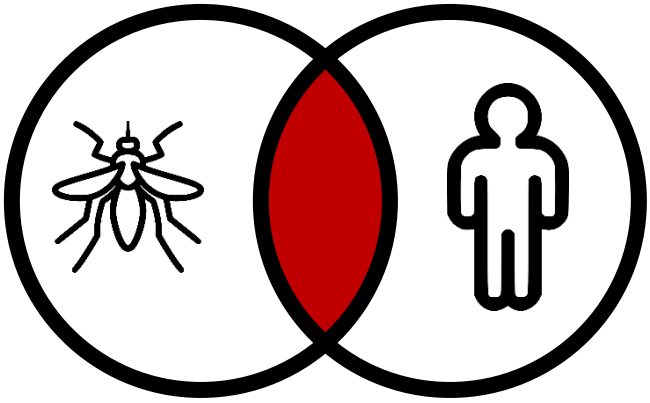
This thesis is dedicated to my beloved Solomon Islands,

with hopes that one day we will be malaria free.



WHEN WORLDS COLLIDE: WHERE AND WHEN ANOPHELINES AND HUMANS INTERACT

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Statement of Contribution

Academic support

Edgar Pollard was primary author of all results chapters and all have been either published or submitted for publication in peer-reviewed journals.

Chapter	Names & nature of assistance
4. Barrier Screen	Edgar Pollard (PhD Candidate) conducted the field trials, analysed the dataset and drafted the manuscript. Tanya Russell (supervisor, James Cook University) and Tom Burkot (supervisor, James Cook University) contributed to the experimental design, data analysis and assisted with writing the manuscript.
5. Mosquito Behaviour	Edgar Pollard and Allan Apairamo (Solomon Islands National Vector Borne Disease Control Programme) conducted the field collections. Edgar Pollard analysed the dataset and drafted the manuscript. Tanya Russell and Tom Burkot assisted with the the experimental design, data analysis and writing the manuscript.
6. Human Behaviour	Edgar Pollard conducted the field data collections, analysed the dataset and drafted the manuscript. David MacLaren (supervisor, James Cook University) contributed to experimental design. David MacLaren, Tanya Russell and Tom Burkot contributed to the data analysis and assisted with writing the manuscript.
7. Serology	Edgar Pollard, Tanya Russell, Tom Burkot, Allan Apairamo and Jance Oscar (Solomon Islands National Vector Borne Disease Control Programme) conducted the field data collections. Bruno Arca (Sapienza Università di Roma) provided salivary antigens. Edgar Pollard and Catriona Patterson (London School of Hygiene & Tropical Medicine) conducted the serological laboratory analyses and drafted the manuscript. Tanya Russell and Tom Burkot, Tanya Russell, Bruno Arca and Chris Drakely (London School of Hygiene & Tropical Medicine) contributed to the experimental design, the data analysis and assisted with writing the manuscript.

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Abstract

Malaria is a disease spread by mosquitoes and kills almost half a million people each year. While substantial gains have been made in combatting the disease, progress has stalled partly because of changes in mosquito behaviour, resistance to the insecticides in vector-control measures, drug resistance in the parasites, inadequate financing, a lack of political support as well as a host of other factors. These vector control measures have been successful since the 1990s in reducing malaria-related mortality; however, their efficacy is now waning. New tools are needed to complement the current vector control measures and combat outdoor biting; however, to develop and roll out new tools the behaviour of mosquitoes and humans needs to be better understood.

Malaria is an endemic problem in the Solomon Islands and is primarily transmitted by *Anopheles farauti*. This study examined the behaviour of *An. farauti* mosquitoes and humans in the Solomon Island and Austrialia in four distinct components. The first component optimized the barrier screen method for mosquito collections. The second component used these optimized barrier screens to collect and record mosquito distributions in Solomon Island villages. The third component used movement diaries to record human behaviour in Solomon Island villagers. The fourth component explored serological techniques to measure mosquito-human interactions.

The characteristics of barrier screens (colour, weight and design) and frequency of inspection were found to be important determinants affecting the collection efficiency. The results for optimising barrier screens indicated that black coloured, medium weighted shade cloth maximized *An. farauti* collection numbers including the first ever-recorded distributions of sugar-fed and male *An. farauti*. *Anopheles farauti* activity including biting in Solomon Islands villages peaked during 7-8pm. During this period of peak biting the majority of people were outdoors in the peri-domestic area, predominantly on the veranda or in adjacent kitchen buildings. Therefore, greatest interactions between the human and the malaria vector populations and therefore the most likely area of malaria transmission is in the early evening in this peri-domestic space. To better evaluate the risk associated with humans being bitten by *An. farauti* the serological response of humans to a mosquito salivary gland antigen was investigated in the Solomon Islands. While of insufficient sensitivity to guide programs when using the gSG6 antigen, this approach holds great promise and might be improved by using antigens from An. farauti in the assay.

The peri-domestic space is identified as the area of greatest risk but also of greatest potential for vector control. The implications of this study indicate that the current vector control measure (insecticide treated nets) are not being used during the peak biting period. Focusing mosquito control on the peri-domestic spaces in villages is needs as this is where the highest transmission potential exists. New tools targeting this area are needed to minimize interactions between the mosquito and human populations.

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List of abbreviations

API: Annual Parasite Incidence

APLMA: Asia Pacific Leaders Malaria Alliance

b/p/h-n: bites/person/half-night

CDR: call data records

EIR: Entomological inoculation rate

GLM: Generalized Linear Model

GLMM: Generalized Linear Mixed Model

GPS: Global Positioning System

gSG6: gambiae salivary protein

HBI: human blood index

HBR: Human biting rate

HDPE: high-density polyethylene

HLC: Human landing catch

IRB: Institutional Review Board

IRS: Indoor residual spraying

ITN: Insecticide treated net

ITWL: Insecticide treated wall-lining

JCU: James Cook University

LLIN Long lasting insecticidal net

LSM: Larval source management

m/c-n: mosquitoes per collection night

MFI Median fluorescence index

PBS: Phosphate-Buffered Saline

PET: polyethylene terephthalate

PNG: Papua New Guinea

SIG: Solomon Islands Government

WHO: World Health Organisation

YAPS: Young Animal Protection Society

1 Introduction

1.1 Background

1.1.1 The state of Malaria

Malaria is transmitted by anopheline mosquitoes, as discovered by British doctor Ronald Ross in 1897 [1]. In 2017, there were an estimated 219 million malaria cases globally, resulting in approximately 435,000 deaths. From 2010 to 2017, global malaria cases declined by 20 million, although between 2015 and 2017 there was a 5 million case increase, indicating stalled progress [4]. The most effective and current World Health Organization (WHO) recommended malaria vector control measures are insecticide treated nets (ITNs) and indoor residual spraying (IRS) that target the indoor behaviours of the mosquitoes [2, 3]. Between 2015 and 2017, 624 million ITNs, mainly long-lasting insecticidal nets (LLINs), were delivered globally. However, 44 per cent of people in malarious areas still do not have access to these nets [218].

Concerns are also growing about possible threats to vector control effectiveness due to increasing resistance (both physiological and behavioural) to these vector control measures [4]. The LLIN and IRS have been hugely effective but complementary tools are needed due to residual outdoor transmission [5, 6]. Currently the only WHO recommended method for controlling malaria transmission outdoors is larval source management, which is only recommended as an supplemental intervention under specific ecological conditions [7]. A growing number of novel vector control strategies are under evaluation to address insecticide resistance and outdoor transmission. However, selection of the most effective control tool will require detailed knowledge of vector behaviours including their susceptibility to a given intervention [4]. The main challenge with LLINs and IRS are physiological and behavioural resistance, misuse of these tools and the behaviour of both humans and mosquitoes [8].

The Solomon Islands has one of the highest malaria transmission and death rates in the Western Pacific region [9]. However, just like global patterns, cases of malaria had dropped, but the rate of reduction has stalled: cases decreased to all-time lows in 2014 but in 2017 went back to 2010 levels. The causes of this stalled progress are unknown but may be explained by improved surveillance, data reporting and test sensitivity, such as the use of more sensitive rapid diagnostic tests rather than an actual increase in cases [10].

1.1.2 Behavioural resistance

Behavioural resistance is when the mosquito changes its behaviour to avoid insecticides [11]. Behavioural resistance was first reported in the Solomon Islands in 1975 in *Anopheles farauti* [12], the predominant malaria vector [13-15]. It quickly adapted to the selection pressure exerted by IRS with DDT by feeding increasingly outdoors and early in the evening, avoiding contact with DDT and thereby

diminishing the effectiveness of the IRS [12]. This shift to outdoor and early blood feeding was further reinforced by the widespread use of ITNs including long lasting insecticide treated nets [4, 11, 15]. Behavioural resistance is not found everywhere IRS or ITNs are used [16], but is being seen increasingly throughout the world [11] with *An. funestus* in Africa now reported to feed during daylight hours in some areas [17].

Tools and techniques to complement current vector control methods are needed, especially in areas where outdoor transmission and behavioural resistance are significant [18, 19]. There will likely not be a "silver bullet" intervention that will work everywhere to control all malaria mosquitoes: as there are at least 41 dominant malaria vector species with unique behaviours [20]. However, a variety of locally suitable strategies working together that target vulnerabilities in the vectors' behaviours are needed if elimination is to be achieved [7]. Underlying the development of novel interventions is the need for a comprehensive understanding of the ecology and biology of anophelines particularly for activities that occur outdoors, including knowledge of distribution and movement behaviour [13, 21, 22].

1.1.3 Mosquito Behaviour

Malaria control and elimination campaigns need to be designed for the local context which means understanding the local ecology and behaviour of mosquitoes and the human host [23]. There is limited knowledge of mosquito ecology at the village level with flight movement particularly poorly understood [22]. While it is known that high winds and rain will limit mosquito movement [24] the analyses of the factors that limit and facilitate mosquito movement have not been precisely quantified. If temporal, spatial and directional movement patterns of mosquitoes are better understood, more efficient placement of control tools that specifically target the vector will be possible.

Knowledge of mosquito movements will also be useful to determine and define the factors that create or enable mosquito foci (areas with significantly higher densities of mosquitoes) in villages. Malaria risk is not evenly distributed with 20% of people receiving 80% of infections: therefore efforts should target those at highest risk [25]. Knowledge of where human movement (see below) intersects with mosquito movement will identify these focal areas for malaria transmission.

A foundation of the response framework in the *Global Vector Control Response 2017-2030* is "increase basic and applied research, and innovation"; one aim of the World Health Organisation is to reduce vector-borne diseases through vector control that is "locally adapted and sustainable" [26]. Bed-nets were born out of basic research that understood that mosquitoes transmit malaria and the dominant vectors species usually bite late at night and indoors when people are sleeping. Bed-nets defend against blood-sucking insects by providing a protective physical barrier and have been observed in many cultures throughout recent history. ITNs were first used in the 1930s and 40s, when it was observed that people who slept under these nets had less malaria [27]. In the Solomon Islands during WWII,

Americans soldiers impregnated bed-nets and jungle hammocks with 5% DDT to create the first insecticide treated nets [28]. Understanding the basic biology of these vectors has been a foundation for the development and success of the bed-net.

The success of ITNs have been enormous, which along with LLINs having reduced child mortality [29]. Dramatic declines in malaria have occurred since 2000 with ITNs thought to be responsible for 68% of the decline in *Plasmodium falciparum* malaria in sub-Saharan Africa with around 500 million cases averted [30]. Insecticide treated nets also reduce transmission by reducing mosquito survival and community-wide protection is achieved as coverage is increased resulting in a mass killing effect on mosquitoes [31]. In the Solomon Islands, ITNs reduced the human biting and inoculation rates of *An. farauti* more than IRS, which is why IRS was discontinued [32-34]. However, as with all tools there are concerns and challenges such as compliance and vector resistance. In Papua New Guinea (PNG), ITNs have also resulted in a behaviour shift in the vectors to earlier biting times. Though LLINs initially reduced mosquito numbers, a rebound in both mosquito abundance and human exposure to biting due to the earlier biting shift occurred, especially in areas of high vector abundance and year round transmission [35].

1.1.4 The Barrier Screen

Mosquito sampling using lures (including human landing catches) give useful insights into mosquito abundance at fixed places but little work has been done to understand how the mosquito moves between places. The use of lures (including humans) in traps also changes the movements of mosquitoes. Ideally, to understand mosquito distributions, one would want to sample mosquitoes without influencing their behaviour and movements. Barrier screens are a relatively new method to study mosquito movement [36]. The barrier screen is an insecticide free neutral net "trap" that intercepts mosquitoes as they fly in pursuit of blood meals, resting and oviposition sites, mating sites or sugar sources. When mosquitoes encounter the barrier screen, they will rest and then can be easily seen and captured. The barrier screen is a passive (e.g., no attractant) means of sampling the mosquito population, designed to understand the time and place where mosquitoes are and their direction of travel. This sampling method can also collect a many mosquito species in a wide variety of physiological states (such as blood- feds, which are used for analysing the proportion of mosquitoes that have fed on humans (human blood index (HBI), gravids, unfeds) and both males and females.

1.1.5 Human Behaviour

Previous human behaviour studies have focused on the knowledge and attitudes of the local people to malaria. Understanding the intersection of human and mosquito populations is critical to vector borne disease control [37] and it is thus critical to understand human behaviour patterns in the places where

malaria is endemic. Concurrent surveys of mosquito biting and human behaviours have been used to calculate the human exposure to vectors [38]. A focus on human behaviour patterns (activities and locations) during times of peak anopheline biting is needed to enable a better sense of where to focus efforts to limit malaria transmission. Thus, if temporal, spatial and directional patterns of both mosquitoes and humans are better understood, more efficient placement of control tools could specifically target vector behaviours for maximum impact in culturally acceptable ways.

1.2 Aim

1.2.1 Overall Aim

The overall aim of this thesis is to better understand how mosquito (specifically the primary malaria vector in the Solomon Islands, *An. farauti*) and human populations interact in Solomon Islands villages. The first objective was to optimise the passive barrier screen for *An farauti* collections. The second objective was to record and observe outdoor, adult mosquito distribution patterns in Solomon Island villages. The third objective was to document the locations of villagers in the Solomon Islands. The fourth objective was to trial a novel method for measuring human exposure to biting mosquitoes. The integration of the findings from the four objectives has enabled mapping of spatial-temporal interactions between humans and mosquitoes to better understand malaria transmission dynamics in Solomon Islands villages. Through greater understanding of human-mosquito interactions, specific targeted control strategies to prevent contract between human and vectors can be designed.

1.2.2 Thesis outline

This first chapter introduced the study by sharing essential background information in which data gaps are identified and the rationale and questions for this study defined. The second chapter will describe the researcher and the research design and relate how all chapters fit together to inform the aims of this thesis. The third chapter is the literature review, giving an in-depth look at the current knowledge on the relevant research themes.

The fourth chapter, the first of four “results” chapters, focuses on maximising barrier screens for documenting mosquito distributions. The specific research questions optimised barrier screen design by evaluating fabric colour, fabric weight, the use of eaves and collecting time periods on numbers of mosquitoes collected. This chapter validated the barrier screens used in the field in chapter five.

The fifth chapter documents *An. farauti* distribution patterns within Solomon Island villages. The specific research question explores the distribution of mosquitoes within villages looking at resting heights, spatial and temporal foci and the relationships of these foci to the location of potential host and larval sites and the relationship between environmental conditions and *An. farauti* densities. Results

from this chapter will be the basis for discussions on the interacting space of mosquitoes and humans (from chapter 6) in chapter eight.

The sixth chapter maps the locations in time and space of human populations in Solomon Island villages. The specific research question explores human movement within and away from villages, locations of humans during peak *An. farauti* biting times and human sleeping behaviours. The outcome is a detailed understanding of human locations in Solomon Island villages and is linked with results from chapter five to discuss the interacting space of mosquitoes and humans in chapter eight.

The seventh chapter explores a novel method for measuring the levels of exposure of humans to biting mosquitoes. Measuring levels of IgG antibody in village resident sera to a mosquito salivary gland peptide, it was hoped to determine biting exposure without collecting mosquitoes (e.g., mosquito-free entomological surveillance). The levels of response to anophelines salivary antigen were compared to human biting rates estimated from human landing catches in sub-villages areas to ascertain the specificity and sensitivity of the technique.

The final eighth chapter is the synthesis where findings from all research chapters are brought together in discussion (Figure 1.1).

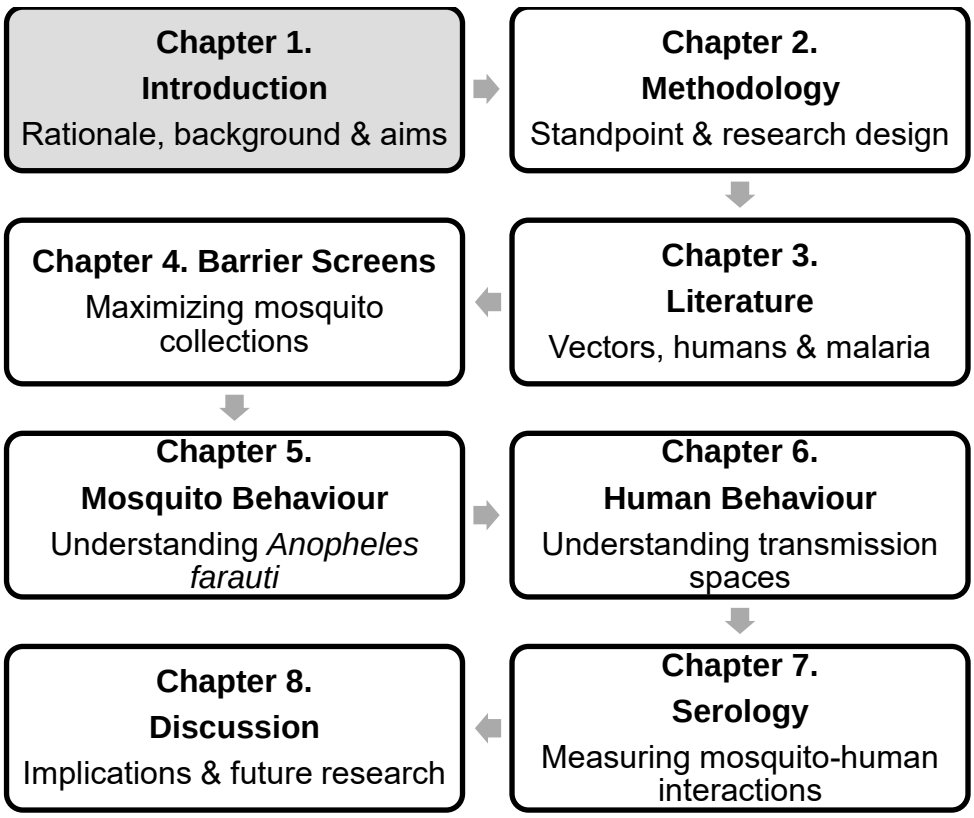
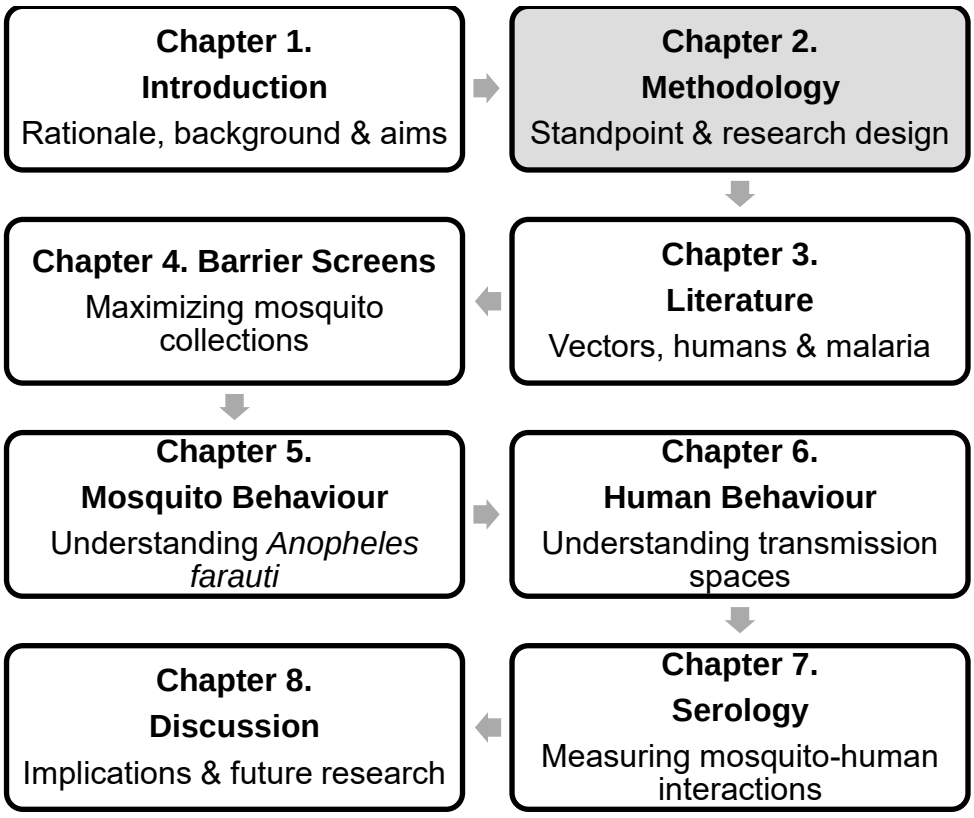


Figure 1.1 Structure of thesis

2 Methodology

2.1 Introduction

The second chapter will describe the researcher and the research design and relate how all chapters fit together to inform the aims of this thesis. The standpoint describes the background, values and related experience of the researcher. The research design explains the philosophical underpinnings of this research along with the specific research questions, setting and processes.



2.2 Standpoint

I was born in the Solomon Islands and am the eldest of 3 boys. My mother is from Malaita, born and raised in the village and is the first female Solomon Islander to graduate with a PhD. Her PhD titled "Gender and Leadership in `Are`Are Society, the South Sea Evangelical Church and Parliamentary Leadership- Solomon Islands" looked at the role of women in leadership in these 3 different spheres. She was originally trained and worked as a teacher before working with women through government and non-government organisations including the founding of the Rokotanikeni West AreAre Women's Association. She has influenced me through her dedication and perseverance and pushing me to achieve more. My father is from New Zealand though born and raised in the Solomon Islands and is a business man. He was also originally trained and worked as a teacher before moving into consulting and business management. He runs a couple of businesses, a HR company and a virgin coconut oil exporting venture. He has influenced me through his integrity and wisdom and enabling me to pursue my dreams.

I was raised in the Solomon Islands but spent substantial years in Australia (primary school), New Zealand (high school) and Fiji (University). I therefore think of myself as a *haf haf* not only racially but also as an insider/outsider being able to straddle the inside Solomon Islands world and the greater outside world. I was raised in a Christian family and still am a Christian. This has helped give me the values of stewardship and the intrinsic value of life.

I am an ecologist by profession. In many ways Melanesians are natural ecologists as we think about the inter-relations of all systems in life. My background in ecology has helped me look at the malaria problem in a different light. I have worked with both local communities and international NGOs in relation to conservation, learning to navigate the complexities at both ends of the spectrum. Understanding perceptions and nuances (cultural and social) from the village level and marrying them with those from national and international levels (policies and systems). Understanding that there are many factors at play and noting that malaria is both a human and mosquito problem. Therefore, if gains are to be made against malaria, challenges in both spheres need to be tackled. Ecology looks at the system, all the factors at play, inputs and outputs and identifies points of interaction and vulnerability. Viewing the malaria challenge through the lens of ecology helps us see the bigger picture but also understand the little complexities that may be context-specific. Eradication will likely not be achieved by a miracle single bullet but by a multi-pronged attack along the wide scope of this disease.

As a Solomon Islander conducting this research, there is a sense of privilege as I am able to approach this problem from the inside. Anyone can collect mosquitoes but from the inside it is easier to communicate the importance of this to local people. Anyone can track people but from the inside there are personal insights as to why this may be happening. However, the villages where we worked still considered me as an outsider (as I am from a different village/tribe/island), the ability to *tok stori* in local *pijin* helped remove barriers and reveal certain insights. On the other hand as an outside researcher

coming into these communities, I am able to understand the greater context of this study and be as objective as possible.

I very nearly lost my life to malaria and am committed to its elimination, this is further strengthened by the knowledge learnt, experiences gained and relationships created throughout this research. Here are a few lessons that I have learnt that will help Solomon Islands reach malaria elimination. There is a need to understand the basic fundamentals of how malaria works and this information then needs to be translated out to communities. The government and relevant stakeholders need to take malaria seriously by ensuring that we are well resourced with personnel, facilities and funds. We then need to take a military-like approach to combat the disease by being more proactive to search, track and treat and to also work strongly with local communities in reducing the mosquito burden.

2.3 Research Design

This thesis uses a quantitative descriptive research approach to study, observe and measure natural phenomena. The assumptions in this study are: that there is single mosquito population that displays regular behavioural patterns and norms within a given environmental context in a given location and time; that there is a single human population that displays regular behavioural patterns and norms within a given social context in a given location and time. The hypothesis of this study is that mosquito and human behaviour patterns overlap in a given location and time to create an optimum space of interaction that sustains malaria transmission. Therefore, to reduce malaria transmission in a given location this overlapping space of interaction needs to be understood to inform malaria elimination in that given location. This required an understanding of both the mosquito and human population behavioural and distributional patterns.

This approach included: experimental trials of different barrier screens (for mosquito collection) and detailed measurements of environmental parameters (temperature, rainfall, humidity, wind speed and direction) and mapped locations of houses and bodies of water that provide mosquito oviposition sites. Surveys were used to quantify and map locations of mosquitoes and humans over time. These methods were designed to be locally appropriate and suitable in this specific context of the Solomon Islands. Therefore, the quantitative methods were situated in a local Solomon Islands context – to inform malaria elimination in that environmental, social and cultural context.

2.4 Research Setting

This PhD focused on mosquito and human behaviours in rural Solomon Island villages. The fieldwork in Solomon Islands was undertaken in Haleta village in Central Province and Saeragi, New Mala, Jack Harbour and Tuguivili villages in Western Province. These villages were selected because they have a

range of mosquito densities, high levels of malaria and preliminary background data had been collected included mapping of the houses, larval habitats and mosquito species identification. Detailed information on specific study settings is provided in Chapters 4, 5, 6 and 7.

The Solomon Islands, an archipelago in the South Pacific, is located at 9ºS and 150ºE and composed of a double chain of nearly a thousand islands extending around 1500 km in a south-eastern direction. The Solomon Islands has 10 provinces: Choiseul, Western, Isabel, Central, Guadalcanal, Malaita, Makira, Temotu, Rennell and Bellona, as well as Honiara City). The Solomon Islands is located 2,000 km northeast of Australia. Most of the Solomon's islands are of volcanic origin as the archipelago is located along the "Pacific ring of fire", in the subduction zone between the Pacific and Indo-Australian plates. Though the total land area of the Solomon Islands is around 30,000 km² the country has a total marine area of around 1.3 million km². The total population of the Solomon Islands is estimated to be over 600,000 with 95% of Melanesian ethnicity speaking over 70 local languages.

2.5 Research Process

The main stages of the research process were review of literature, experimental data collection, data analysis and write-up. A literature narrative review (Chapter 3) summarizes the understanding of this study topic. This included online and library searches for articles, reports and books. To record mosquito behaviour, this study used barrier screens to sample mosquitoes and human landing catches to sample biting mosquitoes. To understand human behaviours at times of peak exposure, questionnaires and movement diaries were used. To determine the specificity of antibody responses, blood was collected from villagers and serum subsequently separated. Detailed environmental recordings were made using portable Kestrel 4500 weather meters. Fieldtrips to the Solomon Islands were conducted throughout the year to collect data and account for patterns of seasonality. ONA (an online data collection platform) was used to collect and store data collection sheets. Microsoft Excel was used to store data on the JCU Tropical Research Hub before statistical analysis with R and ArcGIS used for mapping and geographic analyses. The barrier screens were trialled in four different villages but were not suitable for sites with low mosquito densities so detailed studies were conducted in the two villages with highest densities. Sample movement diaries were also trialled with two households to optimize questions and to determine suitability of the method.

2.6 Ethical considerations

Prior to commencement of fieldwork for this PhD, ethics approval was granted by both James Cook University Human Research Ethics Committee (No. H6840 & No. H6488) and the Solomon Islands Health Research and Ethics Review Board (No. HRE046/16 & HRE002/16). The three main ethical

issues in this thesis relate to the human landing catch, collection of blood samples and the recording of human behaviours.

The human landing catch (HLC) method for collecting mosquitoes does have potential occupational health challenges as potentially exposing villagers to infectious bites. Human landing catches are the standard techniques used by the Solomon Islands National Vector Borne Diseases Control Programme and is necessary as alternative methods of catching the *An. farauti* in the Solomon Islands has, thus far, not been found. Individuals hired as mosquito collectors were recruited from the study villages. Adults greater than 21 yrs of age were recruited, the methods and any risks of the study were explained, and an informed consent form signed prior to initiation of the work. Although only adults who were likely to have some immunity to malaria participated and although they were instructed to capture the mosquitoes before they bite, there was some risk that they might be bitten by an infectious mosquito. We provided malaria medication (Coartem) if needed by participants though through-out the study period no participants recorded having malaria.

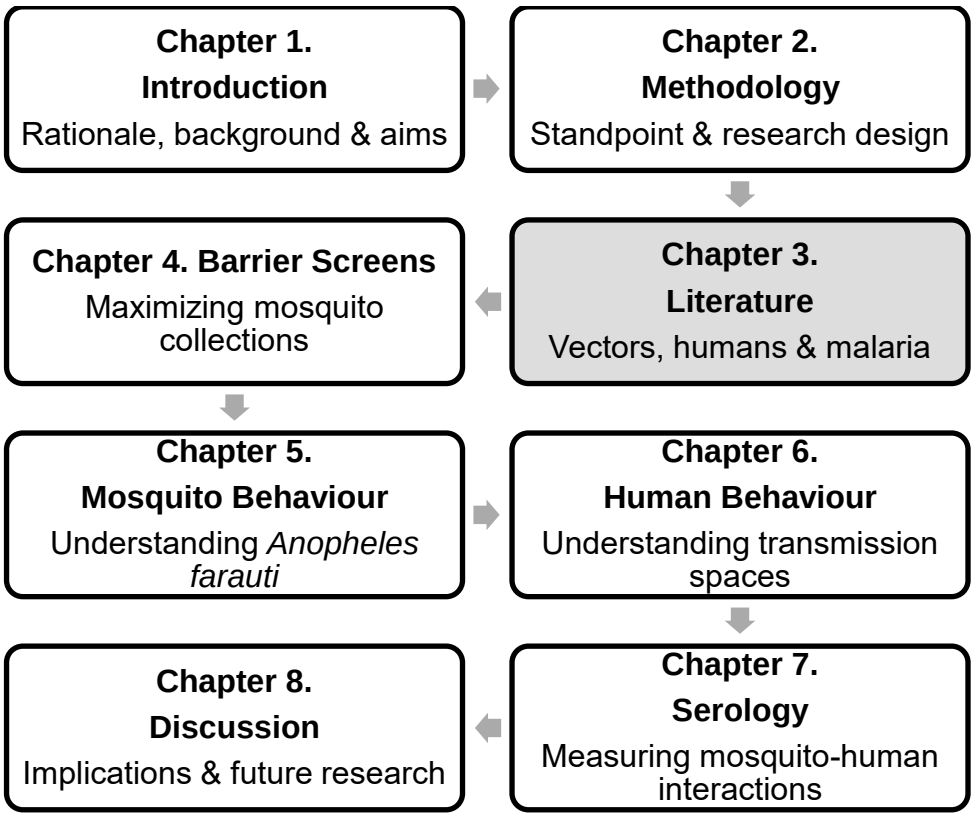
The collection of blood samples causes some discomfort and pain during finger prick sample collection and then the confidentiality of samples taken. To minimize discomfort experienced healthcare staff collected blood samples and were on standby for any complications that may arise. The responses, details and results data were de-identified to ensure the privacy of individuals who consented to the studies. The raw data forms are kept in a secure location at Cairns Campus, James Cook University. Community sensitization measures were undertaken including a general community informational meeting in each village and accompanying printed information was provided to households of each community. These documents included information about the purpose of the study and the procedures. The information sheet and the consent forms were translated into Solomon Island *pijin*. All interviews were conducted by local persons with knowledge of and experience in the communities and languages.

