ANOPHELES VECTORS SPECIES COMPOSITION, THEIR BITING CYCLE AND ROLE OF HUMAN BEHAVIOUR IN MALARIA TRANSMISSION IN AN ENDEMIC REGION FROM NAMIBIA

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ABSTRACT

Any vector control effort aimed at reducing malaria burden should be based on an understanding of the malaria vectors' populations, distribution, and behaviours as they relate to both transmission and response to interventions in place. It is well documented that the composition of malaria vectors changes over time but there has not been up-to-date information about that in Namibia. This study aimed to determine the population dynamics of Anopheline vectors and climatic factors that affect their distribution. It also described their biting behaviour and human social activities and behaviours that might expose them to mosquito bites. To address these objectives, human landing catches were conducted hourly from 19:00 hrs – 07:00 hrs indoors and outdoors for eight consecutive days during 2018/2019 and 2019/2020 malaria seasons in Shadikongoro village, Kavango East region. Mosquitoes collected were identified to species level using both morphological and molecular tools. Concurrently, data on which housing structures offered a high risk of mosquito bites was observed. Meteorological data from the Namibia Meteorological Centre was collected and used to determine the relation between these factors and mosquito abundance. A total of 1958 mosquitoes collected. Of these, 1190 were collected in 2018/19 and 768 in 2019/20. Species identification confirmed the presence of *Anopheles arabiensis*, *An. gambiae* s.s. and *An. funestus* s.s. In the 2018/2019 malaria season, *An. arabiensis* was the most abundant species, predominating both indoors (n = 334) and outdoors (n = 625). *Anopheles funestus* s.s was the least abundant species with 10 mosquitoes collected indoors and only one outdoors. During the 2019/2020 season, only *An. arabiensis* was collected. The statistical comparison showed a difference in species abundance between the two sampling periods ($X^2 = 24.0$, $p < 0.008$).

In the 2018/19 malaria season, both *An. arabiensis* and *An. gambiae* s.s. preferred biting outdoors ($X^2 = 32$, $p < 0.001$; $X^2 = 25.9$, $p < 0.001$, respectively) than indoors while *An. funestus* s.s. preferred biting indoors ($X^2 = 1532.719$, $p < 0.001$). In the 2018/2019 and 2019/2020 malaria seasons, sleeping indoors was associated with a higher risk of mosquito bites (2018/19: OR = 0.62, 95% CI: 0.17 – 0.91, $p = 0.02$; 2019/2020: OR = 0.32, 95% CI: 0.07 – 1.06, $p = 0.008$). In 2019/2020, outdoor chatting (OR = 0.70, 95% CI: 0.31 – 1.58, $p = 0.01$) was a risk factors for mosquito bites. The risk of being bitten by mosquitoes in traditional houses was significantly higher (RR = 0.79, 95% CI: 01.23–6.56, $p = 0.001$) than in modern (RR = 0.48, 95% CI: 0.07–0.93, $p = 0.012$) and zinc (RR = 0.15, 95% CI: 0.04–0.63, $p<0.06$) houses. In 2018/2019, only rainfall had a significant effect on overall abundance (Coeff = 0.33, 95% CI: 0.31 – 1.58, $p = 0.01$) while in 2019/2020, only temperature had an effect (Coeff = 0.6, 95% CI: 0.12 – 0.88, $p = 0.01$) on abundance. Given this evidence, there is a need to provide interventions targeting both indoor and outdoor mosquito biting such as mosquito nets and spatial insect repellents, respectively. Mosquitoes are still abundant even when there is no rainfall. Therefore, it is important to spray insecticides that last throughout the year.

Keywords: *Anopheles* species composition, biting behaviour, human social activities, malaria, Kavango East region, Namibia.

