 5.2*) and life expectancy (*LE, Equation*

5.3) of vectors [451]. A critical assumption of this formula is that mosquito age structure is stable over the period of consideration. To meet this, here data on parity were pooled across a study year to encompass a full annual cycle of population rise and fall. A gonotrophic cycle length (parameter *n* in equation) of 2.5 days was assumed in these calculations, based on previous studies indicating this varies between 2 - 3 days for *An. gambiae* s.l. in Burkina Faso [224].

Finally, the EIR was estimated for each village from the product of village-specific sporozoite rates (Model 2, Table 5.1) and village-specific human biting rates (mean number of *An. gambiae* s.l. biting per night as described in Chapter 3 and named "abundance"; Model 1, Table 3.1). Estimates of the Entomological Inoculation Rate (EIR) were calculated for each study year (first vs second). Annual EIRs were estimated as the product of the mean nightly Human biting rate (HBR) and sporozoite rate multiplied by 365 days.

All analyses were conducted using Generalised Additive Mixed Effect Models (GAMMs) within the 'mgcv package' [335] augmented with the lme4 package [278] named GAMM4 [397] in the R statistical software as described previously (Chapter 3). In brief, a full model was created for each response variable which included all explanatory variables of interest and relevant interactions (Table 5.1). Model selection was conducted by a process of backward elimination by sequentially removing terms, and assessing their significance using the 'anova.gam' function in the 'mgcv package' [336]. After model selection, mean values and 95% confidence intervals for all statistically-significant terms were estimated using the 'predict.gam function' [337] from the 'mgcv package' [335]. Apart from the HBR analysis that was modelled following a negative binomial as described for the abundance in Chapter 3 (Model 3.1, Table 3.1), all the other models were fitted following a binomial distribution. Two variables, mosquito daily survival (S) and life expectancy (LE) were derived from mean values of parity rates obtained from statistical analysis. The confidence intervals associated with these estimates were derived from the lower and upper values of parity as calculated from its 95% confidence intervals.

Table 5.1*:* Maximal models used for investigating spatial, seasonal and longer-term temporal variation in parity, sporozoite rates and human biting rates by the malaria vector *An. gambiae* s.l. in this study. The average temperature and relative humidity were obtained by averaging the records over the course of the collection night. Here, "subset of *An. gambiae* s.l. lab-processed" refers to subset that were individually identified to species level and tested for *Plasmodium falciparum* sporozoite infection including both dissected and non-dissected. *Used for assessing difference between year 1 and year 2

Model	Tests	Response variables	Fixed Effect variables	Random effect variables	Type of data	Distribution
5.1	Parity rate	(Parous/(Parous +Non-Parous))	Village + Location + Sporozoite + Species + Village: Species + Location: Species + Temperature + Humidity + nDate + t2(cDate, bs = cc),	Compound+ Household	All *An. gambiae* s.l. lab-processed, from 12 villages	Binomial
5.2	Sporozoite rate	(Positive/Positive+ Negative))	Village + Location + Species + Village: Species + Location: Species + Temperature + Humidity + nDate+ t2(cDate, bs = cc),	Compound+ Household	Subset of *An. gambiae* s.l. lab-processed from 12 villages	Binomial
5.3*	Sporozoite rate	(Positive/Positive+ Negative))	Village + Location + Species + Temperature + Humidity + Year + t2(cDate, bs = cc),	Compound+ Household	Subset: *An. gambiae* s.l. lab-processed data from 6 villages	Binomial
5.4*	Human biting rate (HBR)	Number of *An. gambiae* s.l.	Village + Location + Temperature + Humidity + Year + Village: Year + t2(cDate, bs = cc),	Compound+ Household	Host-seeking nightly *An. gambiae* s.l. data from 6 villages	Negative binomial

5.3. Results

5.3.1 General results

Within the subset of *An. gambiae* s.l. caught in HLCs and analysed by PCR, 53.8% were *An. coluzzi*i, 45.9% were *An. gambiae* and 0.3% were *An. arabiensis*. A total of 927 (males and females) mosquitoes were collected from the RBTs including four genera of which *An. gambiae* s.l. was the most common (63.32%; Chapter 3 Table 3.4 and 3.5). Overall, 70.5% of *An. gambiae* s.l. dissected had laid eggs at least once (Table 5.2). Of the 7852 *An. gambiae* s.l. samples tested for *P. falciparum* sporozoites, 3.87% were positive (Table 5.3). As described in Chapter 3, of the 584 *An. gambiae* s.l. collected in RBTs, 164 were blood-fed females. The source of blood meal could be successfully identified in only 94 individual samples. Five of these 94 females were infected with *P. falciparum* sporozoites (4 with a human blood meal, 1 with cattle).

Table 5.2: Parity status of the within the subsample of *An. gambiae* s.l. that were individually identified to species level by PCR. Data are presented for different vector species and pooled over sampling location (indoors and outdoors) and the collection period (October 2016 to December 2018).

	An. arabiensis		*An. coluzzii*		*An. gambiae*		
Village	Non-parous	Parous	Non-parous	Parous	Non-parous	Parous	Total tested
Dangouindougou	1	8	80	215	71	168	543
Gouera	0	0	52	71	66	240	429
Nianiagara	0	0	8	20	34	137	199
Nofesso	0	0	9	9	59	189	266
Ouangolodougou	0	0	4	12	43	154	213
Sitiena	1	0	153	396	36	84	670
Tengrela	0	0	539	977	17	28	1561
Tiefora	3	0	355	556	195	455	1564
Timperba	0	0	8	20	73	171	272
Tondoura	0	1	1	17	86	414	519
Toumousseni	1	1	110	285	68	312	777
Yendere	2	4	42	118	110	286	562
Total	8	14	1361	2696	71	168	7575

Table 5.3: Number of *An. gambiae* s.l. tested for the presence of *P. falciparum* sporozoites from 12 study villages in southwestern Burkina Faso displayed by species, pooled over sampling location (indoors and outdoors) and period of collection (October 2016 to December 2018).

	An. coluzzii		*An. gambiae*		
Village	Negative	Positive	Negative	Positive	Total tested
Dangouindougou	323	19 (5.9%)	233	17 (7.3%)	602
Gouera	130	4 (3.08%)	292	20 (6.85%)	447
Nianiagara	32	1 (3.13%)	162	17 (10.49%)	212
Nofesso	18	0 (0%)	238	16 (6.72%)	272
Ouangolodougou	15	2 (1.33%)	209	10 (4.78%)	236
Sitiena	540	10 (1.85%)	118	2 (1.69%)	672
Tengrela	1555	26 (1.67%)	45	0 (0%)	1626
Tiefora	891	37 (4.15%)	644	18 (2.8%)	1593
Timperba	28	2 (7.14%)	242	14 (5.79%)	286
Tondoura	18	1 (5.56%)	477	34 (7.13%)	531
Toumousseni	382	14 (3.66%)	378	10 (2.65%)	786
Yendere	167	7 (4.19%)	381	23 (6.04%)	589
Total	4099	123 (3%)	3419	181 (5.29%)	7852

5.3.2 Parity and survival rates in *Anopheles gambiae* s.l. population

Parity rates in *An. gambiae* s.l. females varied significantly between villages (df = 11, x^2 = 21.04, p = 0.03), trapping locations (df = 1, x^2 = 9.75, p = 0.001), season (edf = 1.94, x^2 = 11.49, p = 0.016) and in relation to malaria infection status (df = 1, x^2 = 49.18, p < 0.0001; Table 5.4). Controlling for these factors, there was also evidence of a longer-term increase in parity rates over the study period (df = 1, x^2 = 20.07, p < 0.0001; Table 5.4 & 5.5). Between villages, mean parous rates varied from a low of ~72% in Tengrela to high of ~86% in Tondoura (Figure 5.3). Parous rates were slightly higher in *An. gambiae* s.l. caught inside rather than outside of houses (Figure 5.4) and varied seasonally, with a peak toward the end of the rainy season (> 80% in September and October), and low in late dry season (~68% in April; Figure 5.5A). There was significant increase in parity rates over the collection period from ~73% at the beginning to ~85% toward the end of the collection (z = 7.2, p < 0.0001; Table 5.5, Figure 5.5B). There was no evidence of variation in parity rates between vector species (df = 1, x^2 = 0.66, p = 0.42), or in relation to temperature (df = 1, x^2 = 0.51, p = 0.47) and humidity (df = 1, x^2 = 1.32, p = 0.25) after controlling for seasonality (Table 5.4).

Based on the mean estimate of PR in the study area (78.57%), the daily survival rate of *An. gambiae* s.l. was estimated as ~91%, corresponding to an average of ~11 days. Estimates of mean daily survival in *An. gambiae* s.l. ranged from a low of ~88% in Tengrela to 94% in Tondoura (Figure 5.6); corresponding to life expectancies of 8 days (95% CI: 6 – 10) to ~16 (95% CI: 12 – 25) days respectively (Figure 5.7).

Table 5.4*:* Significance of explanatory variables included in the model for assessing variation in *An. gambiae* s.l. parity rates (Model 5.1, Table 5.1). Here, df is the degree of freedom and Chi-sq (x^2) represents the values of Likelihood Ratio Test. cDate is a smoothing function on days from 1 - 365 describing a year of collection, for assessing the seasonality in the proportion. nDate a discrete variable from first to the last day of collection (798), describing the long-term trend in the proportion. The temperature and relative humidity were obtained by averaging the records over the course of the collection night.

Explanatory variable	Chi-sq	df	p-value
cDate	11.49	1.94[a]	0.016*
Humidity	1.32	1	0.25
Location	9.75	1	0.001*
Location: Species	0.4	1	0.53
nDate	20.07	1	< 0.0001*
Species	0.66	1	0.42
Sporozoite	49.18	1	< 0.0001*
Temperature	0.51	1	0.47
Village	21.04	11	0.033*
Village: Species	19.35	7	0.055

* indicates the significant terms in the model and

[a] indicate the given df represent the estimate degree of freedom (edf = degree of wigginess) from smoother term.

Table 5.5: Summary of the estimate (β), standard errors, z values and p-value for each explanatory variables included in the final model 5.1 (Table 5.1) used for assessing the variation in *An. gambiae* s.l. parity rates. nDate a discrete variable from first to the last day of collection (798), describing the long-term trend in the proportion.

Parameters	β	Std Error	z value	p value
Intercept	1.049	0.175	5.997	0.000*
Gouera	-0.200	0.248	-0.806	0.420
Nianiagara	0.085	0.306	0.277	0.782
Nofesso	-0.193	0.282	-0.683	0.495
Ouangolodougou	0.087	0.289	0.301	0.764
Sitiena	-0.209	0.252	-0.831	0.406
Tengrela	-0.513	0.207	-2.475	0.013*
Tiefora	-0.528	0.213	-2.475	0.013*
Timperba	-0.378	0.277	-1.363	0.173
Tondoura	0.169	0.248	0.685	0.494
Toumousseni	-0.146	0.245	-0.598	0.550
Yendere	-0.299	0.224	-1.331	0.183
Location Outdoor	-0.172	0.055	-3.106	0.002*
Species An. gambiae	0.001	0.000	4.386	0.000*
nDate	2.083	0.289	7.204	0.000*
Sporozoite positive	1.049	0.175	5.997	0.000*

* indicates $p < 0.05$.

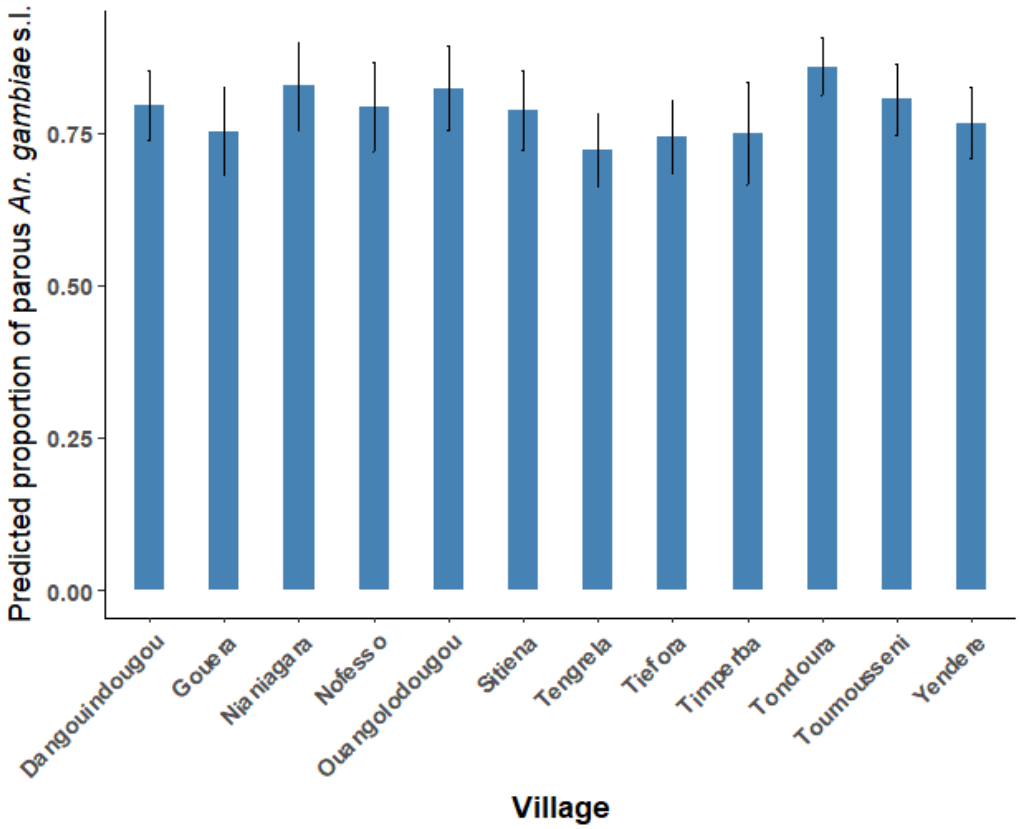


Figure 5.3: Mean predicted parity rates in *An. gambiae s.l.*, collected using Human Landing catches from October 2016 to December 2018 from 12 villages in southwestern Burkina Faso, based on prediction from the final model. The error bars indicate 95% confidence intervals.

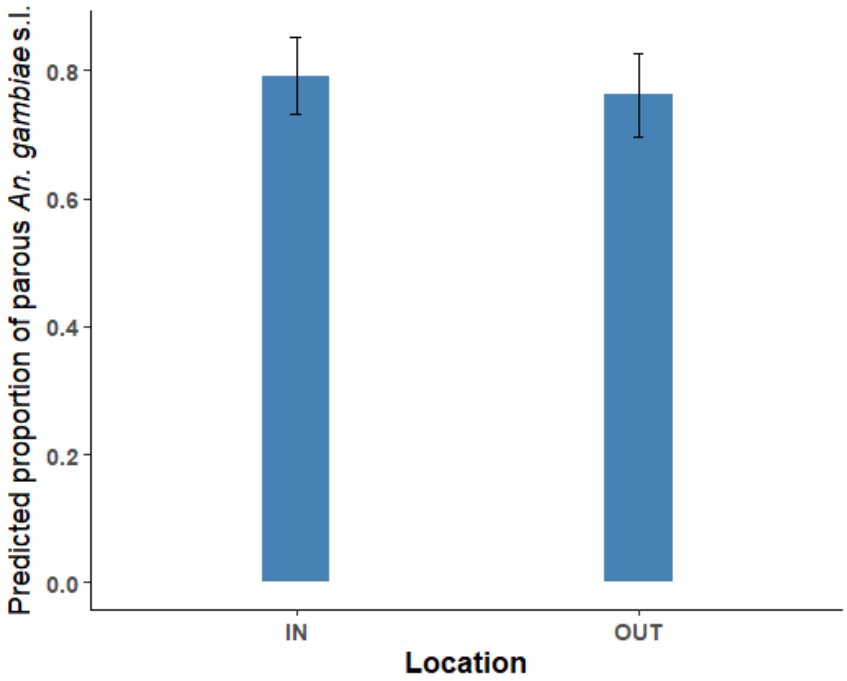


Figure 5.4: Mean predicted proportion of parous *An. gambiae* s.l., collected using Human landing catches from October 2016 to December 2018 in 12 villages in southwestern Burkina Faso, based on the final model prediction at each location (IN= indoor versus OUT = outdoor). The error bars indicate 95% confidence intervals.

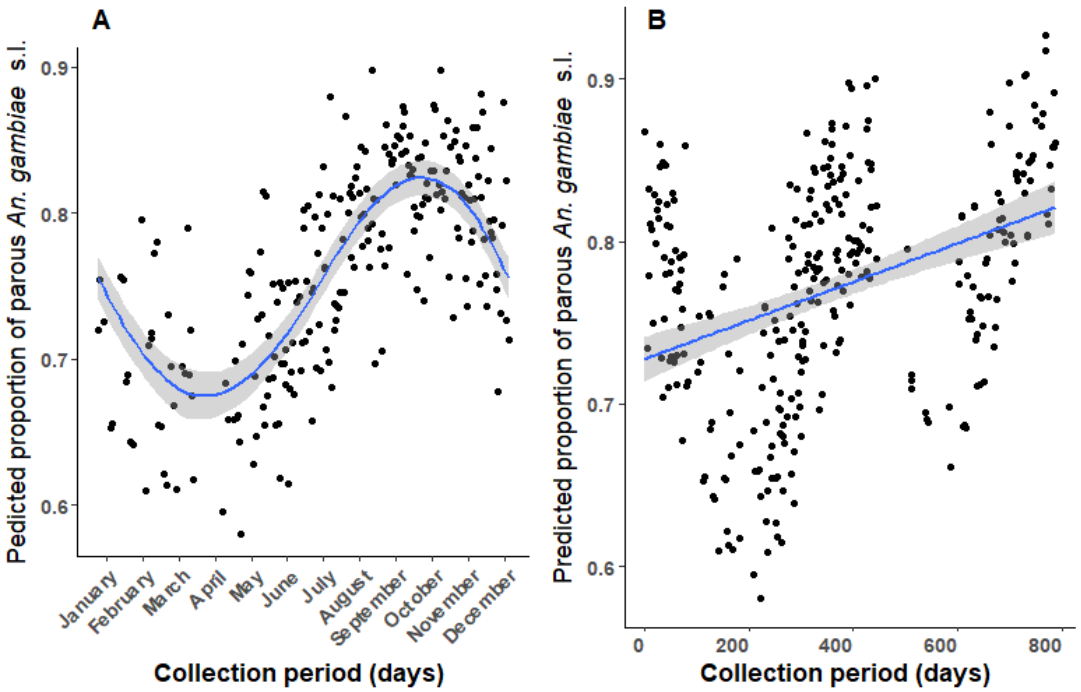


Figure 5.5: **A)** Seasonal and **B)** longer-term trend of the daily mean predicted proportion of parous *An. gambiae* s.l. (dotes) based on the final model prediction. Here, *An. gambiae* s.l. data were collected from October 2016 to December 2018 using Human Landing Catches at 12 villages in southwestern Burkina Faso. The blue lines indicate the regression lines and the grey-shaded areas around them indicate 95% confidence intervals.