The monitoring and recording of people's behaviours and movement required participant written informed consent, confidentiality respected as to privacy of information and consideration of local culture. All information collected has been kept completely confidential. Hard copy questionnaire forms are kept in a secure location at the Cairns Campus, James Cook University. The privacy of the individuals who participated in the study are protected, with participants de-identified in the datasets and published material. Prior to commencing the study, written informed consent from participants was obtained after explaining the nature of the research and any risks associated with the work. All questionnaire surveys were conducted by the principle researcher and experienced health workers from the area (all of whom were Solomon Island citizens). All investigators were fluent in Melanesian *pijin* which was used for all communications with study participants. The studies were carried out with the assistance of a local interpreter to ensure that all cultural customs are respected.

3 Literature Review

3.1 Introduction

This third chapter is the literature review, which examines, in-depth, two critical central themes, mosquito behaviours and human movement as well as a broad overview of essential background data on malaria, vector control and the Solomon Islands. This thesis sought to define the spatial-temporal interface where malaria vectors and human populations interact and where transmission predominantly may occur. It therefore requires a broad understanding of malaria transmission and control, vector biology and human behaviours. Hence, this literature review will cover key aspects of malaria and vector control with a focus on transmission in the Solomon Islands by the dominant vector, *An. farauti*. This review will examine critical publications on mosquito behaviours and human movement studies



3.2 Vector-borne disease

Vector-borne diseases are parasites, viruses and bacteria transmitted to humans mainly by mosquitoes, sandflies, triatomine bugs, blackflies, ticks, tsetse flies, mites, snails and lice. Vector-borne diseases are responsible for 17% of all infectious diseases killing more than 700,000 people per year with 80% of the world's population at risk [26]. The most efficient vector and transmitter of a large range of diseases is the mosquito, attributed to diseases such as dengue fever, lymphatic filariasis, Zika, Japanese encephalitis, West Nile fever and malaria. The most devastating of these diseases is malaria with over 200 million cases and over 400,000 deaths per year. Human malaria is a parasite caused by four species, *Plasmodium falciparum* (*Pf)*, *Plasmodium vivax* (*Pv)*, *Plasmodium malariae* (*Pm)*, and *Plasmodium ovale* (*Po)*, with Pf and Pv the most prevalent. Malaria has a complex multi-stage life cycle across both the mosquito vector and human host and is transmitted by around 41 dominant species of mosquitoes belonging to the *Anopheles* genus [20].

3.2.1 Vector control

Most vector-borne diseases can be controlled effectively with well-implemented vector control. Vector control consists of a range of interventions that reduce human-vector contact, lower vector survivorship or prevent the infection and transmission of vectors with human pathogens [26, 39]. The two recommended core vector control interventions for malaria are insecticide treated nets (ITNs) and indoor residual spraying (IRS). ITNs are bed nets treated with an insecticide. ITNs provide both a physical barrier against biting mosquitoes and an insecticide that kill mosquitoes that land on the net. Long-lasting insecticide treated nets (LLINs) are a new type of ITN in which the insecticide remains bound to the net for up to 20 washes and effective under field conditions for up to 3 years. IRS is the application of a long-lasting insecticide to the walls and ceilings of houses and targets mosquitoes that rest indoors. ITNs and IRS are estimated to be responsible for an estimated 68% and 10% of the malaria clinical cases averted from 2000-2015 in sub-Saharan Africa [30]. The other supplementary malaria vector interventions include larval source management (LSM) and house improvements [39]. Alternative vector control methods to LLINs are needed to eliminate malaria especially in the southwest Pacific including the Solomon Islands [41].

3.3 The Solomon Islands

The Solomon Islands is an archipelago of nearly 1000 islands spread across the southwest Pacific. Malaria burden in the Solomon Islands is one of the highest outside Sub-Saharan Africa with ninety-nine per cent of the population at risk of contracting malaria. The Annual Parasite Incidence (API) significantly declined from a peak of 422 cases per 1,000 people in 1993 to 40 cases per 1,000 people

in 2015. However, the malaria death rate in the Solomon Islands is quite low with only 4 deaths reported in 2017 directly attributed to malaria[10].

Malaria transmission in the Solomon Islands is both temporally stable with little seasonality and quite spatially heterogeneous. Transmission occurs predominantly within 2 km of the coast which correlates with the distribution of the primary vector, *An. farauti*. Endemicity varies considerably between provinces: Rennell and Bellona Province is currently considered non-endemic. Isabel, Western, Choiseul and Temotu provinces are considered to be low endemic. Honiara City, Makira-Ulawa and Guadalcanal provinces are considered to be medium endemic. While Central Islands and Malaita provinces are highly endemic. A third of malaria cases are *P. falciparum* and 62% are *P. vivax*.[10]. *Plasmodium malariae* and *P. ovale* are present but uncommon and rarely diagnosed. The main malaria vector is *An. farauti* (described in detail later) with a secondary vector is *An. punctulatus*, being relatively uncommon with a patchy distribution [42].

Malaria control in the Solomon Islands started during the Second World War in the 1940s with the arrival of foreign troops. Then there was the eradication era of the 1960s and 1970s with IRS using DDT as the primary intervention. During the mid-1970s, the API for malaria was at its lowest ever level with less than 40 per 1,000 people. However, changes in the biting habits of the main vector occurred following the use of IRS [12] reducing the effectiveness of IRS. Subsequently, the withdrawal of vector control measures was followed by a resurgence of malaria to the highest ever APIs in the mid-90s of over 400 API per 1,000 people. The introduction and widespread use of first the ITN in the 1990s and then LLINs resulted in major reductions in malaria to 40 cases per 1000 in 2015, levels equivalent to that seen when IRS with DDT was used [9].

3.3.1 Anopheles farauti

The *An. punctulatus* group is composed of 13 species including the *An. farauti* complex with 8 species including *An. farauti* [13]. The members of the *An. punctulatus* group are generalists with broad feeding and oviposition habits which has aided their dispersal [43]. Dispersal of mosquitoes into the Australian-Pacific region is likely to have happened during the Pleistocene (2mya – 10,000ya) where glacial periods dropped sea levels to more than a 100m below current levels. Long distance mosquito dispersal is mostly by wind with human movement helping relatively recent movement [43]. The ocean is a substantial barrier to dispersal with restricted gene flow between *An. farauti* populations in the Solomon Islands. Hence, it is believed that the behaviour shifts to early biting in *An. farauti* has happened independently in different island sub-populations [44].

Anopheles farauti is the primary malaria vector and the most abundant anopheline in the Solomon Islands [13]. Distance from the ocean strongly influences *An. farauti* distributions giving it a mostly coastal distribution throughout its range from eastern Indonesia through the Solomon Islands to Vanuatu

(and including northern Australia) [45, 46]. *Anopheles farauti* were one of the first species in the world to record behavioural resistance to vector control [12]. Prior to the use of IRS and ITNs, *An farauti* would rest around houses at night before and after feeding, but preferred outdoor resting during the day in cool, humid and shaded areas [47, 48] such as in vegetation, holes and pits [49]. After wide-scale implementation of control measures indoors (i.e., IRS and ITNs), *An farauti* now infrequently bites indoors [12]. The peak biting time of *An farauti* is early evening (between 6 pm and 9pm) as found in 4 provinces in the Solomon Islands (Central, Temotu Western and Guadalcanal Provinces) [18, 50, 51]. The exophagic biting habit of *Anopheles farauti* compromises the effectiveness of IRS and LLINs [15, 52]. However, a small proportion of the *An. farauti* population do enter houses and come into contact with the insecticides in IRS and LLINs [18] and coupled with a short feeding cycle, LLINs and IRS still exert significant control [15].

3.4 Mosquito behaviour

Understanding mosquito behaviours led to the development of vector control tools such as ITNs and LLINs which have saved millions of lives. There is a need therefore, to understand the biology and ecology of vectors better to further identify vulnerabilities that can be targeted by control measures [21]. If temporal, spatial and directional patterns of mosquitoes can be better understood, more efficient placement of surveillance tools to specifically target vectors will be possible. Knowledge of mosquito movements will also be useful to determine and define the factors that create or enable mosquito foci in villages; such areas can be then targeted for control to minimize contact between mosquitoes and people.

3.4.1 Mosquito flight behaviour

Mosquitoes fly for a variety of reasons and have characteristic activity patterns. Most anopheline mosquitoes have a nocturnal pattern of daytime resting and night-time flying [53]. Flight patterns can be basically divided into random flight, targeted flight and long-distance wind dispersal. Random searching flight also known as non-appetitive flight occurs until visual or chemical cues associated with blood sources, oviposition sites, sugar or other behavioural activities are encountered. This flight tries to get the mosquito in the right habitat to encounter cues and may be assisted by the wind. Targeted or appetitive flight then occurs based on the visual or biochemical cues encountered. This flight behaviour may be classed as close range [54] and these flights are mostly active mosquito flight in response to these cues [22, 55].

Mosquito flight altitude ranges from within 1m of the ground to 20m above the ground [56]. In Kenya, *An. gambiae* fly at 1.8m when entering and exiting houses eaves (the gap between the wall and the roof)

[57]. Mosquito flight speeds also vary, with average speeds of 0.25m/s [58] and maximum speeds of host-seeking females between 1.4 and 1.8m/s (recorded in laboratory conditions) [59]. Flight range varies by species, from 50m to more than 50km (wind-aided dispersal) [55]. The average flight distance for anophelines was determined as 542m by a meta-analysis of 18 mark release recapture (MRR) experiments with *An. farauti* having a flight range of up to 1.6km [60]. Flight movement is also thought to be age dependent [61], with older mosquitoes flying further [62]. However, if all the mosquito's needs are met in a particular area then there is little need for longer flights [22, 43]. Mosquitoes will fly to escape droughts and *An. gambiae* will fly to either aestivate (a much longer resting period during warm or dry conditions) or to migrate (longer range flight) [63]. Aestivation is dormancy with reduced activity including reduced reproduction and blood meal frequency; enabling one *An. gambiae* to survive 7 months through a Sahelian dry season [64].

A typical daily anopheline flight pattern is believed to be flight out of diurnal resting areas such as the swamp forest to blood feeding areas such as in a village, as observed in The Gambia [65]. Although there are specific differences between species [60], mosquito flight is in response to six needs; blood-feeding, sugar-feeding, mating, ovipositing, resting [22] and to escape unfavourable conditions [54]. In the following sections these six specific adult mosquito behaviours will be reviewed.

3.4.2 Feeding

Mosquitoes feed on blood and sugar [66, 67]. Blood-feeding (by females only) is required for egg production, the numbers of eggs laid is determined by the quantity and nutritional quality of blood [68] Anophelines generally prefer mammalian blood [69], though some anophelines blood feed on birds, amphibians and reptiles [70, 71]. Detection of a combination of cues associated with potential blood meal hosts including carbon dioxide, heat and odours (volatile chemicals) emanating from skin surfaces drive flight behaviours to locate blood sources [58, 72, 73]. Carbon dioxide is the most important long-range cue that mosquitoes use to find hosts [74, 75], with other odours and heat acting closer to the blood source [58, 76].

Mosquito host selection depends on both extrinsic and intrinsic factors [67, 77]. Extrinsic factors include climate, humidity and host factors such as heat and odours (as above), nutritional quality of blood, host body size, gender and defensive behaviours of the potential hosts. Intrinsic factors are the genetic and physiological makeup of the mosquito [67]. Availability of hosts will also influence feeding behaviour [67, 78, 79] and is an important factor in determining the mosquito population size [80, 81].

Vectors which are both zoophagic and anthrophagic pose challenges to malaria elimination with current control methods: they can maintain residual transmission by evading vector control targeted on humans (e.g., LLIN and IRS) [82] by feeding on animals, though are less efficient vectors. Host selection plasticity (the capacity to select among host species for blood meals based on relative abundance and

availability) can reduce the impact of vector control methods such as LLIN as vectors can feed on alternate hosts. These vectors can therefore maintain a high population size and sustain transmission [41].

Anopheles farauti will feed on a wide variety of warm-blooded vertebrates when available [47, 83, 84]. In Papua New Guinea (PNG), *An. farauti* preferred blood meals on dogs and pigs compared to humans [84]. However, in the Solomon Islands there is a slight preference for human 81 - 91% of blood meals are on humans [71, 85]. The very high proportion of blood meals on humans can be explained by the limited availability of alternate hosts to humans resulting in the majority of blood meals being on humans [85]. Similarly, in Tanzania, *An. arabiensis* feeding behaviour was dependent on the presence or absence of livestock in households [78]. Mixed blood feeding due to interrupted feeding is common in anophelines in PNG villages [84] and also is found in Africa where multiple blood feedings per feeding cycle has been observed [86].

Sugar from flower nectar, honeydew, fruit and other plant materials provides mosquitoes with energy for flight [87]. It is the only food for male mosquitoes but seems to be supplementary for females [70, 88], as was observed in *An. gambiae* females who could replace sugar-feeds by increased blood-feeding [89]. Male mosquitoes sugar feed roughly 8 times more frequently than females (who may sugar feed between blood meals) [88]. Sugar meals seem to affect blood feeding in *An. gambiae* post emergence by reducing the amount of blood and delaying the time of blood feeding [90]. *Anopheles gambiae* females need to feed 24-36hr post emergence and there is a preference for sugar for the first meal. However after 5 days, females change their behaviour to predominantly prioritising blood over sugar [91]. For *An. gambiae* in Mali, peak feeding on plant sugars was between 1930-2200hrs and 0400-0500hrs while the peak human biting time was around midnight [92].

Floral odours are cues to find sugar sources [93] and mosquitoes seem to be more attracted to flowers, as *An. sergentii* was caught 70-130 times more in traps that were baited with flowers than flowerless branches [94]. In Mali, both fruits and flowers were attractive, but the attractiveness index for the most attractive flower was much higher than the most attractive fruit [92]. Some plants are favoured as sugar sources over others [99]. From 40 plants found around larval habitats only 3 were fed upon by *An. sergentii* males [100] and *An. gambiae* fed preferentially on 4 of 13 local endemic plants for sugar [101], with feeding on these preferred plants associated with increased fitness [102]. In Mali, *An. gambiae* showed an attractive preference for 6 of 26 fruits and seedpods and 9 of 26 flowering plants [92]. The availability of sugar sources is also a major determinant of local mosquito population fitness [95] and vector potential [96] with increased survivorship found with greater access to sugar-rich plants for *An. gambiae* [97, 98].

3.4.3 Mating and oviposition

Anopheline mosquitoes mate in swarms formed by males around dusk [103] and the sound from conspecific female wingbeats is believed to influence mate choice [104]. Most *An. gambiae* females blood feed and mate on the second night after emergence but only become gonotrophically active (egg laying) after the third night. Males emerge a day earlier than females which allows for maturation and dispersal thereby limiting cross breeding with siblings [105]. The gonotrophic (time from one oviposition to the next) and feeding (time from one blood meal to then next blood meal) cycles vary among species [106]. Gonotrophic cycle duration is influenced by parity with nulliparous mosquitoes taking twice as long to complete the initial gonotrophic cycle [107]. *Anopheles farauti* has a relatively short feeding cycle of 2.1 days in the Solomon Islands [15] compared with a range of 2-4 days for most anophelines [108]. Peak oviposition time also varies among species and is thought to be regulated by light with *An. aquasalis* preferring between midnight and 4am [109] and *An. gambiae* before midnight, [110, 111].

Anophelines will hover above the water to deposit their eggs on water and *An. farauti* prefers the margins of lagoons, ponds, ground pools and temporary artificial water [112, 113] and will oviposit in multiple sites [114]. Finding and choosing oviposition sites is important for all mosquitoes and oviposition flight, as with all other mosquito flights, is dependent upon weather factors such as rainfall, humidity, temperature and wind speed. Female mosquitoes use chemical cues to differentiate and select oviposition sites [115] including volatiles produced from the soil [116]. Cedrol, a soil volatile, is an attractant chemical cue for gravid *An. gambiae* for orientation towards potential oviposition sites [116]. Microbial activity also produces odours that act as cues to help gravid mosquitoes find suitable oviposition sites [117]. Vision becomes more important during pre-oviposition behaviour once the oviposition site is found [118], visual contrast and substrate moisture are important qualities for *An. gambiae* oviposition sites [119, 120]. Gravid *An. gambiae* were attracted to shiny sticky surfaces that may appear to them as larval habitats [121] and laid less eggs on substrates with grasses [122]. *Anopheles gambiae* also prefers water with fewer impurities such as rainwater over more organically rich water from farms, forests and wetlands [123] and also avoid oviposition in sites that contain predators and competition from other mosquito larvae [111, 124-126]. *Anopheles melas* pre-oviposition behaviour involves short, darting flights around the site to test the substrate [110].

3.4.4 Resting

Mosquitoes fly from larval habitat areas soon after emergence and are believed to stay in cool and humid resting sites close to feeding areas [83]. Fully engorged mosquitoes will rest after blood feeding and do so both indoors and outdoors [79]. *Anopheles stephensi,* in Iran, rests in animal sheds after feeding on humans [127]. Endophilic (indoor liking) mosquitoes tend to rest indoors on walls whilst

exophilic (outdoor liking) mosquitoes are harder to find as they rest on vegetation outside [36]. Indoor released *An. darlingi* were observed to fly up and rest on ceilings in Brazil [128]. In Java, median nocturnal indoor resting heights of anophelines were measured: *An. aconitus, An. subpictus, An. indefinitus* rest less than 38cm off the ground while *An. kochi* was found within 68cm from the ground and *An. barbirostris* was found up to 229cm above the ground [129]. *Anopheles gambiae* was observed to rest outdoors in dense vegetative growth in salt bushes and also in crevices in the ground [130]. Though there is a volume of literature describing mosquito behaviour there is still a lot of unknowns regarding the details, such as specific locations and times for resting especially for *An. farauti*.

3.4.5 Factors influencing flight

Wind, temperature, humidity, illumination and the availability and location of blood and sugar resources are the main physical factors that influence mosquito flight [54] as well as ecology and population dynamics [131]. *Anopheles gambiae* survival is believed to be a function of temperature and relative humidity [132]. Temperature is a sensitive variable for adult mosquito survival [133], with optimum flight performance for *Aedes aegypti* observed at 21°C and flight ability was greatly reduced when temperatures were below 15°C or above 32°C [134]. Optimum humidity for mosquito activity was observed between 70-80% for *An. quadrimaculatus* [135].

For nocturnal flyers, the change in light intensity at sunset is thought to trigger flight [136]. The moon is also thought to influence mosquito behaviour by promoting flight [137, 138] and aids the mosquito in locating sites of interest such as oviposition sites [139]. Moonlight affects the biting density and time of biting of *An. farauti* [140] and mosquito movement is greatest during the full moon [141].

Wind more than any other factor influences the flight of mosquitoes [24, 62]; however, responses of anopheline mosquitoes to wind has been poorly studied and understood [142]. Increased winds will dilute odours that mosquitoes follow [143] with wind speeds above 1.3m/s limiting mosquito flight [141]. Mosquitoes can also be passively carried long distances by the wind [22, 144-146]. Wind direction also plays an important role in mosquito flight behaviour. After oviposition mosquitoes fly upwind and crosswind in search of hosts for blood feeding [58, 147] with and without host cues [148]. It was observed that mosquitoes flew crosswind in non-appetitive flight until picking up an odour before following it upwind [149]. Upwind flight is more pronounced closer to the ground [150], due to visual cues and reduced wind speeds [143]. Higher wind speed disperses the concentration of carbon dioxide plumes, reducing efficiency of host-seeking activity and can contribute to reduced flying in anopheline populations, especially when windspeeds are greater than mosquito flight speeds [142]. Effects of wind around larval habitats are also important as the creation of waves may affect larval mortality [142].

3.5 Measuring mosquito behaviours

3.5.1 Human landing catch

The human landing catch (HLC) is the most useful and universally successful method for collecting mosquitoes including anophelines that blood feed on humans [55], though in various environments and dependent on specific vector behaviour other tools may be useful [151]. The HLC is relatively easy to carry out and does not require expensive equipment or extensive technical expertise and is the most direct method for assessing the human-biting rate (HBR) which is needed to calculate entomological inoculation rates (EIR) [55]. The disadvantages of HLC are the requirement for close supervision, concerns regarding the exposure of collectors to pathogens such as arboviruses, the labour intensive nature of the technique and the expense associated with hiring humans to perform the catches [152]. With continued focus on vector control there is a need for high-quality entomological monitoring and for many mosquitoes, an alternative for HLC that is as sensitive and versatile has not yet been found [152]. A study by Gimnig *et.al* [152] comparing malaria incidence in collectors with malaria chemoprophylaxis to non-collectors in the same village over the same time found that the incidence of malaria was significantly lower in individuals who were HLC collectors. Therefore, with proper prophylaxis, HLC-based studies can be carried out without increased risk of malaria infection in collectors [152]. Other trapping methods that use humans as the lure for mosquitoes includes, tent traps, light traps, human odour baited traps and mosquito net traps such as the Mbita trap [55]. In the Solomon Islands, HLC is still the most efficient method for entomological studies as other methods have reduced catch efficiency.

3.5.2 Non-Attractant & Non-Mechanical Sampling

For many studies, sampling mosquitoes without influencing their behaviour and movements is desirable. The use of lures in traps or the use of live animals or humans to collect mosquitoes changes the natural movements of mosquitoes. Non-attractant methods reduce biases associated with entomological sampling and non-mechanical methods are generally cheaper and easier to deploy in rural isolated areas such as in Solomon Island villages. Malaise traps, ramp traps, stationary nets and sticky traps are non-attractant methods for collecting mosquitoes. In this section methods for collecting mosquitoes that are both non-attractant and non-mechanical will be reviewed.

The Malaise trap was one of the first non-attractant traps for insects [153]. The Malaise trap was first designed to primarily collect wasps and would use the natural tendency of insects to fly up when encountering a barrier into a collecting container. Advantages of this trap are that it can be used anywhere including remote locations, it can be used in any weather condition (except high winds) and also in high mosquito density locations as a prolonged presence of the human collector is not required. It can also be standardized for comparing sites. The recommended placement for Malaise traps should

be at the borders of forests, fields and depressions at right angles to the main direction of mosquito flight [154].

Ramp traps were invented by Gilles [155] in 1969 to study directional mosquito movement. This trap basically guides mosquitoes up a ramp into a collection chamber. The direction of mosquito flight can be deduced by placing traps such that they face one side at a time. However, certain species respond visually, either positively or negatively, to the trap and therefore may bias results [55]. Stationary nets are made of nylon netting in a pyramid shape with the large 2x2m opening facing perpendicular to the ground to allow dispersing and migrating mosquitoes to fly into the pyramid then into a nylon collecting sleeve [156]. Sticky traps use adhesive compounds to create sticky surfaces that immobilize mosquitoes. These traps can be used to study flight direction of mosquitoes and also resting behaviours [55].

The barrier screen [36] is the most recent method for collecting mosquitoes that is both non-attractant and non-mechanical. The barrier screen is an insecticide free neutral (no bait or lure) net "trap" that intercepts mosquitoes as they fly in pursuit of blood meals, resting and oviposition sites, mating sites or sugar sources. The initial purpose for barrier screens was to capture blood fed mosquitoes for blood meal analysis and determination of the human blood index. This method involves constructing a fence up to 20m long of non-attractant durable netting, such as shade-cloth, attached to bamboo poles. Mosquitoes when flying between feeding, oviposition, mating, sugar feeding and resting sites would temporarily stop when encountering the barrier. When stopped and resting on the barrier screen, they are easily visible and can be collected using mouth aspirators. Previously it was very difficult to collect mosquitoes outdoors after blood feeding to determine the human blood index. The barrier screen makes finding recently engorged mosquitoes easier to see and collect. Directional behaviour patterns can also be deduced for mosquitoes of different physiological status (e.g., blood-fed, unfed, gravid) [36]. The direction of movement and the motivation for their movement is inferred from the side of the barrier screen on which the mosquito is collected and the proximity to presumptive targets (oviposition sites or potential blood meals sources) for mosquitoes. The major strength of this method is that it is largely an unbiased method for intercepting flying mosquitoes for collection and gives us valuable insights into adult behaviours.

The major difference between the Malaise trap and the barrier screen is the use of a collecting container in the Malaise trap as opposed to collecting done by humans for barrier screens. However, barrier screens incorporating eaves to minimize the inspection frequency may act as a type of collection container.

3.6 Human behaviour

The three elements of malaria transmission are the parasite, the vector and the human. Human behaviour has received the least attention, one reason eradication was not achieved in the 1960s [157].

Understanding malaria transmission requires the analysis of human mobility in association with parasitological and entomological analyses as all are parts of a complex transmission system. Mobility can be classed by space and time, with time ranging from daily regular to long-term irregular movements [157]. It is essential to understand human behaviour and distribution patterns where malaria is endemic and how human behaviours and distributions affect their exposure to biting mosquitoes [158, 159]. Human movement is a challenge to progress against vector-borne diseases [26] and influences malaria through importation of parasites into lower endemic area and is thus a challenge to elimination [160, 161] [162]. Human mobility contributes to transmission of malaria at scales that exceed mosquito flight limits [163], especially so with asymptomatic individuals who can act as reservoirs of infection by carrying the parasite with them [162] and may accelerate the spread of drug resistant malaria parasites [164].

Transmission of mosquito-borne diseases depends on the degree of human–vector contact. [165, 166]. Thus, it is important to integrate an analysis of human and vector behaviours and movements to understand where and when malaria transmission occurs [165, 167]. Detailed knowledge of human and vector behaviours will allow us to better understand why malaria control strategies have worked and what might work better on the road towards elimination [168]. Further, determining the frequency of human-mosquito contact in time and space may identify potential opportunities to control malaria [169-171].

Planning for control and elimination also depends on identifying and understanding human movements [172, 173]. There is a need to develop new theories and models based on field data that explore mosquito ecology and behaviour and vertebrate host movement [174]. Human movement patterns vary across spatial, temporal, demographic and socio-economic groupings [173]. This information is necessary for models and critical for effective disease prevention programs [167, 175, 176]. Investigating locally and culturally acceptable methods of mosquito control and community responses to malaria and mosquito control in rural villages will also be extremely valuable [177]. This includes detailed mapping of social, cultural and behavioural aspects of village life in the design and implementation of control interventions [178]. Understanding social connections is also important as they influence transmission as movement patterns are based on social ties [179].

The importance of including human behaviour into malaria studies can be seen when the protective efficacy of bed nets is calculated through incorporating the sleeping behaviour of the human population (when people are inside bed nets in relation to the biting density of anophelines) to determine the protective efficacy of bed nets [158, 169, 180]. A study in Cambodia found that a major risk factor associated with malaria infection was staying overnight in the farm plot hut [181]. In Ethiopia, travel from the home village was a statistically significant risk factor for malaria infection [175]. In Thailand, movement into forest areas was related to malaria risk as well as those that travelled more [182].

There have been limited human behaviour studies on malaria in the Solomon Islands. A single study on Isabel Province used focus group discussions (FDG) and key informant interviews (KII) to discuss travel habits, malaria surveillance and management of malaria cases. The most common travel destination identified within Isabel province was to the provincial capital, Buala, and the most common travel destination outside of the province was to Honiara, the national capital [183]

3.6.1 Measuring human behaviour

Human behaviour, distribution and movement is a key factor in vector borne diseases at all spatial and temporal scales [167]. Human movement can be permanent or migratory, periodic or annual, return or short-term and routine or daily movements [173] and scale is important in determining appropriate methods for measuring human movement. [167]. At the local spatial scale, house to house movements play an important role in the spread of vector-borne diseases such as those that place hosts in higher risks [184].

Understanding small-scale heterogeneity in transmission needs high resolution human movement data [173]. Traditional human movement data has come from household travel surveys, cross-border and traffic surveys and census data [173]. Today GPS, mobile phones, satellites, air and shipping statistics and social media can track human movements [185]. Human mobility is dependent on past behaviours, therefore historical movement data can also be useful [186]. Matched surveys of mosquito and human behaviour can determine human exposure to vectors [38].

All technologies have advantages and disadvantages. Issues with GPS are cost, battery life and technical limitations and issues with mobile phones are applicability in low-resource situations and privacy concerns [167]. Census data does not tell us how and where people travel and interviews and surveys including travel diaries are inexpensive but can be limited by recall and interviewer biases [163, 167]. The spatial resolution of GPS loggers and satellite maps limit fine scale movement tracking in and around domestic structures [187]. GPS devices can assess time-location patterns though accuracy errors may exist especially at finer scale resolutions [188]. Call data records (CDR) from mobile phones can be used to follow anonymised individual travel patterns over large areas such as among or within countries or provinces, though probably not applicable to within villages [162]. CDR includes a timestamp, phone number and mobile tower, providing spatial and temporal data [189]. Use of mobile phones can help us identify larger scale ‘source’ and ‘sink’ regions in relation to malaria transmission [163]. Understanding migration episodes at a national level using mobile data is a promising method to monitor, interpret and respond to migration [189]. Android applications can also be used to collect dynamic demographic information using GPS satellite and mobile tower signals. Mobile phones are suited to collecting data in real time and space as they are already a part of people’s daily lives [190].

Many other studies have used unstructured [191] and structured [178] interviews have been used to survey knowledge, attitudes, practices, behaviours and perceptions of mosquitoes and malaria [16, 158, 168, 170, 178, 191, 192]. Information collected in such surveys includes demographic data [192], mosquito-biting prevention practices [178, 191, 192] such as bed net use [16, 168, 170], knowledge of malaria and mosquitoes [168, 191, 192], sleeping patterns [16, 38, 170, 171, 178], mobility between villages [168] and evening social activities [168, 171].

3.7 Serology, measuring interaction

Serological measurements are being developed to provide an alternative method for estimating the exposure of humans to biting mosquitoes [193] without the time and cost of collecting mosquitoes. When the vector injects saliva proteins into the host, the salivary proteins elicit an immune response. The prevalence of people with antibodies to the saliva can be an indicator of the exposure history of that person to mosquitoes. This technique therefore can be an alternative method to measure the potential exposure risk to malaria [194], especially in areas where malaria transmission is low [195].