LIST OF CONFERENCES AND POSTERS

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3. Mwema Tabeth, Seth J. Eiseb, Iitula Iitula, Petrina Uusiku and Mumbengegwi Davis R (2019). *Anopheles* species composition and biting behaviour in Kavango East region, Northern Namibia. Paper presented at the 2nd Annual National Students' Research Symposium 2019, Windhoek, Namibia. Oral presentation (Awarded first prize for oral presentation).
4. Mwema Tabeth, Eiseb Seth J, Iitula Iitula, Petrina Uusiku and Mumbengegwi Davis R (2019). *Anopheles* species composition and biting behaviour in relation to human behaviour in Kavango East region, Namibia. Paper presented at the 4th Multi-disciplinary Research Centre Conference 2019, Windhoek, Namibia. Oral Presentation.

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LIST OF ABBREVIATIONS

ACTs	Artemisinin-based Combination Therapies
CDC	Centre for Disease Control
DDT	Dichloro-diphenyl-trichloroethane
DNA	Deoxyribonucleic acid
GPS	Global Positioning System
ITNs	Insecticide Treated Nets
IRS	Indoor Residual Spraying
Kdr	Knockdown Resistance
LLINs	Long Lasting Insecticide Nets
MoHSS	Ministry of Health and Social Services
NAM-ZAM	Namibia-Zambia
NVDCP	National Vector-borne Disease Control Programme
OPD	Outpatient Department
PCR	Polymerase Chain Reaction
RDT	Rapid Diagnostic Test
TAE	Tri-acetate-EDTA
TKMI	Trans-Kunene Malaria Initiative

WET	Window Exit Traps
WHO	World Health Organisation

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DEDICATION

I dedicate this work to my family. With a heart full of gratitude to my loving mother whose words of encouragement kept echoing and reminding me of my capabilities. My sisters Edith, Yvonne, Cynthia and Omri who always stood by my side.

DECLARATIONS

I, Tabeth Mwema, hereby declare that this study is my own work and is a true reflection of my research and that this work or any part thereof has not been submitted for a degree at any other institution.

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Name of Student	Signature	Date

CHAPTER ONE: INTRODUCTION

1.1. Background of the study

In tropical countries, malaria is a major cause of death and more than 40% of the world's population is at risk (WHO, 2017). In 2018 worldwide, the disease was responsible for 228 million clinical cases and approximately 405 000 deaths. About 93% of those cases and 94% of those deaths were in Africa (WHO, 2019). The main vectors of malaria in Africa are the *Anopheles* mosquito from the *An. funestus* group and *An. gambiae* complex with *An. gambiae* sensu stricto, *An. arabiensis* and *An. funestus* sensu stricto being the principal vectors (Sinka et al., 2012; Chanda et al., 2015). *Anopheles gambiae* s.s. was previously divided into the S and M molecular forms (Weetman, 2012) but were recently named *An. gambiae* and *An. coluzzii*, respectively (Coetzee et al., 2013; Chabi et al., 2019). *Anopheles coluzzii* prefers urban and dry environments and breeds along irrigated fields and permanent or semi-permanent swamps (Kudom, 2015). Contrary, *An. gambiae* is better suited to rural and humid forests and prefers temporary pools such as rice cultivations (Caputo et al., 2008). Dry and peri-urban areas are *An. funestus'* preferred habitats. *Anopheles moucheti* is a rare but efficient vector whose habitat is slow-moving rivers. *Anopheles arabiensis* prefers drier lands which is probably why it is absent from the Democratic Republic of Congo (DRC), Republic of Congo and Angola and abundant in Ethiopia, Kenya, Somalia, Namibia and Botswana (Figure 1) (Sinka et al., 2012). Due to high levels of pollution in urban areas, they are less inhabited by vector species such as *An. gambiae* s.s. which prefers unpolluted water.