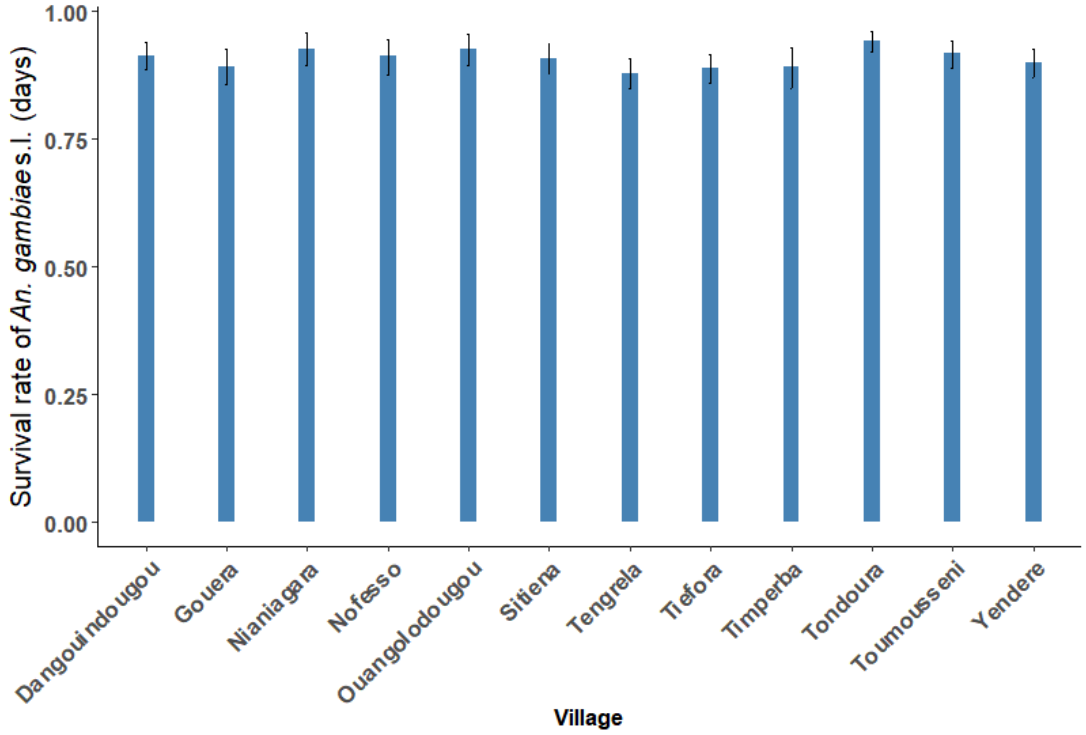


Figure 5.6: The mean predicted daily survival rates of *An. gambiae* s.l. in 12 villages in southwestern Burkina Faso as predicted from parity rates. Here, *An. gambiae s.l.* data were collected from October 2016 to December 2018 using Human Landing Catches at 12 villages in southwestern Burkina Faso. The error bars indicate 95% confidence intervals.

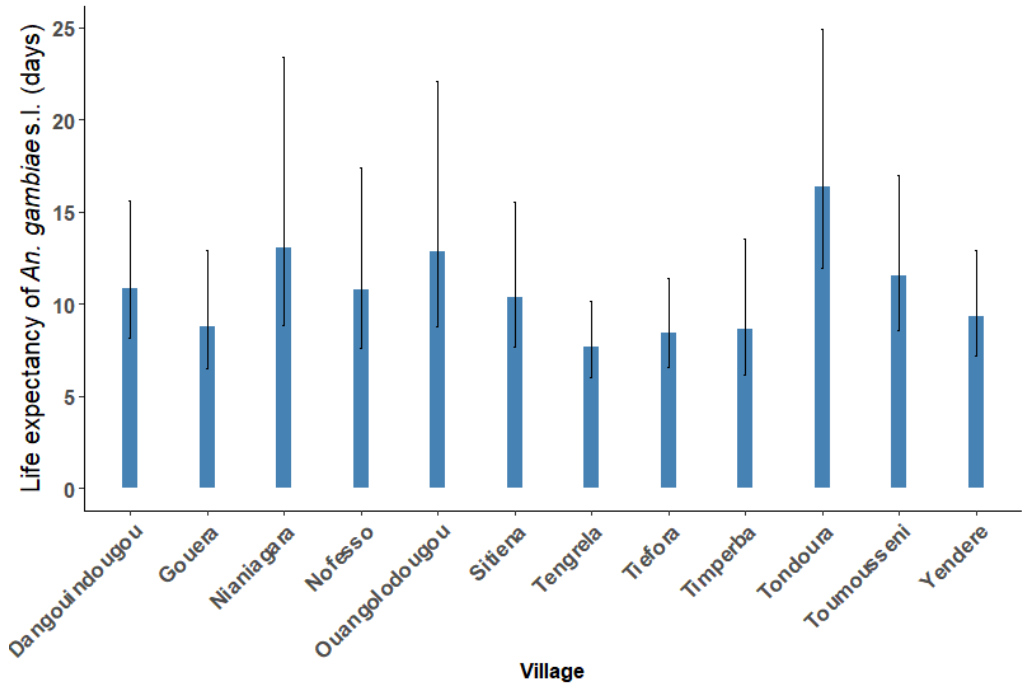


Figure 5.7: Predicted life expectancy (in days) of *An. gambiae* s.l. in 12 villages in southwestern Burkina Faso based on parity data. Here, *An. gambiae* s.l. data were collected from October 2016 to December 2018 using Human Landing Catches. The error bars indicate 95% confidence intervals.

5.3.3 Sporozoite rate in *An. gambiae* s.l. population

The mean SR in *An. gambiae* s.l. in the study area was 3.48% (95%CI: 1.51 - 5.26%). This SR varied significantly varied between villages (df = 11, x^2 = 34.61, p = 0.0002) and seasons (edf = 1.3, x^2 = 3.18, p = 0.03) and showed evidence of a longer-term decrease over the study period (df = 1, x^2 = 6.26, p = 0.01; Table 5.6 & 5.7). Sporozoite rates varied up to 9-fold across villages (Figure 5. 8) and was significantly higher in the rainy (compared to dry season, Table 5.7, Figure 5.9A). Sporozoite rates in *An. gambiae* s.l. fell from a mean of ~5% to ~2% over the study period (z = -2.5, p = 0.01; Table 5.7, Figure 5.9B). There was no evidence of different in SR between the two major vector species (*An. coluzzii* and *An. gambiae)*, or in *An. gambiae* s.l. caught host seeking inside versus outside of houses (Table 5.6). The overall sporozoite rate in year 1 of the study was higher than in year 2 (3.18 vs 1.64%; Model 4, df = 1, x2 = 5.73, p = 0.02).

Table 5.6: Significance of explanatory variables included in the Model 5.2 for *Plasmodium falciparum* sporozoite rates, combining the non-parous and the parous individuals. Here, df is the degree of freedom and Chi.sq (x^2) represents the values of Likelihood Ratio Test.; cDate is a smoothing function on days from 1 - 365 describing a year of collection, for assessing the seasonality in the proportion. nDate a discrete variable from first to the last day of collection (798), describing the long-term trend in the proportion. The temperature and relative humidity were obtained by averaging the records over the course of the collection night.

Explanatory variable	Chi.sq	df	p-values
cDate	3.175	1.3[a]	0.036*
Humidity	0.54	1	0.46
Location	0.02.49	1	0.12
Location: Species	1.02	1	0.31
nDate	6.26	1	0.01*
Species	0.05	1	0.82
Temperature	2.41	1	0.12
Village	34.61	11	0.0002*
Village : Species	3.35	11	0.95

* indicates the significant terms with $p < 0.05$ and

[a] indicate the given df represent the estimate degree of freedom (edf = degree of wigginess) from smoother term.

Table 5.7: Summary of the estimates (ß), standard errors, z values and p-value for each explanatory variables included in the final model 5.2 (Table 5.1) used for assessing the variation in *An. gambiae* s.l. sporozoite rates. nDate a discrete variable from first to the last day of collection (798), describing the long-term trend in the proportion.

Parameters	*ß*	Std Error	z value	p value
Intercept	-2.677	0.247	-10.829	0.000
Gouera	-0.338	0.381	-0.887	0.375
Nianiagara	0.255	0.409	0.624	0.533
Nofesso	-0.093	0.415	-0.225	0.822
Ouangolodougou	-0.222	0.434	-0.510	0.610
Sitiena	-1.335	0.460	-2.903	0.004
Tengrela	-1.348	0.346	-3.899	0.000
Tiefora	-0.457	0.313	-1.461	0.144
Timperba	-0.259	0.422	-0.615	0.539
Tondoura	0.099	0.346	0.286	0.775
Toumousseni	-0.603	0.374	-1.611	0.107
Yendere	-0.016	0.340	-0.048	0.962
nDate	-0.001	0.000	-2.502	0.012

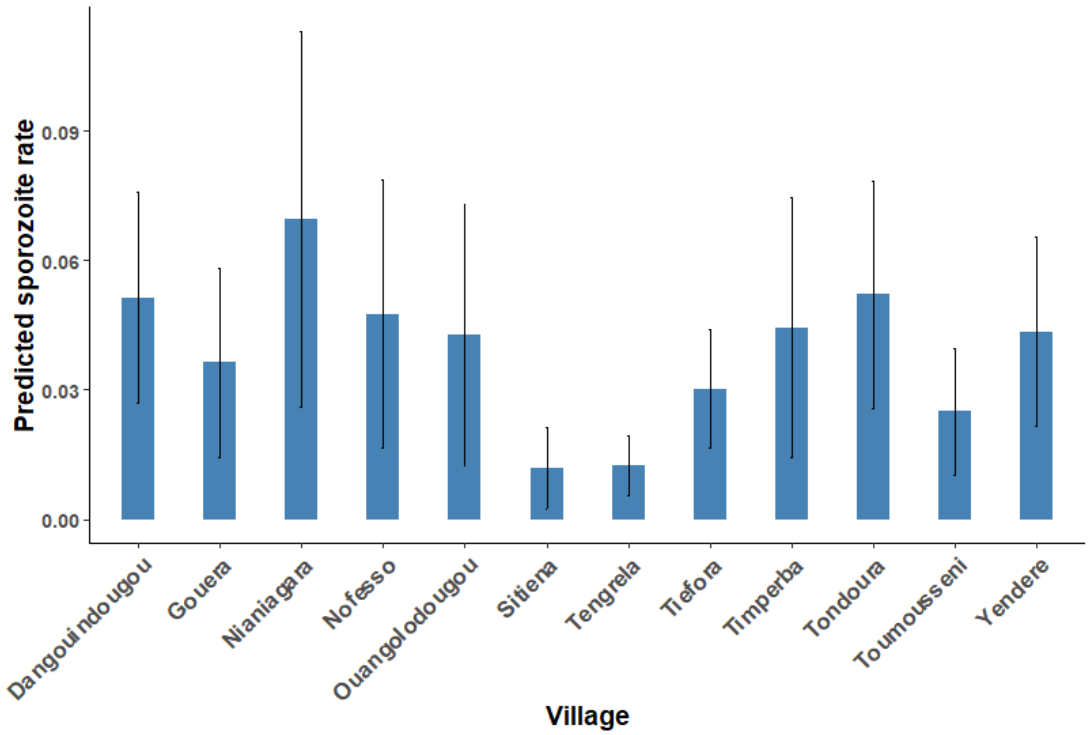


Figure 5.8: Proportion of *An. gambiae* s.l. predicted to be infected with *P. falciparum* sporozoites across 12 villages in southwestern Burkina Faso. Here, *An. gambiae* s.l. data were collected from October 2016 to December 2018 using Human Landing Catches and consisted of parous and non-parous individual samples molecularly analysed. The error bars indicate 95% confidence intervals.

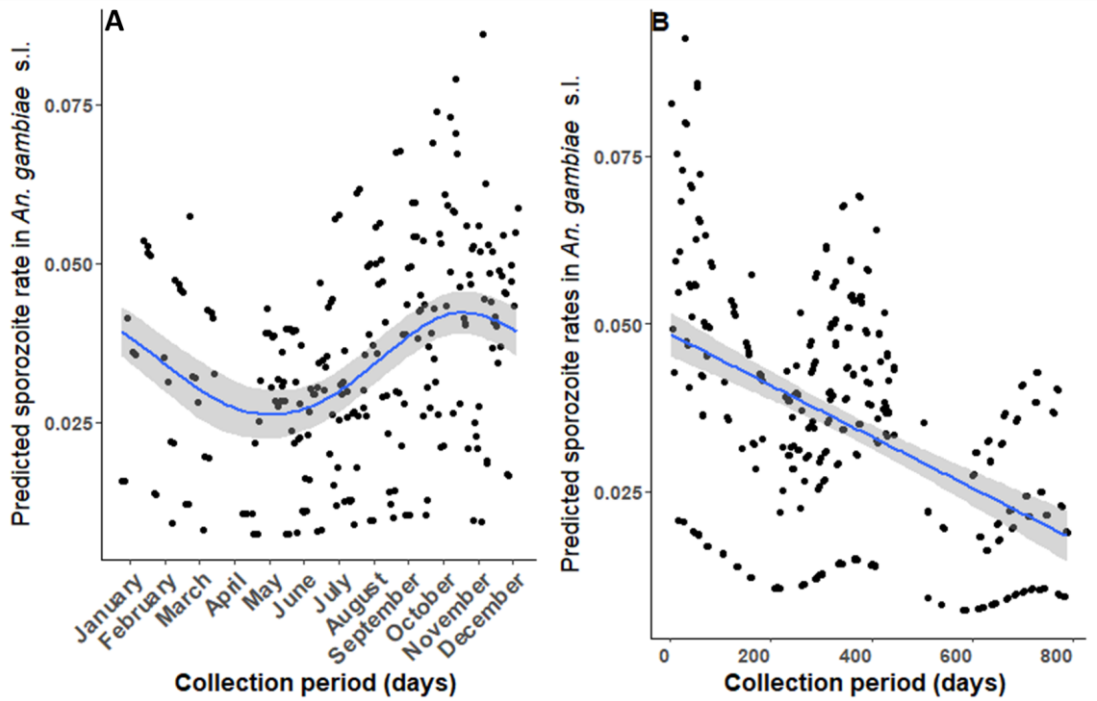


Figure 5.9: **A)** Predicted seasonal trend in the sporozoite rates in *An. gambiae* s.l. **B)** Predicted longer-term trend in in sporozoite infection rates in *An. gambiae* s.l. (dots) sporozoite infected considering the parous and non-parous individuals. Data are from 12 villages in southwestern Burkina Faso using Human Landing Catches from October 2016 to December 2018. The curve blue and line indicate the regression curve and line respectively and the grey-shaded areas around them indicate 95% confidence intervals.

5.3.4 Entomological Inoculation Rates

As described in Chapter 3, the mean human biting rates of *An. gambiae* s.l. varied significantly between villages (df = 11, x2 =230.54, p < 0.0001 Table 3.4 & 3.7), trapping location (indoors versus outside, df = 1, x2 = 21.28, p < 0.0001), seasons (x2 = 1165, edf = 6.84, p < 0.0001; Table 3.7) and appeared to decline over the study period (df = 1, x2 = 6.63, p = 0.01; Table 3.7). The predidtced mean of *An. gambiae s.l.* biting rates (HBR) from Model 5.4 (Table 5.1) and sporozoite rates (Model 5.3, Table 5.1) were combined to estimate annual Entomological Inoculation Rates (EIR) for each of the 12 study villages (Table 5.6). This revealed substantial heterogeneity in exposure risk, with EIR ranging from a low of ~27 infective bites/person annually in Sitiena, to > 200 infective bites per person annually in the three villages with the

highest exposure (Table 5.6). Restricting analysis to the subset of 6 villages that were monitored over two years (year 1: Oct 2016 - Sept 2017, year 2: Oct 2017-Sept 2018), EIR was estimated to be higher in the first than second year (year 1 EIR = 320.15; year 2 EIR: 104.75). After adjusting for the proportion of bites that predicted to be preventable by use of effective LLINs (~85%, as described in Chapter 3), people in most communities (8 out of 12) were still predicted to be exposed to ≥ 10 infected bites per person per year (Table 5.6).

Table 5.8: Predicted *Plasmodium falciparum* sporozoite rates, human biting rates (HBR, number of bites per night) in *An. gambiae* s.l. collected in human landing catches in 12 villages in southwestern Burkina from October 2016 to December 2018. The product of sporozoite rates and nightly mean human biting rate was multiplied by 365 to generate an annual Entomological Inoculation Rate (EIR, number of infective bites per person per year).