The gSG6-P1 peptide from *An. gambiae* was proposed as a useful candidate for indicating anopheline exposure as antibodies to this peptide showed strong correlations with malaria rates and by extension, malaria transmission intensity and thus the densities of mosquitoes [196-198]. The SG6 salivary gland protein has been found in 17 *Anopheles* mosquitoes [199]. Evidence from Africa and South America suggests that the antibody response to this *An. gambiae* peptide is genus specific, not being found in other culicinae subfamilies, and can therefore indicate exposure to *Anopheles* bites [195]. Therefore, human antibodies recognizing this particular peptide might be used to estimate biting rates to other anopheline species in other areas, including the Solomon Islands (i.e., human antibodies generated in response to *An farauti* saliva may recognize the salivary gland protein of *An gambiae*). Furthermore, the immune response does not appear to elicit immunological memory [195]. The lack of immunologic memory means that antibody responses are short-lived and thus may reflect the short-term cumulative exposure to anopheline bites. Thus, this serological assay may track seasonal biting exposure where anophelines in the subgenera *Cellia* and *Anopheles* which have a SG6 protein are endemic.

3.8 Knowledge Gap Summary

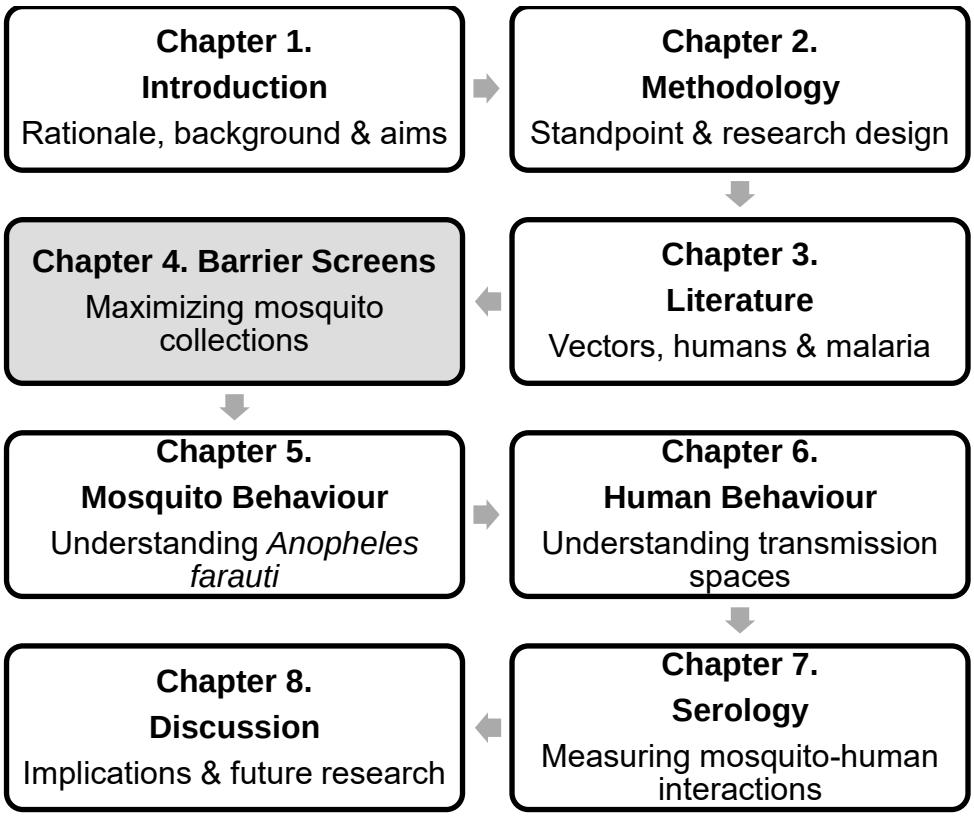
The road towards malaria elimination requires greater understanding of the vectors that transmit malaria [21]. Elimination campaigns need to be designed for the local context which means understanding the local ecology and behaviour of mosquitoes and the human host [23]. We need to better understand vector and human behaviours and how these interact to further combat malaria. To help us better understand these human-mosquito interactions, new and novel methods to give us new insights into

malaria transmission and mosquito human interactions are needed. Malaria risk is not evenly distributed - 20% of people receive 80% of infections; therefore efforts should be targeted to those at highest risk [25]. Knowledge of mosquito movements will also be useful to determine and define the factors that create or enable mosquito foci (areas with significantly higher densities of mosquitoes) in villages in order to minimize interaction between mosquitoes and people. If temporal, spatial and directional patterns of mosquitoes and humans are better understood, more efficient placement of control tools could specifically target vector behaviours for maximum impact in culturally acceptable ways.

4 Barrier Screens

4.1 Introduction

This fourth chapter is the first of four results chapters and is written as a standalone paper. It focuses on maximising barrier screens for mosquito collection as a tool to observe mosquito distributions. The specific research question is to optimise barrier screen design by evaluating fabric colour, fabric weight, the use of eaves and inspection frequencies with the output being numbers of mosquitoes collected. These experiments were conducted in Cairns, Australia just offsite from the JCU campus. This chapter has been published in *Parasites & Vectors* [200] and can be read in the appendix.



4.2 Background

Mosquito sampling using long-range odorant lures (including human landing catches) give useful insights into mosquito densities attracted to fixed locations [55] but little work has been done to understand the movement of mosquitoes between locations. Observing insects in their natural flight patterns without influencing their behaviour requires using capture methods with minimal long-distance attractants [201]. Almost all sampling techniques are prone to biases and different techniques will be better suited to collecting different subsets of insect populations [202]. Sampling techniques without odorant attractants (hereafter referred to as passive tools) are useful for representative sampling of entire populations (e.g., males and females of all physiological states (unfed, sugar fed, blood seeking females, recently engorged females as well as gravid females seeking oviposition sites)). Often the utility of traps is constrained by requirements for attractants or trap designs (size) or power requirements for light or fans. Passive tools without moving parts or power requirements are simpler, cheaper, more robust and easier to deploy in isolated areas.

There are two functional types of passive tools; tools that provide estimates of resting mosquito numbers and tools that infer mosquito movement. Passive tools that target resting mosquitoes include pit shelters, resting pots and boxes such as the sticky resting box [203]. Common passive methods for collecting mosquitoes and inferring movement patterns include malaise traps, ramp traps, stationary nets and sticky traps [202]. The malaise trap was one of the first passive tools for insects [153] and is advantageous because it can be used in remote locations, in almost any weather condition (with the exception of high winds), where mosquito densities are high, and a human collector is not required. While the malaise trap can capture insects approaching from all directions, it generally does not allow the direction of flight to be determined. Ramp traps were invented by Gillies [155] in 1969 to study directional mosquito movement. The ramp trap guides mosquitoes into a collection chamber by a ramp with the direction of mosquito flight inferred by the orientation of the trap as insects can only enter the trap from a single direction. However, some mosquito species respond visually, either positively or negatively, to the ramp trap, thus potentially biasing the estimates of species densities [55]. Stationary nets are similar to the ramp trap but are made of nylon netting in a pyramid shape which allows flying mosquitoes to enter a large opening into a collecting sleeve [156]. The stationary net is usually oriented to capture mosquitoes flying in a single direction. Sticky traps use a sticky surface to immobilize mosquitoes [55] with movement inferred from the direction the trap is facing.

Collection of outdoor blood fed resting mosquitoes can be extremely difficult and time-consuming [202]. The barrier screen is the newest passive attempt at collecting outdoor resting mosquitoes [36]. The barrier screen was developed to determine the frequency of mosquito blood feeding on different host species by capturing an unbiased sample of blood fed mosquitoes outdoors. The barrier screen is a passive tool that intercepts mosquitoes as they fly while tracking odorant cues of blood meal sources,

resting, oviposition sites, swarming sites or sugar sources. The barrier screen allows information on the flight direction of the mosquitoes collected to be inferred from the side of the screen on which the mosquito was collected in relation to the proximity of houses, larval habitats, sugar sources and likely resting sites [36]. Barrier screens have been constructed from a variety of durable materials, such as shade-cloth, made from materials including polyvinylchloride-coated polyester, polyethylene and cotton [36]. Flying mosquitoes will encounter the screen and temporarily rest. When resting on the barrier screen, mosquitoes are easily visible and can be collected using aspirators. The impact on numbers of mosquitoes collected on barrier screens as a function of the barrier screen materials and design has not been evaluated.

To maximise mosquito numbers collected using barrier screens, different attributes of barrier screen construction (e.g., cloth weight, cloth colour, design and inspection frequency) were systematically evaluated by comparing the numbers of mosquitoes captured when each individual attribute was employed. Optimising barrier screens will facilitate this tool to be utilised to better understand the natural distribution and movements of mosquitoes in time and space and thus to better inform how to monitor and control mosquitoes.

4.3 Methods

4.3.1 Study site

The study was conducted in Smithfield, 15 km north of Cairns, Queensland, Australia (16.8221° S, 145.6972° E) (Figure 4.1). The study site is situated proximal to an extensive swamp that provides larval habitats for a range of mosquito species near the Young Animal Protection Society (YAPS) Animal Refuge and the Smithfield Recycling Transfer Station. The specific location borders the recycling station and swamp forest with a tree canopy dominated by *Melaleuca*, *Archontophoenix* palms and *Ceriops* mangroves [204]. The closest house was ~500 m away. The site has a tropical climate with hot, humid summers and cooler, drier winters. Average annual rainfall is 1992 mm and temperatures range from 23-31 °C in the summer and 18-26 °C in the winter [205]. Although malaria was eliminated from Australia in 1981 [206], the former dominant vector, *Anopheles farauti*, is a common mosquito in northern Australia.

4.3.2 Barrier screen design and mosquito sampling

Barrier screens were constructed of 6 m straight lengths of 1.8 m high, high-density polyethylene (HDPE) UV stabilised shade cloth (Coolaroo® Gale Pacific Ltd) attached to poles and erected parallel to the swamp forest. On the side of the barrier screen opposite to the swamp, 150 g of dry ice was placed within a 2 litre cooler jug with four small holes to release CO_2. This was then placed 1 m behind each

barrier screen to simulate a blood meal source. Although not usual, the dry ice was used here to maximise collections on the barrier screens and to facilitate a more powerful direct comparison of the different construction attributes.

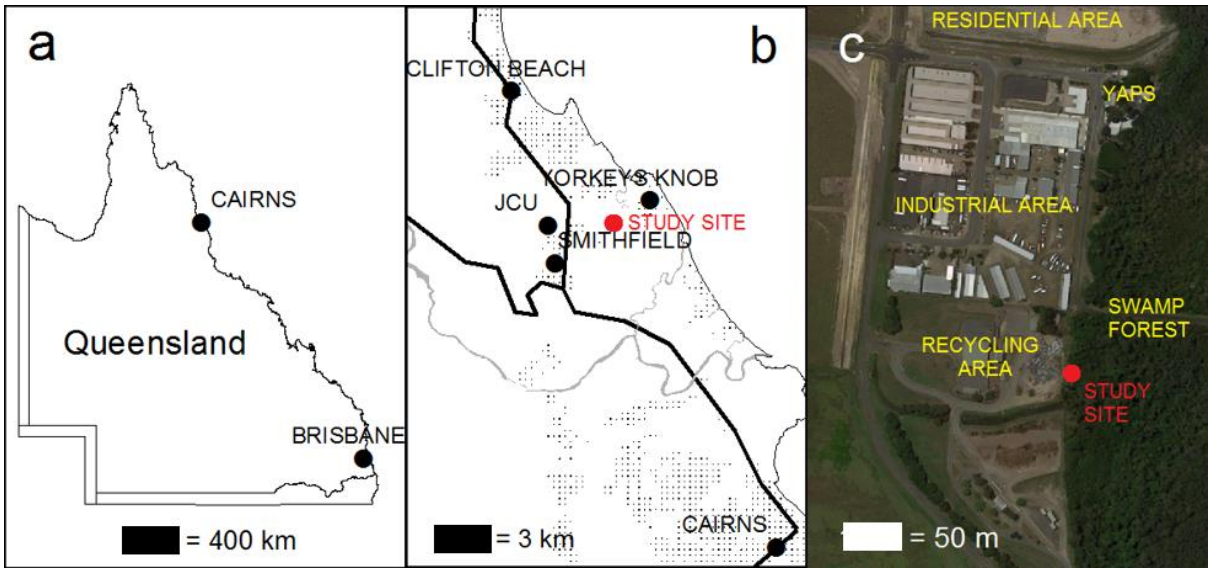


Figure 4.1 Map of Queensland (a) showing Cairns and Smithfield (b) as well as the layout of the Young Animal Protection Society (YAPS) study site (c)

The impact of four basic barrier screen parameters on numbers of mosquitoes collected were evaluated individually as follows: shade cloth weight, shade cloth colour, construction design and frequency of inspection. After the optimal shade cloth weight was determined by comparing different shade cloth weights of identical colour, that weight was then used to determine optimal colour. The optimal weight and colour was then used to evaluate optimal designs and inspection frequencies (see Results).

Cloth weight is defined by grams per square meter (g/m^2) with green cloth of 135 g/m^2, 160 g/m^2 and 214 g/m^2 tested, corresponding to 50 %, 70 % and 90 % shading, respectively. The impact of colour was then evaluated using white, green and black cloths of optimal weight (determined as described previously). Barrier screen construction design was varied to determine if eaves of 25 cm depth could improve collection efficacy. It was hypothesized that mosquitoes would remain on the screens for longer periods by baffles or eaves; therefore, three barrier screen designs were evaluated using the optimal weight and colour cloth as previously determined. Screens without eaves (a straight 1.8 m x 6 m flat shade cloth), screens with perimeter eaves (e.g. vertical eaves along both sides and a horizontal eave along the top) and a screen with complete eaves (e.g., vertical screens on both sides and three horizontal eaves at heights of 60 cm, 120 cm and 180 cm from the ground) Figure 4.2. The frequency of inspections on mosquito numbers collected was evaluated by inspecting sets of identical screens (optimised for weight and colour as described above) at intervals of 30 min, 60 min and 90 min during 3 hour sampling periods.

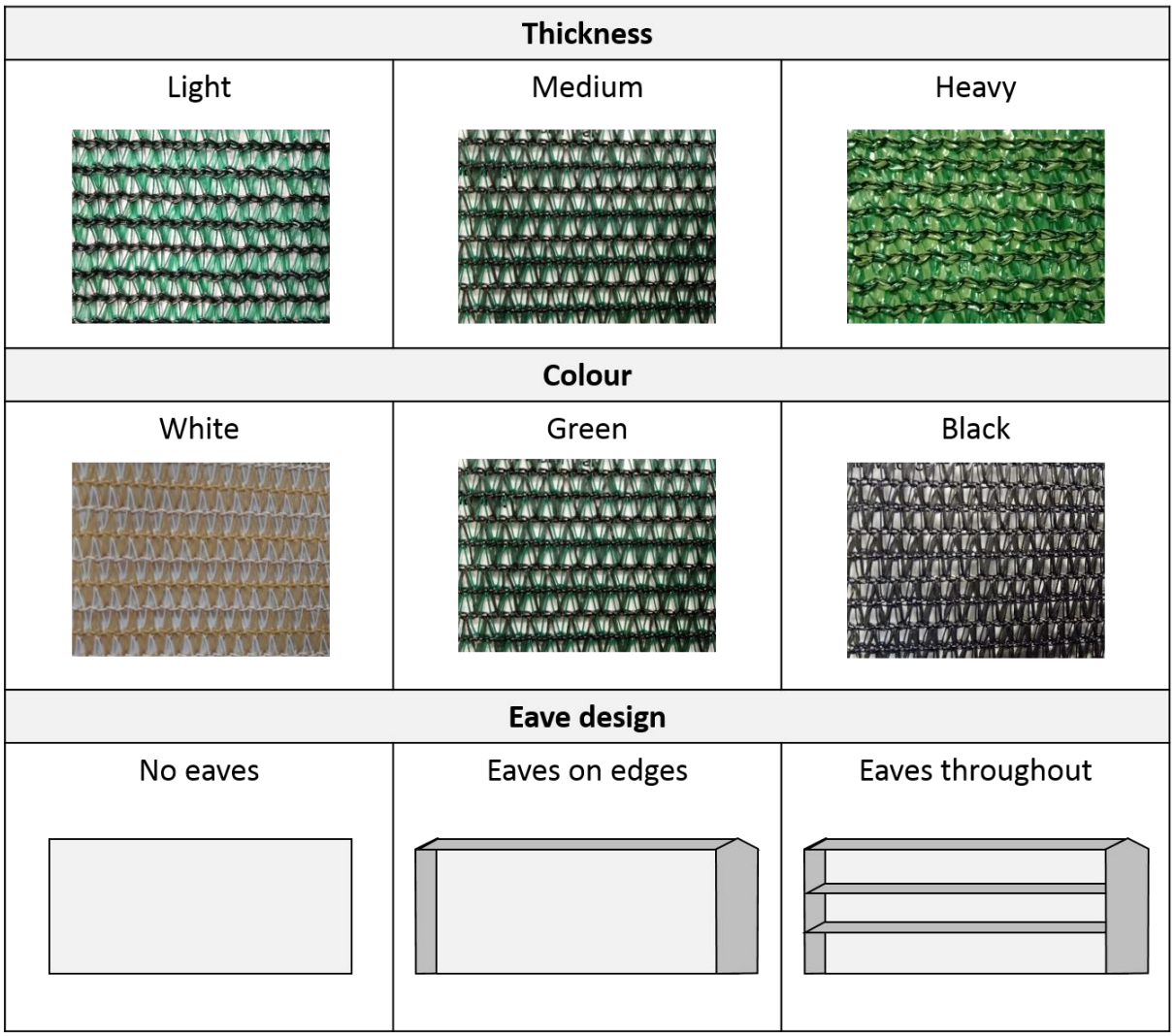


Figure 4.2 The factors that were evaluated to optimise the barrier screens included weight, colour and eave design.

A balanced Latin square design (3 × 3) was used to compare each of the experimental parameters. Triplicate barrier screens were erected in a row (separated by 2 m gaps). Each variable tested was rotated through all three potential spatial positions over three consecutive nights to eliminate any location associated bias (a full rotation of positions). For each variable three full rotations were completed unless specified otherwise.

Replicate barrier screens were examined for mosquitoes by a single collector from 1800 h until 2100 h using a mouth aspirator to remove resting mosquitoes from the screens. The collector applied mosquito repellent (active constituent 92.8 g/L Picaridin Aerogard®) and unless stated otherwise, mosquitoes were collected hourly with each searching event lasting approximately10 min per screen with the swamp side searched first. Mosquitoes from the same screen and hour were stored in separate labelled polyethylene terephthalate (PET) holding cups. The resting height on the screen, low (0-60 cm from the

ground), middle (60-120 cm above the ground) or high (120-180 cm above the ground) was also recorded for 3 nights. All mosquitoes were morphologically identified to genera and sex [207]. The study was conducted between March 2016 and February 2018.

Cloth weight was tested over three rotations where light, medium and heavy cloth was compared, an additional 2 rotations with only light and medium weighted cloth (to ascertain if there was a significant difference in numbers of mosquitoes collected between light and medium cloth) was carried out. The influence of cloth colour on mosquito numbers was tested during four rotations over 12 nights: with white, green and black barrier screens.

Screens without eaves were initially compared to screens with perimeter eaves during 2 nights (1 rotation). During the initial testing period, differences between the two designs were not found so screens with complete eaves were added for an additional 2 rotations (6 more nights of testing). The impact of the frequency of inspection on mosquito numbers collected was tested over 9 nights (3 rotations with inspections at 30 min, 60 min and 90 min).

4.3.3 Statistical analysis

The effect of barrier screen variables on resting female mosquito densities was analysed with a Generalized Linear Mixed Model (GLMM) with a negative binomial distribution and a random factor for the rotation of the Latin square (glmer.nb; package = *lme4*) with a sequential post hoc analysis to clarify any statistical differences between the experimental factors (glht; package = *multcomp*). By incorporating the random factor for rotation into the GLMM, the model accounts for natural fluctuations in mosquito densities observed during sampling periods while increasing the power of the model. Separate analyses were conducted for each experimental parameter and for the *An. farauti* group (although *An. farauti* is the dominant species, *An. hinesorum* is found in the study area) as well as for mosquitoes in the *Aedes* and *Culex* genera. This analysis was conducted using R statistical software (ver.3.1.2).

4.4 Results

A total of 6395 female unfed mosquitoes were captured over 24 nights. Of these 2668 were *An. farauti sl*, 2807 were *Culex* mosquitoes (predominantly *Culex gelidus, Culex annulostris* and *Culex pullus*) and 920 were *Aedes* mosquitoes (made up of *Aedes vigilax,* and *Aedes kochi*). Only 46 males were collected and blood fed and gravid mosquitoes were not captured.

4.4.1 Influence of cloth weight

Cloth weight had an impact on the average number of female mosquitoes collected with fewer females captured on heavy netting (5 mosquitoes/collection-night (m/c-n)) compared to medium (43 m/c-n) or light cloth (43 m/c-n). Cloth weight significantly influenced the number of female *An. farauti* resting on the barrier screens (β = -0.7249, se = 0.2591, p = 0.005) with the heavy shade cloth having fewer resting mosquitoes than barrier screens made with light and medium weighted shade cloth (Figure 4.3 and Table 4.1). The same effect of cloth weight was also found for *Culex* females (β = -0.7139, se = 0.2253, p = 0.001) and *Aedes* females (β = -0.9555, se = 0.2813, p = 0.0007).

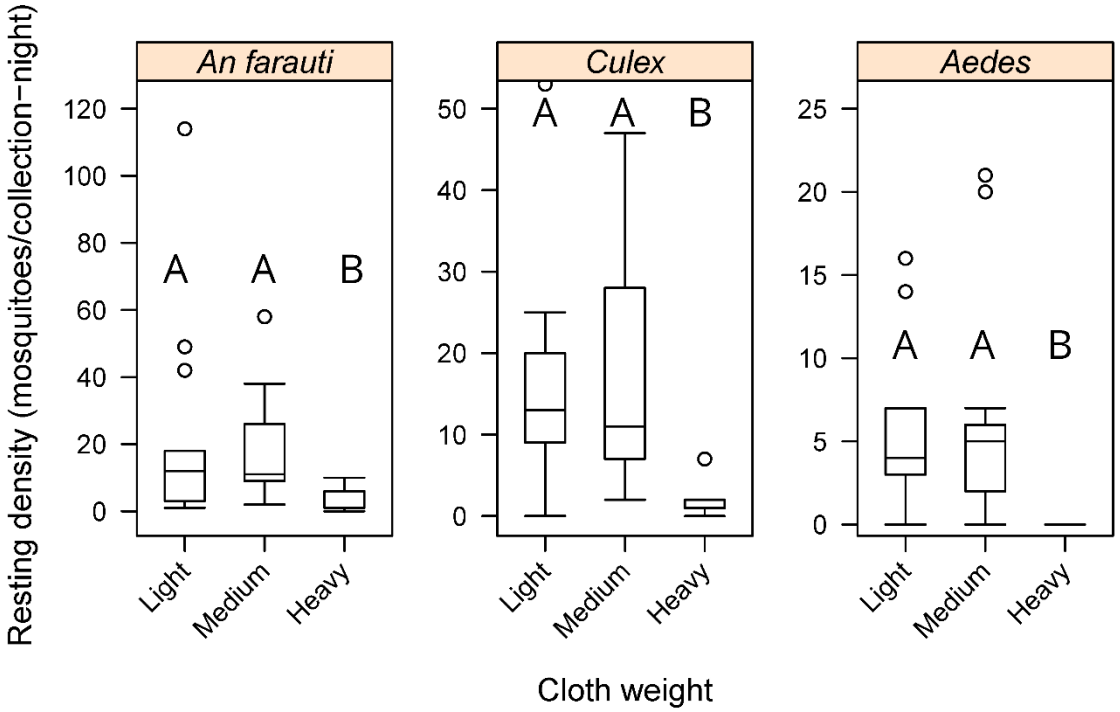


Figure 4.3 Comparison of the densities of female *Anopheles farauti, Culex* and *Aedes* mosquitoes caught resting on barrier screens with different weights (Legend: Different letters signify p< 0.05 difference).

4.4.2 Influence of cloth colour

The influence of cloth colour was compared using material of medium weight (160 g/m^2). Cloth colour significantly impacted the average number of all female mosquitoes collected with fewer mosquitoes captured on white cloth (64 m/c-n) compared to green (118 m/c-n) or black cloth (103 m/c-n). Similarly, cloth colour significantly influenced the number of female *An. farauti* resting on the barrier screens (β = 0.3504, se = 0.1757, p = 0.05) with the white colour having fewer *An. farauti* than green or black screens (Figure 4.4 and Table 4.1). Numbers of resting *Aedes* and *Culex* were not statistically

significantly influenced by cloth colour (β = 2.1718, se = 0.3879, p = 0.08; β = 0.2026, se = 0.1723, p = 0.2, respectively).

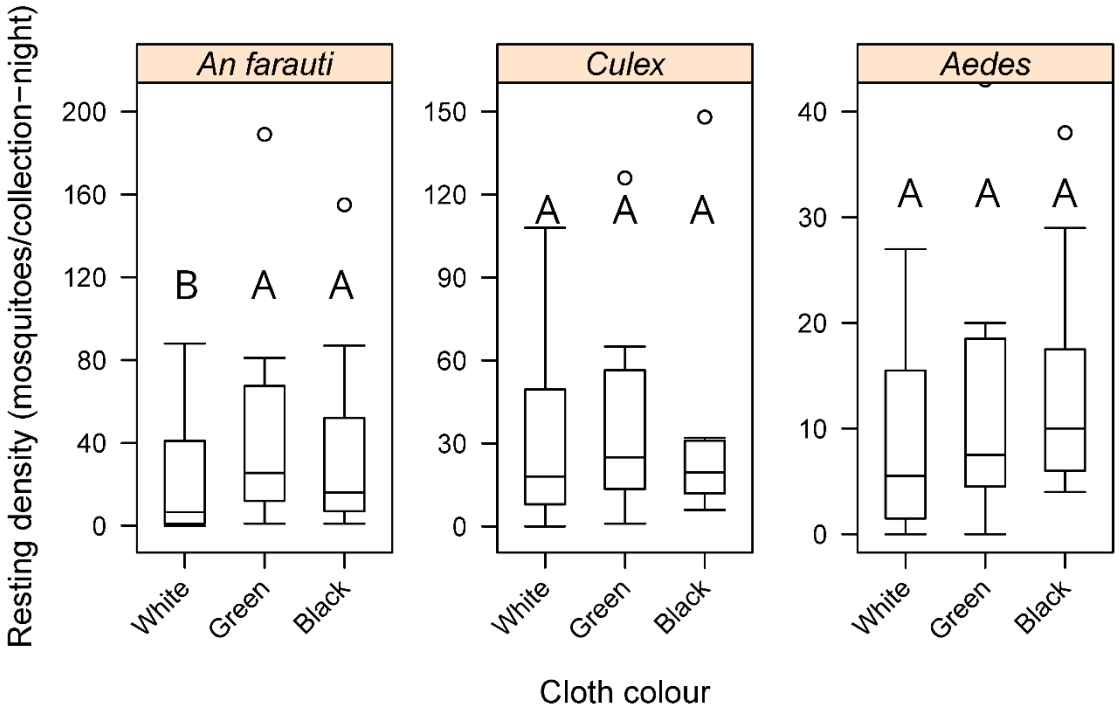


Figure 4.4 Comparison of the densities of female *Anopheles farauti, Culex* and *Aedes* mosquitoes caught resting on different colour barrier screens (Legend: Different letters signify p< 0.05 difference).

4.4.3 Influence of screen design (eaves)

The influence of screen design was compared using green material of medium weight. Eaves impacted the average number of all female mosquitoes collected with fewer mosquitoes captured on screens with complete eaves (6 m/c-n) compared to perimeter eaves (38 m/c-n) or no eaves (38 m/c-n). Similarly, the presence of eaves significantly influenced the average number of female *An. farauti* collected resting on the barrier screens (β = -0.4444, se = 0.2021, p = 0.03), with the complete eave design having fewer mosquitoes captured on it than screens with perimeter eaves screens or screens without eaves (Figure 4.5 and Table 4.1). The same effect of eave design was also observed for *Culex* females (β = -0.9047, se = 0.2419, p = 1.83e-04), and *Aedes* females (β = -0.5747, se = 0.1891, p = 0.002).

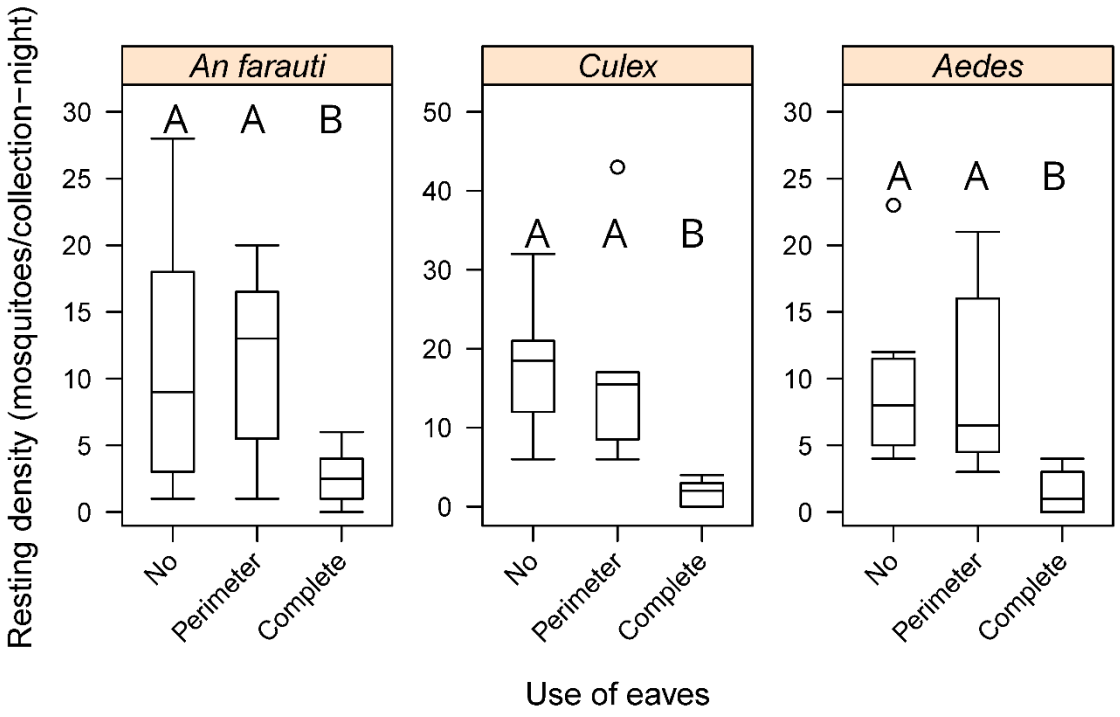


Figure 4.5 Comparison of the densities of female *Anopheles farauti, Culex* and *Aedes* mosquitoes caught resting on differing barrier screens designs (no = no eaves, perimeter = eaves on sides and top, complete= 3 horizontal eaves and vertical eaves on the sides) (Le

4.4.4 Influence of frequency of inspections

The influence of the frequency of inspections was compared using green material of medium weight and constructed without eaves. The average number of all female mosquitoes caught was inversely related to the length of time between inspections; inspections at 30 min intervals captured more mosquitoes (67 m/c-n) than inspections at 60 (36 m/c-n) and 90 min (26 m/c-n). Similarly, the length of the time period between inspections significantly influenced the average number of female *An. farauti* collected on the barrier screens, with inspections every 30 min collecting more resting *An farauti* than inspections every 60 and 90 min (β = -0.6268, se = 0.1974, p = 0.001) (Figure 4.6 and Table 4.1). This pattern was also found for *Culex* resting females (β = -0.4500, se = 0.1860, p = 0.01) and *Aedes* resting females (β = -0.5187, se = 0.2183, p = 0.02).