Village	Sporozoite rate	HBR	Annual EIR	Annual EIR not preventable by LLINs
Dangouindougou	0.053	25.78	502.00	75.3
Gouera	0.037	6.33	85.56	12.85
Nianiagara	0.072	3.53	93.11	13.97
Nofesso	0.048	1.93	34.12	5.12
Ouangolodougou	0.044	3.77	60.72	9.11
Sitiena	0.012	5.94	26.82	2.52
Tengrela	0.013	62.03	285.27	43.24
Tiefora	0.031	43.58	494.02	74.10
Timperba	0.046	4.70	78.43	11.76
Tondoura	0.054	4.13	81.05	12.16
Toumousseni	0.026	7.87	74.24	11.14
Yendere	0.044	11.75	190.06	28.51

5.3.5 Human blood index

Of the 94 *An. gambiae* s.l. specimens from which blood meals could be identified, 56 samples were *An. coluzzii,* 36 were *An. gambiae* and 2 were *An. arabiensis*. Fifty two percent of blood meals were human-only, 35.11% were cattle only, and 12.77% were a mixture of cattle and human blood (Table 5.7). Counting all blood meals that tested positive for human blood (single and mixed meals), this corresponds to an overall HBI of 64.9%. Sample sizes were too low for robust analysis of differences between sampling locations (villages, indoors versus outdoor), however some general observations are noted. For *An. coluzzii,* the HBI for mosquitoes resting outdoors was 82% compared to 60% indoors. For *An. gambiae*, the HBI was 67% in mosquitoes caught outdoors compared to 71% indoors (Table 5. 8). However, the proportion of *An. gambiae s.l.* collected that have taken blood-meal, whether on human or cattle, was higher indoor (Table 5.9).

Table 5.9: Total numbers of blood-fed female *An. gambiae* s.l. caught using Resting Bucket Traps in the 12 villages, from October 2016 to December 2018 (RBT) and display by species and trapping location pool over villages, according to the blood source. % = proportion of blood source collected indoors and outdoors in the total collection.

Species	Location	Cattle	Human	Human - cattle	Total	HBI
An. arabiensis	Indoor	1	0	0	1	0
	Outdoor	1	0	0	1	0
An. coluzzii	Indoor	18	23	4	45	0.6
	Outdoor	2	7	2	11	0.82
An. gambiae	Indoor	7	11	6	24	0.71
	Outdoor	4	8	0	12	0.67
Total		33	49	12	94	0.65

5.4. Discussion

The study showed a substantial spatial, seasonal and longer-term variation in several entomological predictors of malaria transmission across two years following a mass LLIN distribution in south west Burkina Faso. Overall, parous rates in *An. gambiae* s.l. within study area were high (~79%), corresponding to expected life span of 11 days. There was also evidence of a gradual rise in parity rates across the study period; indicating mosquito survival may have been increasing through time. The human blood index of the *An. gambiae* s.l. was relatively low at 65%, revealing these vectors regularly feed on cattle as well as people. Approximately 3.5% of *An. gambiae* s.l. were infected with malaria sporozoites; but infection rates varied up to 9-fold between villages and by 60% between seasons. Both sporozoite infection rates and mean human biting rates (Chapter 3) declined over the study period, resulting in a predicted reduction of the Entomological Inoculation Rate by ~ 67% between the first and second year of study. However, given the EIR in both years was still very high (> 100 infected bites per person per year), with only ~85% of exposure expected to be preventable by effective use of LLINs (Chapter 3); it is clear that high levels of residual transmission can be maintained by these vector populations.

The mean parous rate for *An. gambiae* s.l. females in the study area (~79%) was higher than previously reported in studies in the central-west (~70% [223]) and southwest of Burkina Faso (~ 60%, [452]). Further, the PR here is relatively high compared to that reported in recent studies from northern and south-eastern Benin (~72% for each, [344, 453]). The PR in *An. gambiae* s.l. from this setting corresponds to a life expectancy of ~ 11 days, which is sufficient for the extrinsic incubation period of *P. falciparum* in *An. gambiae* at 29°C (9-11 days [422]). Thus, a relatively high proportion of vectors in the study area have potential to live long enough to transmit malaria. In contrast, the mean sporozoite rate in *An. gambiae* s.l. in the study area (3.5%) was somewhat lower than the average of ~5% reported in other areas of Burkina Faso [217, 223, 454, 455]. However, there was substantial spatial and seasonal heterogeneity in sporozoite rates within the current study, with

rates going as high as ~7% in some villages. However, the mean SR across sites here is in line with that reported for other areas of West Africa (e.g south eastern Benin: 3%, [453]; central Benin: 8%, [344], and Cote d'Ivoire: 6%, [357]). Additionally, it is noted that previous studies have generally estimated sporozoite rates only from mosquitoes caught indoors using CDC Light traps indoors. There is conflicting evidence on whether sporozoite rates in *An. gambiae s*.l. vary [456, 457] or not [458, 459] between HLC and CDC collections.

This mean EIR averaged over all sites and years was ~215 bites/person/year. This is within the higher range of values reported from other African settings (e.g. 100 to 156 infectious bite per person per year; [434, 460]); but below other settings in Cote d'Ivoire where extreme values of up to 897 infective bites per person per year were recently reported in Cote d'Ivoire [357]. This local and nationa variation in EIR is likely due to variation in ecological conditions such a temperature and humidty which can impact mosquito population dynamics [461, 462]. Current EIR is however higher than that from Senegal ~70 infective bites per person per year from collection done over six months during the rainy season [463]. Whilst the EIR values reported here at not the highest recorded for Africa, they are amongst the top range. Considering that a maximum of 85% exposure (described in Chapter 3) is expected to be preventable by LLIN use in this study area, residents may be still be exposed to average of ~25 infective bites per person per year even under conditions of 100% LLIN coverage and use. This is more than sufficient to sustain high levels of residual transmission.

The relatively high survival of malaria vectors in the study area may be explained both by their high level of insecticide resistance (Chapter 4), and/or their ability to obtain bloodmeals from humans outside of sleeping hours or unprotected animal hosts. Approximately 48% of *An. gambiae* s.l. tested positive for cattle blood, either on its own or in combination with a human blood meal. The Human Blood Index (65%) of these vectors is lower than previously reported in Burkina Faso (> 77%; [223, 331, 454]) and Benin (> 90%, [356, 370]). Although, reported differences in HBI between studies

may also be due to variability in collection methods (CDC-Light Trap used outdoor; [331]) and sampling location in previous studies (e.g. [356, 370]) that may have preferentially targeted the anthropophagic population [464]. Through sampling both the indoor and outdoor resting population, these results confirm the *An. gambiae* s.l. population has plasticity in host choice and can readily feed on animals when people are not accessible.

Through molecular analysis of a subset of *An. gambiae* s.l., it was possible to conduct species level analysis of mosquito demographic and transmission traits (between *An. gambiae* and *An. coluzzii*). There was no difference in parity or sporozoite rates between these species. Other studies in southwest Burkina Faso [452] and Cameroon [465] also reported similar parity rates in *An. coluzzii* and *An. gambiae*. Similarly, no difference in sporozoite rates between *An. coluzzii* and *An. gambiae* was detected in other part of Burkina Faso [223]. However, sporozoite rates were somewhat higher in *An. gambiae* than *An. coluzzii* in other parts of West Africa (e.g. from Burkina Faso [340] and Senegal [466]). However, these studes were relatively short-term (2 to 5 months) and occurred only during the rainy season where *An. gambiae* is more abundant than *An. coluzzii*. Thus so, it is unclear whether these apparent species-specific differences were confounded by seasonal dynamics. Thus, I conclude that there are no major differences in transmission between these vector species within the Cascades region of Burkina Faso, with both contributing to residual transmission

There was considerable variation in *An. gambiae* s.l. demographic and transmission traits across the 12 villages investigated here. For example, parity rates and consequently survival and life expectancy in *An. gambiae* s.l. were much lower in Tengrela and Tiefora compared to Nianiagara and Tondoura. Additionally, sporozoite infection rates varied by up to 9-fold across villages. This spatial variation may be due to differences in local ecology and human population characteristics. For example, both parity and sporozoite rates were lower in Tengrela, a village where there is year-round rice irrigation on flooded lands. In contrast, infection rates were higher in Nianiagara and Tondoura where conditions are relatively drier with no

irrigation scheme. Sporozoite rates were also reported to be lower in *An. gambiae* s.l. at an irrigated site in Mali compared to surrounding areas [362]; highlighting how local variation in the availability and type of larval habitat may contribute to focal transmission.

Variation in these mosquito demographic and infection rates corresponded to variation in EIR from a high of ~500 infectious bites per person per year in one village (Dangouindougou) compared to a low of ~27 infectious bites/person/year in another (e.g. Sitiena). Such variation was also documented in northern Benin where EIR varied substantially sites (from 120 to ~216 infectious bites per person per year) [344]. This heterogeneity could be driven by variation each of the composite parts of EIR (e.g vector abundance and sporozoite) alone or in combination [76]. Here, the highest EIRs occurred in villages with semi-permanent/permanent breeding sites (e.g Dangouindougou, Tengrela, Tiefora and Yendere). Local variation in malaria vector biting rates and transmission of the nature described here has been previously associated with environmental factors such as temperature, level of urbanization, rainfall, temperature and altitude [467, 468], housing type, human population density [434, 468] and LLIN coverage and usage [373]. Though, these and other factors likely account for the spatial variation in transmission observed here, with further studies need to elucidate the relative contribute of different factors to EIR.

Vector demography and transmission potential followed distinct seasonal patterns. Parity rates and corresponding estimates of daily survival and life span were considerably higher in the wet than dry season. In principle, parity rates may be lower during the wet than dry season, as the wet season population may be characterized by high numbers of newly emerged (and thus nulliparous) females [469, 470]. For example, a previous study in Burkina Faso [452] reported that parity rates in vectors was somewhat higher in the dry (> 75%) than wet season (~ 67%). Higher parity rates in the dry (~ 87%) than wet (~ 70%) season have also been reported in Benin [344]. However, only a few individuals were dissected for parity rate in this study. Sporozoite rates increased from the late dry season (~2.5%) to a maximum

at late rainy season (~5%; October-November), as has been reported in other west African settings [52, 54]. Similar results showing seasonality in SR were described in *An. gambiae* s.l. population in central [455] and in southwestern Burkina Faso [52, 54] at the end of the rainy season (October - December). The seasonal variation in the PR, SR and HBR gives rise to the characteristic seasonal profile of malaria transmission in west Africa, with the majority of human infections occurring during the wet months of July- October [471, 472].

By comparing vector demographic and transmission traits over 2 years following a mass LLINs distribution, this study aimed to test for possible signals of a rebound or increase in transmission due to either emergence of mosquito behavioural (Chapter 3) or insecticide resistance (Chapter 4). Evidence for this was mixed. Consistent with the hypothesis of intensifying insecticide resistance (Chapter 4), parity rates gradually increased over the study period indicating an increase in their survival. The increase in insecticide resistance over the study period may have enhanced the survival of vectors. However, both the sporozoite rate and human biting rates in *An. gambiae* s.l. populations fell over the study period, culminating a reduction in EIR from 320 to 105 between the first and second year. Similar declines in EIR have been described in other African settings following the introduction of control measures [428, 433, 473-475]. For example, an analysis of data collected over 10 years of successive LLIN deployment in Senegal showed a huge decrease (by > 92%) in the EIR [349]. This fall in vector density and EIR at the same time as insecticide resistance is rising implies that the LLIN distribution is having a sufficient impact on vector populations to reduce transmission and create strong selection for resistance. At present, the negative impact of insecticide resistance may be outweighed by the larger impact of LLINs in reducing vector abundance. However, this trade-off may be altered as vectors develop more diverse and effective resistance strategies. Careful long-term monitoring of vector resistance and transmission traits will be needed to assess this, and ideally identify tipping points in advance.

Based on values of EIR and the proportion of exposure that can could be prevented by consistent use of effective LLINs (Chapter 3), it was estimated that people in these communities would be exposed to between ~3 to ~75 of infected bites per year. EIR is positively correlated with malaria incidence [427] with previous epidemiological analyses indicating that EIR values of 1.5 or higher are sufficient to sustain transmission [476]. Consequently, even with 100% coverage and usage of highly effective LLINs, high levels of transmission are expected to persist in this setting. Extrapolating this observation to country level, this may explain why malaria prevalence has been reported to increase in Burkina Faso between 2016 and 2017 [13]. Clearly the current vector control strategies being carried out by the National Malaria Vector Control Programme are not sufficient to progress control.

While the current study provides useful insights on the stability of malaria transmission after LLIN distribution; the methods used have several limitations. First, the method used to estimate survival from parity rates depends on several crucial assumptions that may be unrealistic or unknown. For example, it was assumed that the *An. gambiae* s.l. population had a fixed gonotrophic cycle length of 2.5 days, and bite only once per cycle. There may have been exceptions to this as gonotrophic cycle also varies with environmental (larval sites) and temperature conditions [46, 477, 478]. Additionally, estimates of the HBI were based only on testing for human or blood meals in *An. gambiae* s.l., with a large proportion of specimens having unidentified blood meals (70 out of 164). It is possible that these *An. gambiae* s.l. populations were also feeding on other domestic animals (e.g. chickens, dogs, goats) as showed elsewhere [306, 479]; and if so failure to test for these host types in blood meal analysis would lead to an overestimation of the HBI. However the high sporozoite rates and malaria incidence within the study area [217, 218] do suggest a relatively high degree of human feeding. Additionally, the ELISA method [273] used here may have underestimated the sporozoite rate and thus EIR as it is considered less sensitive than the PCR method [480, 481]. Thus, further improvement in molecular methods and entomological sampling methods, including more

reliable methods to age grade mosquitoes [482], are needed to improve estimation of EIR , VC and other malaria transmission parameters.

5.5. Conclusions

This study revealed relatively high rates of survival and sporozoite rates within this population of highly insecticide resistant *An. gambiae* s.l. in Burkina Faso. On this basis, people in the study area are expected to be exposed to ~26 to 502 infective bites per person in the absence of LLINs. Accounting for the proportion of transmission that could be theoretically prevented by consistent use of highly effective LLINs (85%), residents are still expected to receive ~25 infective bites per person per year, which is more than enough to sustain transmission. As expected, these mosquito vector demographic and transmission traits showed considerable spatial and seasonal variation; highlighting possible value of temporally and spatially targeted control measures.

Chapter 6: General discussion

6.1 Overview of the principal findings

Despite several mass distribution campaigns of Long-Lasting Insecticide-Treated Nets (LLINs) in Burkina Faso, malaria incidence is still increasing every year making it one of the highest malaria burden countries in Africa [13]. My PhD research was embedded within a larger multidisciplinary collaborative project entitled "Improving the efficacy of malaria prevention in an insecticide resistant Africa (MIRA" funded by the Wellcome Trust. This project consisted of 5 work packages focussed on investigating different factors that may explain the increasing trend of malaria incidence in Burkina Faso. The overall aim was to understand the limited impact of current malaria control strategies in Burkina Faso with a focus on the Cascades region. Here, my role was to assess the relative contribution of entomological factors such as vector ecology, behaviour and insecticide resistance to this problem. This was achieved through conducting an intensive, large-scale and longitudinal surveillance of malaria vectors (*Anopheles gambiae sensu lato* (s.l.) complex). Through this, I addressed the following specific objectives as described in the four data chapters in this thesis (i) evaluation of the performance of a new mosquito sampling method, the Mosquito Electrocuting Trap, for measuring spatial and temporal variation in human exposure to malaria vectors (Chapter 2); and characterization of spatial, seasonal and longer-term trends in (ii) vector abundance and behaviours (Chapter 3), (iii) insecticide resistance within *Anopheles gambiae* s.l. (Chapter 4) and (iv) malaria vector survival and transmission potential (Chapter 5). This research was conducted in the two years following a mass LLIN distribution occurring in the Cascades Region of Burkina Faso (October 2016 - December 2018). Principal findings are briefly summarised below.

6.1.1 Evaluation of mosquito electrocuting traps as a safe alternative to the human landing catch for measuring human exposure to malaria vectors in Burkina Faso.

Malaria vector control requires routine monitoring of vector population to assess intervention efficacy. There is a lack of detailed data on malaria vector biting behaviour in Burkina Faso because gathering this type of information is difficult and not routinely collected. The most commonly used and gold standard approach for measuring mosquito biting activity indoors and outdoors is the Human Landing Catch (HLC). This method involves having volunteers expose themselves to mosquitoes and trying to catch them before they bite. As this procedure involves some risk of exposure to infected mosquitoes it is increasingly prohibited thus there is a need for a safer alternative. Here data collected over 324 nights were used for evaluating the exposure-free "Mosquito Electrocuting Trap (MET)" as an alternative to the HLC in 12 villages in Burkina Faso. Results indicated that the MET collected fewer *An. gambiae* s.l. than the HLC, with the relative sampling efficiency of the MET being higher outdoors than in indoors. Although the MET was less sensitive than the HLC, there was a high correlation of *An. gambiae* s.l. catches between these methods across a range of seasons and mosquito densities. Furthermore, the MET provided a consistent representation of vector species composition, behaviour (e.g. biting location and time) and malaria infection rates relative to the HLC. Thus, although the MET may underestimate the absolute density of malaria vectors compared to the HLC, it does provide a reliable characterization of seasonal and spatial variation in vector biting, ecology and infection rates. Considering the MET's substantial advantage of preventing exposure of collectors, this consistent performance suggests it could be a useful alternative to the HLC.

6.1.2 Spatial and temporal variation in the abundance and behaviour malaria vectors following scaling up of LLINs in rural Burkina Faso

Vector control interventions such as Long-Lasting Insecticide-Treaded Nets (LLINs) and Indoor Residual Spraying (IRS) have been shown to drive changes in malaria vector behaviour in several sub-Saharan African countries [119, 310, 483]. These behavioural changes may allow vectors to reduce their contact with insecticides deployed inside houses; phenomenon defined as "behavioural avoidance". Here, surveillance of vector biting and resting behaviours were carried out over in 12 villages using the gold standard HLC method and resting bucket traps (RBTs). Aims were to assess spatial (between villages) and temporal (seasonal and longer-term) shifts in *An. gambiae* s.l. biting and resting behaviours in the ~2-year period following a mass LLIN distribution. Nearly fifty thousand mosquitoes were collected using HLC (N= 49 482), and 1000 in RBTs (N= 927) over 26 months. The malaria vector group *An. gambiae* s.l. was most abundant in collection (~81% and ~63% of mosquitoes in HLC and RBTs respectively). There was substantial variation in vector abundance between sites and seasons, and evidence of a longer-term decline over the study period (~23% fall from start to end of study). *Anopheles coluzzii* (~ 54%) and *An. gambiae* (~ 45%) were the predominant species within the *An. gambiae* s.l. group. There was also evidence of substantial variation in malaria vector species composition between sites and seasons; and a longer-term shift with the proportion of *An. coluzzii* relative to *An. gambiae* declining over the study period. A higher proportion of outdoor biting (~54%) was detected than expected based on previous studies, but there was no evidence of spatial, seasonal or longer-term changes in exophagy over the collection period. Malaria vectors had a similar pattern of biting time in outdoor and indoor environments with most activity occurring late at night during hours when residents were indoors (between midnight to 4 am). Analysis of the subset of *An. gambiae* s.l. identified to species level suggests that the peak biting time of *An. coluzzii* is one hour earlier than *An. gambiae,* and that *An. gambiae* is slightly more likely to bite outdoors than *An. coluzzii* (~55% vs 51%). There was some evidence of seasonal variation in malaria vector resting behaviour; with a

higher proportion of *An. gambiae* s.l. resting inside houses in the dry compared to wet season. Considering human and mosquito behaviour, I estimated that ~85% of exposure to malaria vectors could be preventable by use of effective LLINs during typical sleeping hours (10 pm – 5 am). Overall, the proportion predicted to be preventable by LLIN use appeared to decline by 10% over the study.

6.1.3 Spatial and temporal variation in insecticide resistance within *Anopheles gambiae sensu lato* (s.l.) populations following scaling up of LLINs in rural Burkina Faso

Insecticide resistance (IR) has been implicated as main entomological factor responsible for the reducing impact of malaria interventions such as LLINs and Indoor Residual Spraying [329, 484]. To understand the relative contribution of IR to the failure of LLINs in Burkina Faso, I measured the magnitude and rate of increase in resistance to deltamethrin, the known insecticide used in the LLINs distributed in 2016, in nine *An. gambiae* s.l. populations in the Cascades region of Burkina Faso. According to the criteria set in the WHO guidelines, all the surveyed populations were confirmed to be highly resistant to deltamethrin. There was evidence of some variation in the lethality of deltamethrin between vector populations and seasons (resistance appears to be higher in dry that wet season). In addition, IR increased over the study period and was generally higher than reported in previous studies from the same area. There was no evidence of variation in IR between *An. gambiae* and *An. coluzzii*.

6.1.4 Spatial and seasonal variation in malaria vector survival and transmission following scaling up of LLINs in rural Burkina Faso.

Results presented in Chapter 3 and 4 confirmed a high rate of outdoor biting and intense IR within malaria vector populations in the Cascades region in Burkina Faso. In Chapter 5, I explored the potential epidemiological consequences of these traits by assessing the transmission potential of local vector populations. This was done through measurement of key predictors of vectorial capacity (vector survival) and human exposure (SR = sporozoite

rates in vectors, EIR = entomological inoculation rates). Based on assessment of mosquito parity rates, malaria vectors in this area were estimated to have a high daily survival rate (> 90%), with some variation between villages and seasons (higher in dry than wet season). Overall after controlling for this spatial and seasonal variation, there was evidence of a longer-term increase in vector survival rate over the study period. Overall, ~4% of host seeking *An. gambiae* s.l. were infected with malaria sporozoites, but with variation between villages from a high of ~7 % to < 2%. In addition, the SR declined from ~5% to ~2% over the course of the study. The annual EIR in the study area was estimated to be ~320 infective bites per person per year in the first year, compared to ~105 in the second. This reduction in EIR between years is due to longer-term decline in vector abundance and sporozoites rates over the study period. Using estimates of derived in Chapter 3, these values of EIR were used to predict the number of bites expected after adjusting for the proportion of exposure that could be prevented by using effective LLINs (~85%). Even though most bites could be prevented by LLINs, the remainder equates to ~48 and ~16 infective bites per person per year in the first and second year respectively. Finally, analysis of mosquito blood meals indicated that *An. gambiae* s.l. in this area take a smaller proportion of blood meals from humans (65%) than expected based on previous description of these species being highly anthropophilic [57].

6.2 Implication of the findings

6.2.1 Potential suitability of the Mosquito Electrocuting Trap

An effective malaria control programme requires routine vector surveillance. Several methods have been proposed for estimating human exposure to malaria vector bites, with the HLC remaining the gold standard. Given the risk involved with this method, there is an urgent need to find a safer yet similarly reliable alternative. In considering alternative approaches, it is not necessarily a requirement for a new method to capture a similar number or more malaria vectors than the HLC. Some reduction in relative numerical performance may be acceptable as long as catches with alternative methods are consistent with the HLC across space and time. Recently, the MET was

developed and demonstrated to be a promising alternative to the HLC in Tanzania. Here, I evaluated the MET for first time in west Africa in a study that also allowed me to asses local and seasonal variation in trap performance. I found the MET has lower but consistent sensitivity with the HLC. However, overall the MET had lower relative performance in Burkina Faso compared to that reported in recent studies in Tanzania [240]. In addition, I also found some evidence of local variation in MET performance within study sites in Burkina Faso. These two points highlight the need for robust trap evaluation in different epidemiological and ecological settings (e.g. west versus east Africa) to get a sense of generalizability and value of method. In addition, there is need for wider scale and long-term evaluation to consolidate the evidence base on when and where new methods will be of value. Results presented here will fit in with wider evidence being collected on use of METs for malaria and dengue vectors [485].

6.2.2 Understanding malaria control in Burkina Faso

The current work characterised the malaria vector population in the Cascades region of Burkina Faso as being highly resistant to insecticides (Chapter 4), with a higher than expected proportion of outdoor biting (> 50%, Chapter 3). However, there was evidence of a reduction in malaria transmission (EIR) over the 2-years following a mass LLIN distribution; indicating this intervention may still be having a substantial impact on control. However, as this was observational study with no control sites (e.g. areas without LLINs), I cannot rule that other factors such environmental changes may have been responsible for this decline.

Even though LLINs may still be partially effective in this area, results collected here indicate there is high, ongoing transmission in this area. The persistence of transmission is likely reinforced by behavioural and physiological resistance in vectors that allows them to both minimize contact with LLINs, and their lethality. A cohort study carried out at the same time as my entomological surveillance indicated that the overall malaria incidence was ~53% in children (5 - 15 years old) in the study area [218]. This

confirms the predictions from entomological results that people in this study area are exposed to high ongoing transmission. Here, based on detailed studies of mosquito behaviour, and community surveys of resident's sleeping behaviour; I estimated that ~85% of total exposure to malaria vectors could be prevented if people consistently used high quality and effective LLINs throughout standard sleeping hours of 10 pm – 5 am. However, there are several reasons why this estimate represents the "best case scenario". First, a health anthropology study carried out within the MIRA found that there was substantial variation in people's sleeping hours throughout the study area according to their age and gender [378]. Additionally, there was pronounced temporal variation in the amount of time people spend outdoors during the evening in response to seasonal activities and events [378]. Thus, certain individuals may receive substantially less protection from LLINs, at different times of year. Additionally, my estimates assume that people are fully protected by LLINs throughout sleeping hours. This assumes that LLINs are completely intact, consistently used, and correctly fitted to beds. Several studies from different part of Africa (e.g. central [486] east [124] and west [377]) have shown that LLIN durability decreases with time. Assessing the net integrity in the study area during the same period, [218] found that only 63% of the LLINs were in good quality and that 23% were torn by end 2018 (~17 months following distribution). Furthermore, a study in the same region found that only ~13% of the LLINs are still in good condition 3 years after deployment [487]. Therefore, the proportion of transmission that can be prevented by LLINs is probably lower than estimated here when accounting for these additional sources of inter-individual and temporal changes. As even the "best case" scenario of prevention estimated here indicates a substantial amount of transmission will persist even with full LLIN coverage. Therefore, it is not surprising that the malaria burden in this area continues to be so high.

The persistence of high ongoing malaria transmission in the Cascades region highlights the urgent need for supplementary control methods to effectively tackle malaria in Burkina Faso and other high burden African countries. Both IR and outdoor biting are likely contributing to ongoing transmission; with

modelling work suggesting that IR may have a bigger role [484]. Consequently, an important part of the solution may be to switch the chemicals used on LLINs to those that mosquitoes have no resistance. This could include switching from pyrethroid-only LLINs to those using a combination of different chemical (two different insecticides or a non-insecticide plus an insecticide) on the same net, known as "next generation nets" [89], or to different net designs that allow other classes of chemicals to be safely used on them (e.g. barrier nets [488] to mitigate the impact of the resistance. For example a country could use Pyrethroid-PBO nets (Pyrethroid and the non-insecticide: Piperonyl Butoxide) nets, or Interceptor G2 (a pyrethroid plus different class of insecticide= chlorfenapyr, [89]) that have recently shown good performance in experimental huts trials compared to pyrethroid-only nets in west Africa [489-492]. Fortunately in the most recent mass LLIN distribution in Burkina Faso (2019), the National Malaria Control Programme distributed some Interceptor G2 and pyrethroid-PBO nets in addition to the standard pyrethroid nets (Permanet® 2.0); with allocation based on resistance mechanisms [493]. Results obtained here also suggest incorporating tools that target outdoor biting and resting mosquitoes will be an important component of an enhanced control strategy. This could include novel methods such as attractive target sugar baits [149, 150, 443, 494], spatial repellents [82, 495] and genetically-modified mosquitoes [496-498], as well as well-established methods such as larviciding [141-143, 499, 500]. However, given that the bulk of transmission still occurs inside houses, there is also a need to find alternative insecticides that can be used to tackle highly pyrethroid-resistant mosquitoes inside houses. Animals such as cows are often used in farming by residents in the study area and kept within household compounds (outside but next to house) at night. Here, I found a relatively high degree of cattle feeding in vector populations (~ 48% of blood meals). Thus, it is possible that integrated vector control strategies including targeting cattle (e.g. through application of insecticides or endectocides) could also be effective [501, 502]. Finally, methods that do not rely either on vector behaviour or susceptibility to insecticides (e.g. genetically modified mosquitoes, larval control) could be useful in this setting.

6.3 Limitations in the study

This study provides detailed insights into spatial and temporal variation in malaria vector ecology, behaviour and insecticide resistance in the Cascades region. However as detailed in the previous chapters, it also has several important limitations that should be taken into account when considering implications. Here I comment on a few additional limitations that apply to the work as a whole. First, I considered the potential contribution of different entomological factors (e.g. mosquito behaviour, resistance) to transmission independently; and did not investigate potential interactions between them. For example, one factor such as IR may also be correlated with changes in vector behaviour. Therefore, considering these traits separately may not be informative of their combined epidemiological impact. Additionally, I interpreted long-term changes in mosquito behaviour and IR across the study area as evidence of adaptation to vector control. However, no corresponding investigation of mosquito genetics was conducted to assess whether these changes were due to selection (e.g. change in frequency of genes associated with traits) or phenotypic plasticity. It has been shown that some epidemiologically relevant malaria vector behaviours have a genetic basis. For example, there is evidence that differences in host choice of *An. arabiensis* is associated with chromosomal inversions however, no genetic difference between indoor or outdoor resting and biting behaviours was found [63, 205]. Additionally, there are many known genetic markers of insecticide resistance (reviewed in Chapter 1), but I did not measure these in the current study. Confirming whether the changes in vector behaviour and IR described here are due to evolution or plasticity would help understand the potential consequences of changing control strategies (e.g. if vector populations would return to susceptibility, and how quickly).

Another limitation is that changes in vector populations were interpreted as being related to LLIN distribution; however, there was no "temporal" (e.g. data from before the recent LLIN distribution) or "spatial" (areas without LLINs) controls in the study design. Thus, although it is reasonable to

hypothesize the patterns of vector behaviour, resistant and transmission potential described here may have been influenced by the LLIN distribution, I cannot rule out the possibility of additional impacts from environmental or other changes that co-occurred with the LLIN distribution.

A final limitation is the accuracy and reliability of the bioassays used to measure mosquito behaviour and insecticide resistance. For example, the bioassays used to measure IR here only assessed short-term mortality after insecticide exposure (e.g. mortality in 24 hours following exposure); with mosquitoes being exposed for sixty minutes. This method may not be ideal as under natural conditions where mosquitoes may only be in contact with LLINs for ~3 minutes [503] during feeding, and thus receive a much smaller dose of insecticide. Further it is known that even mosquito vectors classified as "highly resistant" may experience delayed mortality (e.g. evident 24 hours after exposure [393]) which could help maintain LLIN effectiveness. Thus, it may be worth considering these effects when assessing the impact of IR (from bioassays) on LLIN efficacy.

6.4 Further work

The key entomological factors measured during this PhD programme could help understand why malaria is still having high burden in Burkina Faso. However, it will be important to assess how these estimates interact with each other and clinical data. As a follow up to this PhD, I plan on conducting further analysis to test for associations between mosquito population-level traits (e.g. insecticide resistance, outdoor biting) and transmission potential (VC, EIR). Additional analysis will also include assessment of interactions between entomological data and malaria incidence that were collected during the same period as part of the same MIRA project. Furthermore, as part of a new project, data collected here will be used to assess whether there is any genetic basis for the resistance traits between vector populations from different sites, locations (indoor versus outdoor) or through time. There is also a possibility that these data will be used to test for genetic variation between indoor and outdoor host seeking and resting

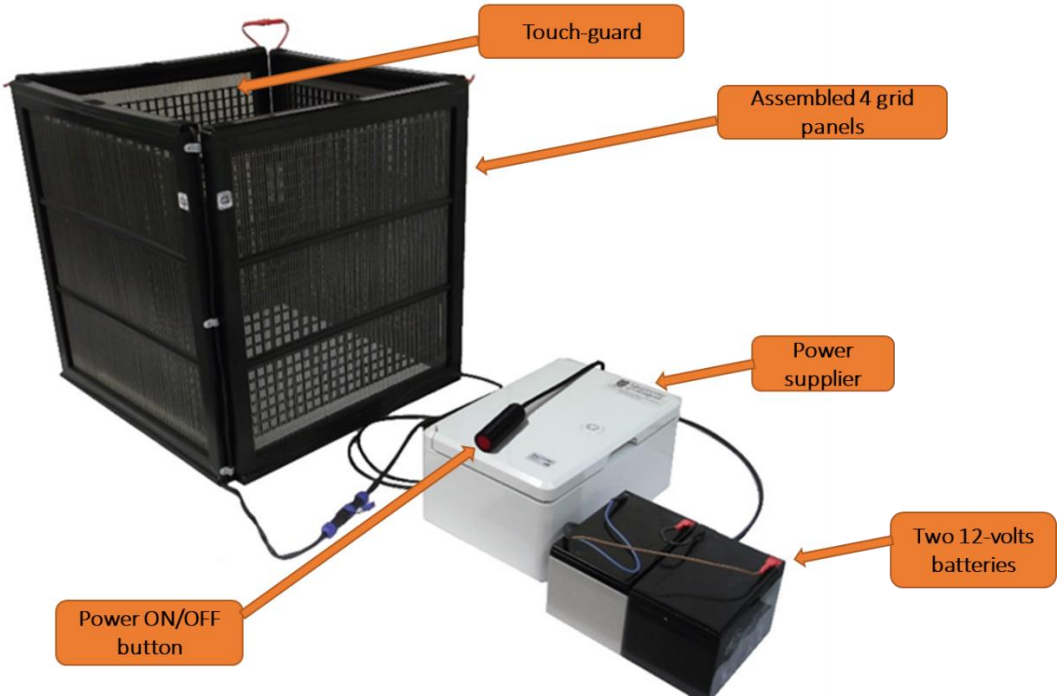
populations. I would also like to continue longer-term surveillance of vector populations in this area to confirm how vectors are responding to different LLIN types distributed in the most recent distribution (2019), and their impact on malaria transmission.

6.5 Conclusions

LLINs and IRS in addition to the deployment of the artemisinin based-combination therapies have made great contributions by reducing malaria burden in many countries. However, Burkina Faso and 10 other countries are showing an increase in malaria burden and contributing to the stalling of malaria control since 2015. Taking the case of Burkina Faso as a representative example, results obtained here suggest that both IR and outdoor biting by malaria vectors are contributing to the persistence of transmission in high burden African countries. Consequently, a successful vector control programme in this context need a clear insecticide resistance management plan and supplementary tools targeting vector outdoors blood-feeding activities.

7. Appendices

7.1. Appendix 1: Assembled Mosquito Electrocuting trap used for mosquito collections, connected to the power supplier and the 12-volt batteries.



7.2. Appendix 2: Information on the Mosquito Electrocuting Trap

Each Mosquito Electrical trap is made of four frames, size 30cm by 30 cm

Each frame is made of PVC with 1.5mm holes in 5mm pitch on the top and bottom of frames.

Each electric grid is mounted within PVC frame, grids are manufacture such each wire (Stainless steel size 1.2 mm diameter) are separated from each other by 5mmm and fitted to PVC frames through the drilled holes. Adjacent wires are not connected to each other but every odd numbers are connected permanently, this is similar to even ones, this is done by welding a horizontal rod to each odd wire on the top and similar on the bottom of frames for even ones.

Grids have two spacers in the middle of the frames, again drilling hole for each wire and inserting wires to spacers, this technique ensures that no adjacent wires will touch each other, if do touch, it will cause short circuit which could cause damage to power control, although there is a short circuit protection built into power control.