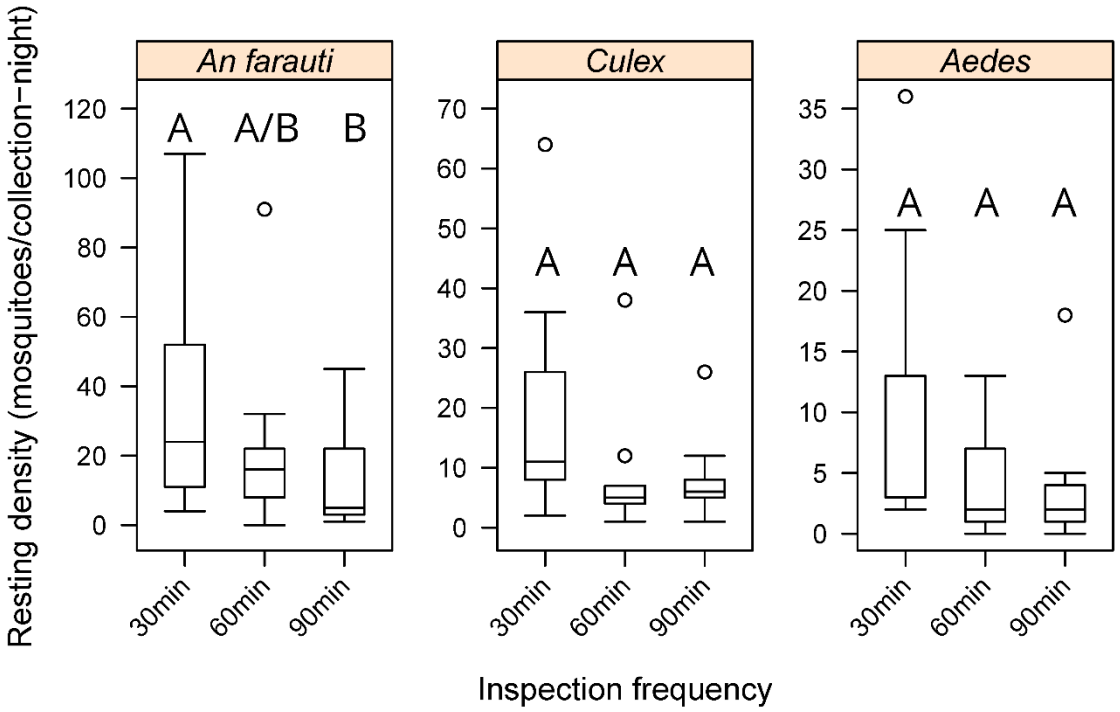


Figure 4.6 Comparison of the densities of female *Anopheles farauti, Culex* and *Aedes* mosquitoes caught resting on barrier screens with different intervals between inspection events (Legend: Different letters signify p< 0.05 difference).

4.4.5 Comparison of resting heights

The average number of all female mosquitoes caught per night was fewer on the high (59 m/c-n) section than the middle (132 m/c-n) and low (142 m/c-n) sections. Similarly, the difference between heights of the barrier screen for resting *An. farauti* was significant with low and middle areas having more resting mosquitoes than the high areas (β = -0.5122, se = 0.2237, p = 0.02) (Figure 7). The same pattern of resting heights was also observed for *Culex* (β = -0.3301, se = 0.1595, p = 0.04). Height of resting *Aedes* was not analysed statistically due to insufficient numbers.

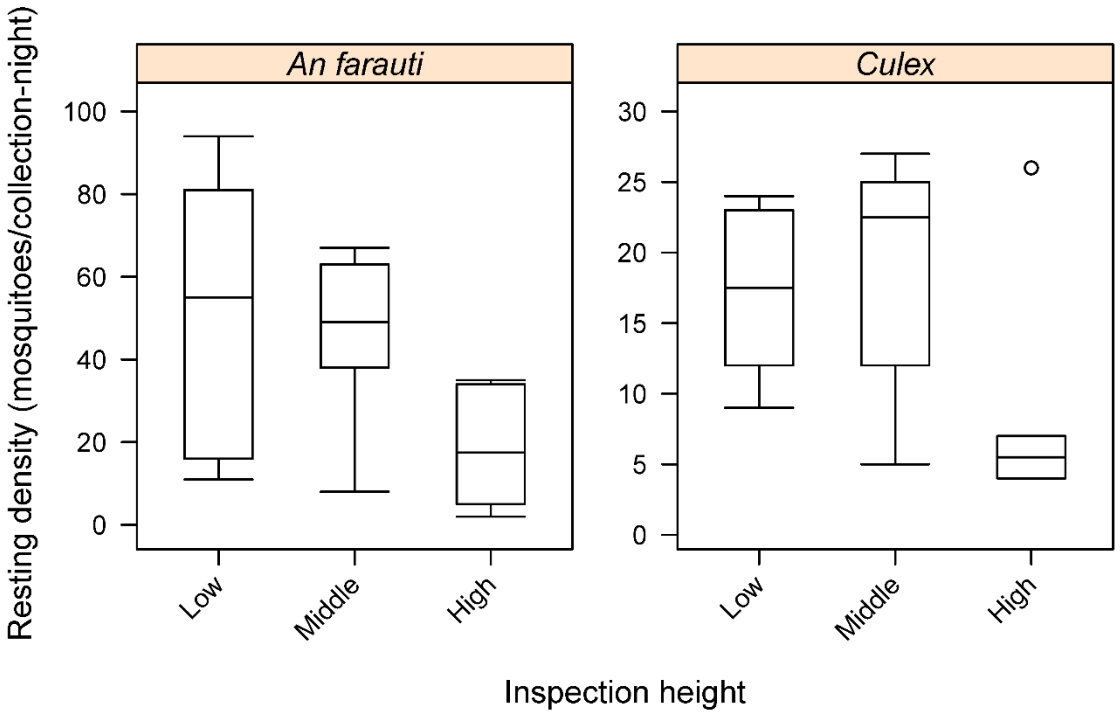


Figure 4.7 Comparison of the densities of female *Anopheles farauti, Culex* and *Aedes* mosquitoes caught resting on barrier screens on different resting heights (low= within 60 cm of the ground, middle= resting between 60 and 120 cm from the ground, high= resting more than 120 cm above the ground)

4.5 Discussion

The barrier screen is intended to be used in the field as a passive mosquito collecting method (without addition of a lure or attractant) to record natural mosquito densities and movements. As mosquito numbers in many location are often low, a systematic assessment of some basic parameters of barrier screens were undertaken with a hypothesis that numbers of mosquitoes collected could be increased. While each experiment was designed to assess the impact of a single specific parameter on numbers of mosquitoes collected, it is important to note that all these factors interact and influence each other. The experimental design targeted host seeking females by providing dry ice as a carbon dioxide source on one side of the screen to simulate the presence of a potential vertebrate host. The colour and density of screen material used as well as construction design and frequency of collection were all significantly impacted the numbers of mosquitoes captured.

Fewer mosquitoes were collected resting on the heavy (90 % - 214 g/m^2) weight cloth; this may be due to the denser material limiting the amount of carbon dioxide passing through the heavy netting compared to the light and medium weight netting. Cloth material corresponding to 70 % shading (160 g/m^2) had comparable numbers of mosquitoes captured on it relative to the 50 % (135 g/m^2) shading.

The 70% shade cloth would be advantageous for field use due to the increased strength and durability relative to 50% shade cloth.

Table 4.1 Statistical test summary for each parameter variable and experimental factors

Mosquito group	Experimental factors	Parameter Variable	Test values
An. farauti	Weight	Medium – Light	(β = 0.1775, se = 0.3173, p = 0.84)
		Heavy – Light	(β = -1.8271, se = 0.4070, p = 1.9e-05)***
		Heavy – Medium	(β = -2.0046, se = 0.4125, p = < 1e-05)***
	Colour	Green – White	(β = 1.0615, se = 0.3022, p = 0.0013)**
		Black – White	(β = 0.7271, se = 0.2928, p = 0.035)*
		Black – Green	(β = -0.3344, se = 0.2881, p = 0.48)
	Design	Perimeter – None	(β = 0.0703, se = 0.2435, p = 0.95)
		Complete – None	(β = -1.2207, se = 0.3542, p = 0.0015)**
		Complete –Perimeter	(β = -1.2910, se = 0.3561, p = 0.00075)***
	Search frequency	60min – 30min	(β = -0.7285, se = 0.3871, p = 0.14)
		90min – 30min	(β = -1.2519, se = 0.3987, p = 0.0048)**
		90min – 60min	(β = -0.5235, se = 0.3917, p = 0.37)
Culex	Weight	Medium – Light	(β = 0.0765, se = 0.2643, p = 0.95)
		Heavy – Light	(β = -1.8854, se = 0.3857, p = 2.2e-06)***
		Heavy – Medium	(β = -1.9619, se = 0.3866, p = < 1e-06)***
	Colour	Green – White	(β = 0.5665, se = 0.3206, p = 0.18)
		Black – White	(β = 0.4353, se = 0.3196, p = 0.36)
		Black – Green	(β = -0.1312, se = 0.3056, p = 0.90)
	Design	Perimeter – None	(β = -0.0736, se = 0.2578, p = 0.95)
		Complete – None	(β = -2.2632, se = 0.3981, p = < 1e-06)***

Cloth colour was a significant factor influencing the effectiveness of barrier screens for collecting mosquitoes. Previously, darker barrier screens (black or green) were successfully used in the Solomon Islands and Papua New Guinea [36] whereas in Indonesia, black [36], white [Lobo, University of Notre Dame USA, 2018, unpublished] and grey [208]barrier screens were used to collect mosquitoes. In general, mosquitoes prefer darker coloured substrates [209]. Anophelines are more frequently found in small resting boxes lined with darker fabrics than in boxes with lighter colours [210]. *Aedes* are also more attracted to darker coloured, lower reflective materials with black and red being more attractive than lighter yellows and whites [211-213]. Results from this study confirmed the observations of the original studies in Indonesia, Solomon Islands and Papua New Guinea, in that the higher colour contrast

between the black screen and mosquitoes made it easier to see resting mosquitoes and thus capture them. Substrate reflectance may influence mosquito numbers collected and the physiological state of the mosquito may also affect preferences [209, 214].

It has been hypothesized that the frequency at which screens need to be inspected could be lessened if mosquitoes remained on the screens for longer periods by baffles or eaves; and preliminary data collected in Indonesia supports this hypothesis (N Lobo, University of Notre Dame USA, 2018, personal communication). Hence, screens with eaves were created to test this hypothesis in Australia (e.g., the number of mosquitoes retained on barrier screens was hypothesized to be greater on barrier screens with eaves). However, the barrier screens with eaves in this experiment did not increase the number of resting mosquitoes collected. This may be due to the fact that resting mosquitoes could have been more difficult to see in edges or corners and/or the construction of the eaves described in this paper may function to make the screen thicker or denser (which was shown to be associated with fewer numbers of mosquitoes collected). The results from the experiments reported here suggests that simple linear barrier screens without eaves are recommended for, at least unfed, *An farauti* collections, as eaves did not increase the numbers of mosquitoes collected despite the increased search time necessitated by the presence of eaves. Eaves also increased the likelihood of acquiring unwanted fauna such as spiders and associated webs. However, additional modifications to the design of barrier screens does deserve further attention, though the simplicity of the current design is one of its strengths.

All mosquitoes caught in these experiments were unfed and there was a significant increase in mosquitoes caught with more frequent inspections. Mosquitoes in different sex and physiological states might be expected to have different resting durations. While increasing the frequency of inspections to every 20 or even every 10 min might increase the numbers of mosquitoes collected, the increased presence of the collector inspecting the screen may serve as a lure to attract mosquitoes and thus could bias collection results. While topical repellent applied to the collector will prevent mosquito bites, the body heat and CO_2 of the collector will act as attractants to mosquitoes and could lure mosquitoes to the vicinity of barrier screens. Based on our results, a collection interval of 30 min is recommended for collectors using a recommended topical repellent. However, this can be adapted based on the physiological status of mosquitoes being targeted for collection; therefore, if blood-fed mosquitoes are the focus of mosquito sampling, inspection events every 60 min might be sufficient, but this will require evaluation to confirm.

More mosquitoes were collected resting below 1.2 m than above it. Using suction traps in Africa, most mosquitoes (80 %) were collected within a metre of the ground with catch number decreasing with increased height [215]. In Java, median nocturnal indoor resting heights of anophelines were measured: *An. aconitus, An. subpictus, An. indefinitus* rest within 38 cm of the floor while *An. kochi* was found within 68 cm of the floor [129] which agrees with the outdoor resting heights observed in this study for

An. farauti. Using the barrier screens in Amazonian Peru most *An. darling* were collected less than 1m from the ground [216] which is similar to the results found in this study. There is limited data regarding resting and flying heights of mosquitoes and the barrier screen does provide a simple method for collecting such useful information. Barrier screens of increased height (up to 3 m) do deserve further attention to see if mosquitoes fly and rest at higher altitudes; however, this may also increase the difficulty in collecting mosquitoes from the barrier screens.

Since the barrier screen method was published in 2013, several studies have used this method to collect a combination of outdoor resting blood-fed, unfed, gravid and sugar-fed mosquitoes. The original barrier screen collections in Indonesia, the Solomon Islands and Papua New Guinea by Burkot *et al* [36] used black or greens screens without eaves of 70 % shading weight, that were checked every 60 min. Russell *et al* [85] also used green 70 % shading weight screens in the Solomon Islands. Moreno *et al* [216] in Amazonian Peru successfully captured *An. darlingi* using green, 'lightweight' barrier screens checked every 60 min. In Papua New Guinea, Keven *et* al [41] used green, 70 % shading without eaves and checked their screens every 20 min. The results of the experiments reported here suggest simple (without eaves) barrier screens at least 120 cm tall constructed with black 70 % shading weight netting can effectively be used to collect mosquitoes with collections every 30 min. Further experiments are recommended that would record mosquito behaviour upon encountering the barrier screen and to visualize initial responses to answer questions such as how long mosquitoes of different species, sex and physiological states rest on the barrier screen.

4.6 Conclusion

The barrier screen is a relatively new adaptable tool that can answer a number of behavioural questions relevant for the surveillance and basic understanding of vectors by sex or physiological status. Data collected by barrier screens can then be translated to inform control strategies. Although publications from 4 countries report data collected using the barrier screen, this is the first paper seeking to maximise the barrier screen method. Barrier screens were developed to collect anophelines outdoors, and this method has demonstrated diversity in its capability to collect a wide range of other mosquito species as well as flexibility and compatibility in the numbers and locations in which barrier screens can be deployed to explore mosquito movements within rural and domestic environments.

4.7 Summary

Traditional methods for collecting outdoor resting mosquitoes are generally inefficient with relatively low numbers caught per unit effort. The barrier screen, designed to intercept mosquitoes as they fly between areas where blood meals are obtained and oviposition sites where eggs are laid, was developed

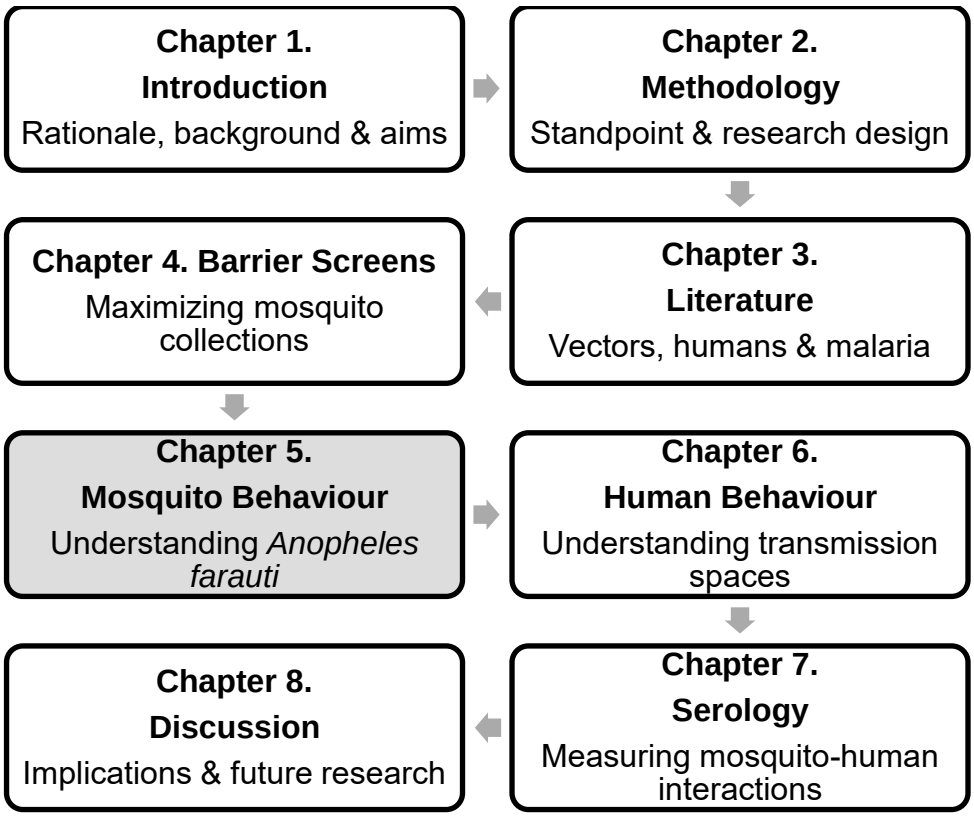
in 2013 as a novel method of sampling outdoor mosquito populations. Barrier screens do not use an odorant lure and are thus a non-mechanical, simple, low maintenance and passive sampling method for use, even in isolated locations. To maximise mosquito collections from barrier screens, multiple Latin square 3 × 3 experiments were conducted in Smithfield, Queensland, Australia. Parameters of barrier screens were varied including the effects of construction materials (net weight and colour), screen design and frequency of inspections. Significantly more mosquitoes were collected on simple dark coloured screens of 50 % or 70 % shading weight with collections every 30 min. Sixty percent of mosquitoes were found on barrier screens within 60 cm of the ground.

The barrier screen is a relatively new adaptable tool that can answer a number of behavioural, ecological and epidemiological questions relevant for the surveillance and basic understanding of the movement and resting habits of mosquitoes by sex or physiological status. This method has demonstrated robustness in collecting a wide range of mosquito species as well as flexibility in where barrier screens can be deployed to explore mosquito movements within rural and peri-domestic environments.

5 Mosquito Behaviour

5.1 Introduction

The fifth chapter documents *An. farauti* distribution patterns within Solomon Island villages. The specific research question explores the distribution of mosquitoes within villages looking at resting heights, spatial and temporal foci and the relationships of these foci to the location of potential host and larval sites and environmental conditions. Results from this chapter will be the basis for discussions on the interacting space of mosquitoes and humans in chapter eight. This chapter has been published in *Parasites & Vectors* and can be read in the appendix.



5.2 Background

Mosquito ecology remains inadequately understood for many species [21, 22], including *Anopheles farauti*, a dominant malaria vector in the southwest Pacific from western Indonesia through Papua New Guinea and the Solomon Islands to Vanuatu [15, 46]. Though there are behavioural differences among species [60], in general, mosquitoes fly to satisfy five basic behaviours: to blood feed, to find favourable resting sites, to lay eggs, to mate and to sugar feed [22]. Much is known about the blooding feeding of *An. farauti* [84, 85] but less is known about resting [47-49, 84] and oviposition behaviours [45, 113, 217]. These behaviours directly impact the efficacy of the three WHO recommended interventions of insecticide treated nets (ITNs), indoor residual spray (IRS) and larval source management (LSM) [39]. Further, very little is known about sugar feeding and male *An. farauti* behaviours which are behaviours being targeted by novel vector control tools; there are no published data on where or on what plants *An. farauti* prefer to take sugar meals and *An. farauti* swarms have also not yet been observed.

There are significant variations in activity patterns among species and these patterns are changing as mosquitoes respond differently to selection pressures induced by changing environmental conditions and human attempts to control them [12, 218]. Prior to IRS with DDT, *An. farauti* sought blood meals throughout the night, both indoors and outdoors. After the malaria elimination campaigns using IRS with DDT, a shift to earlier and more outdoor blood feeding occurred. This behavioural shift was reinforced by the widespread deployment of ITNs to the point where 76 % of biting now occurs outdoors before 21.00 h [15].

Knowledge of mosquito behaviours have been dominated by the use of traps with lures (including the use of humans and animals as baits) to define densities and distributions of species. Data generated in most traps provide useful “snapshots” on numbers of mosquitoes in specific physiological states, but these numbers may be biased by the lures used with traps. Thus, such data provides only limited insights into mosquitoes transitioning from one state to the next or where these behaviours take place (as lures induce mosquitoes to move towards the traps) [55]. There is a need to track mosquito behaviours without influencing the behaviours themselves to understand how best to monitor and control vector populations.

The barrier screen is an insecticide free neutral (no bait or lure) net “trap” that intercepts mosquitoes as they fly in pursuit of blood meals, resting and oviposition sites, mating sites or sugar sources [36, 200]. Mosquitoes when flying between feeding, oviposition, mating, sugar feeding and resting sites temporarily stop to rest when encountering a barrier screen, from which they can be collected. This approach is advantageous to studies of mosquito distributions in that it does not alter the natural locations of mosquitoes with lures and it samples both males and female mosquitoes of all physiological states. The natural outdoor temporal and spatial distributions of *An. farauti* subpopulations by gender and physiological status were mapped within villages in the Solomon Islands using barrier screens.

5.3 Methods

5.3.1 Study sites

The study was conducted in Jack Harbour village on Kolombangara Island in Western Province (8.059°S, 157.196°E) and Haleta village on Ngella Sule Island in Central Province (9.098°S, 160.115°E) in the Solomon Islands (Figure 5.1) [51]. Both coastal villages are on mountainous, rain-forested islands; with a mean daily temperature of 27 °C and annual rainfall between 3000 – 5000 mm [219]. Haleta had a population of 366 in 70 households and Jack Harbour had a population of 151 in 38 households. Central Province had an API (Annual Parasite Incidence) of 280 malaria cases per 1000 persons while Western Province had an API rate of 30 malaria cases per 1000 persons in 2016 [220].

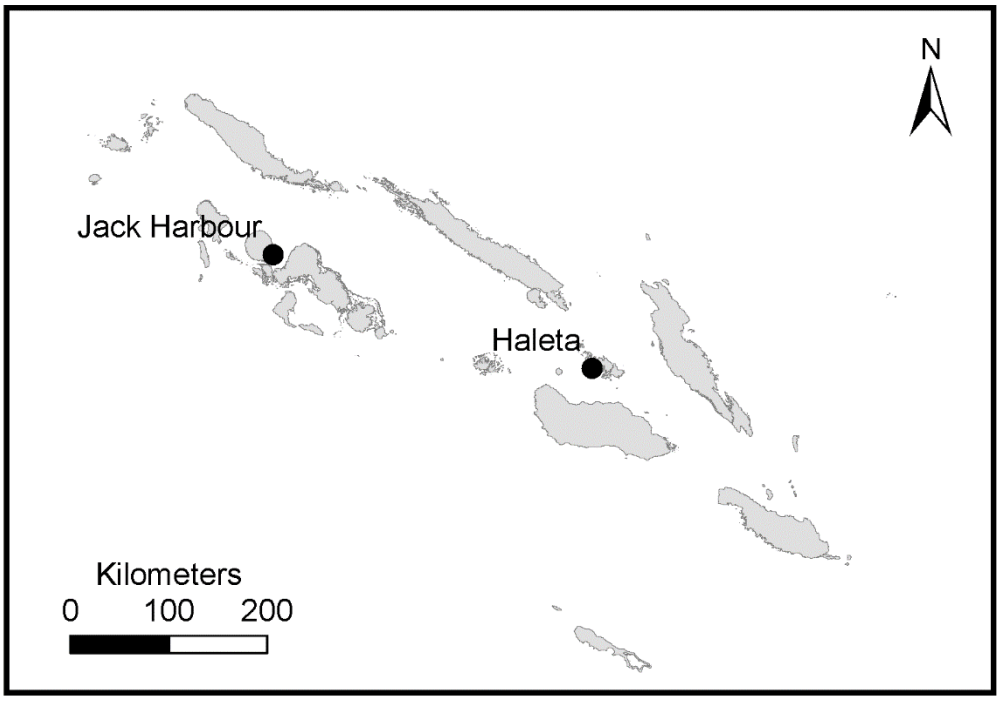


Figure 5.1 Map of Solomon Islands and showing Haleta village on Nggela Sule Island in Central Province and Jack Harbour village on Kolombangara Island in Western Province.

Anopheles farauti, the dominant malaria vector in the Solomon Islands, is the only human biting anopheline found in both villages with a mean of 14.8 bites per person per half-night (b/p/h-n) in Haleta village for 2011 – 2014 [15] and 26.3 b/p/h-n in Jack Harbour for 2014 – 2016 [51].

5.3.2 Sampling adult mosquitoes

During each sampling period, mosquitoes were sampled simultaneously over 4 – 6 sequential nights from 18.00 to 00.00 h using both barrier screens and human landing catch (HLC) in May, August and November 2016 and February 2017. In addition, Haleta was sampled in July 2017.

Barrier screens were constructed from 20 m long black high-density polyethylene shade cloth of 70 % shading (160 g/m^2 ; Coolaroo® Gale Pacific Ltd, Melbourne, Australia) [36, 200]. Mosquitoes resting on barrier screens were collected by mouth aspiration for 15 minutes every hour. For each mosquito, the time of collection by hour; the side of the barrier screen and resting height above the ground (using 3 broad categories of low: 0 – 0.6 m, medium: 0.6 – 1.2 m and high: 1.2 – 1.8 m) were recorded. On any given night, 8 barrier screens were deployed across a village, and the distance of barrier screens to the nearest house and primary larval habitat measured. Locations of barrier screens were relocated to sample a wide range of habitats/locations. Host-seeking females were also captured by HLC outdoors at 10 sites distributed throughout each village. The same locations were used for all HLC sampling efforts during all sampling periods as described previously [15, 51].

Captured anophelines were held by hour and collection station until identified to species by morphology [47], and categorised to sex (male or female) and physiological state at the field sites. Unfed, blood-fed and gravid state mosquitoes were identified according to Detinova [221]; mosquitoes with a distended abdomen with a clear, likely sugar meal will hereafter be referred to as sugar-fed.

Weather Meters (Kestrel 4500) with wind vanes recorded the temperature, humidity and wind speed and direction at ground level and at 1.8 m above the ground nightly during collections.

5.3.3 Statistical analysis

Generalised linear models (GLMs) with a negative binomial distribution were used to analyse differences in (1) the temporal density of mosquitoes compared between physiological states; (2) the distance from nearest house and physiological state; and (3) the resting density of mosquitoes with average temperature, humidity and wind speeds during collections. The significance of the interaction was analysed using a Chi-square test (ANOVA) that compared the fit of two nested Poisson GLM models. The effect of barrier screen height on resting female mosquito densities was analysed with a Generalized Linear Mixed Model (GLMM) with a negative binomial distribution and a random factor for the date (glmer.nb; package = *lme4*). Samples without any resting mosquitoes were removed from the analysis. Incorporating date as the random factor into the GLMM model accounted for natural fluctuations in mosquito densities observed while increasing the power of the model. This analysis was conducted using R statistical software (ver.3.1.2).

5.3.4 Geospatial analysis

Vector foci (areas with higher than mean densities) were determined using FleXScan (v3.1.2), a spatial Poisson distribution model to identify aggregated clusters by identifying spatial windows with greater ratios of observed to expected cases (relative risk). A single cluster detection was based on a spatial

matrix [33] defined using triangular irregular networks created based on Delaunay Triangulation, with Euclidian distance, limited to 10 stations with $p < 0.01$. The FleXScan identified foci were then mapped in ArcMap 10.1 with a 10 m buffer.

5.4 Results

A total of 3,411 mosquitoes resting on barrier screens were collected: 2,345 from Jack Harbour during 21 half nights and 1,066 from Haleta during 28 half nights of collections. The positions of barrier screen positions in Haleta and Jack Harbour are shown in Figure 5.2.

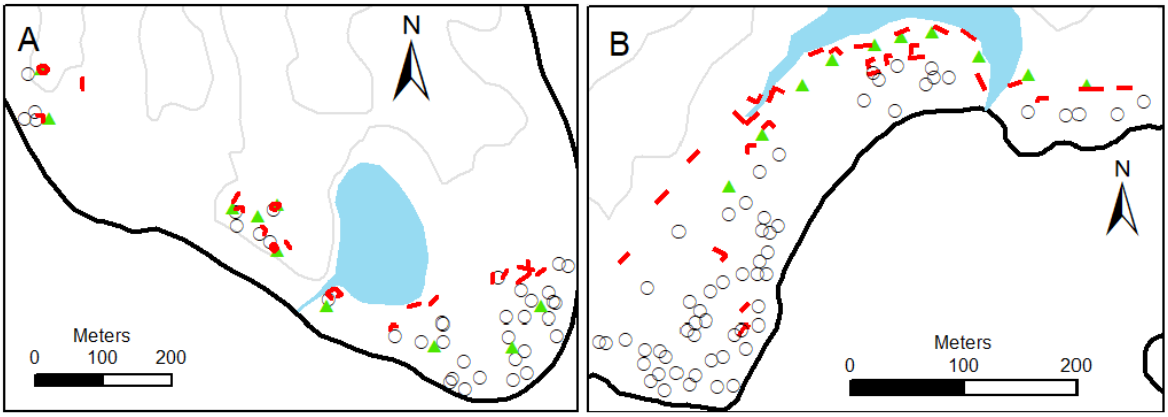


Figure 5.2 Village maps of (A) Jack Harbour and (B) Haleta showing all locations where barrier screens (red) and human landing catch stations (green) were located, as well as houses (circles) and primary larval habitat (blue).

Of these, 2,292 were *An. farauti* and 1,283 were culicine *spp*. Ninety-two percent of the *An. farauti* collected were females (n = 2,121) and 87 % of the culicine *spp*. were females (n = 1,119). Culicines were composed of a mix of species in the genera *Culex* (*Cx. sitiens, Cx. quinquefasciatus*) and *Verallina spp.* There were also occasional rare collections of *Aedes* (*Stg scutellaris)* and *Armigeres spp.* on the barrier screens. Of the female *An. farauti,* 67 % were unfed (n = 1,421), 23 % blood-fed (n = 484), 8 % sugar-fed (n = 173) and 2 % gravid (n = 43). Mean number of resting female *An. farauti* per barrier screen per half-night (r/bs/h-n) during sampling periods ranged from 0.9 r/bs/h-n in the dry season to 11.0 r/bs/h-n in the wet season.