The Grids shield must be installed before traps are connected to power supply.

The Electric Mosquito Electrocuting Trap (MET) is connected to Variable Power Supply Unit (VPSU) which is a DC to DC high voltage converter.

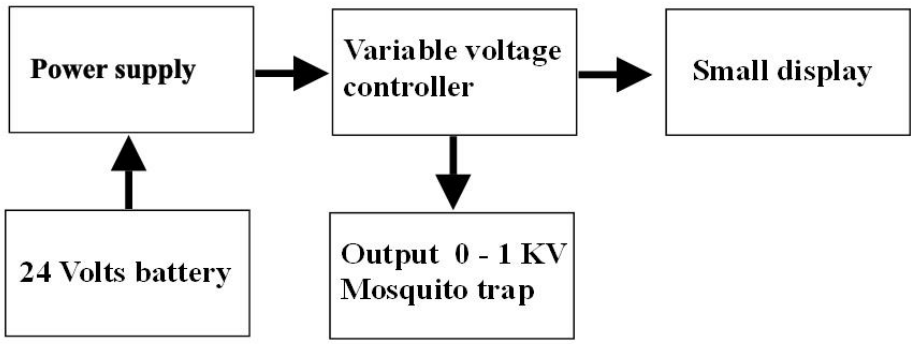
Variable power supply for Mosquitoes Grids

This variable power supply unit (VPSU)is tailored for demanding applications in Mosquitoes traps. It is very clean, quiet signal, low output ripple and solid-state polarity switching in voltages up to 1 kV.

It is a small package and has following features:

- Up to 1 kV output
- Easily modified for adjustable output (Programmable output voltage)

- Solid state polarity switching
- Short circuit protected (small circuit at the output of power supply unit is fitted to act as short circuit protection
- Input protected against polarity reversal
- High output stability
- Output fly lead is RG58
- Operating temperature 0℃ to +50℃
- Maximum relative humidity 80% for temperature up to 31 ℃, decreasing linearly to 50% relative humidity at 40℃



High Variable & regulated power supply

The power supply is supplied from a current limited supply providing 24 Volts dc from 2 batteries each of 12 Volts connected in series to give 24 Volts with capacity of 11Amp per hour.

Variable voltage programming is a small circuit inside a small box with external potentiometer setting to adjust the output voltage from 0 volts to maximum of 1 KV with output current capability of 10 mA.

Small display indicates the selected output voltage to the traps.

The entire system is fitted in waterproof box (IP65,66) for field work.

Special safety:

Care should be taken in the installation of this device.

- The power supply unit has no user serviceable parts and should not be dismantled

- This unit should not be handled or touched when the supply is connected. After disconnection from the supply (batteries), allow 30 seconds before handling so that all the charged components can be discharge.

- Grids are shield from human touch, guards for traps (grids) needs to be installed at all time.

8. References

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Evaluation of mosquito electrocuting traps as a safe alternative to the human landing catch for measuring human exposure to malaria vectors in Burkina Faso

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Abstract

Background: Measuring human exposure to mosquito bites is a crucial component of vector-borne disease surveillance. For malaria vectors, the human landing catch (HLC) remains the gold standard for direct estimation of exposure. This method, however, is controversial since participants risk exposure to potentially infected mosquito bites. Recently an exposure-free mosquito electrocuting trap (MET) was developed to provide a safer alternative to the HLC. Early prototypes of the MET performed well in Tanzania but have yet to be tested in West Africa, where malaria vector species composition, ecology and behaviour are different. The performance of the MET relative to HLC for characterizing mosquito vector population dynamics and biting behaviour in Burkina Faso was evaluated.

Methods: A longitudinal study was initiated within 12 villages in Burkina Faso in October 2016. Host-seeking mosquitoes were sampled monthly using HLC and MET collections over 14 months. Collections were made at 4 households on each night, with METs deployed inside and outside at 2 houses, and HLC inside and outside at another two. Malaria vector abundance, species composition, sporozoite rate and location of biting (indoor *versus* outdoor) were recorded.

Results: In total, 41,800 mosquitoes were collected over 324 sampling nights, with the major malaria vector being *Anopheles gambiae* sensu lato (s.l.) complex. Overall the MET caught fewer *An. gambiae* s.l. than the HLC (mean predicted number of 0.78 *versus* 1.82 indoors, and 1.05 *versus* 2.04 outdoors). However, MET collections gave a consistent representation of seasonal dynamics in vector populations, species composition, biting behaviour (location and time) and malaria infection rates relative to HLC. As the relative performance of the MET was somewhat higher in outdoor *versus* indoor settings, this trapping method slightly underestimated the proportion of bites preventable by LLINs compared to the HLC (MET = 82.08%; HLC = 87.19%).

Conclusions: The MET collected proportionately fewer mosquitoes than the HLC. However, estimates of *An. gambiae* s.l. density in METs were highly correlated with HLC. Thus, although less sensitive, the MET is a safer alternative than the HLC. Its use is recommended particularly for sampling vectors in outdoor environments where it is most sensitive.

Keywords: Mosquito electrocuting trap, Human landing trap, Malaria, *An. gambiae*, Host-seeking behaviour, Outdoor biting

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Background

Measurement of malaria transmission and evaluation of vector control requires estimation of human exposure to malaria-infected mosquitoes [1]. This exposure is often estimated in terms of the Entomological Inoculation Rate (EIR [2]) defined as the mean number of malaria-infected mosquito bites a person would be expected to receive in a given setting [1, 3]. Accurate estimation of exposure to mosquito bites is crucial for evaluating interventions, thus there is an urgent need for reliable and robust methods to give unbiased estimates of exposure in a range of settings [3]. Several methods have been used to measure mosquito host-seeking behaviour and human exposure to mosquitoes. Historically, the human landing catch (HLC) has been the most commonly used method for African malaria vectors and is considered a gold standard approach for direct measurement of human-mosquito contact in both indoors and outdoors settings [4]. In this method, human volunteers expose part of their body, usually the lower legs, to lure host-seeking mosquitoes that are then collected upon landing [4].

Although HLC provides a direct measurement of human exposure to bites, its estimates can be biased due to variation in the skill of mosquito collectors and their attractiveness to mosquitoes [5–8]. HLC also raise ethical concerns as collectors are exposed to potentially infectious mosquito bites [9]. While this risk can be minimized by providing malaria prophylaxis to collectors, protection cannot be guaranteed in areas of drug resistance or where mosquitoes are carrying other pathogens, such as arboviruses [10, 11]. One African study indicated that HLC participants had no increased risk of malaria [12], but there remains a concerns about disease exposure in areas where other mosquito-borne pathogens are circulating.

Due to these limitations of the HLC, a range of alternative "exposure-free" methods have been developed. Most common is the CDC light trap [4, 13–15], a trap that can be placed next to a person sleeping under a bed-net and used to collect mosquitoes that would have otherwise have fed on them [14]. Although effective and easy to use in indoor environments [16], this method is harder to implement outdoors and may not accurately reflect human exposure in this setting [16–18]. Furthermore, CDC light catches can be affected by variation in the trap-light intensity [19, 20] and colour [16]. Other "exposure-free" methods include the human-baited double net trap (HDN) [18], Suna Trap [21], Host Decoy Trap (HDT; [22]), Ifakara tent trap design C (ITT-C) [23] and the Mbita trap [11]. Of these the last two have the same limitation as the CDC light trap of not being suitable or representative for measuring exposure in outdoor environments. For example, the tent trap only samples mosquitoes that are capable of entering a small enclosed structure, therefore, disproportionately catches indoor biting mosquito species [24]. The HDN was as efficient as the HLC in collecting outdoor anthropophilic mosquito. However, like the Tent Trap, it may also be selectively biased towards indoor biting mosquitoes, or sample vectors that enter the net to rest instead of biting [18, 25]. Similarly the Mbita trap had poor performance relative to the HLC in a setting where most vectors were exophilic and zoophilic [26]. Both the SUNA and Host Decoy Trap have shown promise for sampling outdoor biting malaria vectors [21, 22]; although may under [27] or overestimate [22] human exposure relative to the HLC. Given the growing recognition of outdoor biting as a major source of residual transmission in Africa [28–30] there is a clear need for improved methods that can reliably and safely measure exposure outside of homes.

The mosquito electrocuting trap (MET) has been developed as a representative and safer alternative method to the HLC for measuring human exposure to mosquito vectors both indoors and outdoors [17, 31, 32]. As previously described [31], the MET builds on previous work using electrified nets and grids to trap flies [33, 34] and mosquitoes [35–39] attracted to hosts or their odours. This trap consists of four panels that can be assembled into a box around the lower legs of seated human [17, 31] (Additional file 1: Figure S1), or an entire host (human or cow) [32]. Each panel consists of an electrified surface that allows free air movement and is safe to use in close proximity to a human volunteer, and intercepts and kills mosquitoes just before they land on hosts. An advantage of this method is that in addition to protecting participants from mosquito bites, it can be used in a standardized way in both indoor and outdoor environments. This method has shown promise as alternative to the HLC for sampling malaria vectors in Tanzania [17, 31, 32]. For instance, the first prototype achieved a sampling efficiency of ~ 60% relative to the HLC for sampling *Anopheles arabiensis* outdoors in rural Tanzania, falling to 20% when used indoors [31]. Further study on an improved prototype carried out in an urban area indicated the MET had a similar performance to the HLC [17]. A recent study evaluated a further prototype of the MET in which the electrified trapping panels were expanded to encompass the whole body of a human volunteer or calf [32], with the performance of the MET exceeding that of the HLC. The MET has not been tested yet outside Tanzania thus its effectiveness in different ecological settings is unknown. There is a need to evaluate the MET in west African settings where vector species composition, ecology and biting behaviour is often markedly different from East Africa and to see how its performance varies between sites and seasons.

This study aimed to evaluate the performance of the MET relative to the HLC in a longitudinal study in south-western Burkina Faso. Sampling was conducted over a 14-month period in 12 villages, where malaria vector abundance and species composition are known to vary considerably between seasons and sites (unpublished data). The aims were to test the performance of the MET relative to the HLC for estimating vector abundance, and location of biting (indoor vs outdoor): (i) over the study period, (ii) over the course of the night, and iii) in relation to mosquito density. Additional aims were to compare estimates of mosquito vector species composition and infection rates between HLC and MET collections and assess if they produce comparable estimates of exposure to *Anopheles gambiae* sensu lato (s.l.), based on human behaviour.

Methods

Study site

This study took place in 12 villages within the Cascades Region of south-western Burkina Faso (Fig. 1), where mosquito sampling was conducted over 14 months between October 2016 and December 2017. Residents of these villages live within compounds consisting of one or more households. Most residents are subsistence farmers whose primary crops are cereals, vegetables, rice and cotton. Domestic animals including dogs, cattle, sheep, goats, pigs, donkey and poultry are usually kept within compounds. The area has two distinct seasons: a rainy season (May to October) and a dry season (from November to April) [40, 41]. Annual rainfall in the area ranges from 600 to 900 mm, with a mean temperature of 26.78 °C (range: 15.7–38.84 °C) and mean humidity of 61.89% (range: 15.11–99.95%) during the study period. *Anopheles gambiae* s.l. is the most abundant malaria (> 90%) vector in this area [42, 43].

Trapping methods

Mosquitoes were collected using HLCs [44] and METs [31]. The MET used was an improved prototype of the version used previously [17, 31]. In brief, it consists of four 50 cm × 50 cm grid panels that can be assembled into a square with the bottom and top open. Panels are

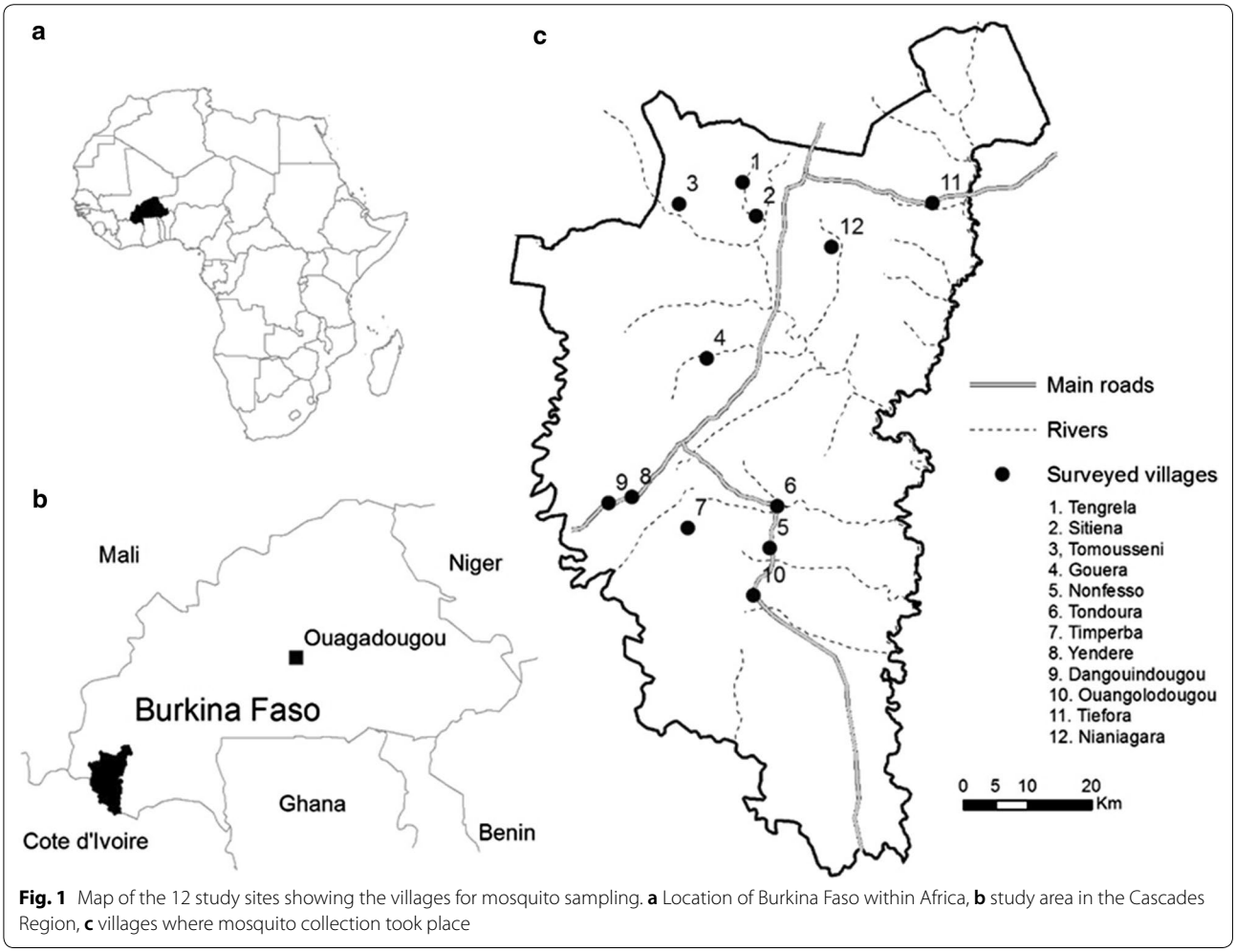

Fig. 1 Map of the 12 study sites showing the villages for mosquito sampling. **a** Location of Burkina Faso within Africa, **b** study area in the Cascades Region, **c** villages where mosquito collection took place

made from polyvinyl chloride (PVC) frames. Stainless steel wires (1.2 mm thick) were embedded to run from the top to bottom of each frame at a spacing of 5 mm. Adjacent wires were differentially charged as negative or positive, such that an insect would be shocked on contact with both. The assembled grid panels were connected to a power supply sourced by two 12-V batteries in series (Additional file 1: Figure S1). A protective shield made from PVC was fitted into the interior side of each panel to prevent any accidental contact between users and the electrified surface.

Experimental design

Across the study period (Oct 2016–2017), adult mosquitoes were collected twice a month in each of the 12 villages with the occasional breaks for holidays and team training. Additionally, only one night of sampling was conducted in each village during the first month. This resulted in mosquitoes being sampled from 4 households at each village for approximately 14 months. The same group of four households was sampled on 2 nights each month; with a different group of households being selected the following month to maximize the spatial coverage of sampling within villages. There was a minimum distance of 30 m between houses sampled on the same night. This culminated in a total of 672 households being sampled over 14 months. Collections were made both inside houses and, in the peri-domestic area (within 8–10 m of the house). Indoor collections were usually conducted in the sitting rooms of houses or in single-room houses.

Mosquito collection

On each night, host-seeking mosquitoes were collected using the HLC and MET. On the first night of sampling during each 2-day period, two houses were randomly allocated for collections with HLC and two others with METs. On the second night, these methods were rotated between households in a cross-over design. Participants involved in mosquito collections also rotated between indoor and outdoor trapping stations each hour to avoid confounding location with individual differences in attractiveness to mosquitoes.

When collecting mosquitoes by HLC, the volunteers sat on a chair with their legs exposed up to the knees. Mosquitoes landing on their legs were sucked into pre-labelled papers cups using a mouth aspirator and a torch (Fig. 2a). For MET sampling volunteers sat on a chair with their legs up to their knees placed inside the trap (Fig. 2b, c), while the remaining part of their body was protected from mosquito bites using protective clothing (first 6 months, Fig. 2b) or a netting screen (from April 2017, Fig. 2c). The METs were placed on top of a plastic mat, which was covered with a white cloth to make it easier to see electrocuted mosquitoes that fell off the trap and onto the ground.

Each night, the HLC and MET collections were run from 7 p.m. to 6 am, with participants conducting trapping for 45 min of each hour followed by a 15-min rest break. During the break period, the MET was switched off and technicians collected mosquitoes trapped on the outer surface and those that had fallen on the white cloth using tweezers. All mosquitoes collected using METs were stored in pre-labelled Petri dishes while those collected by HLC were transferred into paper cups labelled to identify the household and trapping location (indoors or outside, trap type and collection hour).