A total of 12,733 female, blood-seeking *An. farauti* were collected by HLC: 7,296 from Jack Harbour (14 half nights) and 5,437 from Haleta (20 half nights) villages over 34 half nights. Mean number of host-seeking female *An. farauti* per sampling period ranged from 1 b/p/h-n to 13 b/p/h-n).

5.4.1 Dynamics of mosquitoes outdoors

The hourly numbers of mosquitoes collected on barrier screens varied by physiological status (χ^2 = -205.37, df = -36, p = < 0.0001). Numbers of unfed female and male *An. farauti* resting on the barrier screens peaked at 19:00 – 20:00 h, decreasing to 00:00 h when sampling ceased (Figure 5.3). The number of blood-fed female *An. farauti* on barrier screens maintained a longer peak (from 19:00 – 21:00 h) and had a more gradual decline in numbers to 00:00 h. Sugar-fed female *An. farauti* were collected earlier in the evening, peaking at 18:00 – 19:00 h. Blood-seeking *An. farauti* females from HLC had a similar temporal patterns to resting unfed females on the barrier screens as also recorded in previous studies in the same villages [15].

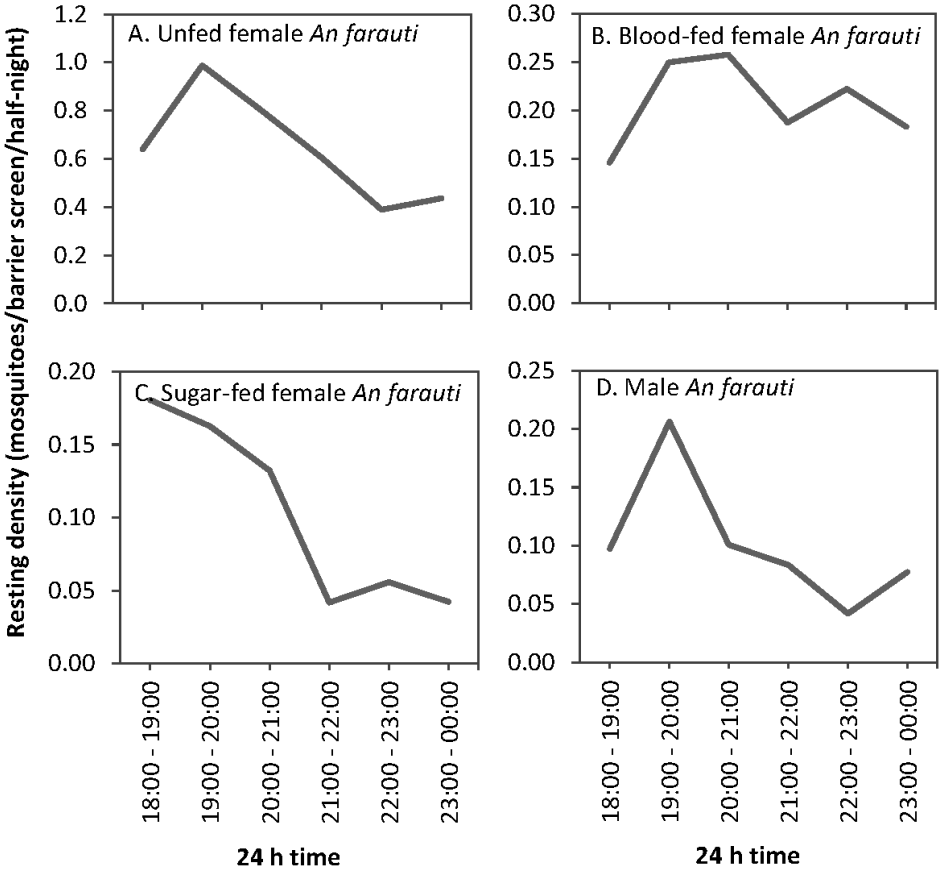


Figure 5.3 Densities of *An. farauti* on barrier screens by time: A. unfed females, B. blood-fed females, C. sugar-fed females, D. males.

There was a significant inverse association between the height above the ground where mosquitoes were collected and the mean numbers collected (β = -0.3596, se = 0.1331, p = 0.007): 57% of *An. farauti* females were found within 60 cm of the ground with a mean of 3.8 r/bs/h-n. In contrast the mean resting density between 60 and 120 cm above the ground was 2.3 r/bs/h-n. Above 120 cm, only 1.2 r/bs/h-n *An. farauti* were collected.

5.4.2 Geospatial resting locations

There is a significant interaction between the distance to the house and the physiological state of resting female *An. farauti* (χ^2 = -136.82, df = -4, p = < 0.001). Unfed and blood-fed *An. farauti* were most commonly found within 10 m of a house, while more sugar-fed female *An. farauti* were found 11 – 20 m from houses (Figure 5.4). Male *An. farauti* numbers were highest at distances > 20 m from houses and also most (62%) were found within 10 m of a large swamp especially evident in Haleta village.

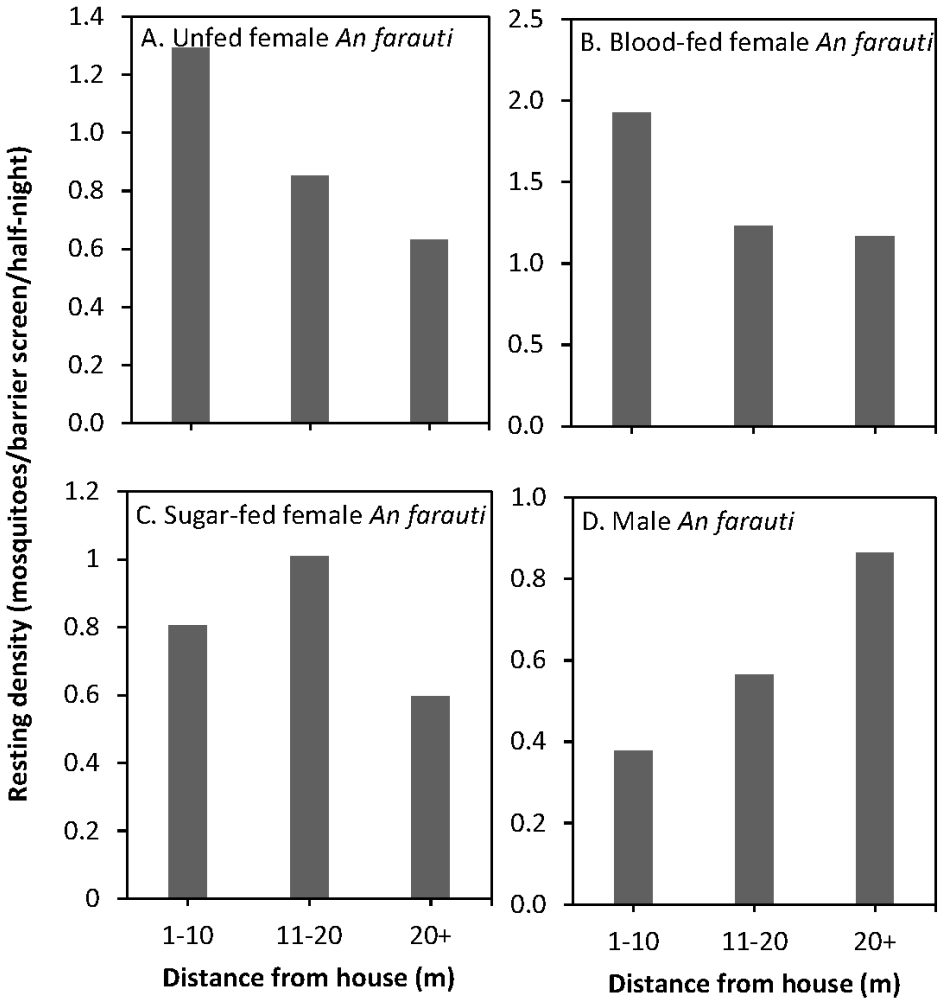


Figure 5.4 Densities of *An. farauti* on barrier screens in proximity to the closest house: (A) unfed females, (B) blood-fed females, (C) sugar-fed females and (D) males.

Significant foci of mosquitoes by physiological states and species were identified within each village (Table 5.1). In Haleta and Jack Harbour villages, there was high spatial overlap for where unfed female *An. farauti* and sugar-fed and blood-fed female *An. farauti* were found (Figures 5.5, 5.6). Sugar-*fed An. farauti* females and *An. farauti* males were also found in close proximity, particularly evident in Jack

Harbour. Blood-seeking *An. farauti* foci also tended to resemble patterns of unfed and blood-fed female *An. farauti.* Although there were differences between the villages, the male *An. farauti* foci was always smaller than the female foci. The populations of female culicines and anophelines (e.g., *An. farauti*) were largely segregated into different parts of the villages.

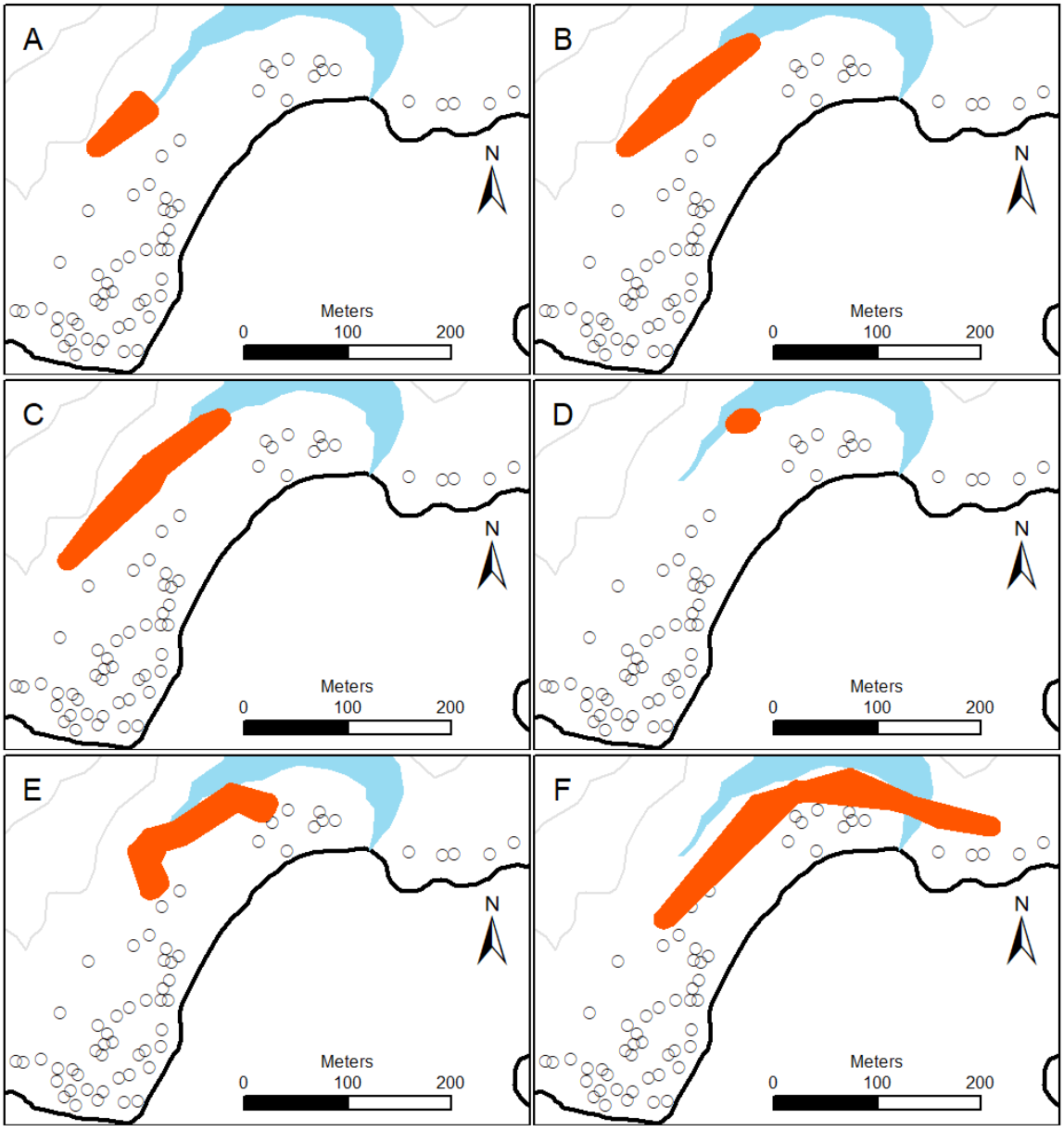


Figure 5.5 Locations of significant mosquito foci in Haleta village, Central Province, collected by barrier screens ((A) unfed female *An. farauti*, (B) blood-fed female *An. farauti*, (C) sugar-fed female *An. farauti*, (D) male *An. farauti*, and (E) female culicine species) and human landing catch ((F) blood-seeking female *An. farauti*) are shown in orange.

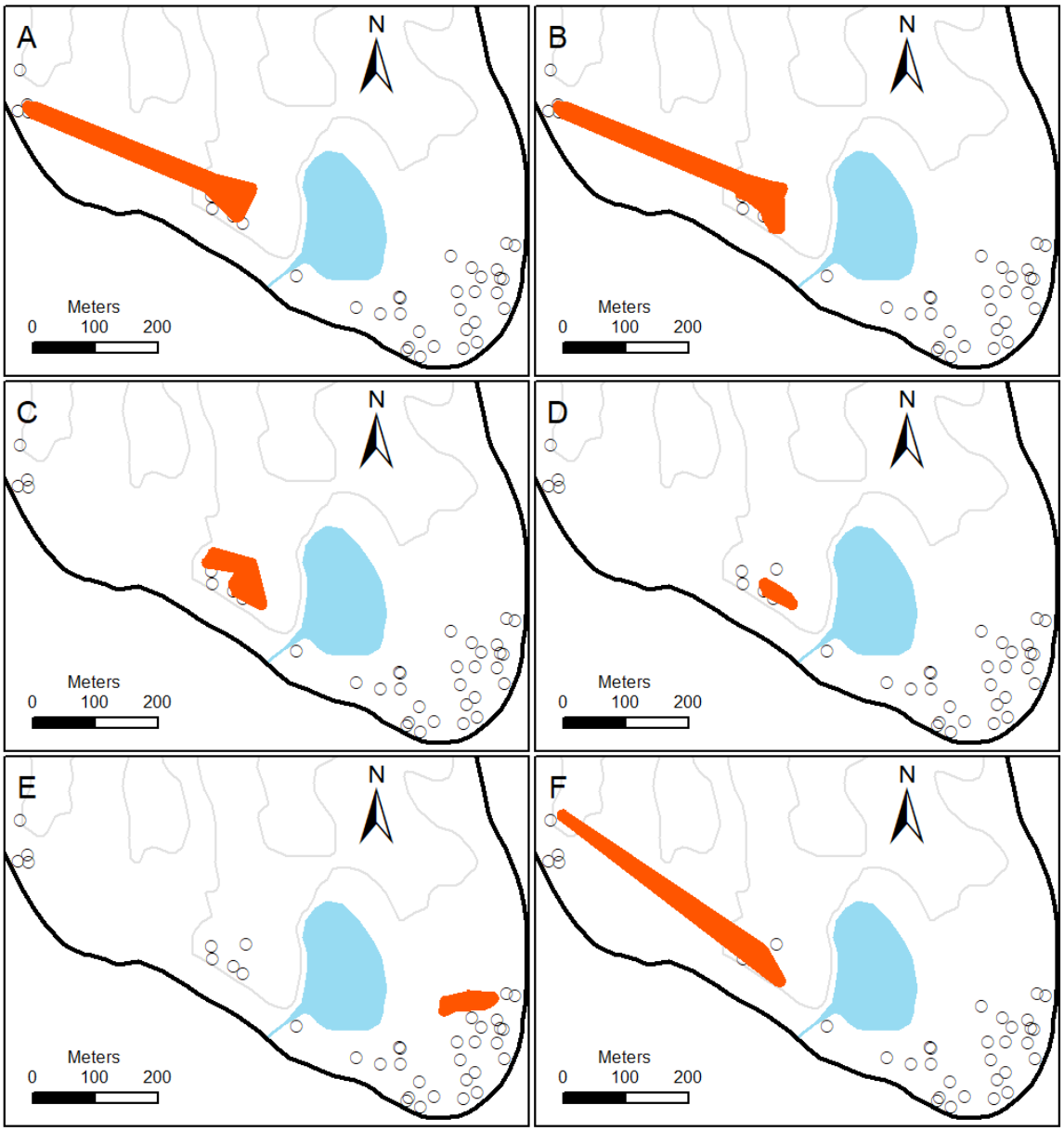


Figure 5.6 Locations of significant mosquito foci in Jack Harbour village, Western Province, collected by barrier screens ((A) unfed female *An. farauti*, (B) blood-fed female *An. farauti*, (C) sugar-fed female *An. farauti*, (D) male *An. farauti*, and (E) female culicine species) and human landing catch ((F) blood-seeking female *An. farauti*) are shown in orange.

5.4.3 Weather

Temperature, humidity and wind speed strongly influenced mosquito numbers on barrier screens (temperature: $\beta = -0.4398$, se = 0.0340, $p = < 0.001$; humidity: $\beta = -0.1570$, se = 0.0073, $p = < 0.001$ and wind speed: $\beta = -2.7890$, se = 0.1558, $p = < 0.001$) and with HLC (temperature: $\beta = -0.2922$, se = 1.8985, $p = < 0.001$; humidity: $\beta = 0.0516$, se = 0.0108, $p = < 0.001$ and wind speed: $\beta = -1.9914$, se = 0.2026, $p = < 0.001$). Higher average wind speeds were associated with lower *An. farauti* collections

on barrier screens and with human landing catch (Figure 7). Collection densities above 1 female *An. farauti* per night on barrier screens never occurred when average wind speeds were > 0.2 m/s. Lower average humidity during mosquito sampling was associated with lower numbers of *An. farauti* found on barrier screens. Higher densities of female *An. farauti* on barrier screens were associated with lower average temperatures.

Table 5.1 Spatial clusters (foci) of *An. farauti* and *Culex* within Jack Harbour and Haleta villages

Species	Physiological state	Maximum distance (m)	Percent of locations (census areas)	Observed percent of mosquitoes	Expected no. of mosquitoes	Relative risk (obs/exp)
Haleta village						
An. farauti	Unfed female	52	7 (2/29)	28 (107/381)	30	3.61
An. farauti	Blood-fed female	141	14 (4/29)	33 (54/165)	31	1.75
An. farauti	Sugar-fed female	185	17 (5/29)	47 (28/60)	12	2.26
An. farauti	Male	0	3 (1/29)	39 (53/136)	11	4.74
Culex spp.	Female *Culex*	128	21 (6/29)	74 (211/287)	48	4.44
An. farauti	Blood-seeking female (HLC)	341	70 (7/10)	76 (4123/5437)	3806	1.08
Jack Harbour village						
An. farauti	Unfed female	368	18 (4/22)	59 (583/987)	223	2.61
An. farauti	Blood-fed female	385	32 (7/22)	61 (172/280)	99	1.75
An. farauti	Sugar-fed female	103	23 (5/22)	71 (80/113)	30	2.64
An. farauti	Male	40	9 (2/22)	70 (21/30)	4	4.90
Culex spp.	Female *Culex*	71	18 (4/22)	64 (525/824)	89	5.92
An. farauti	Blood-seeking female (HLC)	407	30 (3/10)	58 (4268/7296)	2189	1.95

Note: Foci were detected with a flexible scan statistic using FleXScan software and were significant at $P < 0.05$.

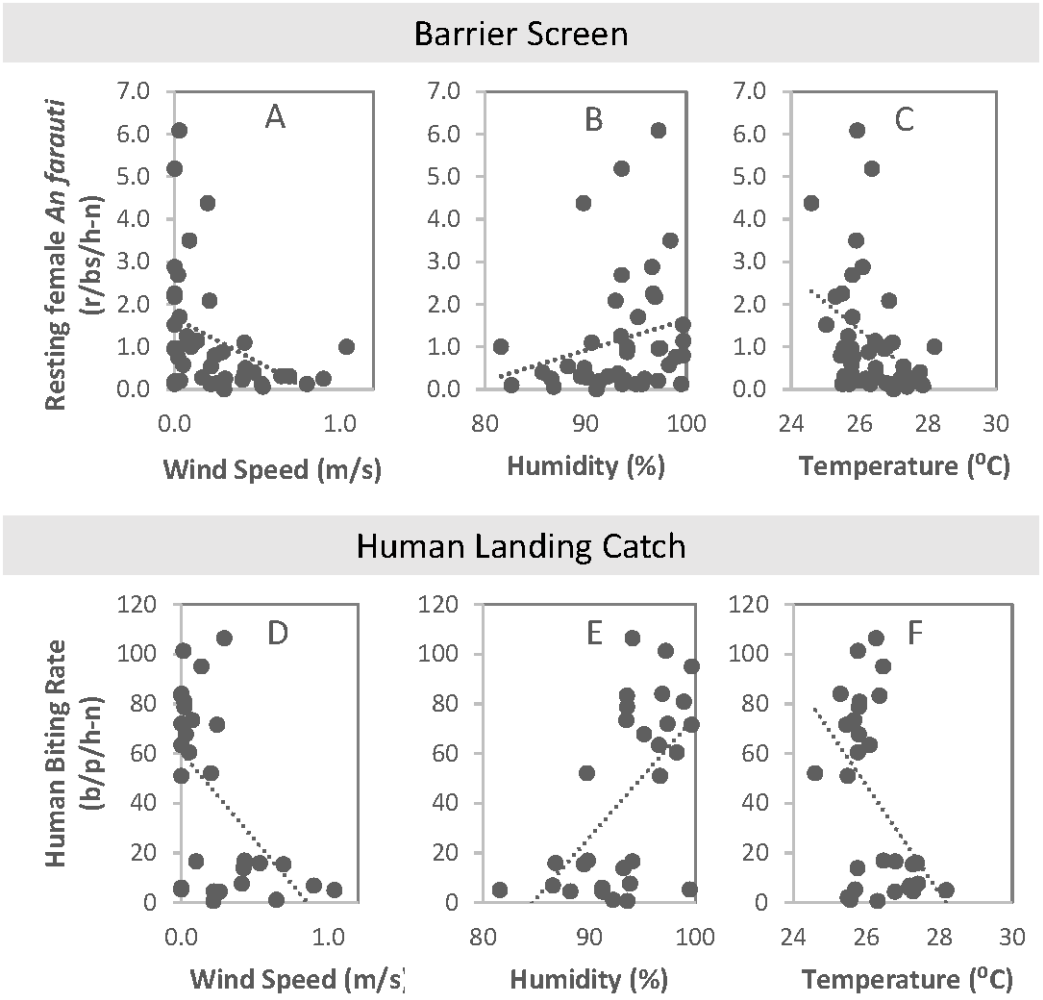


Figure 5.7 Relationships between weather parameters and average total female resting *An farauti* per half night with linear trendline (A) Average wind speed and average total female resting *An farauti* per half night, (B) Average humidity and average total female resting *An farauti* per half night, (C) Average temperature and average total female resting *An farauti* per half night (D) Average wind speed and average total female biting *An farauti* per half night, (E) Average humidity and average total female biting *An farauti* per half night, (F) Average temperature and average total female biting *An farauti* per half night.

5.5 Discussion

In villages, exposure to anophelines and malaria transmission is unevenly distributed [222]. Previous studies documented heterogeneity in the distribution of biting *An. farauti* in villages in the Solomon Islands and proposed that the risk of malaria was best estimated by biting rates in low transmission villages [51]. This study expands our understanding of the distributions of mosquitoes to include other species, physiological states, genders and behaviours. Analogous to the temporal activity patterns of *An. farauti*, the spatial activity patterns for *An. farauti* in the Solomon Islands also differed by physiological states and sex. Whereas biting rates estimate the risk of malaria transmission, the distributions of other vectors by physiological states or behaviours may enable optimising control and

monitoring strategies. This study also highlights the suitability of the barrier screen for collecting non-anopheline mosquitoes.

The complexity of the environment influences the locations where mosquitoes were sampled, with barrier screens near potential blood sources (houses) intercepting more unfed (potentially blood meal seeking) and blood fed *An. farauti*. The barrier screens are most efficient at sampling unfeds (63 – 67 %), followed by blood-feds (23 – 36 %) with only 1 – 2 % being gravid, as also seen from previous studies in in PNG, Indonesia and the Solomon Islands [208, 223].

Here, the sugar-fed *An. farauti* were predominantly collected in the early evening, indicating that sugar feeding is predominantly an early evening activity. This is similar to studies of the *An. gambiae* complex in Africa showing that sugar feeding occurs early in the evening and morning (prior to blood feeding) [27]. Generally sugar-feds females and males, are collected but in low numbers (<8 %) on the barrier screens [208, 223], but with a high sampling effort, sufficient numbers can be collected. A limitation here is that due to the non-standardized approach to determine sugar status, the real number of sugar-feds may either be under or over-represented.

Across both villages, male *An. farauti* were found in more geographically confined areas. Male *An. farauti* were mostly found on barrier screens near larval habitats shortly after sundown suggesting that emergence of males or swarming occurs in the early evening. This is the first indication of possible times and locations of *An. farauti* mating as swarms in this species have yet to be documented.

Mosquito population dynamics and mosquito sampling efficiencies are strongly impacted by weather [224-226]. Wind, temperature and humidity are major factors influencing mosquito flight [54]. Despite the limited range in temperature, humidity and wind speed recorded during this study, significant impacts on the densities of resting and biting *An. farauti* were found: increased densities of biting and resting *An. farauti* were associated with higher humidities and lower temperatures (within the 24-30 ºC range). The finding that wind speeds greater than 1 km/h can significantly reduce *An. farauti* flight is consistent with impact of wind on other species [227] and suggests that reductions in exposure to biting *An. farauti* can be obtained by avoiding sheltered areas in the early evening when most *An. farauti* bites occur.

Data from outdoor barrier screens and indoor resting behaviours suggest many anophelines fly and rest predominantly within a meter of the ground [200, 208]. The height above the ground where *An. farauti* were found on barrier screens suggests that *An. farauti* predominantly flies within a metre of the ground in the Solomon Islands. These observations are consistent with the observation that bites from *An. farauti* in Papua New Guinea were significantly reduced even at elevations of 35 cm [228].

These data defining the heights at which *An. farauti* fly and the influence of wind on flight suggest that significant protection from biting *An. farauti* can be afforded by two simple human behaviours:

avoidance of protected areas in the early evening to maximise wind exposure (and thereby minimising mosquito bites) in the evening before sleeping and then sleeping in elevated houses under a LLIN. Spatial foci of *An. farauti* within the village were clearly different from culicine spp. and also differed in having different peak times of activities, signifying different ecological niches. The differing distributions of culicines and anophelines suggests that interventions for controlling these different mosquitoes may require different distribution strategies.

5.6 Conclusion

Anopheles farauti subpopulations, as defined by physiological state and gender, were found to be heterogeneously distributed in Solomon Island villages. This heterogeneity is observed to be a function of proximity to blood and sugar sources, as well as resting and oviposition sites with the density of mosquitoes at any given location moderated by weather parameters (temperature, humidity and wind). Understanding the basis for mosquito heterogeneities in villages will lead to more accurate surveillance of mosquitoes and greater efficiency and effectiveness of vector control tools. In the absence of new control tools, there are simple measures that individuals can take to protect themselves from mosquito bites based on an understanding of the factors that determine the distributions and densities of biting mosquitoes.

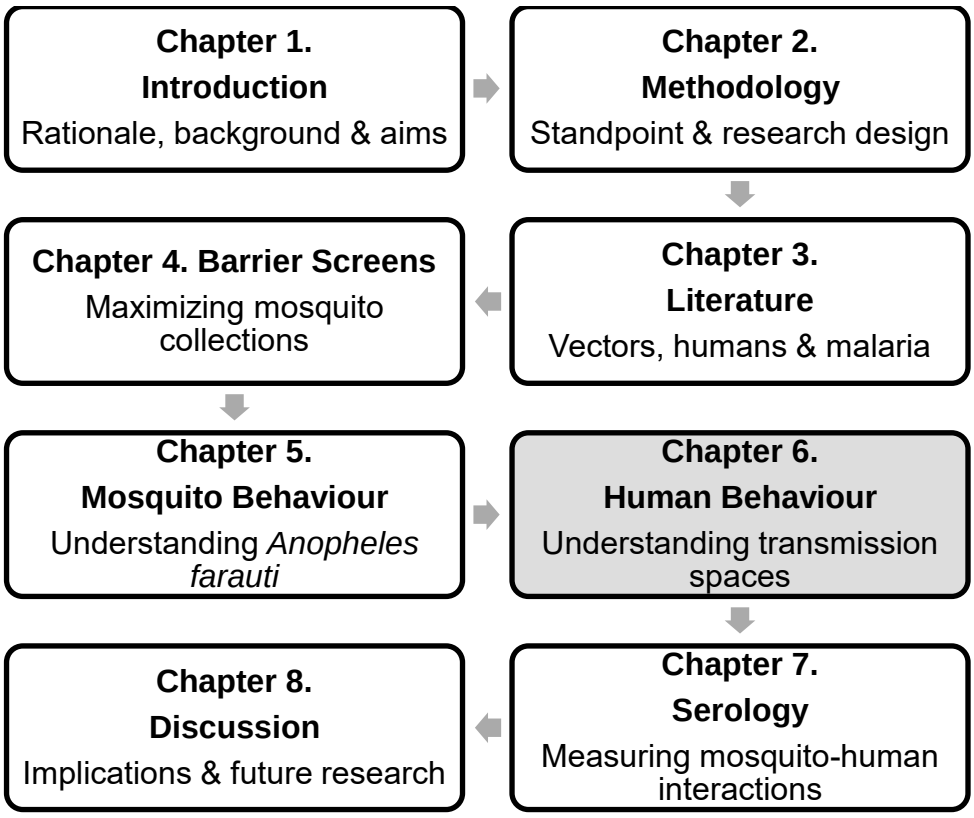
5.7 Summary

Mosquito ecology remains inadequately understood for many mosquitoes including *Anopheles farauti*, the dominant malaria vector in the southwest Pacific including the Solomon Islands. Studies to map fine scale vector distributions are biased when trapping techniques use lures that will influence the natural movements of mosquitoes by attracting them to traps. However, passive collection methods allow the detailed natural distributions of vector populations by gender and physiological states to be revealed. Temporal and spatial distributions of over 15,000 mosquitoes, including males as well as unfed, host blood seeking, blood-fed, non-blood fed and gravid females were mapped in two Solomon Island villages from May 2016 to July 2017. Spatial and temporal patterns varied by species, sex and physiological state. Sugar-fed *An. farauti* were mostly found between 10 – 20 m away from houses with peak activity from 18.00 – 19.00 h. Male *An. farauti* were mostly found greater than 20 m from houses with peak activity from 19.00 – 20.00 h. *Anopheles farauti* subpopulations, as defined by physiological state and gender, are heterogeneously distributed in Solomon Island villages. Understanding the basis for these observed heterogeneities will lead to more accurate surveillance of mosquitoes and will enable spatial targeting of interventions for greater efficiency and effectiveness of vector control.

6 Human Behaviour

6.1 Introduction

The sixth chapter maps the locations in time and space of individual humans in Solomon Island villages. The specific research questions explore human movement within and away from villages, locations of humans during peak biting times and human sleeping behaviours. The outcome is a detailed understanding of human behaviour and is linked with results from chapter five to discuss the interacting space of mosquitoes and humans in chapter eight. This chapter has been written as a standalone paper and is under review by *Scientific Reports*.



6.2 Background

Transmission of mosquito-borne diseases depends on human–vector contact[165, 166]. The concurrent movements and activities of both the human and vector populations that bring these two populations in contact will define the intensity of malaria transmission[158, 163, 165, 167, 174]. After universal access and use of malaria preventive services is achieved, eliminating residual malaria transmission requires appropriate interventions to disrupt the remaining human-vector contact[168-174].

Malaria transmission in a specific location is determined by the lifestyle and movements of the residents (among houses, workplaces, neighbourhoods, villages, towns), coupled with mosquito biting patterns through space[51] and time[18]. People move in and around the specific local areas where they live and work and also beyond their local environs. When people move beyond the immediate range of a mosquito's flight, wider dispersals or acquisitions of malaria and other human vector-borne pathogens can occur[162, 163, 229]. Investigating how people move on a broad scale, which can be periodic and/or seasonal across regional, intra-national or international scales[167, 173, 185] helps to understand broad scale malaria transmission and risk. Investigating how people move within more specific local areas such as in/around households, villages or neighbourhoods is critical to understanding and preventing local malaria transmission in specific locations. This understanding of local level transmission is of particular importance when striving to eliminate residual malaria transmission.

As overall malaria transmission is reduced at a provincial, national or regional level, residual transmission becomes highly heterogeneous and local transmission foci emerge[230, 231]. In the South Pacific nation of the Solomon Islands[51], malaria transmission varies at two scales, an inter- and at an intra-village scale. Within villages, mosquito biting is highly heterogeneous and understanding the locations where residents spend the majority of their time during periods of peak mosquito activity will define their local risk of malaria. In particular, the amount of time that people spend inside households directly relates to protection from biting mosquitoes and malaria in the Solomon Islands. This protective house effect is a function of two factors. Firstly, the two WHO recommended malaria vector control tools, long lasting insecticidal nets (LLINs) and indoor residual spraying (IRS), only provide protection to individuals inside houses, either when sleeping under an LLIN or inside a room that was sprayed with insecticides (e.g., IRS). The second factor relates to the biting behaviour of the malaria vector, *Anopheles farauti*. *Anopheles farauti* is temporally and spatially heterogeneous [51] and bites predominantly outdoors and early in the evening [18]. Thus, individuals are far less likely to be bitten when they are inside a house, even if the house is without screening or other mosquito exclusion methods. People nearby but outside the house, in the peri-domestic area, do not receive the protective benefits of LLIN/IRS and are exposed to higher biting rates by *An. farauti* due to the mosquito's preference for biting outdoors

At the Solomon Islands provincial level, two provinces (Isabel and Temotu) are nearing malaria elimination while other provinces continue to have high rates of transmission. People moving out of Isabel and Temotu provinces are at increased risk of malaria infection. Movement of infected individuals into these provinces threatens elimination efforts by introducing malaria parasites. To date, only one study has explored human movement in the Solomon Islands. This study focused on Isabel Province, and documented broad-scale travel between villages/towns within and beyond the province[183]. However, fine scale movement of people within villages remains undocumented.

To inform a better understanding of how people move within specific residual transmission foci in the Solomon Islands, this study documented how people moved at both the specific local scale (in/around households within villages throughout the day) and at a broader scale (beyond the village) using individual movement diaries. The study took place over a 14-day period in two villages. One village is in a high transmission area (Central Province) and the second in a low transmission area (Western Province). From the data, human-vector interactions[169] quantified where and when humans are exposed to mosquito bites.

6.3 Methods

6.3.1 Study site

The study was conducted in two typical Solomon Island coastal villages (Haleta and Tuguivili; Fig. 6.1). Haleta village (population 366 people) is located on Ngella Sule Island in Central Province (9°5′56″ S, 160°6′56″ E). Central Province had an Annual Parasite Incidence (API) of 280 cases per 1,000 persons during 2015[220]. The average human biting rate of *An. farauti* was 15 bites per person per night (b/p/n) during 2011 – 2014[15].

Tuguivili village (population 167 people) is on New Georgia Island in Western Province (8°11′49″ S, 157°12′54″ E). Western Province had an API of 30 cases per 1,000 persons during 2015 [220]. The human biting rate of *An. farauti* was 3 b/p/n during 2015 – 2017[51].

Houses in the Solomon Islands are predominantly constructed on stilts with timber frames and timber or leaf-thatched walls, and with roofs of iron sheet or leaf-thatch. The houses have large open eaves. The mean daily coastal temperature for the Solomon Islands ranges between 24°C and 30°C with a mean of 27°C and rainfall between 3000 – 5000 mm[219]. *Anopheles farauti* is the dominant malaria vector in the Solomon Islands as has been documented in previous studies [50].

6.3.2 Interviews and movement diaries

The lead author (EJMP) conducted the recruitment and enrolment process after permission was obtained from the village chief to conduct research activities within the village. The village was divided into

geographic zones, and households meeting the inclusion criteria were selected from each zone (see Fig. 6.1). The inclusion criteria included household heads having basic literacy, all household members being permanent residents of the study village and being willing to provide informed consent before participating. Prior to the start of the study, data collection and recording were trialled for 2 days under the supervision of the lead author and translator to ensure comprehension of the data recording instructions and to ensure accurate recording of data. During the study, the lead author and the translator lived in the study villages and visited households in the evening to answer questions and to check progress including daily inspections of the movement diaries to ensure complete data capture for all recording periods for all household members.

A total of 86 people were enrolled from 16 households distributed across each village (Fig. 6.1). The location of each resident was recorded for 14 days during July 2017. The demographic information of each household was captured with an initial questionnaire that included: number of household occupants, their age, gender and use of LLINs (number/household). The location of each resident was recorded by the household heads using daily movement diaries for the 14-day period. During the day (06:00 – 18:00 h), data were recorded in 3 blocks of time, each of 4 h duration. During the evening (18:00 – 00:00 h), data were recorded hourly. A single block of time was recorded for the night (00:00 to 06:00) when limited people movement occurred. For each time period, the household head observed the location of the household members. The predominant location of each participant was recorded as short open text answers which were subsequently categorised into 4 main broad geographic areas ("Inside House", "Peri-domestic area", "Village" and "Beyond Village", see Table 1).

6.3.3 Statistical analysis

The age distribution of participants was described and compared with the national baseline average using a chi-squared contingency table (*chisq.test*). Baseline population data was accessed from projected figures for 2017 based on the 2009 census data[232].

Generalised linear models (GLMs) with Gaussian distribution were used to analyse differences in: 1. the temporal location of participants compared between villages; 2. the number of nights that participants spend away from their home village by gender and village; 3. the age of participants who travelled compared with the age of all participants; 4. the location of all participants across different times of the day and into the evening (06:00 – 22:00 h); 5. the location of all participants across different hours of the evening (18:00 – 00:00 h), 6. the location of participants between weekend and weekday days; and 7. the participants locations inside the house between age groups throughout the evening (18:00 – 00:00 h). The significance of the interaction between location of the participants and time of the day was analysed using a Chi-square test (*anova*) that compared the fit of two nested poisson GLM models. This statistical method was chosen because both the factors of location and time of the day

were categorical. The eventual sleeping location and the usage of LLIN compared by age group used a chi-squared contingency table (*chisq.test*).

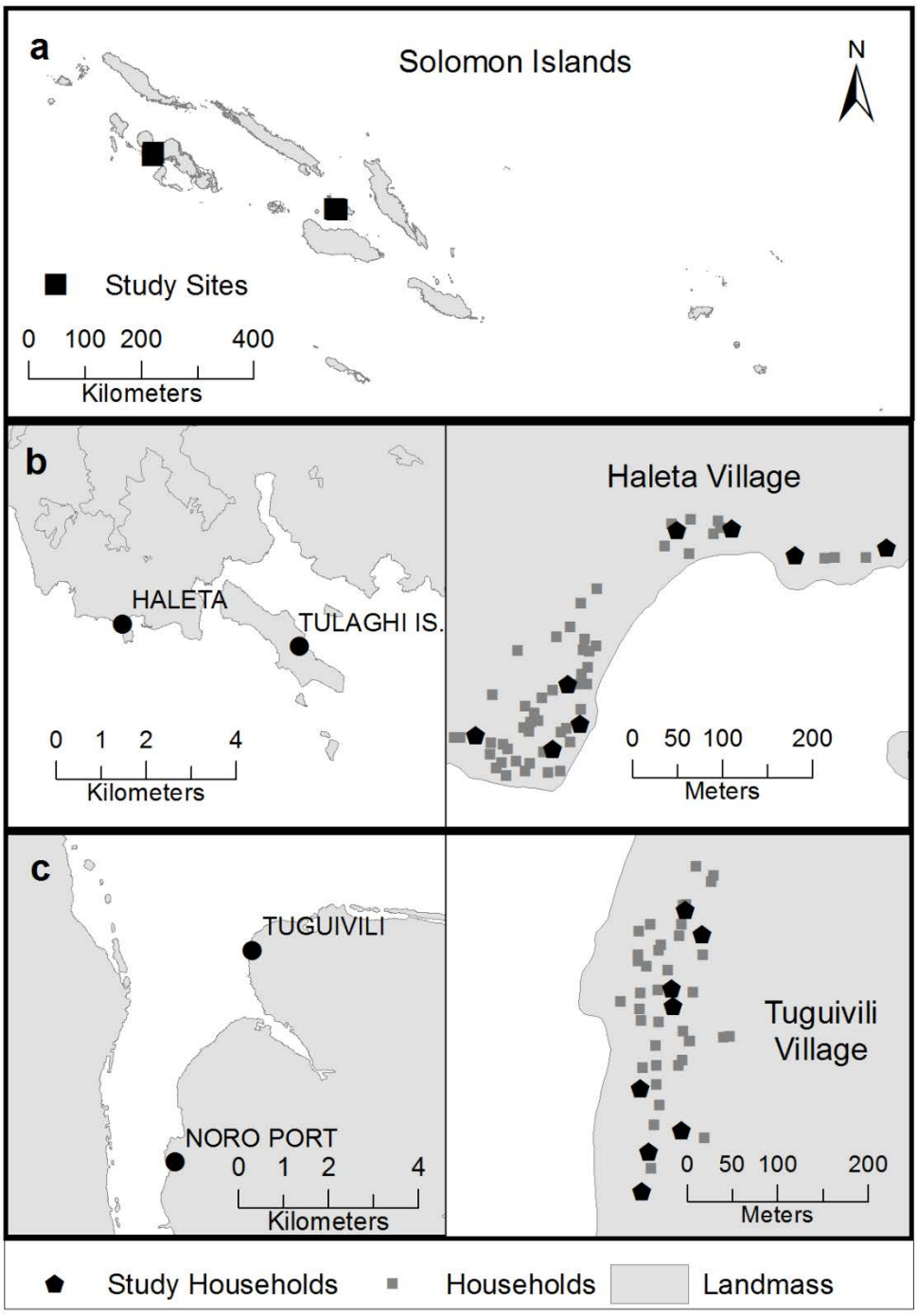


Figure 6.1 Map of (a) the Solomon Islands showing (b) location of Haleta village on Nggela Sule Island in Central Province (9°5′56″S, 160°6′56″E) on the right and detailed map of the positions of all households and study housholds on the left and (c) location of Tuguivili village on New Georgia Island in Western Province (8°11′49″S, 157°12′54″ E) on the left with the locations of the all households and study households on the right.

Table 6.1 Categorisation of locations were people were located

Broad locations	Sub-locations	Descriptions
Inside house	*The interior of the building (often elevated on posts) used by a family as the primary residence with four walls enclosing one or more rooms under a roof, and may have, as follows:*	
	Bed room	An area of a house defined by four walls that is used primarily for sleeping
	Living room	An area of a house defined by four walls that is used for purposes other than sleeping
Peri-domestic	*A cluster of outbuildings and the associated area of land used by the residing family. Here, the peri-domestic is associated with a primary residence (is external to the nested category inside house) and includes the following:*	
	Kitchen	A roofed structure that may have walls, separated from the house and used primarily for cooking and dining
	Outside areas	The land adjacent to the house not covered by a roof
	Under house	An open sheltered area beneath the floor of the house
	Veranda	A sheltered platform along the outside of the house, level with house flooring.
Village	*The smallest administrative unit in a rural area encompassing a cluster of residential houses (but external to the nested category peri-domestic) and other buildings including:*	
	Another house	Any house that is not the residence of the participant and is situated within the confines of the village
	Church	The building and adjacent area of land where religious activities are held
	Freshwater	Areas where water is collected for consumption or washing (e.g., well, water tank)
	School	The building and adjacent area of land where educational activities take place
	Seaside	The coastal areas bordering the ocean
	Store	A building that sells a variety of food and household items
Beyond Village	*All geographic areas beyond the village which may be proximal (and frequented by day trips) or distal to the village (whose visitation often necessitates being away overnight from the home village) and includes the following:*	
	Another village	Clusters of rural houses away from the home village
	Forest	Uncultivated or forested areas where hunting and gathering occurs
	Garden/Farm/ Plantation	A cultivated area of land, often near the village, where food is grown

	Town or City	Urban areas characterised with larger populations, denser housing, commercial activities and government services
	Sea	Marine area where saltwater fish are caught

6.3.4 Quantifying human-vector interactions

Prior to conducting the human movement surveys, the biting behaviour of the local *An. farauti* population was quantified and published[15]. The proportion of human contact with mosquito bites occurring indoors (π_i) was calculated by weighting the mean indoor and outdoor biting rate of *An. farauti* throughout the night by the proportion of humans indoors and outdoors at each time period (indoors being humans inside the houses) and outdoors being humans in the peri-domestic area to match with the mosquito data collected "inside" and "outside" of houses): $\pi_i = \sum[I_t S_t] / \sum[O_t(1 - S_t)] + I_t S_t$; where S = the proportion of humans indoors, I = the total number of mosquitoes caught indoors, O = the total number of mosquitoes caught outdoors [see [169] for more detail].

6.4 Results

Human movement data was collected from a total of 1204 person-days. Participants were children ≤5 years (11%), youth 5 to 18 years (37%) and adults > 18 years (52%). The age structure of survey population was representative of the national population, and there was no difference in the age structure between either population ($\chi^2 = 1.276$, df = 2, p = 0.528). All households had LLINs with an average of almost 4 LLINs per household. There were 1.4 persons/LLIN. Although 84% of people sleep under an LLIN, only 7% were under an LLIN during the 18:00 to 21:00 h peak mosquito biting period. Window screens were present in only one house.

6.4.1 Overall people movement

People's movements were designated as being within one of four nested categories, of increasing scale: inside the house, the peri-domestic area around the house (including the veranda and external kitchen building), the residential village and all areas beyond the village of residence (Fig. 6.2). Each nested location category was composed of finer scale sub-locations (Table 1). There were significant changes in the location of people during a 24 hr period with most people in or near their homes in the early morning as well as in the late afternoon and at night; however, in the middle of the day more people were outside their village or within their village but away from the house and peri-domestic area and

these daily changes in locations over time were statistically different (χ^2 = 768.17, df = 6, p = < 0.0001; Fig. 6.3).

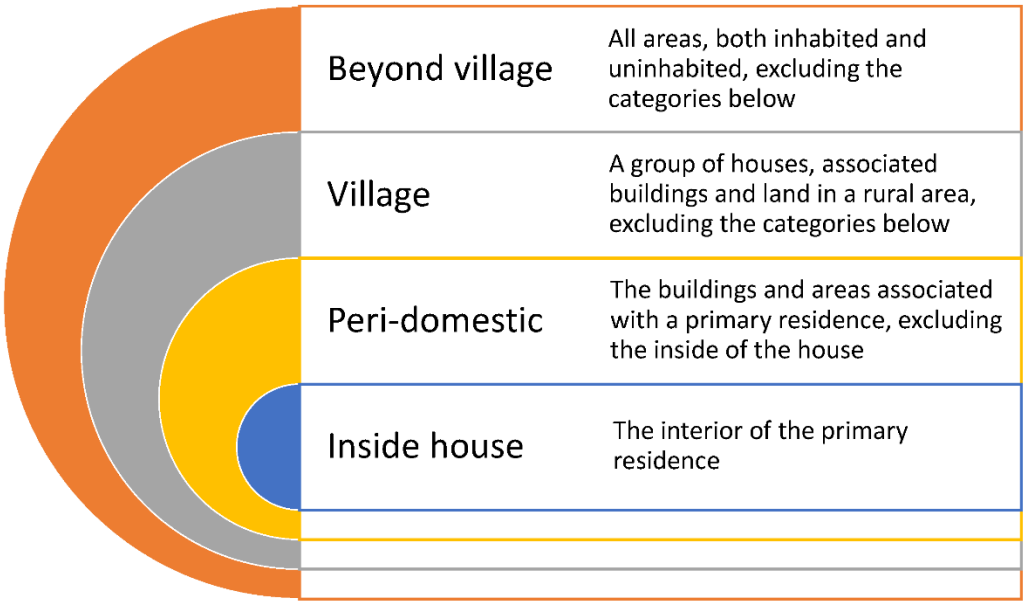


Figure 6.2 Schematic detailing the set of nested categories where people were located

6.4.2 Overnight people movement

The overnight period was analysed to document if people spent the night within their home village or outside their village. During the 14-day study, 34% (n = 29) of participants spent at least one night away from the village. Almost equal numbers of males (n = 15) and females (n = 14) spent at least one night away from their village. The average number of nights away from the village was 3.6 per fortnight (range = 1 to 12) with no significant differences by gender (females μ = 3.0 and males μ = 4.1 nights away; β = 1.066, se = 1.245, p = 0.399) or age (compared with the baseline age distribution of all participants) (β = 1.122, se = 3.601, p = 0.756). The average number of nights that participants spent away from the village differed by village (Haleta village μ = 1.2 and Tuguivili village μ = 4.6 nights away; β = 3.378, se = 1.197, p = 0.008). The most frequent overnight travel location was to another village (59%) followed by a town or city (24%) and for employment on ships (14%).

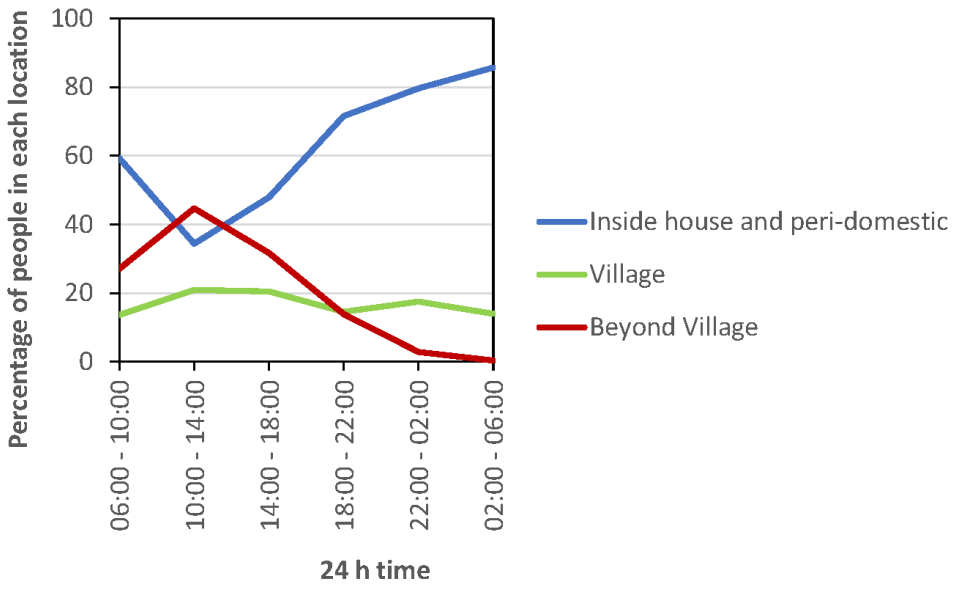


Figure 6.3 The 24 h profile of human movement within the three categories of at home (includes both inside house and peri-domestic), within village and beyond village

6.4.3 Daytime people movement (06:00 – 18:00 h)

The daytime period was analysed for people's movement in and around their villages in 4 h blocks: morning (06:00 – 10:00 h); middle of the day (10:00 – 14:00 h); and afternoon (14:00 – 18:00 h). People's daytime movement was recorded in 3 categories (inside the house and the peri-domestic area were combined into a single category because over any 4 h block people frequently moved back and forth between inside the house and peri-domestic areas). In the morning (06:00 – 10:00 h), 59% of people were in the house/peri-domestic area, 14% were in the village and 27% were beyond the village. During the middle of the day (10:00 – 14:00 h), 35% of people were in the house/peri-domestic area, 21% were in the village and 44% were beyond the village. In the afternoon (14:00 - 18:00 h), 48% of people were in the house/peri-domestic area, 20% were in the village and 32% were beyond the village. The most frequented day trips made beyond the village were to urban areas (51%, such as the provincial capital) and also to gardens/plantations (33%).

6.4.4 Night-time people movement (18:00 – 06:00 h)

The night-time period (18:00 – 06:00 h) was analysed in 1-hour blocks between 18:00 – 00:00 h and then a single 6-hour block between 00:00 – 06:00 h. Locations at specific times were recorded in 3 categories: the house, the peri-domestic area and the village (people who were beyond the village for the evening were not included in the 'internal village' movement analysis). There were significant differences in the locations of participants during the evening hours between the three location categories (18:00 – 00:00 h) ($\chi^2 = 1762.3$, df = 10, p = < 0.0001), across all days and ages. At 18:00 h

12% were inside the house, 65% were in the peri-domestic area and 23% were in the village (Fig. 6.4a). During 18:00 – 21:00 h, half (53%) of participants were in two peri-domestic locations, the kitchen (25%) and the veranda (28%) (Fig. 6.4c). During 21:00 – 00:00 h, 62% of people were inside the house (with almost 80% in a bedroom) (Fig. 6.4b), 29% in the peri-domestic area and 9% in the village. At 00:00 h 80% were inside the house, 17% were in the peri-domestic area, 3% were in the village. Between 00:00 h and 06:00 h, 94% were inside the house, 5% were in the peri-domestic area, 1% were in the village.

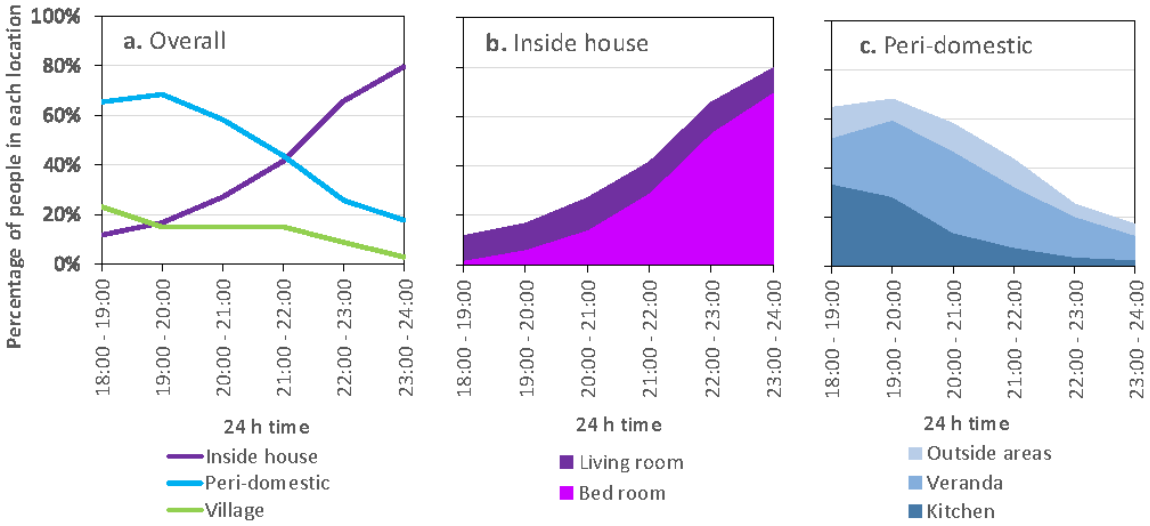


Figure 6.4 The profiles of human movement across the evening for: a) within the three nested categories of inside the house, the peri-domestic area and the village; b) the sub-categories for inside the house; and c) the sub-categories for the peri-domestic environment. Note that the stacked profiles for graphs b and c are calculated as a breakdown of the percentages presented for each category in graph a.

6.4.5 Differences between age groups

The number and proportion of participants inside the house across the evening varied by age (χ^2 = 39.526, df = 10, p <0.0001). Ultimately, 100% of under 5 year olds, 99% of 6-18 year olds and 89% of adults (χ^2 = 39.3, df = 2, p <0.0001) slept inside the house, however the time of entering the house differed. Half of under 5 year olds were in the bedroom by 20:30 h, half of 5-18 year olds by 22:00 and half of people >18 year by 23:00 h (Fig. 6.5). LLINs were used by 100% of under 5 year olds, 85% of 5-18ys and 80% of >18 year olds which was statistically different (χ^2 = 29.4, df = 2, p <0.0001).

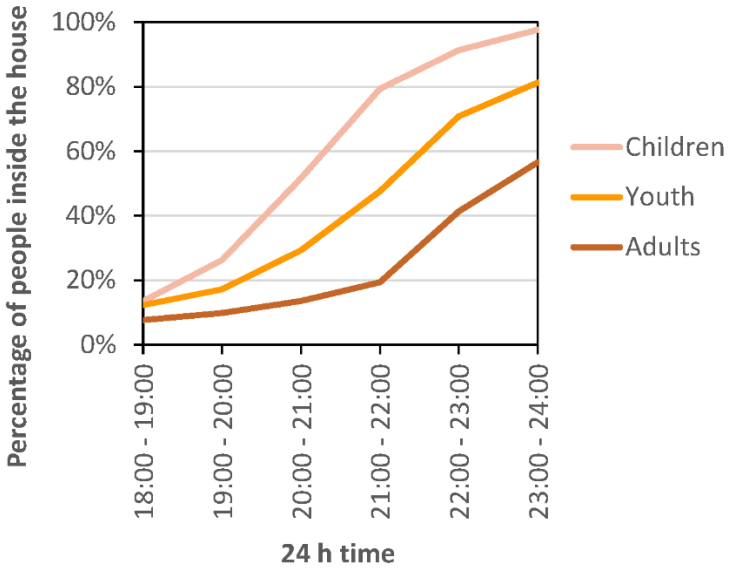


Figure 6.5 The evening profile of participants located inside the house for each age category

6.4.6 Difference between weekdays and weekends

Overall the daytime movement of people differed significantly between the weekdays and weekends ($\chi^2 = 4841.3$, df = 20, p = <0.0001). During weekdays, more people made day trips beyond their village of residence compared to weekends, particularly during the midday (10:00 -14:00 h) period (weekdays = 49% and weekends = 33%). However, there was no statistical difference between weekends and weekdays for the percentage of people that were inside the house over the evening (eg. people inside house at 20:00 - 21:00: weekdays = 23% weekends 25%).

6.4.7 Difference between villages

The movement of people differed significantly between the villages (β = 111.76, se = 12.34, p = 0.0004). In Tuguivili village, more people moved beyond the village on any given 24-hour period compared to Haleta village. This was particularly relevant for overnight trips (as mentioned earlier). For those who remained in the village, there was no statistical difference between the two villages in the percentage of those inside the house (Tuguivili = 15%, Haleta = 19%) and inside the peri-domestic area (Tuguivili = 68%, Haleta = 72%) during 20:00 – 21:00 h (middle of the evening).

6.4.8 Quantifying human-vector interactions

Between 18:00 – 21:00 h, 18% of people were inside the house and 82% were outside (64% in peri-domestic areas, 18% in village) (Fig. 6.6). Of the 18% indoors only 7% were in the bedroom with access to an LLIN. Previous entomological studies from these villages documented that *An. farauti* is highly

outdoor and early biting, with 0.8 bites/hour indoors and 2.1 bites/hour outdoors and 76% of all bites occurring between 18:00 h and 21:00 h[15]. Using this data to quantify human-vector interactions, on any given night 3.2 bites per person occurred inside and 8.2 bites per person occurred outside. Across the entire study population and across the entire night (18:00 to 06:00 h), 16% of all bites occurred inside and 84% outside. The proportion of bites that occurred inside tended to differ by age, with 24% (± 18%) of bites for under 5 year olds, 21% (± 17%) of bites for 5-18ys and 12% (± 12%) of bites for over 18-year olds occurring inside the house, noting the wide standard errors around these estimates.

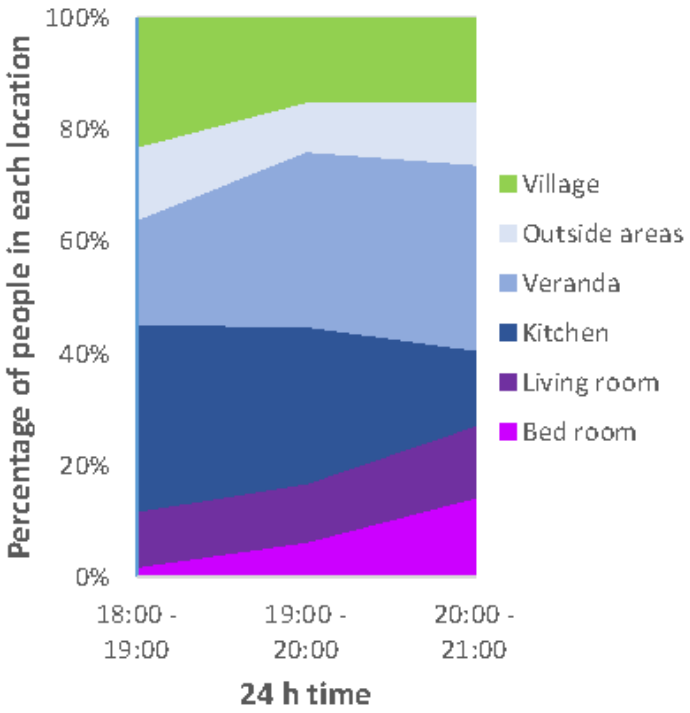


Figure 6.6 Villager locations during peak mosquito biting times (1800 – 2100 h)

6.5 Discussion

This study documented significant movement of people both within and beyond two villages in the Solomon Islands. Although almost everyone in the study had access to and ultimately slept under an LLIN, only 7% of people were under an LLIN during the 18:00 – 21:00 h peak biting period when 76% of *An. farauti* bites occur[15]. Transmission risk is a product of both the location of people and the biting activity of mosquitoes. By quantifying human-vector interactions in this manner, the greatest exposure to mosquito bites was determined to occur early in the evening when over half of people were on the veranda or in the adjacent external kitchen building, neither of which offer protection from mosquitoes. Thus, a large protection gap exists despite universal access and near universal use of LLINs

for most people by virtue of their location within the outdoor peri-domestic area when most malaria mosquitoes are seeking blood meals.