Overall mosquitoes were sampled on 324 nights in the 14 months of data collection, culminating in a total of 1296 HLC. According to the experimental design, a similar number of HLC and MET collections should have been performed. However, due to problems with the functioning of METs and rainfall on some nights (battery problems and short circuiting) only 1080 MET collections were conducted (outdoor = 531, indoor = 549).

Mosquito processing

Cups containing mosquitoes collected by HLC were placed into a cool box. Cotton pads soaked in a 10% sugar solution were placed on top of collection cups to feed any survivors and transferred to the laboratory. Once in the laboratory, mosquitoes were killed by putting them in a freezer, then sorted to species complex level using morphological keys [45] and stored in labelled 1.5 mL Eppendorf tubes containing silica gel. A subsample of 3199 females (36.3% of total), morphologically identified as *An. gambiae* s.l., were selected to provide a representative sample from each month, village, trapping location (indoor vs outdoor) and method (HLC, MET). The subsampling strategy was guided by consideration of the minimum sample size likely to be required to detect malaria infection in one unique mosquito collection (e.g. permutation of night, trapping method and location). Based on previous data for the study area, this was estimated as a subsample of 40 individuals. Further explanation of the rationale and strategy for this subsampling are provided in the Additional file 2: Additional information S1. Legs from individual mosquitoes from this subsample were analysed by PCR analysis to confirm their species following [46]. Likewise the head and thorax of the same specimens were tested for *Plasmodium falciparum* sporozoite infection using Enzyme-Linked Immuno-Sorbent Assay (ELISA) [47].

Environmental data collection

During the mosquito collection, temperature (°C) and humidity (%) were recorded using Tiny Tag data loggers

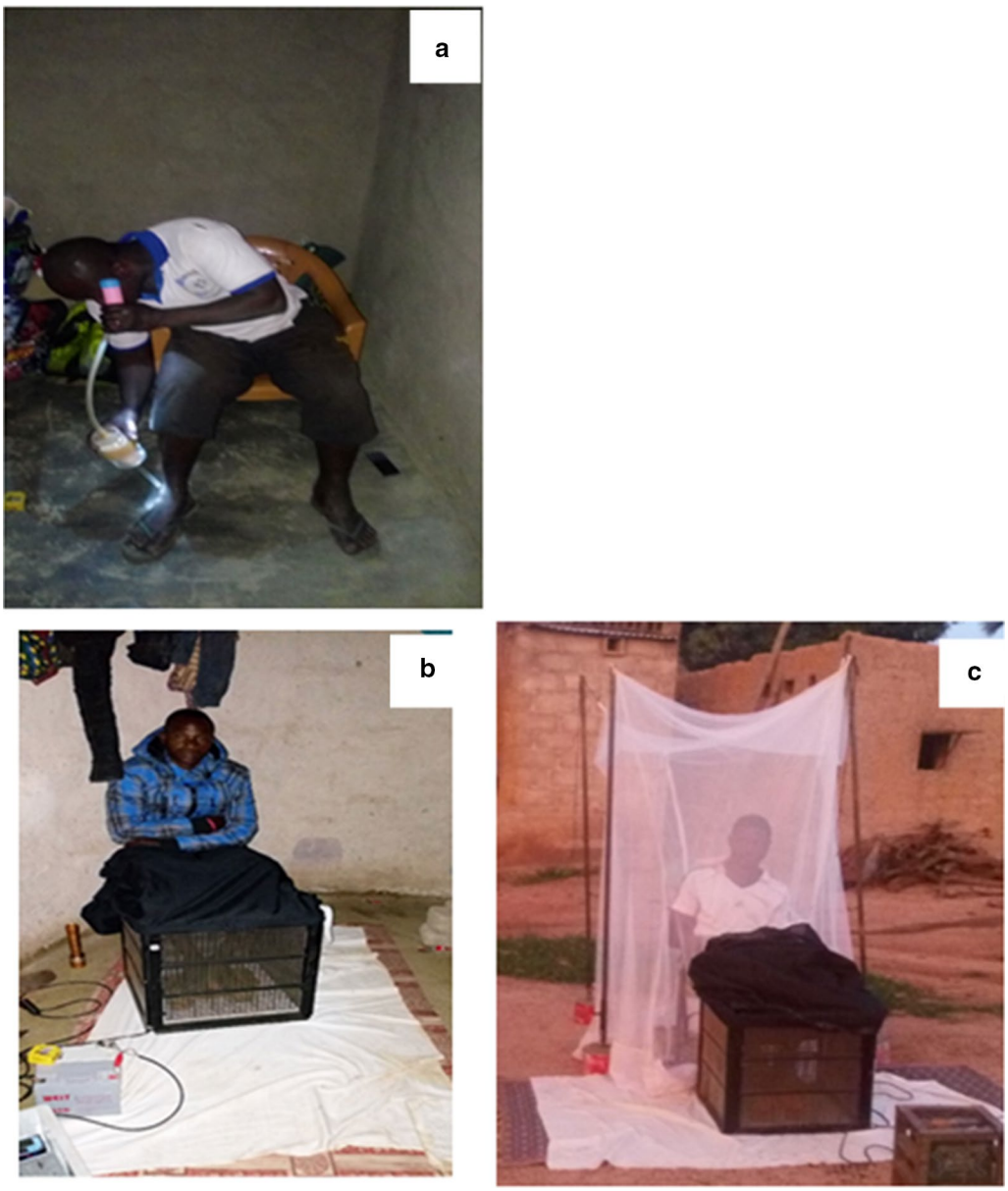

Fig. 2 **a** A volunteer collecting mosquitoes landed on his leg using the human landing catch (HLC) method. **b**, **c** Volunteers using mosquito electrocuting traps (METs)

(Tiny Tag application Explorer 4.9) at each trapping location. Additionally, the time at which residents form the houses where the sampling is taking place go to and get out of their houses were also recorded alongside the mosquito collection.

Statistical analysis

Analysis was conducted to test for: (i) variation in mosquito abundance between traps (per night, per hour and across the study period), (ii) density dependence in the performance of the MET relative to the HLC (iii) variation in malaria vector species composition between trapping methods (defined by the proportion of *Anopheles coluzzi* within the *An. gambiae* complex), and (iv) variation in *An. gambiae* s.l. sporozoite infection rate between traps. Additionally, (v) estimates of hourly and location-dependent (indoor vs out) produced were used to calculate and compare three key metrics of human exposure to bites generated from different trapping methods as described below [48–50]. Generalised Linear Mixed

Effect Models (GLMMs) were constructed within R statistical software version 3.5.0 (2018-04-23) [51] augmented with the lme4 packages for statistical analysis [52] except for the analysis on density dependence and the variation in trap performance across the study period.

The relative efficiency of the MET compared to the HLC was assessed in terms of the number of *An. gambiae* s.l. caught per night. Mosquito abundance data were highly over-dispersed so they were modelled using a negative binomial distribution [53]. Initially, trapping method and its interaction with village and trap location were included in the maximum model of *An. gambiae* s.l. abundance along with other covariates (Model 1, Additional file 3: Table S1) to allow testing of whether trap performance varied between sites and trap location.

The variation of the relative efficiency of MET to HLC in predicting *An. gambiae* s.l. throughout the collection period was assessed separately for outdoor and indoor collection using Generalized Additive Models (GAM) with a negative binomial distribution [54]. This package allowed estimation of the nonparametric function by using a smoothing spline on week. In the full model, the response variable consists of the number of *An. gambiae* s.l. caught per night whilst the explanatory fixed effect variables were method and its interaction with the smoothing splines. To assess whether the interaction was significant in each location (indoor and outdoor), the model with interactions was compared to the basic model without interaction using the Akaike Information Criteria (AIC). Here, no random effect was included as only variation in the seasonal variation of *An. gambiae* s.l. abundance was of interest.

In addition, to test whether the relative performance of the MET compared to HLC changed over the course of night, a model was constructed with the response variable of the proportion of *An. gambiae* s.l. caught in METs in each hour of sampling out of the total in MET and HLC combined (Model 2, Additional file 3: Table S1). Here sampling "hour" was defined as a continuous variable where 1 corresponded to the first hour of collection (7 p.m. to 8 p.m.) and 11 being the last hour (5am to 6am).

Density dependence in MET performance was assessed by testing for linearity between *An. gambiae* s.l. catches in the MET and HLC following the method described in [17] using Markov Chain Monte Carlo (MCMC) in the programme Jags [55, 56]. Here the response variable was the number of *An. gambiae* s.l. collected using the MET and the explanatory variable the number collected using HLC.

Further statistical analyses relating to *P. falciparum* sporozoite rate were performed on the same subset of *An. gambiae* s.l. (n = 3199) that were individually identified to species level. In the analysis related to species composition the response variable was the proportion of *An. coluzzi* in the *An. gambiae* s.l. complex per night with explanatory variables for trapping method, location, temperature and humidity (Model 3, Additional file 3: Table S1). A similar model was constructed to analyse variation in the sporozoite rate of *An. gambiae* s.l. with the explanatory variables being mosquito species, trapping method, interaction between species and location, village, temperature and humidity (Model 4, Additional file 3: Table S1). It was not possible to include analysis of seasonality in these models because of sample sizes of mosquitoes in the dry season at some of the villages. Both data on % *An. coluzzi* and infection rate were modelled using a binomial distribution.

Finally, data on the time and location of biting (indoors vs outside houses) were used to estimate three standard epidemiological parameters of relevance for estimating human exposure to mosquito bites and the impact of Long-Lasting Insecticide-Treated Nets (LLINs) [50, 57]. These are defined as the (i) proportion of *An. gambiae* s.l. host-seeking indoors (P_i), (ii) proportion of mosquito bites occurring when most people are inside (time spent inside estimated based on observations, Additional file 4: Figure S2) their dwellings and likely asleep (P_{fl}) and (iii) proportion of human exposure to *An. gambiae* s.l. bites occurring indoors π_i). The π_i metric estimates the proportion of exposure to malaria transmission that occurs indoors and could be prevented using LLINs [50, 57]. These proportions were used as response variables in analyses that tested whether these exposure estimates varied between trapping methods and in response to season, temperature and humidity (Model 5–7, Additional file 3: Table S1).

In all the analysis, random effects were incorporated at the intercept to capture the baseline variability by day, compound, household and village excepted for the Model 1 (Additional file 3: Table S1). For each variable of interest, model selection was conducted through a process of backward elimination starting from a maximal model (Additional file 3: Table S1) in which likelihood ratio tests (LRTs) were used to evaluate the significance of individual terms. Mean values and 95% confidence intervals for all statistically-significant effects in the minimum model ("best model") were obtained from the GLMMs using the effects package [58].

Results

A total of 41,800 mosquitoes were collected over 324 trapping days, of which 41,395 were females (Additional file 5: Table S2). Most of the female mosquitoes were anophelines (86.4%), with the remainder being culicines (Additional file 5: Table S2). *Anopheles gambiae* s.l.

represented 97.7% of all anophelines, (Additional file 5: Table S2). Within the subset of *An. gambiae* s.l. individually analysed to species level (n = 3199, 36.3% of total), *An. gambiae* constituted 41.58%, *An. coluzzi* 58.17% and *An. arabiensis* 0.25%. No molecular identification of species within the *Anopheles funestus* group was performed because of the small number collected indicated this is not a major vector in the area (n = 35). There was seasonal variation in vector species composition, with the proportion of *An. coluzzi* within the *An. gambiae* s.l. complex varying from ~ 75% to ~ 44% between the dry and wet season (Additional file 6: Table S3).

Trap sampling efficiency

Overall, there were notable differences in *An. gambiae* s.l. abundance between villages, trapping methods and locations (Table 1). In addition, *An. gambiae* s.l. abundance also varied notably across the collection period, with peaks during the rainy season (May –Oct) followed by decline in the dry season (Nov-April, Additional file 7: Figure S3).

The mean abundance of *An. gambiae* s.l. was best explained in a final model that included the interaction between trapping method and village (df = 11, $\chi^2 = 59.7$, $p < 0.0001$), trapping method and location (df = 1, $\chi^2 = 4.20$, $p = 0.04$), season (as dry or wet season, (df = 1, $\chi^2 = 244.42$, $p < 0.0001$)) and humidity (df = 1, $\chi^2 = 9.71$, $p = 0.002$). The significance of these interactions indicates that there is a spatial variability in trap performance (Table 1, Fig. 3) as well as between outdoor and indoor locations (Table 1, Fig. 4). Overall the relative performance of MET compare to HLC was 46.88% (95% CI 46.20–47.42%), but there was considerable variation between villages from a low of ~ 17% relative sensitivity in Sitiena to a high of ~ 100% in Toumousseni (Fig. 3). Similarly, there was variation in trap performance between indoor and outdoor settings. However, regardless of location (in or outside), the number of *An. gambiae* s.l. collected using METs was less than the HLC (indoor: $z = -5.93$, $p < 0.0001$; outdoor: $z = -5.42$, $p < 0.0001$) with the performance of the MET relative to HLC being slightly higher in outdoor (Fig. 4, 51.47%;95% CI 50.89–52.22%) than indoor settings (Fig. 4, 42.86%; 95% CI 42.0–43.44%). In general, mean nightly temperatures were higher and humidity lower inside of houses than outdoors (Additional file 8: Table S4). Accounting for other significant variables in the model, *An. gambiae* s.l. abundance was positively associated with humidity ($z = 3.33$, $p = 0.001$, Additional file 9: Figure S4), and significantly higher in the wet than dry season (df = 1, $\chi^2 = 244.42$, $p < 0.0001$, Additional file 10: Figure S5), irrespective of trapping method.

Relative performance of trapping methods across seasons

Analysis by GAM indicated there was significant seasonal variation in *An. gambiae* s.l. abundance based on both indoor and outdoor collections indoors (edf = 6.697, $\chi^2 = 700.3$, $p < 0.0001$) and outdoors (edf = 6.346, $\chi^2 = 624.3$, $p < 0.0001$). However, seasonal trends in *An. gambiae* s.l. abundance were indistinguishable as predicted from MET and HLC collections. The simple model (at both indoor and outdoor) with no interaction has the lower AIC compare to model including interactions between variable method and the smoothing spline

Table 1 Number of *An. gambiae* s.l. females collected using different trapping methods, and at different locations (indoor *versus* outdoor) across the 12 study villages between October 2016 and December 2017

Village	HLC			MET		
	Indoor	Outdoor	HLC total	Indoor	Outdoor	MET total
Dangouindougou	787	784	1571	334	454	788
Gouera	762	866	1628	113	370	483
Nianiagara	477	480	957	125	149	274
Nofesso	338	540	878	103	206	309
Ouangolodougou	268	407	675	73	82	155
Sitiena	1588	1609	3197	313	267	580
Tengrela	3407	3104	6511	1457	1323	2780
Tiefora	2276	2389	4665	1174	1125	2299
Timperba	444	414	858	225	353	578
Tondoura	550	575	1125	197	161	358
Toumousseni	787	893	1680	309	520	829
Yendere	546	676	1222	185	359	544
Total	12,230	12,737	24,967	4608	5369	9977

HLC human landing catch, *MET* mosquito electrocuting trap

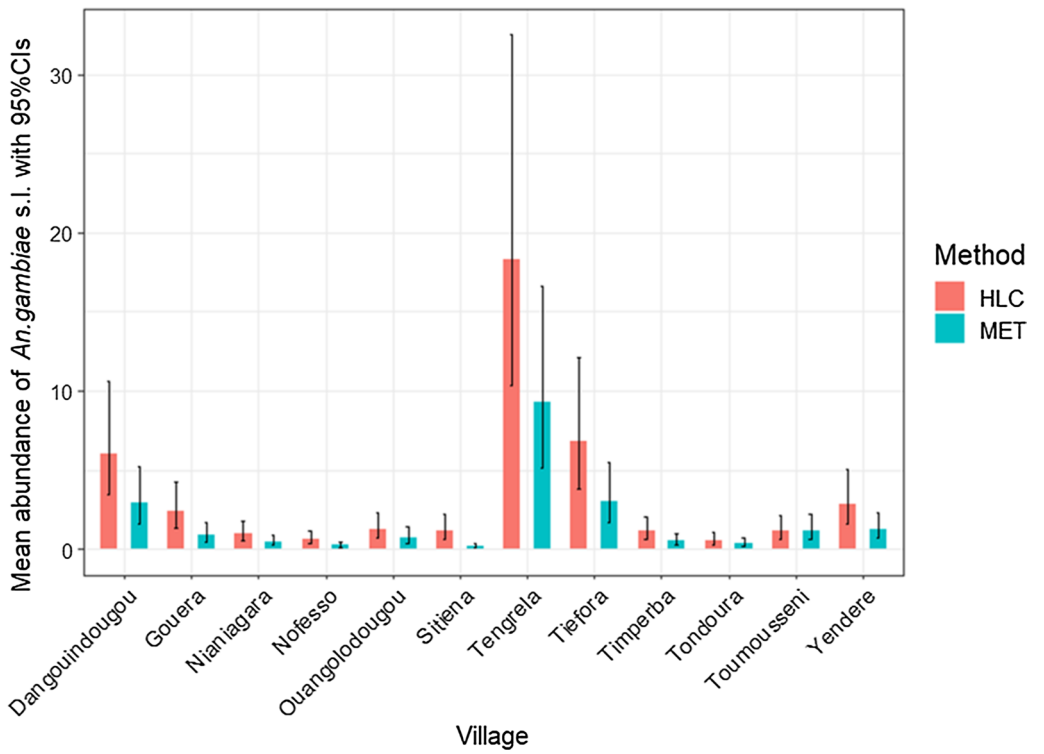

Fig. 3 Mean predicted abundance of *An. gambiae* s.l. caught per night using different trapping methods in 12 villages in southwestern Burkina Faso. Data are pooled across trapping location (inside houses or outdoors) and the study period (October 2016 to December 2017). Error bars are with 95% confidence intervals. Here pink bars indicate HLC collection, and blue bars MET collections

(difference in AIC are 0.55 indoor and 5.66 outdoor); indicating both methods predict similar trends (Fig. 5).