Understanding small-scale heterogeneities in transmission requires high resolution human spatial data[173]. This is the first study to record the daily patterns of where and when people are, within and beyond villages, in the Solomon Islands. The study population was representative of the Solomon Island population: 80% of the Solomon Islands population live in rural areas in a nuclear families (mean of 5.3 people), 5 % of whom are formally employed (by the government or in the private sector [232]). Households consist of single family homes constructed on stilts with 99% of roofs constructed with iron sheets or leaf-thatch and 78% of walls made of wood or leaf-thatch with 96% of households having at least one LLIN [233]. Of the household residents participating in the study, 10% were formally employed and resided in households with a mean of 5.4 people per house, 100% of which were constructed on stilts with roofs of iron or leaf-thatch and with walls made of wood or leaf-thatch. All (100 %) of participating households had LLINs. The methodology employed in this study (questionnaires and movement diaries) was able to collect high quality fine-scale data on human movements by time and locations[234], and focused on specific areas around and within the home in remote village settings. In contrast, mobile phone records and GPS technologies are unlikely to capture human movement with such fine granularity [185], and further there is limited cell phone coverage in rural Solomon Islands. This study documented both frequent human movement within a village, and travel beyond the village boundaries in which a third of villagers spent an average of 3.6 nights away from the home village over a 2-weeks period. Malaria control programmes therefore need to protect people within villages and when travelling beyond the village, in particular between high and low transmission areas.

This study identified verandas and kitchens in the peri-domestic space as where most people are when malaria vectors are most actively seeking blood meals. These areas of high vector-human contact are open and exposed and are where residual transmission is maintained. These areas should be the focus of additional vector control strategies. Malaria programs can consider extending the usual application of residual insecticides to beyond the inside walls of house to include targeted spraying of the exterior walls of verandas and kitchens. World Health Organisation guidelines recommend that countries determine the locations where vectors bite and rest to determine locations for residual insecticide applications [235]. The findings of this study suggest that IRS should include kitchens and verandas in addition to the inside walls of houses. Alternatively, novel control methods such as insecticide-treated durable wall linings[236], spatial repellents,[237-239] insecticidal paints or screening to mosquito-proof verandas and kitchens could be evaluated.

6.6 Study Limitations

The study had several limitations. A limitation is that a 14-day sample over 2 villages is used to draw conclusions about movements at other times, and in similar villages across the Solomon Islands. To ascertain the degree of variation in daily movement (e.g., if the data set captured a range in the location patterns of individuals by time), we plotted the mean and interquartile range in the numbers of locations individuals within the study occupied by hour of the night. During each of the first four one-hour periods, the mean number of locations recorded for the study participants was 5 with the third quartile ranging up to 9 locations, demonstrating the wide range of activities and locations of individuals captured in the dataset. During the later hours of the night, the participants tended to frequent fewer locations, as they were more usually sleeping inside at this time. Thus, despite the data being recorded over a 14 day period, we are confident that the data captured both typical and atypical behaviours. A second potential limitation was not documenting mosquito biting rates during the 14-day study period. However, there were substantial mosquito biting rate data generated over several years immediately preceding the study. Relative mosquito biting rates at locations (both indoors or outdoors and within different village areas) and time of biting by *An. farauti* was consistence giving confidence that relative exposure to bites during the study could be predicted with confidence and precision.

6.7 Conclusion

This study linked entomological data with fine scale human movement studies to define the locations where residual transmission occurs and to identify the locations where future strategies with the potential for preventing mosquito bites should focus. Gaps when LLINs are not protective were identified during the early evening when most vectors seek blood meals. During this time, most people are outside, in peri-domestic areas (e.g., on verandas or in the kitchen area) near their houses. The size of this protection gap will vary depending on the behaviours of the human and vector populations[169]. While LLINs continue to provide significant protection from malaria, supplemental vector control strategies are needed to accelerate transmission reduction. Even after malaria is eliminated, vector surveillance and control along with human behaviour research needs to be maintained in receptive areas where there is significant risk of importation of parasites by infected people[51, 240].

6.8 Summary

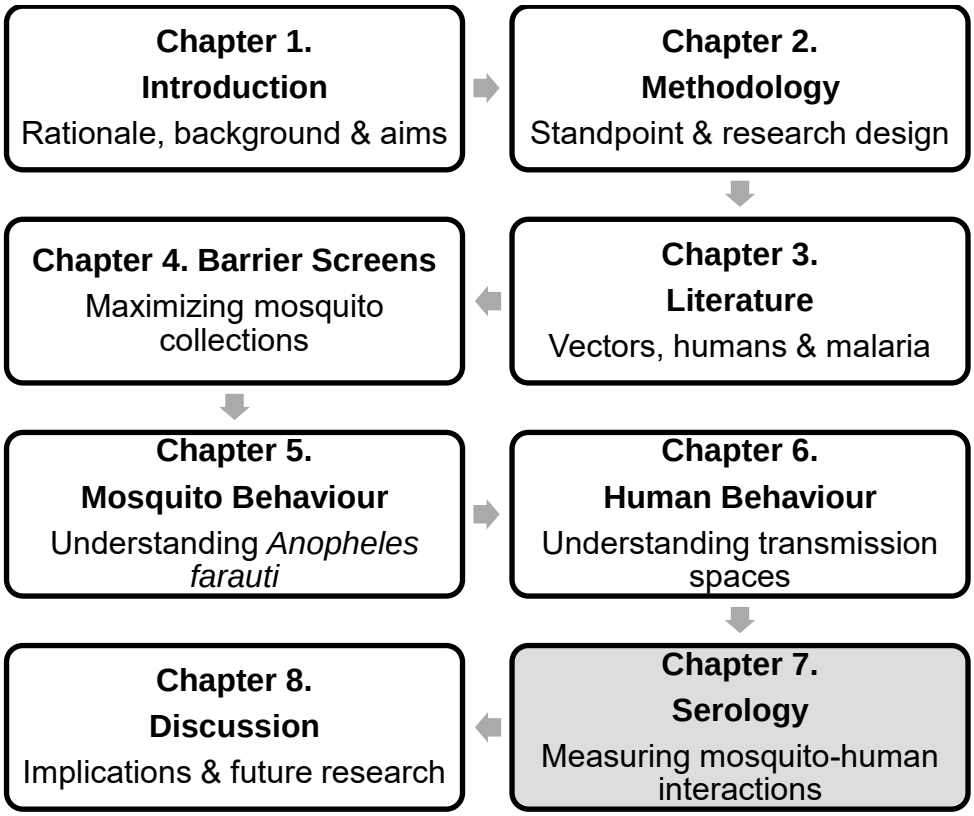
Malaria transmission after universal access and use of malaria preventive services is known as residual malaria transmission. The concurrent spatial-temporal distributions of people and biting mosquitoes in malaria endemic villages determines where and when residual malaria transmission occurs. Understanding human and vector population behaviors and movements is a critical first step to

preventing mosquito bites and eliminating residual malaria transmission. This study identified where people in the Solomon Islands are over 24-hour periods. Participants (59%) were predominantly around the house but not in their house when most biting by *Anopheles farauti,* the dominant malaria vector, occurs. While 84% of people slept under a long-lasting insecticide-treated bed net (LLIN), on average only 7% were under an LLIN during the 18:00 to 21:00 h peak mosquito biting period. On average, 34% of participants spend at least one night away from their homes each fortnight. Despite high LLIN use while sleeping, most human biting by *An. farauti* occurs early in the evening before people go to sleep when people are in peri-domestic areas (predominantly on verandas or in kitchen areas). Knowledge of people movement across a 24-hour period also helps understand broader social and environmental movements as well as exposure to other vectors such as the *Aedes* vectors of dengue, Zika and chikungunya viruses which are active during the day. Novel vector control tools that protect individuals from mosquito bites between sundown and when people sleep are needed for peri-domestic areas.

7 Serology

7.1 Introduction

The seventh chapter explores a novel method for measuring the levels of interaction between mosquitoes and humans. By measuring levels of IGg antibody in village resident sera to an anopheline salivary gland antigen, we hope to estimate biting exposure of individuals without the risk of exposing people to mosquitoes. The levels of IgG response and prevalence to the gSG6 a salivary antigen of *An. gambiae* were compared to human landing catch data for *An. farauti* at 10 locations in villages to analyse the specificity and sensitivity of the technique. This chapter has been published in *Malaria Journal* and can be read in the appendix.



7.2 Background

As malaria transmission diminishes, endemic areas become stratified with foci of residual transmission appearing based on receptivity, vulnerability and access and use of malaria prevention strategies (e.g., long-lasting insecticide treated nets and indoor residual spraying) [230, 241]. Defining foci and receptivity becomes increasingly challenging in areas with small vector populations. The classic entomological measure of transmission intensity, the entomological inoculation rate (EIR), requires estimating two parameters (the biting rate and the sporozoite rate), both of which can vary widely and rapidly in time and space. Hence, the EIR best estimates transmission intensity in high malaria transmission settings with high mosquito biting populations [242, 243]. Due to the lack of precision in estimating sporozoite rates in low transmission settings, vector biting rates remain the best entomological proxy to estimate receptivity despite the logistical challenges associated with spatial-temporal heterogeneity in biting rates [51].

Recently, human antibodies recognizing the *Anopheles gambiae* salivary protein gSG6 [244] or the gSG6-P1 peptide [245] were shown to be associated with recent exposure to anopheline bites in tropical Africa [196, 246]. A similar, although weaker, association was later documented in a number of geographic areas including South America and South-East Asia where other anopheline species are responsible for malaria transmission [247, 248]. In the South Pacific nation of Vanuatu, seroprevalence to gSG6 was correlated to reactivity to *P. falciparum* and *P. vivax* antigens which was hypothesized to be related to exposure to the bites of *An. farauti*, but biting rates were not estimated [249]. These observations suggested that anti-gSG6 antibody levels or seroprevalence might serve as an entomological proxy to estimate anopheline biting intensity by reflecting recent exposure to anopheline bites [246]. This approach has advantages compared to estimating biting rates by mosquito counts, as antibody prevalence would be less affected by short-term fluctuations in transmission intensity relative to estimates of the number of biting mosquitoes and would therefore be more cost-effective and potentially more precise.

In the Western Province of the Solomon Islands, malaria transmission is heterogeneous with a wide range in *An. farauti* biting rates documented [51]. We investigated the potential of anti-gSG6 antibodies to be a biomarker of human exposure to *An. farauti* bites in the Western Province, Solomon Islands.

7.3 Methods

Mosquito and human blood surveys took place during the dry and wet seasons in the villages of Jack Harbour, Tuguivili, Saeragi and New Mala in Western Province, Solomon Islands [51, 250]. Human biting rates were estimated twice in the dry season (May and August 2016) and twice in the wet season (November 2016 and February 2017). Because *An. farauti* biting densities are heterogeneous within and among villages, biting rates were estimated by outdoor human landing catches (HLC) at 10

locations (stations) within each village from 18:00 h to 00:00 h. During this time period, 93% of bites by *An. farauti* occurs [15]. Hence, this collection period closely approximates the number of bites by *An. farauti* nightly and will be referred to hereafter as the nightly biting rate. During each village vector survey, sampling was conducted for four nights at each of 10 sampling locations (Figure 7.1). The mosquitoes were identified morphologically in the field [47] and then a subsample was further identified by PCR amplification of the internal transcribed spacer region 2 of the ribosomal DNA for molecular confirmation of species [251].

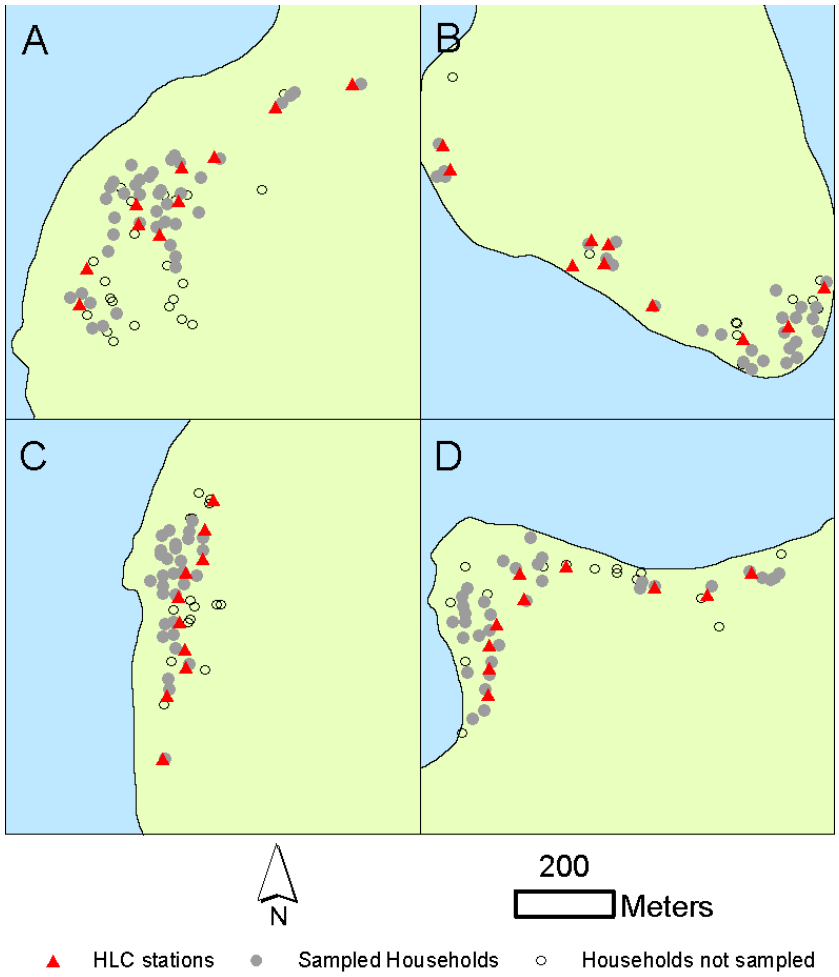


Figure 7.1 Proximity of stations where *An. farauti* biting rates were estimated by human landing catch (red triangle) to households of individual providing serum (grey circles) are shown for the villages of New Mala (A), Jack Harbour (B), Tuguivili (C) and Saeragi (D)).

Human blood surveys were conducted in September 2016 and March 2017. After explaining the purpose of the survey including potential risks, blood samples were taken from all individuals providing written consent (consent for individuals <18 years was provided by a parent or guardian). Demographic data (name, age, gender, house location, travel history, anti-malaria and bednet use histories) were recorded prior to collection of blood samples by finger prick. Samples were allowed to clot before

centrifugation to separate serum. Serum was removed and transferred into 2 ml micro-vials labelled with a unique identifying number. Sera was initially stored at 4 ºC, and within 4 days moved to a central location and frozen before shipping on dry ice with subsequent storage at -80 ºC.

Anti-gSG6 antibody titres were measured using an IgG detection quantitative suspension bead array on the Luminex xMAP© platform (Luminex Corp, Austin TX) alongside a panel of *Plasmodium falciparum* (PfAMA1, PfMSP119 and GLURP2) and *Plasmodium vivax* (PvAMA1 and PvMSP1-19) derived recombinant antigens. The Luminex assay for gSG6 antibodies was optimized by testing a range of gSG6 concentrations coupled to Luminex microspheres against 4 control pools for reactivity, specificity and optimal coupling concentration. Assay conditions were validated against serum samples from Burkina Faso with high and low mosquito bite exposure along with British negative controls and malaria positive controls. Serum dilutions of 1:200 were used in subsequent assays to minimize background cross-reactivity with an optimal concentration of 18.28 ug/ml of gSG6-P1 coupled to microsphere beads.

Diluted samples (1:200) were co-incubated with gSG6-coated microspheres, and after washing, a detection antibody was added followed by a final wash. The median fluorescent intensity (MFI) emitted by a reporter molecule on the detection antibody was measured using a Luminex MAGPIX© analyzer. All antigens underwent pre-assay optimization to identify a coating concentration that captured a dynamic range of responses [252]. Control sera consisted of hyperimmune serum samples to *P. falciparum* and *P. vivax*, and British malaria-naïve individuals.

Positives, negatives and blanks were present on each plate and compared to each other and pre-existing data to test for variability and accuracy. The positive control panel consisted of 10/198 (WHO *P. falciparum* positive reference standard curve), two duplicate wells of CP3 (a *P. falciparum* positive pool sourced from hyperimmune Tanzanian individuals) and two duplicate wells of 72/96 (a *P. vivax* positive control) at 1:200 dilutions. Negative controls consisted of four malaria-naive samples per plate (from a panel of forty samples provided by Public Health England). Two wells of each plate contained only dilution buffer to test for background reactivity. A seropositive sample was defined as having an MFI value greater than the mean value of the negative samples plus three standard deviations.

The dataset was analyzed by pairing individual gSG6 antibody MFI values with biting rate estimations based on the locations of individuals' home residences as 82 % of exposure to biting *An. farauti* occurs either in the home or the adjacent peri-domestic area (Pollard et al. (submitted)). Each participant providing a blood sample was assigned an anopheline biting rate based on the mean nightly HLC of the nearest HLC station to that individual's house. Participants living more than 100 m from an HLC station were excluded in the analyses for associations between biting rates and antibodies to gSG6. The relationship between biting exposure (estimated from HLC) and antibody titres was analyzed using Spearman's rank correlation. The differences in biting rates between villages and seasons were analyzed

using generalized linear models (GLM). The difference between the gSG6 MFI values for each village was analyzed using a GLM with the British control group as the reference. For the residents that provided serum during both the dry and wet season, a paired dataset was constructed and the change in the gSG6 antibody MFI values over time was compared with a Wilcoxon signed ranked test. Statistical analysis was conducted using R statistical software (ver.3.1.2).

7.4 Results

A total of 10,110 female anophelines were sampled during 660 HLC collections at 10 stations in each of 4 villages (Figure 7.1). Species identification was confirmed by PCR in a sample of 630; 95% of the confirmed species identifications were *An. farauti* (601 of 630), 3.5% were *An. hinesorum* (22 of 630) and 1% were *An. lungae* (7 of 630). The mean biting rate of *An. farauti* varied significantly by village (Table 7.1; $\beta = 24.740$, se = 1.1216, $p < 0.0001$) and season ($\beta = 6.4063$, se = 1.1917, $p < 0.0001$). The average nightly biting rate of anophelines during the dry season across all 4 villages was 6.5 b/p/n (bites/person/night) compared to an average of 19.0 b/p/n during the wet season. Highest biting rates were in Jack Harbour, where only *An. farauti* was found. Mean biting rates among 10 sampling stations in Jack Harbour ranged to 190 (Table 7.1).

Table 7.1 The mean and range in Anopheles farauti human landing rates from 10 sampling sites within each village during 4 surveys.

Season	Date	Village [Mean (Range)]			
		Jack Harbour b/p/n	**New Mala b/p/n**	**Saeragi b/p/n**	**Tuguivili b/p/n**
Dry	May 2016	44.0 (7 – 120)	2.7 (0 – 14)	0.2 (0 – 2)	1.8 (0 – 8)
	Aug 2016	1.1 (0 – 6)	1.8 (0 – 10)	0.1 (0 – 2)	0.3 (0 – 4)
Wet	Nov 2016	67.4 (0 – 367)	0.2 (0 – 3)	0 (0 – 0)	12.9 (0 – 73)
	Feb 2017	64.6 (2 – 279)	0.6 (0 – 4)	0 (0 – 0)	6.7 (0 – 26)
	Mean	**47.7**	**1.2**	**0.1**	**5.7**

A total of 791 serum samples were collected from residents of the four villages. From each village, Jack Harbour, New Mala, Saeragi and Tuguivili, the number of samples collected in the dry season (September 2016) were 74, 117, 110 and 85, respectively; and in the wet season (March 2017) were 69, 137, 83 and 116, respectively. Overall, a total of 210 samples were paired being collected from the same 105 residents during the dry season and again in the wet season.

Typical age-specific patterns of long-term exposure markers, PfAMA1, PfMSP1-19, GLURP2, PvAMA1 and PvMSP1-19 were observed for the serum samples from the Solomon Islands (results for PfMSP1-19 shown in Figure 7.2), confirming the assay performed as expected for the parasite antigens. When gSG6 MFI values were analyzed by age categories (<5 yrs, 6-15 yrs and >16 yrs), no significant association was seen (Fig. 3; β = -18.35, se = 22.32, P = 0.411). The gSG6 responses of the negative control panel of forty malaria-naïve samples fell within a range of the mean plus three standard deviations (MFI value of 253).

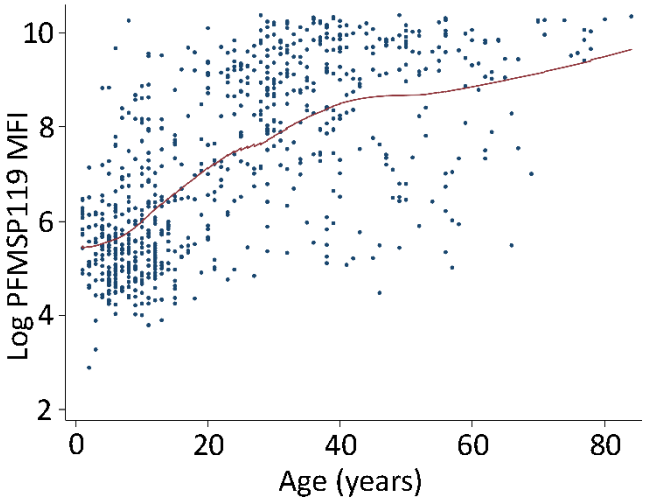


Figure 7.2 Age-specific patterns of long-term *P. falciparum* exposure of residents in Western Province of the Solomon Islands represented by a scatter plot of log-transformed antibody PfMSP1-19 median fluorescence index (MFI) by age with loess regression line (red line).

Estimates of mean *An. farauti* bite exposures of individuals were based on human landing catches at the nearest mosquito collection station to an individual's house (Figure 7.1). Participants living within 100 m of the nearest mosquito collection station (n = 733 samples) were included in the analyses for associations between biting rates and antibodies to gSG6. The mean distance from houses to closest HLC station for participants was 29 m (mean distances by village were 23 m, 36 m, 33 m and 25 m in Jack Harbour, New Mala, Saeragi and Tuguivili, respectively).

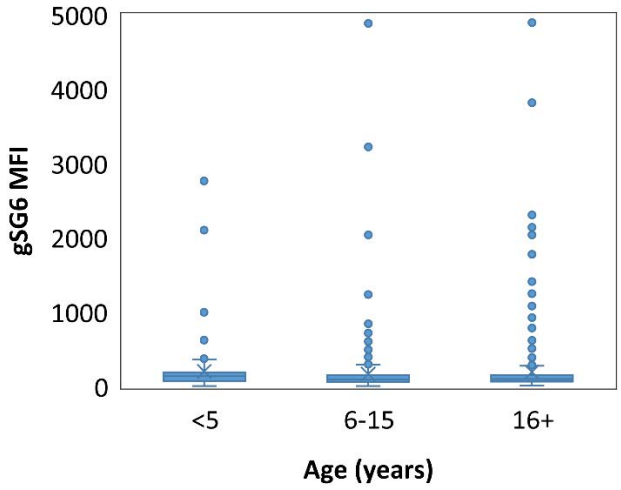


Figure 7.3 Age specific patterns of gSG6 MFI recognition for serum of residents of Western Province in the Solomon Islands (sample sizes: <5 yrs, n = 98; 6-15 yrs, n = 260; >16 yrs, n = 424). Differences in mean gSG6 MFI reactivity by age were not significantly different.

Sera from 11 % of 791 samples were classified as seropositive to gSG6. A significant relationship between the prevalence of anti-gSG6 antibody MFI and intensity of exposure to *An. farauti* bites in the month preceding blood surveys was not found (Figure 7.4) ($\rho = 0.0276$, $p = 0.4$). In fact, residents with high nightly *An. farauti* exposure (>10 bites/night), did not generate high levels of antibody titers: the highest gSG6 MFI value observed was 634 for individuals exposed to high biting rates, while residents exposed to more moderate biting rates (<10 bites/night) had gSG6 MFI values ranging up to 4,897. Analyses comparing the prevalence of anti-gSG6 antibody MFI and intensity of exposure to *An. farauti* bites in both the wet and dry seasons as the mean of HLC catches in surveys one and four months preceding each blood survey also did not reveal any significant relationships ($\rho = -0.0702$, $p = 0.05$).

Differences in the population level antibody titres for each village were analysed both in the dry and wet seasons using the British population as a reference group (Figure 7.5). There were only two Solomon Island populations (Tuguivili village during both the dry and wet seasons when nightly biting rates were 0.3 and 6.7) that were statistically different from the British control serum samples ($P = 0.022$ and $P = 0.002$). In the other villages, MFI values were not significantly different from the British controls, including Jack Harbour village with an almost 10-fold greater nightly number of biting *An. farauti* than Tuguivili village (Figure 5).

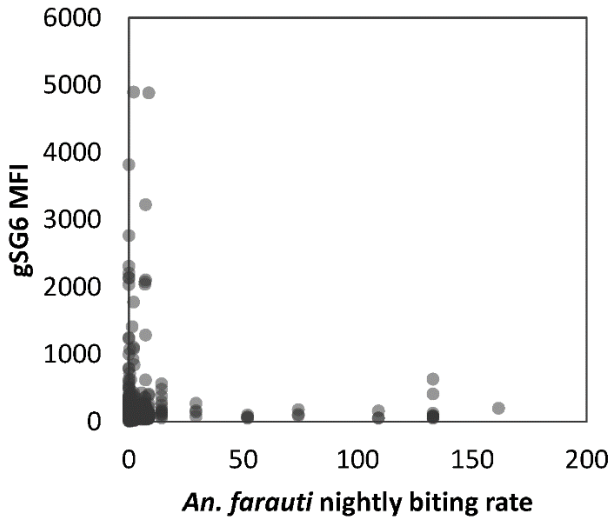


Figure 7.4 The relationship between the intensity of anti-gSG6 antibody responses and exposure to the mean number of *An. farauti* bites per person per half-night (estimated from the nearest collection station to participants homes during the month preceding blood surveys).

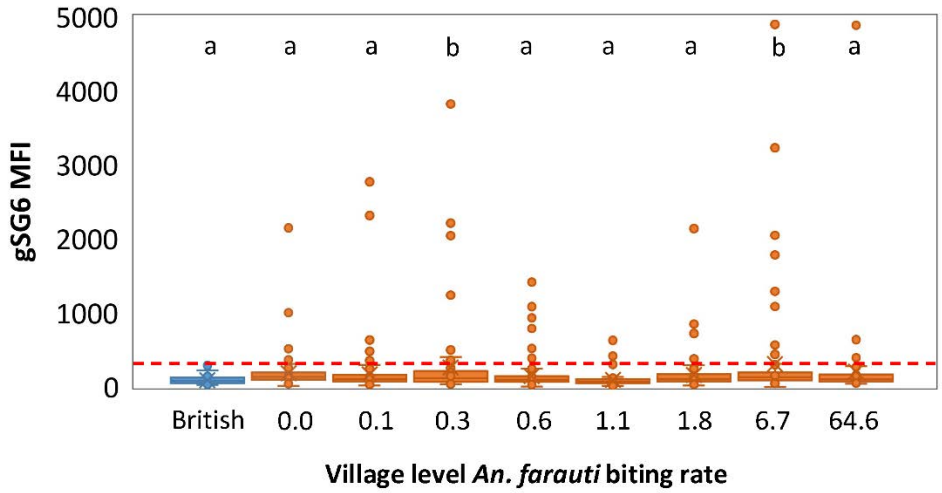


Figure 7.5 The relationship between the intensity of anti-gSG6 MFI antibody responses of individuals by estimated *An. farauti* biting rates from the biting surveys a month before the blood survey. Seropositives were defined as sera generating an MFI greater than the mean plus 3 standard deviations of the MFI values from 40 malaria naïve British (shown as blue). Seropositive cutoff line is shown as a red dashed line. Significantly different mean MFI values (at the 002 level) of participants of villages are identified by different letters above the village specific MFI values. Significantly elevated MFI values relative to British sera were found in two village surveys when biting rates were low (0.3 bites/night) and high (6.7 bites/night).

Overall, significant differences in the mean MFI to gSG6 of all serum samples between the dry and wet seasons were not found. However, when paired sera of individuals (n=105) from the dry and wet seasons were compared, there was a slight but significant increase in MFI values to gSG6 from the dry (mean

=189) to the wet season (mean = 202.9) (Wilcoxon signed ranked test: V= 1982.5, p = 0.01542). The MFI values to gSG6 of residents of Jack Harbour showed a significant increase between the dry and wet seasons, increasing from a mean of 74.08 to 125.8 (Wilcoxon signed ranked test: V= 17, p = 0.0001) when the mean nightly *An. farauti* biting rates in the dry and wet seasons increased from 22.5 to 66.5 (based on the mean of two vector surveys for HLC preceding the blood survey).

7.5 Discussion

An association between human anti-gSG6 antibody titres (expressed as MFI values) and exposure to *An. farauti* bites as determined by HLCs was not observed in this study. While some individual serum samples strongly recognized gSG6, the reactivity of most serum samples from the malaria endemic Solomon Islands were not significantly greater than the British negative controls. Failure to find significant recognition of anti-gSG6 antibodies in the majority of residents tested could be due to epidemiological factors (e.g., inadequate exposure to *An. farauti* bites, or variations in the attractiveness of individuals to *An. farauti* not captured by the estimates of human biting rates) or immunological effects (e.g., limited understanding of the kinetics of the anti-gSG6 response in humans, inadequate assay sensitivity or insufficient similarity of saliva antigens of *An. farauti* to the gSG6 antigen of *An. gambiae*). Previous studies indicated that antibody levels and/or seroprevalence to vector salivary antigens can reliably estimate malaria transmission in a number of countries, especially in tropical Africa (i.e., seroprevalence to gSG6 or gSG6-P1 and malaria antigen markers are significantly associated). However, validating the IgG response to gSG6 as a reliable marker of mosquito exposure requires comparing human antibody reactivity to gSG6-P1 or to gSG6 with estimates of mosquito biting rates on the same individuals or by comparing the reactivity of populations in defined areas with estimates of biting rates in the same areas, as was done in Senegal and Cambodia using single estimates of biting rates for each census district or village, respectively [196, 253]. In this study in the Solomon Islands, biting rates within villages were estimated at 10 locations per village by human landing catches to estimate the exposure of residents of nearest houses to biting mosquitoes.

In Cambodia, despite a 9-fold range in the size of *An. dirus* populations between two villages, little difference was seen in the corresponding antibody recognition of gSG6 [253]. In this study, a greater than 100-fold range in exposures to *An. farauti* bites was documented amongst 4 villages without finding an association with the titre or prevalence of antibodies to the gSG6 antigen.

Previous work in Senegal found an association between antibody prevalence to whole *An gambiae* saliva or to the gSG6-P1 peptide and *An. gambiae* nightly biting rates up to 124 b/p/n [194] [196]. This biting intensity is comparable to the nightly exposure of people to *An. farauti* bites in the high exposure village in this study (191 b/p/n). While a minority of individuals had a wide range in seropositive antibody titres recognizing gSG6 in the Solomon Islands, most individuals (89%) were seronegative.