Relative performance of trapping methods across the night

The proportion of *An. gambiae* s.l. caught in METs relative to HLC was significantly influenced by the interaction between the sampling hour and trapping location ($df=1$, $\chi^2=10.83$, $p<0.001$). In indoor environments, the performance of the MET relative to the HLC stayed constant over all hours of the night ($df=1$, $\chi^2=0.13$, $p=0.71$). However, MET relative performance significantly declined ($df=1$, $\chi^2=27.63$, $p<0.0001$) between the first to the last hour of collection in outdoor settings (Fig. 6).

The density dependence between the trapping methods

The number of mosquitoes collected using HLC ranged from 0 to 575 indoors, and 0–672 outdoors, compared to 0–385 indoors and 0–542 outdoors for the MET. The degree of dependence (β) between HLC and MET collections across this range was estimated to be 0.92 (CI 0.79–1.06) indoors and 1.00 outdoors (CI 0.68–1.14). These values indicate there was no density-dependence as the credible intervals of estimates include 1 at each location Fig. 7). There was also a strong linear correlation between the number of *An. gambiae* s.l. caught in MET and HLC collections both indoors ((r) = 0.84 (CI 0.79–0.89)) and outdoors ((r) = 0.86 (CI 0.81–91).

Proportion of *Anopheles coluzzi* in host seeking collections

The composition of *An. gambiae* s.l. varied substantially across villages ($df=1$, $\chi^2=95.4$, $p<0.0001$), with *An. coluzzi* representing more than 75% of the complex at 4 villages, *An. gambiae* dominating at 6, and a roughly equal composition of *An. coluzzi* and *An. gambiae* at the remaining two sites (Additional file 11: Figure S6). The proportion of *An. coluzzi* did not vary between trapping methods ($df=1$, $\chi^2=0.027$, $p=0.87$), location ($df=1$, $\chi^2=0.12$, $p=0.72$) or in relation to the mean temperature ($df=1$, $\chi^2=2.84$, $p=0.09$). However, the proportion of *An. coluzzi* in collections was negatively associated with humidity ($z=-4.67$, $p<0.0001$; Additional file 12: Figure S7) with *An. gambiae* being more prevalent as humidity rose.

Malaria infection

A total of 157 out of 3199 *An. gambiae* s.l. tested were positive for *P. falciparum* sporozoite infection (4.9%

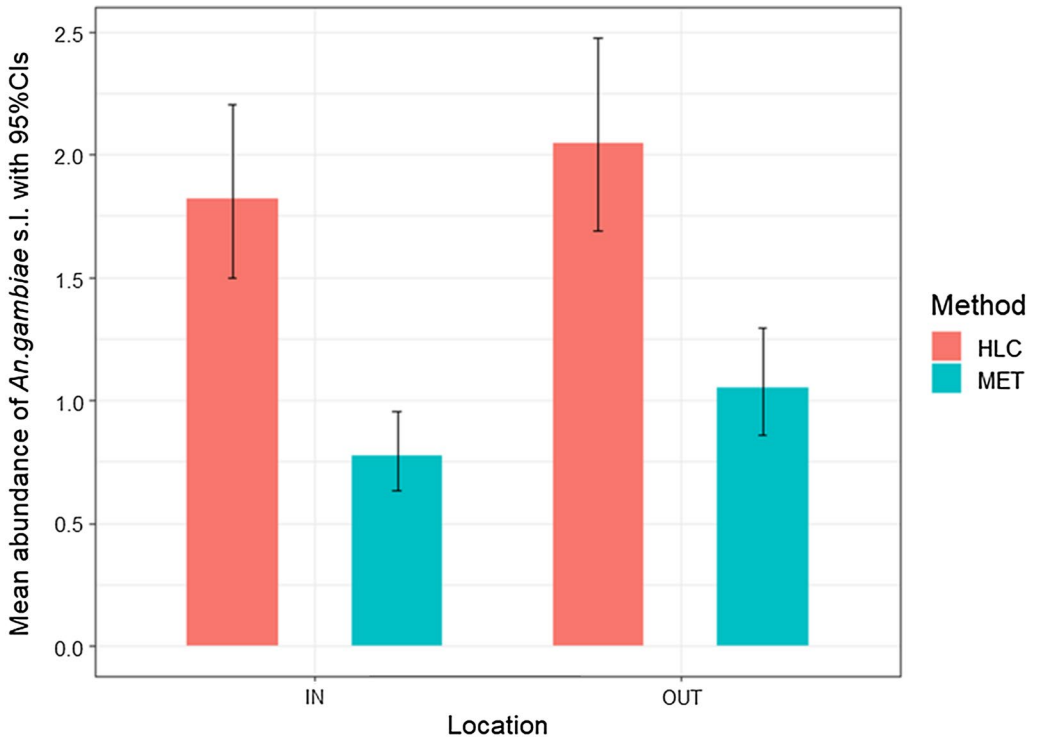

Fig. 4 Mean predicted abundance of *An. gambiae* s.l. per night made at different trapping locations (IN = inside houses, OUT = peri-domestic area outside of houses) using two different trapping methods (pink bars = HLC; blue bars = MET) between October 2016 and December 2017. Errors bars are 95% confidence intervals

infection rate). Sporozoite rates varied significantly between villages (df = 11, $\chi^2 = 27.63$, p = 0.003), (Additional file 13: Figure S8), and in association with the interaction between vector species and trapping location (df = 1, $\chi^2 = 6.15$, p = 0.013). The *P. falciparum* sporozoite infection rate in *An. gambiae* was similar at indoor (5.16%; 95% CI 3.64–7.26%) and outdoor trapping locations (5.67%; 95% CI 4.17–7.66%), whereas sporozoite rates were higher in *An. coluzzi* caught indoors (5.91%; 95% CI 4.2–8.28%) than outside (2.8%; 95% CI 1.78–4.39%). However, sporozoite rates in the overall *An. gambiae* s.l. sample did not vary between trapping methods (df = 1, $\chi^2 = 0.78$, p = 0.38), temperature (df = 1, $\chi^2 = 0.02$, p = 0.88) or humidity (df = 1, $\chi^2 = 0.08$, p = 0.77).

Vector behaviour and human exposure

The *An. gambiae* s.l. population in the study area was relatively exophilic, with numbers host-seeking outdoors being similar or slightly higher than those indoors (Fig. 8). However, estimates of the proportion of indoor biting (P_i) varied somewhat between trapping methods (df = 1, $\chi^2 = 4.25$, p = 0.039); with the HLC predicting a slightly higher degree of outdoor biting (45.73% (95% CI 43.2–48.27%) compared to the MET (43.42% (95% CI 40.47–46.4%), Fig. 8). Similarly, estimates of the proportion of *An. gambiae* s.l. caught during times when most people are indoors ($P_{fl,}$ $\chi^2 = 11.28$, p < 0.001), and the proportion of human exposure to *An. gambiae* s.l. estimated to occur indoors (πi, $\chi^2 = 21.03$, p < 0.0001) were slightly but significantly higher in HLC than MET collections (Fig. 8). There was no significant additional effect of temperature, humidity or season on these human exposure traits t traits (P_i, P_{fl}, and πi; Additional file 14: Table S5).

Discussion

Here the performance of the METs was evaluated as an alternative to the gold standard "HLC" for estimating human exposure to malaria vectors. This was the first time that the trap was evaluated outside Tanzania and in a West African setting. In general, the MET caught fewer *An. gambiae* s.l. than HLC with relative performance being higher in outdoor (52%) than indoor environments (43%). The overall efficiency (combining in and outdoors) of the MET (~ 46%) was similar to that described for first prototype trialled in rural Tanzania by [31], but below the near 100% relative performance reported with further prototypes tested in Tanzania [17, 32]. However, estimates of vector species composition, seasonal dynamics, biting behaviour (indoor vs outdoor) and malaria

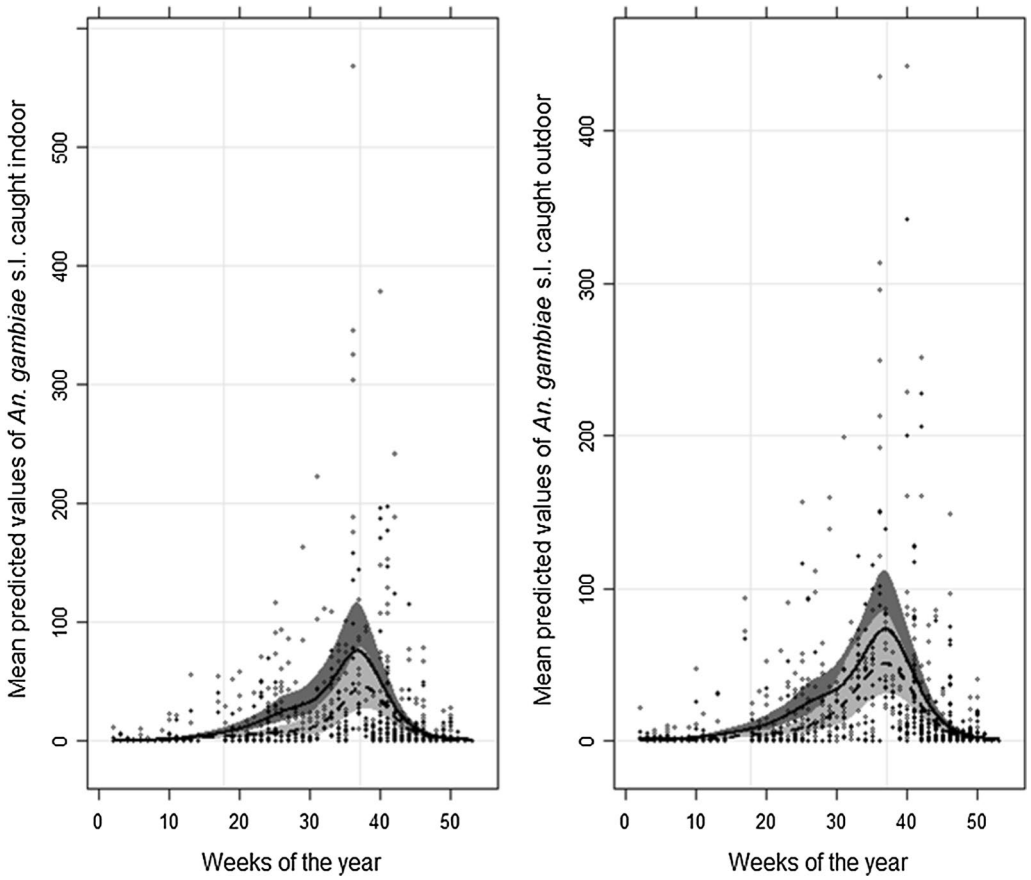

Fig. 5 Mean predicted values of *An. gambiae* s.l. from a generalized additive model (GAM) with a negative binomial distribution. The full and open dots indicate respectively the observed number of *An. gambiae* s.l.in mosquito electrocuting trap and human landing catch through the course year indoors (left panel) and outdoors (right panel). The grey areas are the 95% confidence bands for the splines. The solid line and the dark grey indicate the data from HLC whilst the dashed-line and the light grey represents the MET. Week "1" represents the first week of January, with weeks running consecutively up to week 52 (last week of December)

infections rates were generally similar between MET and HLC collections. This strengthens evidence that METs can provide a safe alternative to the HLC for characterizing attributes of malaria vector populations; even though they may require location-specific calibration for prediction of vector density.

It is unclear why MET performance was relatively lower in this study. However, several factors may account for this. One possibility is that the current study incorporated more intra-site variability. All previous work in Tanzania has involved evaluation at a limited number of fixed sampling points in a few sites. Here the METs were tested at multiple households across 12 different villages and noted considerable variation in MET relative performance between sites (17–100%). Thus, local characteristics of the study site may have a significant impact on trap performance. The relatively lower sampling efficiency of the MET here compared to Tanzania could also be due to operational problems that arose after the first batch of METs had been in continuous use for several months, exacerbated by wear and tear during the regular transport between villages (up to 100 km apart, on poor roads). These operational problems included short-circuits, and power supplier failure in addition to dipping in current/voltage, some of which may not have been noticed until traps failed. Although only data from days in which both MET and HLC collections were conducted was used for analysis, these faults indicate that the MET prototype may need further improvement for stable use over long periods of time. Additionally, there were small differences in trap design between the prototype used here and in Tanzania, which may have contributed to the reduced performance. For example in contrast to previous studies in Tanzania [17, 31], the MET prototype here used white non-treated net to protect the part of participant's bodies that were not in the trap. It has been shown that *An. gambiae* s.l. are more attracted to traps with high visual contrast [22], and the use of white

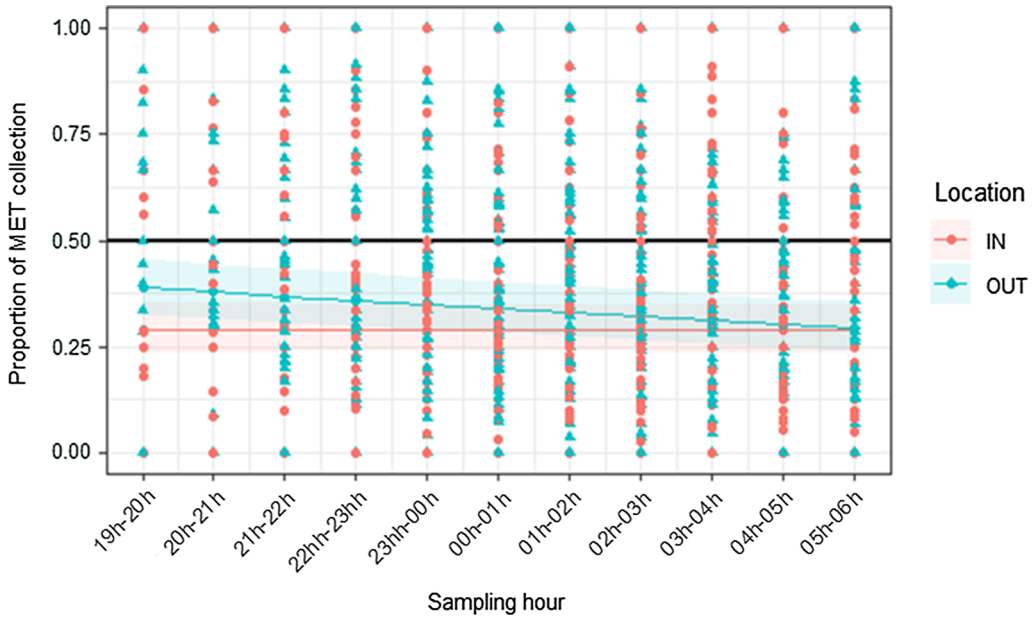

Fig. 6 Mean proportion of *An. gambiae* s.l. caught in mosquito electrocuting trap (MET) collections relative to the human landing catch (HLC) over the course of the night (7 p.m.–6 a.m.). The red dots and blue triangles indicate the ratio MET/(MET + HLC) from the actual raw data respectively collected at indoor and outdoor sampling points. The black solid line indicates the scenario in which MET and HLC catch rates were equivalent. The red and blue lines represent the predicted regression line from models fit on data collected inside houses (IN) and outdoors (OUT). The shaded areas around the predicted lines represent 95% confidence intervals

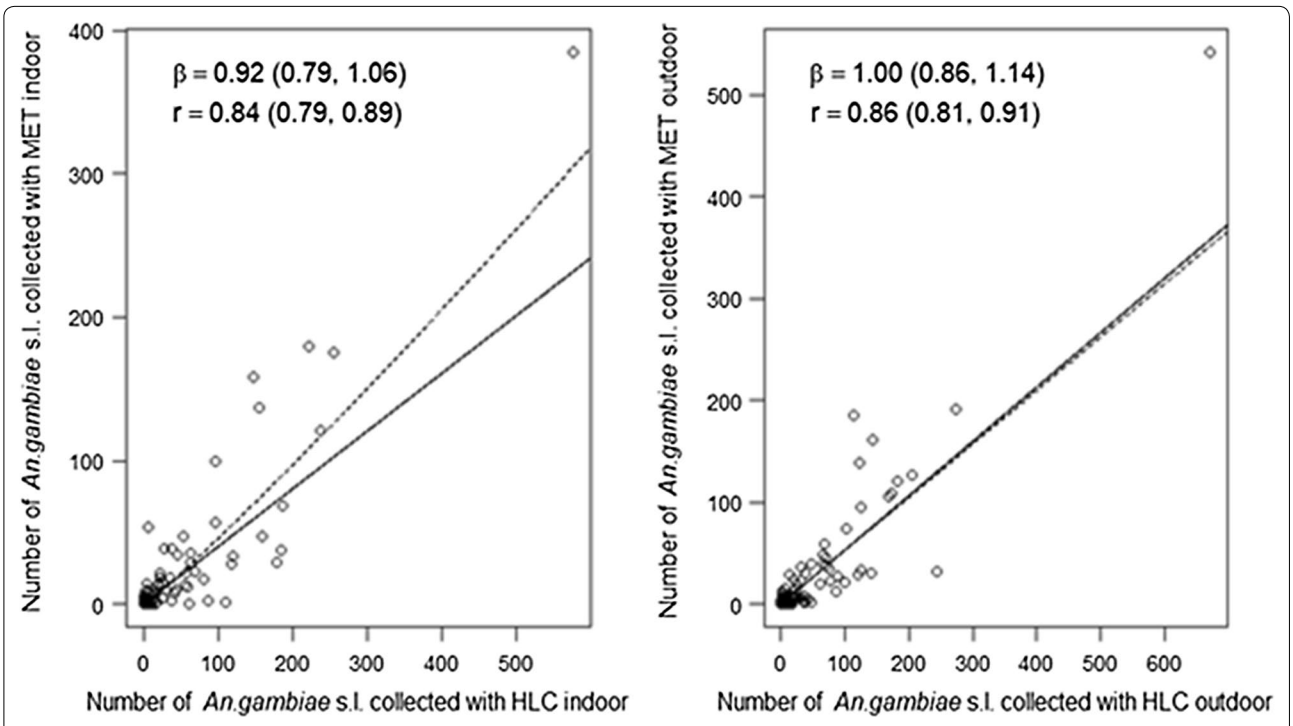

Fig. 7 Observed values (open dots) and predicted relationships between the density of *An. gambiae* s.l. caught in mosquito electrocuting trap (MET) collections and human landing catches (HLC) at indoor and outdoor locations. In each graph, the dashed-lines indicate the model-predicted relationship between the traps and the black solid lines show the density independence relationship between MET and HLC collections

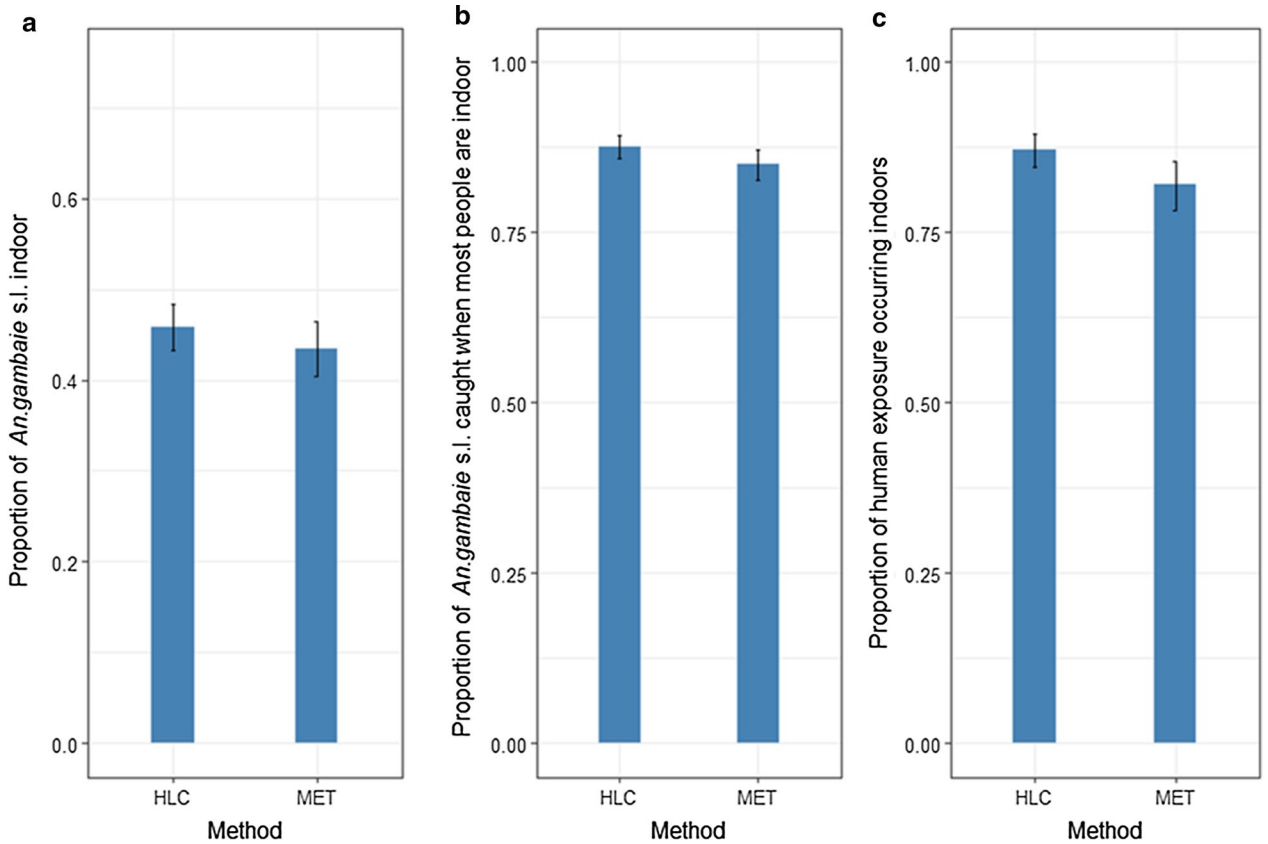

Fig. 8 Estimates proportion of *An. gambiae* s.l. **a** caught indoor, **b** bites occurring when most people are inside their dwellings and likely asleep and **c** the proportion of human exposure to *An. gambiae* s.l. bites occurring indoors from human landing catch (HLC) and mosquito electrocuting trap (MET)

netting to protect participants here may have diminished the contrast between the trap and host bait compared to previous versions. Another factor that can make difference is the vector ecology and species composition. The major vectors in areas where the MET has been used in Tanzania is *An. arabiensis* [17, 31] whereas *An. gambiae* and *An. coluzzi* were the main vectors in our study area in Burkina Faso [42, 43]. Cuticular hydrocarbon composition (CHC) varies between Anopheles species [59–61], and it is known that the electrical conductivity of insects can vary with their CHC, water content and body size [62]. Therefore, the variation in the MET performance between the current study and those carried out in Tanzania could also be due to local variation in vector species composition.

The results from the present study suggested METs performed better in outdoor ~ 52% relative sensitivity compared to the HLC) than indoor (~ 43%) settings. Earlier trials in Tanzania also found MET performance to be higher outdoors than inside houses [31]. It is unclear why MET sampling efficiency tends to be higher outdoors, with further work required to address this bias. Given the growing recognition of the importance of outdoor biting in maintaining residual malaria transmission [28–30] and current lack of satisfactory alternatives to the HLC for measuring this, the MET can serve a useful purpose even if only suitable for use outdoors. The relatively good performance of the MET relative to the HLC for sampling malaria vectors outdoors reported here and elsewhere [17, 32] indicate that it is suitable for monitoring exophagic and zoophilic vector [32] populations.

The relative efficiency of the MET for collection of *An. gambiae* s.l. across dry and wet seasons was evaluated, and its ability to reflect seasonality in vector abundance relative to the HLC standard. Both trapping methods confirm strong temporal variability in vector abundance, likely due to seasonality and meteorological conditions as has been widely documented in Burkina Faso and other parts of West Africa [63, 64]. The current results indicate that the relative performance of the MET compared to the HLC stays constants across seasons, and that both methods predict similar seasonal trend in vector

abundance. Additionally, there was no evidence of density dependence in the sampling efficiency of METs over a wide range of *An. gambiae* s.l. density. This contrasts with results from an earlier prototype where MET performance showed signs of density dependence indoors but not outside [17], but another study also found no density dependence [31]. However, this previous study was conducted over a relatively short period (21 nights) and did not encapsulate the seasonal extremes in vector density incorporated here. Based on the current and previous studies, it can be concluded that the MET can provide relatively accurate estimates of vector population dynamics that are unbiased by season or underlying density. An investigated was also undertaken to assess whether the performance of the MET relative to the HLC decreased over the course of a sampling night as could be indicative of battery drain. Consistent with previous studies [17, 31], there was no detection of any difference in MET sampling efficiency throughout the night when it was used indoors. However, there was a reduction in relative MET performance throughout the night when used outdoors. Such a decrease in MET sampling efficiency outdoors was reported with an early MET prototype in Tanzania [31], but not in a follow up with a new version [17]. It is unclear why MET sampling efficiency falls during the night in outdoor but not indoor settings. One possibility is variation in microclimatic conditions like humidity, which is generally higher outdoors than indoors. Humidity can trigger more rapid discharge of batteries [65]. To maintain consistent MET performance when used outdoors, batteries could be changed during the sampling night.

The malaria vector species composition in this study area varied notably compared to that of previous MET trials in Tanzania. Specifically *An. coluzzi* and *An. gambiae* were the dominant vector species here compared to *An. arabiensis* and *An. funestus* in Tanzania [17, 32, 66, 67]. Previous work in Tanzania indicated MET capture efficiency varied between malaria vector species (e.g. *An. arabiensis* and *An. funestus* [31]). However, vector species composition was similar in collections made by HLC and MET here; indicating no differential sampling performance between *An. coluzzi* and *An. gambiae*. Further calibration may be required to ensure the MET gives unbiased estimates of composition of malaria vector species in new settings. Similar to previous studies [17, 32], we found no difference in malaria sporozoite rates between vectors in HLC and MET collections. Thus, the MET also appears to yield unbiased estimates of appropriate for estimating of *An. gambiae* s.l. infection rates and transmission potential.

Finally, Three key human-mosquito exposure metrics were evaluated to assess whether they were reliably predicted by the MET: the proportion of (i) indoor biting (P_i), (ii) *An. gambiae* s.l. bites occurring during times when most people are indoors ($P_{fl,}$) and (iii) human exposure to *An. gambiae* s.l. bites that would occur indoors in the absence of personal or household physical protection ($\pi_{i)}$ [50]. A higher proportion of outdoor biting by *An. gambiae* s.l. was found than previously reportedly in Burkina Faso [68–70]. In general, estimates of these three exposure-metrics were similar between HLC and MET collections. However, the MET tended to slightly underestimate all three metrics likely because of its slightly lower sampling performance in indoor *versus* outdoor settings. However even this with bias estimates of exposure as calculated by the different trapping methods were generally within a few percentage points of one another. For operational use, estimates of exposure derived from MET collections could be adjusted to compensate for this bias.

The multi-site nature of this study allowed assessment of wider aspects of MET feasibility for programmatic sampling. In contrast to previous trials in Tanzania where the MET was used in fixed, single locations [17, 31]; here was carried out in 12 villages requiring the MET to be moved every few days and sometimes as far as 100 km. The integrity of electrified surfaces on the METS were checked before and after transport in the field. The output voltage was also regularly checked during collections to ensure it was meeting the necessary target. On occasions where voltage output was suboptimal (~0.4% of days), MET operation was stopped and the problem reported to technical support team. Overall, MET collections were performed on ~17% fewer sampling hours than the HLC. However, this does not represent the proportion of times that the MET failed. Most of these MET hours (~9%) were lost while waiting for a replacement unit to be made and delivered (~4-week period). The most frequent problem encountered with MET use was power failure due to short-circuiting (~6% of time) with occasional sparking on the frame. Therefore, further improvements in MET design are needed to resolve this issue. In addition, it was noted that short-circuiting was more likely to occur when there was high level of moisture in the environment (e.g. rainy season, times of high humidity). This was probably due to small water droplets condensing on the frame and occasionally running down the wires. Regular wiping of the MET surface (e.g. during 15 min break periods from sampling) could help avoid a build-up moisture of trap surface. Alternately, redesigning the trap with wires running horizontally instead of vertically will prevent droplets from running down into the frame. METs were subjected to heavy use in this study, under challenging field conditions. It is perhaps not surprising that traps exhibited some degree of physical damage and breakage under these intense

circumstances. These issues could be resolved by making future prototypes more robust, and/or keeping METs in fixed locations rather than in constant transport. In addition, on some other nights, MET sampling was intentionally stopped (~1% of the sampling hours) due to high wind and rainfall that was anticipated to drive water onto the MET surface and cause short-circuiting. Even with these difficulties, the METs still performed relatively well and consistently with the HLC in this study. To increase the protection of volunteers from bites of very small biting insects (those with wingspan less than 5 mm) that may be present at some study sites, we recommend fitting fine-mesh insecticide-free netting on the inner panel of MET surfaces with very small holes.

An additional consideration is the relative expense of doing collections with METs *versus* HLC. Currently, MET are individually built to order by a small team; with the combined cost for all components and manufacture of ~£ 650–700 per unit. This cost is prohibitively high for large-scale surveillance (e.g. by comparison, a standard CDC light trap costs ~$ 100 USD per unit). However, it is anticipated that the production cost would significantly decrease if produced at scale. While costs of MET collections may always be more expensive than a simple HLC where no equipment is required, we believe this additional expenditure is justified in terms of the improved safety to human subjects that it can provide.

Conclusions

This is the first-time that the MET was evaluated outside of East Africa. Overall, the MET collected proportionately fewer malaria vectors than the HLC, and slightly overestimated the proportion of outdoor biting. However, the performance of METs relative to the HLC was consistent over time, and provided similar estimates of seasonal dynamics, biting behaviour, species composition and infection rates in malaria vector populations. Thus, despite some technical problems arising after prolonged MET usage under field conditions, we conclude it presents a promising and safer alternative for monitoring human exposure to malaria vectors in outdoor environments.

Supplementary information

Supplementary information accompanies this paper at https://doi.org/10.1186/s12936-019-3030-5.

Additional file 1. Assembled Mosquito Electrocuting trap used for mosquito collections, connected to the power supplier and the 12-volt batteries.

Additional file 2. Subsampling strategy.

Additional file 3. Maximal models used for the modelling including the primary response variable, explanatory variables and statistical distribution used.

Additional file 4. Graphs indicating the proportion of residents (at households where mosquitoes were being collected) that were observed to be inside their houses during different hours of the night.

Additional file 5. Number of mosquitoes collected pooled over the collection methods (Human landing catch and mosquito electrocuting trap) and displayed by species and per village over 15 months (October 2016 to December 2017). Totals include both female and male mosquitoes.

Additional file 6. Number of *An. gambiae* complex females that tested positive for the presence of *P. falciparum* sporozoites during dry (November 2016 to March 2017 and November to December 2017) and wet season (October 2016) with different trapping methods (HLC, MET) and at different trapping locations (indoors versus outside of houses). Numbers represent totals pooled over study villages and the collection period (October 2016 to March 2017).

Additional file 7. Number (raw data) of *An. gambiae s.l.* collected per month from (October 2016 to December 2017 by trapping methods **a** indoor and **b** outdoor using mosquito electrocuting trap (MET) and human landing catch (HLC).

Additional file 8. Range of average temperature (°C) and relative humidity (%) recorded at the mosquito collection point using data logger.

Additional file 9. Effect of the humidity on the mean predicted number of *An. gambiae s.l.* collected per night according over the trapping methods, location and village. The solid black line indicates the regression line based on the model and grey-shaded area indicates the 95% CIs. Humidity data were only available for part of the sampling period (e.g mostly during the dry season months [Nov 2016 to April 2017, and Nov to Dec 2017], and a few months in wet season [October 2016 and May to October 2017]. The predicted relationship between relative humidity and vector abundance is thus based on months in which matched data were available.

Additional file 10. Mean predicted number of *An. gambiae s.l.* collected per night and season over the trapping methods, location and village with 95% CIs. Dry season indicates *An. gambiae s.l.* collected from November to April whilst wet season corresponds to period between May and October.

Additional file 11. Mean predicted proportion of *An. coluzzii* relative to *An. gambiae* collected per village from October 2016 to December 2017, pooled over the trapping location and methods, with 95% CIs.

Additional file 12. Effect of the mean relative humidity on the estimation of the proportion of *An. coluzzii*. The solid black line is the regression line of the predicted proportions and the grey-shaded area indicate the 95% CIs.

Additional file 13. Mean predicted *Plasmodium falciparum* infection rate in *An. gambiae s.l.* collected per village from October 2016 to December 2017, pooled over the trapping location and methods, with 95% CIs.

Additional file 14. Non-significant term excluded from the best models where LRT represents likelihood ratio test and degree of freedom of 1 for all the terms.

Abbreviations

AIC: Akaike Information Criterion; CDC-LT: Centre Disease Control Light Trap; EIR: entomological inoculation rate; HDN: human-baited double net; HDT: host decoy trap; ELISA: enzyme linkage immuno-sorbent assay; GAM: generalized additive models; GLMM: generalized linear mixed models; HLC: human landing catches; ITT-C: Ifakara tent trap design C; LLINs: long-lasting insecticide-treated nets; LRT: likelihood ratio tests; MCMC: Markov chain Monte Carlo; MET: mosquito electrocuting trap; PCR: polymerase chain reaction; PVC: polyvinyl chloride.

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data analysis, and Jakub Czyzewski at the Bioelectronics Unit at the University of Glasgow for producing the MET and providing technical assistance.

Authors' contributions
AS designed the study, conducted the field data collection, did the molecular analysis, performed the data analysis, interpreted the results and drafted the manuscript. KHT contributed to the field data collection. WMG, NFS, and HMF contributed to the design of the study and the sampling protocol and provided comments upon the manuscript. HMF was a major contributor in writing this manuscript. SZ, PO and FC contributed to the molecular analysis in the labs. LN, JM, contributed to the data analysis. NM, designed, developed assisted the METs. All authors read and approved the final manuscript.

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Availability of data and materials
Material & Data Transfer Agreements (MDTA) will be used when sharing data between members of the consortium who did not generate original data. Public release of data will be timed to follow publication. Earlier use for access to the data will be considered according to the conditions of the National/ Institutional Ethics Review Boards. Data can be made available after a written expression of request for data with no apparent competing interest and in compliance with the "MIRA project", the Centre National de Recherche et de Formation sur le Paludisme (CNRFP) and University of Glasgow data sharing agreements.

Ethics approval and consent to participate
Ethical clearance was obtained from the Ethical Committee for research in Health of the Ministry of Health of Burkina Faso (EC V3.0_CERS No 2016-09-097) and the Institutional Bioethical Committee of the local research institution (National Malaria Research and Training Centre, CNRFP) under EC V3.0_ No 2016-026/MS/SG/CNRFP/CIB) and the Liverpool School of Tropical Medicine (Certificate 16-038). Prior to starting the research, the project aims, and objectives were explained to community leaders in each village. Signed informed consent was also obtained from all household owners where mosquitoes were collected, and from volunteers who took part in mosquito collections by HLC and MET.

Consent for publication
Written consent was obtained from volunteers for publication of their images in publications.

Competing interests
The University of Glasgow has filed a joint patent application for the MET with the Ifakara Health Institute, in which NM, and HMF are named as investigators. All other authors declare no competing interests.

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