Amongst the 11% of individuals who were seropositive, significant associations with *An. farauti* bite exposure were not seen. In Vanuatu where *An. farauti* is also the primary vector, a decrease in seroprevalence to gSG6 with malaria transmission was correlated to reactivity to *P. falciparum* and *P. vivax* antigens [249]. A significant difference between the study in Vanuatu and this study in the Solomon Islands was that the *An. farauti* biting rates in Vanuatu were not estimated entomologically whereas the biting rates in the Solomon Islands were estimated at a fine scale enabling specific estimates of the exposure of individuals to biting *An. farauti* with the intensity of antibody recognition of gSG6 by those same individuals.

These results could be explained, in part, by a lack of assay sensitivity, as hypothesized by the studies in Cambodia [253] and Vanuatu [249]. Previous studies employed gSG6-P1 or gSG6 as antigens with sera diluted from 1:20 to 1:200. The studies in Cambodia and Vanuatu measured responses in an ELISA to 5ug/ml gSG6 at a serum dilution of 1:200. This study also measured antibody responses of serum diluted 1:200 to gSG6, but in a Luminex platform. While anti-gSG6 antibody prevalence may be lower due to the serum concentration used in the assays (1:200), any relationship between exposure to high levels of anopheline bites and corresponding highly reactive sera to gSG6 should still have been evident. Furthermore, a typical age-related increase in antibodies to specific malaria antigens was observed in our study in the Solomon Islands suggesting the assay performed as expected. A lack of age-related antibody response for gSG6 was observed in Burkina Faso but was hypothesized to be immune tolerance generated after intense and prolonged exposure to bites of Afrotropical malaria vectors [254, 255].

Another plausible explanation for the lack of association between anti-gSG6 antibodies and *An. dirus* biting rates in Cambodia [253] and in this study with *An. farauti* may be the limited sequence homology between *An. gambiae* and both *An. dirus* and *An. farauti* SG6 proteins. The *An. gambiae* gSG6 (used as antigen in all reported studies) shares only 54% and 52% identity with *An dirus* and *An. farauti* SG6 proteins, the primary malaria vectors in Cambodia and the Solomon Islands, respectively [199]. The limited similarity (70%) to *An. farauti* gSG6 was hypothesized as likely responsible for a low assay sensitivity in the Vanuatu study [249].

In the study in the Americas, antibody recognition of the gSG6-P1 antigen was reported as significantly correlated with malaria infection status and mosquito bite exposure history of Columbians and Chilians [247]. In that study, residents of Columbia may have been exposed to the bites of the main malaria vectors, *An. albimanus, An. darlingi*, and *An. punctimacula*; Chilean soldiers stationed in Haiti would have been potentially exposed to *An. albimanus*, the only malaria vector in Haiti. However, the SG6 antigen is absent in both *An. albimanus* and *An. darlingi* as well as in all other species belonging to the *Nyssorhynchus* subgenus analyzed [199, 256], suggesting that the antibodies to gSG6 in Columbians may represent exposure to *An. punctimacula* (a member of the *Anopheles* subgenus) or minor vectors.

For the Chilean soldiers, previous exposure to anopheline bites in Chile including *An. pseudopunctipennis* (also belonging to the *Anopheles* subgenus) may have generated the antibody recognition of gSG6 [257]. While *An. atacamensis* and *An. pictipennis* are also endemic to Chile [258], these species are in the same subgenus (*Nyssorhynchus*) as *An. albimanus* or *An. darlingi* which lacks the SG6 protein coding gene [199, 256].

The use of antibodies against anopheline salivary proteins as markers of human exposure to malaria vectors has multiple advantages over longitudinal collections of anophelines by human landing catches; in fact, serological analyses are both faster (requiring a single cross-sectional survey to estimate recent exposure to biting mosquitoes) and less expensive while minimizing exposure of survey teams to anophelines bites.

In the Southwest Pacific where *An. farauti* is the dominant vector, a number of challenges to using and interpreting antibody recognition of the gSG6 antigen as a proxy for measuring biting exposure were identified that limits the potential of this assay for program or research applications. Firstly, the overall low reactivity of most sera of Solomon Islands residents to gSG6 will require large numbers of serum samples to detect significant changes in biting intensity. Consequently, the assay will not be applicable for monitoring small-scale heterogeneities in biting rates (a feature of low transmission scenarios in general and in the Southwest Pacific in particular ([51, 259]). Secondly, finding highly reactive serum in villages with very low *An. farauti* biting rates and, conversely, unreactive serum in village residents with very high biting rates is perplexing. This will require well-characterized studies to compare serum reactivity to saliva antigen with concurrent biting rates to understand the relationship between bite exposure and the development (and loss) of antibodies to saliva antigens to allow epidemiologically relevant interpretations of changes in the prevalence and intensity of antibody recognition of saliva antigens.

7.6 Conclusion

The use of the gSG6-P1 peptide is currently not suitable for measuring biting exposure to *An. farauti.* Despite the fact that significant levels of anti-gSG6 antibodies were not found in individuals exposed to significant numbers of *An. farauti* bites in this study, the potential utility of human antibodies as markers of biting exposure shows great promise. However, as pointed out by the results reported here, the use of anopheline salivary antigens as a proxy for estimating human exposure to bites of malaria vectors may require the use of salivary antigens from local mosquito species and validation by correlation of antibody reactivity with concurrent entomological measurements.

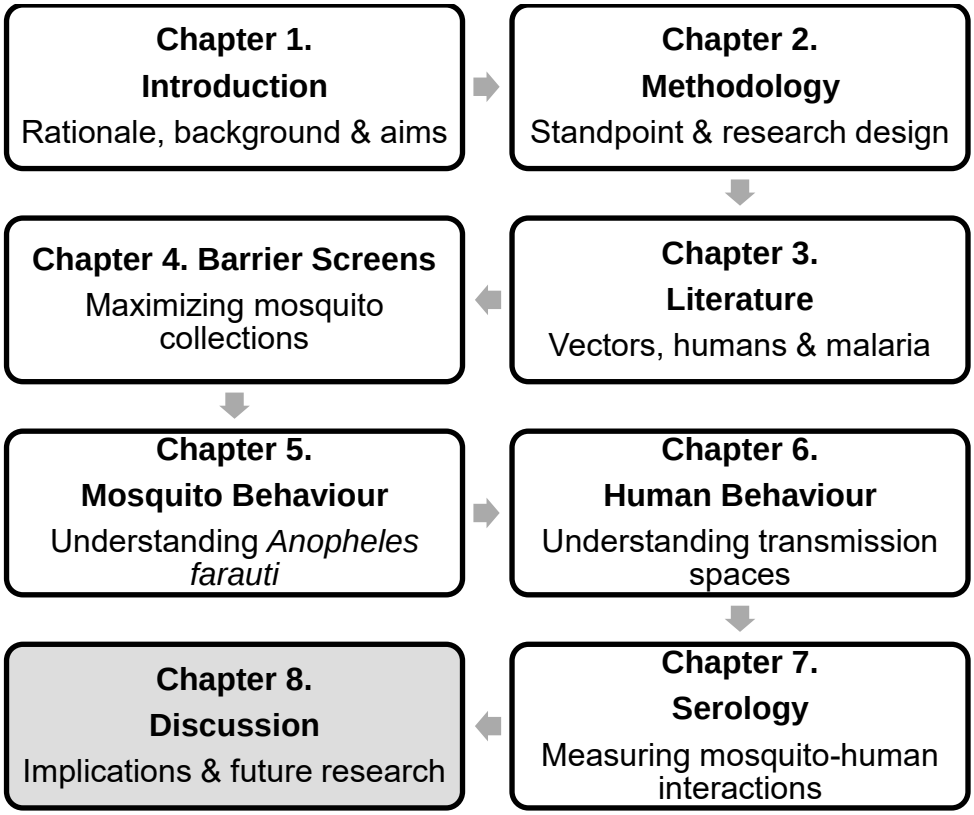
7.7 Summary

Mosquito saliva elicits immune responses in humans following mosquito blood feeding. Detection of human antibodies recognizing the *Anopheles gambiae* salivary gland protein 6 (gSG6) or the gSG6-P1 peptide in residents of Africa, South America and South East Asia suggested the potential for these antibodies to serve as a universal marker to estimate human biting rates. Validating the utility of this approach requires concurrent comparisons of anopheline biting rates with antibodies to the gSG6 protein to determine the sensitivity and specificity of the assay for monitoring changes in vector populations. This study investigated whether seroprevalence of anti-gSG6 antibodies in humans reflected the relative exposure to *An. farauti* bites in the Solomon Islands as estimated from sympatric human landing catches. In our study in the Solomon Islands only 11% of people had very high anti-gSG6 antibody titres, while others did not recognize gSG6 despite nightly exposures of up to 190 bites by *An. farauti*. There were clear spatial differences in the human biting rates, but associations between anti-gSG6 antibody titres and biting rates were not found. Few studies to date have concurrently measured anopheline biting rates and the prevalence of human antibodies to gSG6. The lack of association between anti-gSG6 antibody titres and concurrently measured human biting rates suggests that the assay for human anti-gSG6 antibodies lacks sufficient sensitivity to be a biomarker of *An. farauti* exposure at an epidemiologically relevant scale. These findings imply that an improvement in the sensitivity of serology to monitor changes in anopheline biting exposure may require the use of saliva antigens from local anophelines, and this may be especially true for species more distantly related to the African malaria vector *An. gambiae*.

8 Discussion

8.1 Introduction

This final chapter provides a synthesis of key findings, the implications of these findings and the associated challenges and opportunities arising from these findings. The purpose of this thesis was to better understand the interacting space between anophelines and people in the Solomon Islands and how this data can inform both our understanding and control of malaria. Through better understanding of this overlapping space we can make informed decisions in the battle against malaria.



8.2 Key research findings

The risk of malaria transmission is primarily governed by the interaction between humans with malaria transmitting mosquitoes [169, 178]. As malaria transmission declines, as in the Solomon Islands, malaria prevalence becomes highly heterogeneous in both space and time [51]. There is a great need to understand the heterogeneous transmission patterns to ensure that vector control tools are deployed to the most at-risk populations. Thus, this thesis focused on understanding the behaviour patterns of both mosquitoes and people, and the interactions between the populations, and in so doing, identified potential opportunities to interrupt malaria transmission. Of importance is defining the times and locations where residents are at the greatest risk of being bitten by anopheline vectors and the associated risk of malaria transmission, as this directly relates to implementing effective vector control. At present, the only WHO recommended vector control tools are LLINs and IRS that are implemented inside houses [39].

The main findings of each research component in this thesis are:

1) *Anopheles farauti* can be effectively sampled using barrier screens, an easy to use passive mosquito collection tool. Numbers of mosquitoes captured are enhanced by constructing the barrier screens from material that is dark coloured and of medium thickness, using a simple design without eaves and inspected regularly to collect mosquitoes, i.e. every 30-60 minutes.

2) *Anopheles farauti* of different physiological states and gender were heterogeneously distributed, both spatially and temporally, within villages. Specifically, female mosquitoes tended to be closer to households (up to 20 m away), while males were mostly found further from houses but within 10 m of larval habitats. Unfed, host blood seeking or blood-fed female *An. farauti* mosquitoes were found across larger geographic foci, compared with both sugar-fed female *An. farauti* and male *An. farauti*. The majority (60%) of unfed, host seeking and blood-fed female *An. farauti* were collected prior to 21:00 h.

3) During the period of peak mosquito biting, most people were around their houses, but not inside their houses. While 84 % of people slept under a LLIN, on average only 7 % were under an LLIN during the 18:00 to 21:00 peak mosquito-biting period. During this time period, over half of village residents were in these outdoor kitchens or on verandas within the peri-domestic area of the household. There was also high movement of people in and out of villages.

4) The most sensitive tool available to estimate the exposure of humans to mosquito bites remains direct measures of the human biting rate, usually conducted with human landing catches (HLC). An alternative proposed method is to measure the prevalence of human antibodies to the *An. gambiae* salivary gland protein 6 (gSG6) in residents exposed to host seeking *Anopheles*. In the Solomon

Islands only 11 % of people had high anti-gSG6 antibody titres and a significant relationship between human biting rates by *An. farauti* and anti-gSG6 titres was not found.

8.3 Implications of findings

These key findings have implications for malaria elimination globally and within the Solomon Islands, especially at the village level. The key findings define mosquito and human distributions in both time and space and thus identify where and when these populations interact. The spatial-temporal defined interactions have implications for vector control which aims to reduce these interactions.

8.3.1 Understanding mosquitoes

The main physiological states of an adult mosquito are unfed (searching for blood or sugar meals), fed (either sugar or blood), resting (following feeding and/or preceding oviposition) and gravid (searching for an oviposition site) [55]. Much work to date has focused on the female in blood-seeking and blood-fed physiological states as this is when transmission of malaria from mosquito to person or person to mosquito occurs. This study described the distribution characteristics of all the main physiological states of the female *An. farauti* as well as males.

The results obtained in this study were only possible if mosquitoes distributions could be mapped by a passive method of sampling. This study has proven that the barrier screen is a useful tool to answer basic biological questions as it is passive (minimal influence/attraction to mosquitoes) but also able to monitor the entire adult mosquito population, including males, sugar-feds and blood-fed females of multiple species. Understanding the distributions of these lesser known physiological states may lead to development of novel tools targeting them.

This study has shown that mosquitoes are heterogeneously distributed by physiological states and genders. Defining the space and time of host blood seeking is the first step towards understanding transmission risk; the next step is understanding where humans are, and then calculating the nexus between the mosquito and human populations.

8.3.2 Understanding people

As human behaviour is a key component of malaria transmission dynamics, there is a need for understanding the activities and locations of individuals during peak times of potential mosquito exposure. Important human behaviour studies have included sleeping behaviour and bed-net use [260, 261], acceptability to new vector control tools [177, 262] human mobility [260, 261, 263] and socio-cultural practices [264]. This study documented human behaviour including bed-net use and human distribution both at a fine scale around the home and also at the larger village and provincial scales. At

the village/household scale, the peri-domestic area is the area where the greatest risk of being bitten by malaria vectors occurs. This area has a gap in protection but is also an opportunity for control/protection, especially as the majority of this mosquito protection gap is confined to two specific locations (the veranda and the kitchen). Knowing where people and mosquitoes interact can enable action through push-pull strategies to reduce this unprotected space.

8.3.3 Reducing overlap

This study identified a protective coverage gap where people are during peak mosquito biting times. Biting exposure is predominantly in the peri-domestic area, specifically in the veranda (area 1, figure 8.1) and kitchen (area 2, figure 8.1) (Chapter 6) with increased risk of exposure to *An farauti* bites greater in selected areas of the village (Chapter 5). These are sheltered areas but are not completely walled and are therefore exposed as outdoor environments.

Figure 8.1 The peri-domestic area (1. veranda and 2. kitchen)

Mosquitoes behaviours are guided by chemical cues which can either attract or repel; in combination repellents and attractants can be used as a push-pull technique to move mosquitoes away from high risk areas [265]. Potential novel tools to assist with malaria elimination in the Solomon Islands village context are spatial repellents, attractive toxic sugar baits and insecticide treatments. Spatial repellents [237-239] may be used in the peri-domestic area to limit outdoor transmission by pushing away mosquitoes [266]; however, the openness of the veranda area would likely limit the effectiveness of any spatial repellent as chemical odours would be rapidly diluted by prevailing breezes. Attractive toxic sugar baits (ATSB) target mosquitoes searching for a sugar meal which are needed daily and also by males and work by pulling away mosquitoes [267]. This study found foci of sugar-fed mosquitoes which

would be great opportunities for placement of such ATSBs. Insecticide treatment in the form of insecticide-treated durable wall lining (ITWL) is a method for vector control by attaching to inner walls of housing [236] and maybe used for walls of kitchens or verandas. In addition, in line with WHO IRS guidelines [268] the kitchen, under the house and veranda are recommended sprayable structures. This is a tool that is proven and approved and should be re-introduced in the Solomon Islands to protect the peri-domestic space.

This study also recorded that elevated wind speeds (>1km/h) are associated with lower mosquito densities. This may lead to reduced biting effect when people are located in exposed areas such as the veranda with the sea breeze keeping mosquitoes away with a dual effect of less mosquitoes flying about, and the dispersal and dilution of human odours.

8.3.4 Malaria Elimination

The greater goal is to achieve malaria elimination for the Solomon Islands. Great gains have been made since the 1990s in reducing malaria in the Solomon Islands; however, since 2010, progress has stalled [10]. Solomon Islands signed the Asia Pacific Leaders Malaria Alliance (APLMA) malaria elimination by 2030 declaration and to do that: "Investments in personnel, infrastructure, surveillance and tracking systems" are needed, along with "leadership and collaboration" to then remove the final parasite through "map, prevent, test and treat" [269]. The four pillars of the Global Vector Control Response 2017-2030 are a) strengthened collaboration, b) engaged communities, c) enhanced surveillance and monitoring and d) scale up of tools and approaches [26]. To achieve elimination in the Solomon Islands there will need to be an arsenal of new tools, improved technical capacity and engaged communities.

New tools

Despite both high coverage of LLINs and their use, there is still a large protection gap in the evenings in the peri-domestic area and therefore new tools and techniques are needed. Innovative technologies are under evaluation and may soon receive WHO recommendations for programmatic implementation on the road to elimination [270]. LLINs and IRS still have an important role in controlling malaria [271] but it must be a priority to develop new products that operate synergistically with indoor interventions by minimising transmission outside of houses [5]. Integrated vector control needs to maximize community wide coverage [169] and focus on; optimization of vector control delivery, monitoring of vector populations, development of effective and eco-friendly tools, careful evaluation of new vector control tools, continuous monitoring of the environment around vectors and the cross-disciplinary cooperation [272]. Factors affecting use of protection against mosquito bites are affordability, effectiveness, availability, social factors and practicability. These factors are important to keep in mind while we are looking for new, novel tools for vector control [273] especially relevant in the rural villages

of the Solomon Islands. These new vector intervention combinations need to be tailored to the local ecology, vector biology, epidemiology and local technical capacity [274], but must also be based on evidence and require strong evaluation and monitoring [275].

Technical capacity

This study succeeded due to the expertise of a few people, but ultimately eliminating malaria will need more 'good' people, entomologists, social scientists, epidemiologists and public health specialists to translate research into programmatic tools. There is also a need for improved technical capacity of people and the training of problem solvers. Malariologists need to be problem solvers as they were prior to the discovery of the insecticidal properties of DDT, by using all resources available to combat malaria in their own unique context [271]. There is a need to train entomologists who can identify vulnerabilities of vectors to interventions and solutions to complement current action based on local entomological assessments of vector characteristics and local conditions [276]. It is therefore advantageous to develop local capacity in the fight against malaria as they best understand the local context, challenges and opportunities especially in working with the local community.

Community engagement

Working with the local communities made this study possible, malaria is everybody's business and everybody needs to work together. There is a need for greater community engagement, awareness and knowledge sharing, with community engagement identified as a pillar of action in the Global Vector Control Response 2017 – 2030 [26]. Local people in the village need to be a part of the elimination discussions and to also take ownership of the malaria problem. Helping local people to fully understand the challenges of malaria elimination will lead to greater cooperation in the efforts to stamp out malaria. Malaria and mosquito control in rural villages need to be locally and culturally acceptable [177]; national vector control authorities engaging with communities will ensure adoption and ownership of evidence based control strategies . A simple fact such as home improvement which has been shown to greatly impact malaria transmission is significant [277]. The location, construction and interaction of people with the home will impact transmission.

8.4 Future Research

This section will discuss the realities, challenges and opportunities that arose from this thesis. Fieldwork in remote islands is highly dependent on local conditions such as the weather, and village social environments. Future studies may focus on different Solomon Island villages to compare with the patterns of mosquito and humans described in this study. The five main areas of future research arising

from this thesis are; barrier screens, mosquito ecology, human behaviour, the peri-domestic space and epidemiology.

8.4.1 Barrier Screens

Future research arising would be to better understand the specific interacting behaviours of mosquitoes and the barrier screen. The barrier screen is a passive tool that intercepts mosquitoes as they move. However, it is unclear what exact behaviours these mosquitoes exhibit when they encounter these barrier screens: we know that mosquitoes will rest temporarily on barrier screens but not exactly for how long. Understanding how vectors interact with and rest on barrier screens may open the door to using the barrier screen as a vector control tool. Future studies may look at specific mosquito behaviours on and around barrier screens such as the proportion that do stop and rest, how long they stop and rest and the direction of flight away from and toward barrier screens. Experiments using high tech cameras in laboratory conditions may be able to answer these questions. Further barrier screen studies may add additional factors such as extra colours, different material types and different designs to maximise retention of mosquitoes landing on them to minimise the need for human labour.

8.4.2 Mosquito ecology

Future research arising would be to better understand the ecological conditions that lead to increased mosquito numbers or reductions in mosquito densities. This study observed a significant relationship between the weather variables of temperature, humidity and wind. Further research with a permanent weather station, and regular mosquito collections would enable finer-scale relationships between these weather variables and mosquito density, such as what is the lag time period after significant rainfall or drop in temperature for mosquito numbers to be affected. It would also be interesting to characterize specific microclimates and optimal resting conditions of mosquitoes in and around the home and peri-domestic area. A randomized sampling approach will also enable the creation of contour distribution maps to better define environmental relationships and mosquito densities.

8.4.3 Human behaviour

Future research arising would be to compare and contrast different human movement monitoring tools. The current movement diary methodology proved to be very useful as we could define very fine scale movement of people, and quantify the amount of time that people are spending inside the house relative to being in the peri domestic area. GPS could map larger scale movement and general mobility of people with this data integrated with fine scale data from movement diaries. Another possible research area would be to study human behaviour using qualitative research methods to better understand knowledge, attitudes and perceptions of villagers around behaviour and movement during peak biting times. There

is also a need to better understand local people's perceptions toward malaria, vector control and the use of current or new tools.

8.4.4 The peri-domestic space

The peri-domestic area was determined to an important space and a research question arising would be to look at specific mosquito behaviours in this space as well as specific conditions such as wind speed and flow in this space. Other possible research areas are the impacts and useability of various vector control tools in the peri-domestic space. Trialling different tools previously discussed in this area would provide useful information. Tools that could be trialled are spatial repellents and insecticide treated material in the form of a barrier screen. Trials for IRS in this space should use prequalified insecticides, as this is something that program operators will be able to implement immediately and in the short term. Also interesting would be exploring home designs that might increase ventilation and make these spaces less attractive for mosquitoes.

8.4.5 Epidemiology

Future research can also map not only the foci of mosquitoes and people but also of the malaria parasite. Having a tool that could quickly identify very low levels of parasitemia in individuals would be powerful to locate parasite foci. Especially with a high proportion of malaria infections being afebrile/asymptomatic. Following the serology study next steps would be to identify and use *An. farauti* salivary gland protein to explore whether an improved serological response is generated from a protein belonging to the same species. The purpose of such tools would be to develop one that is programmatically useful with sensitivities akin to HBR data.

8.5 Conclusions

The hypothesis of this study was that mosquito and human behaviour patterns overlap in a given location and time to create an optimum space of interaction that sustains malaria transmission. This area of overlap was identified as the peri-domestic space in the early evening. Therefore, to achieve malaria elimination this peri-domestic space where mosquitoes and human interactions overlap needs to be reduced. This may be done by informing local people to adjust their behaviour, reducing the mosquito population or creating barriers of protection between mosquitoes and humans in this time and place. To ultimately shrink the mosquito protection gap in the peri-domestic space, the area where the vector and human worlds collide.

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10 Appendices

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Parasites & Vectors

RESEARCH Open Access

Maximising mosquito collections from barrier screens: the impacts of physical design and operation parameters

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Abstract

Background: Traditional methods for collecting outdoor resting mosquitoes are generally inefficient with relatively low numbers caught per unit effort. The barrier screen, designed to intercept mosquitoes as they fly between areas where blood meals are obtained and oviposition sites where eggs are laid, was developed in 2013 as a novel method of sampling outdoor mosquito populations. Barrier screens do not use an odorant lure and are thus a non-mechanical, simple, low maintenance and passive sampling method for use, even in isolated locations.

Methods: To maximise mosquito collections from barrier screens, multiple Latin square 3 × 3 experiments were conducted in Smithfield, Queensland, Australia. Parameters of barrier screens were varied including the effects of construction materials (net weight and colour), screen design and frequency of inspections.

Results: Significantly more mosquitoes were collected on simple dark coloured screens of 50% or 70% shading weight with collections every 30 min. Sixty percent of mosquitoes were found on barrier screens within 60 cm of the ground.

Conclusions: The barrier screen is a relatively new adaptable tool that can answer a number of behavioural, ecological and epidemiological questions relevant for the surveillance and basic understanding of the movement and resting habits of mosquitoes by sex or physiological status. This method has demonstrated robustness in collecting a wide range of mosquito species as well as flexibility in where barrier screens can be deployed to explore mosquito movements within rural and peri-domestic environments.

Keywords: Outdoor mosquito collections, *Anopheles farauti*, Barrier screen, Mosquito movement, Passive tool

Background

Mosquito sampling using long-range odorant lures (including human landing catches) give useful insights into mosquito densities attracted to fixed locations [1] but little work has been done to understand the movement of mosquitoes between locations. Observing insects in their natural flight patterns without influencing their behaviour requires using capture methods with minimal long-distance attractants [2]. Almost all sampling techniques are prone to biases and different techniques will be better suited to collecting different subsets of insect populations [3]. Sampling techniques without odorant attractants (hereafter referred to as passive tools) are useful for representative sampling of entire populations, e.g. males and females of all physiological states (unfed, sugar-fed, blood-seeking females, recently engorged females as well as gravid females seeking oviposition sites). Often the utility of traps is constrained by requirements for attractants or trap designs (size) or power requirements for light or fans. Passive tools without moving parts or power requirements are simpler, cheaper, more robust and easier to deploy in isolated areas.

There are two functional types of passive tools; tools that provide estimates of resting mosquito numbers and tools that infer mosquito movement. Passive tools that target resting mosquitoes include pit shelters, resting pots and boxes such as the sticky resting box [4]. Common passive methods for collecting mosquitoes and inferring movement patterns include malaise traps, ramp traps, stationary nets and sticky traps [3]. The malaise trap was one of the first passive tools for insects [5] and

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Parasites & Vectors

RESEARCH Open Access

Unique fine scale village spatial-temporal distributions of *Anopheles farauti* differ by physiological state and sex

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Abstract

Background: The ecology of many mosquitoes, including *Anopheles farauti*, the dominant malaria vector in the southwest Pacific including the Solomon Islands, remains inadequately understood. Studies to map fine scale vector distributions are biased when trapping techniques use lures that will influence the natural movements of mosquitoes by attracting them to traps. However, passive collection methods allow the detailed natural distributions of vector populations by sex and physiological states to be revealed.

Methods: The barrier screen, a passive mosquito collection method along with human landing catches were used to record *An. farauti* distributions over time and space in two Solomon Island villages from May 2016 to July 2017.

Results: Temporal and spatial distributions of over 15,000 mosquitoes, including males as well as unfed, host seeking, blood-fed, non-blood fed and gravid females were mapped. These spatial and temporal patterns varied by species, sex and physiological state. Sugar-fed *An. farauti* were mostly collected between 10–20 m away from houses with peak activity from 18:00 to 19:00 h. Male *An. farauti* were mostly collected greater than 20 m from houses with peak activity from 19:00 to 20:00 h.

Conclusions: *Anopheles farauti* subpopulations, as defined by physiological state and sex, are heterogeneously distributed in Solomon Island villages. Understanding the basis for these observed heterogeneities will lead to more accurate surveillance of mosquitoes and will enable spatial targeting of interventions for greater efficiency and effectiveness of vector control.

Keywords: *Anopheles farauti*, Barrier Screen, Solomon Islands, Males, Sugar-feds

Background

Mosquito ecology remains inadequately understood for many species [1, 2], including *Anopheles farauti*, a dominant malaria vector in the southwest Pacific from western Indonesia through Papua New Guinea and the Solomon Islands to Vanuatu [3, 4]. Although there are behavioural differences among species [5], in general, mosquitoes fly to satisfy five basic behaviours: to blood feed, to find favourable resting sites, to lay eggs, to mate and to sugar feed [2]. Much is known about the blood-feeding of *An. farauti* [6, 7] but less is known about resting [6, 8–10] and oviposition behaviours [11–13]. These behaviours directly impact the efficacy of the three WHO recommended interventions of insecticide-treated nets (ITNs), indoor residual spray (IRS) and larval source management (LSM) [14]. Further, very little is known about two behaviours of *An. farauti*, sugar-feeding and mating, both of which are targets of novel vector control tools. There are no published data on where or on what plants *An. farauti* prefer to take sugar meals and *An. farauti* swarms have also not yet been observed.

There are significant variations in activity patterns among species and these patterns are changing as mosquitoes respond differently to selection pressures induced by vector control and changing environmental conditions [15, 16]. Prior to IRS with DDT, *An. farauti* sought blood

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Malaria Journal

RESEARCH Open Access

Human exposure to *Anopheles farauti* bites in the Solomon Islands is not associated with IgG antibody response to the gSG6 salivary protein of *Anopheles gambiae*

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Abstract

Background: Mosquito saliva elicits immune responses in humans following mosquito blood feeding. Detection of human antibodies recognizing the *Anopheles gambiae* salivary gland protein 6 (gSG6) or the gSG6-P1 peptide in residents of Africa, South America and Southeast Asia suggested the potential for these antibodies to serve as a universal marker to estimate human biting rates. Validating the utility of this approach requires concurrent comparisons of anopheline biting rates with antibodies to the gSG6 protein to determine the sensitivity and specificity of the assay for monitoring changes in vector populations. This study investigated whether seroprevalence of anti-gSG6 antibodies in humans reflected the relative exposure to *Anopheles farauti* bites in the Solomon Islands as estimated from sympatric human landing catches.

Methods: Human biting rates by *An. farauti* were estimated by landing catches at 10 sampling sites in each of 4 villages during the wet and dry seasons. Human serum samples from these same villages were also collected during the wet and dry seasons and analysed for antibody recognition of the gSG6 antigen by the Luminex xMAP© platform. Antibody titres and prevalence were compared to HLCs at the sampling sites nearest to participants' residences for utility of anti-gSG6 antibodies to estimate human exposure to anopheline bites.

Results: In this study in the Solomon Islands only 11% of people had very high anti-gSG6 antibody titres, while other individuals did not recognize gSG6 despite nightly exposures of up to 190 bites by *An. farauti*. Despite clear spatial differences in the human biting rates within and among villages, associations between anti-gSG6 antibody titres and biting rates were not found.

Conclusions: Few studies to date have concurrently measured anopheline biting rates and the prevalence of human antibodies to gSG6. The lack of association between anti-gSG6 antibody titres and concurrently measured human biting rates suggests that the assay for human anti-gSG6 antibodies lacks sufficient sensitivity to be a biomarker of *An. farauti* exposure at an epidemiologically relevant scale. These findings imply that an improvement in the sensitivity of serology to monitor changes in anopheline biting exposure may require the use of saliva antigens from local anophelines, and this may be especially true for species more distantly related to the African malaria vector *An. gambiae*.

Keywords: gSG6, Human biting rate, *Anopheles farauti*, Saliva antigens

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