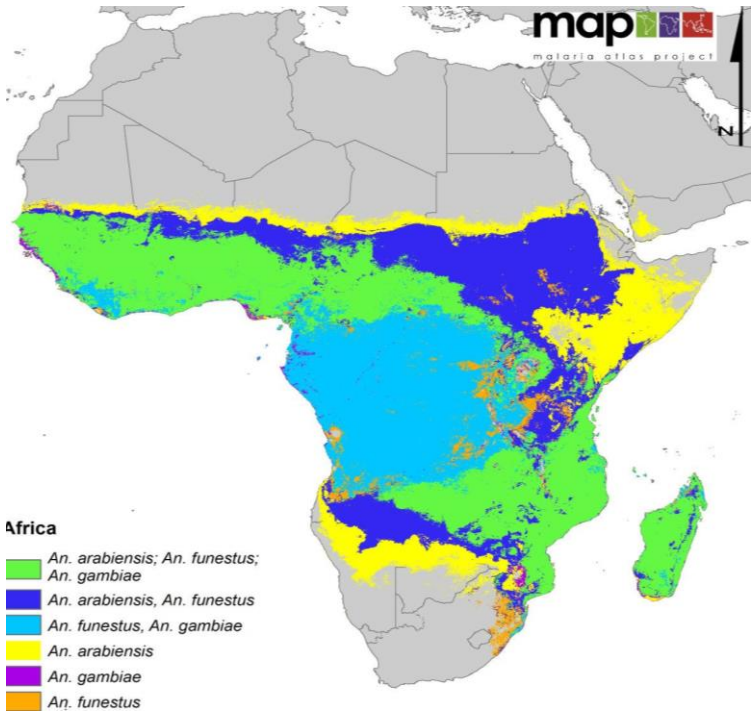

Figure 1. Distribution of the major malaria vectors in Africa (Sinka et al., 2012).

The distribution of mosquito vectors is affected by the temporal and spatial changes in temperature, precipitation and humidity which occur under different climate change scenarios (Parham and Michael, 2010). According to Afrane et al. (2012), an increase in temperature and rainfall is the main outcome of global warming of which temperature is expected to rise by 1.0 to 3.5 °C by the year 2100. Temperature affects mosquito biting frequency, pathogen development rate as well as the survival of larval and adult mosquitoes. This might cause the transmission of malaria to heighten in subsequent years. Gubler (2010) and Shanks et al. (2005) further stated that for the *Plasmodium falciparum* in the mosquito to be infective, it takes nine days at 30°C, 10 days at 25°C, 11 days at 24°C, and 23 days at 20°C. So, as temperatures drop below 20 °C, parasite development is prolonged resulting in a reduction of mosquitoes surviving long enough to develop

infective parasites, thereby affecting malaria transmission. Therefore, despite the availability of malaria vectors, the transmission is terminated.

1.2. Statement of the problem

There was a reduction in the number of malaria incidences in Namibia from 249.7 cases per 1000 in 2002 to 1.4 cases per 1000 in 2012 (MoHSS, 2019). This was due to the increased coverage of vector control interventions such as long-lasting insecticide-treated nets (LLINs), indoor residual spraying and widespread adoption of artemisinin-based combination therapies (ACTs) as well as the use of rapid diagnostic tests (RDTs). However, 14.8 confirmed malaria cases per 1000 population were reported nationally in 2018 and 81% of those were recorded from the Kavango East region and partly from the Kavango West region (MoHSS, 2019). Despite having high malaria cases, there is a lack of information on vectors responsible for ongoing malaria transmission. The last detailed information on malaria vectors from the Kavango East region was described by Kamwi (2005). This makes it difficult to formulate malaria control strategies aimed at prevention as vector composition and behaviour change over time due to climate or vector interventions. Information on vector bionomics is important in designing vector control strategies. However, the deployment of vector control in Namibia is not based on knowledge of the vector, but on malaria cases in previous malaria seasons.

1.3. Objectives of the study

a) To determine the current malaria vectors and their abundance in the Kavango East region in Namibia in the 2018/2019 and 2019/2020 malaria seasons.

b) To determine the biting behaviour of the major malaria vectors indoors and outdoors in the Kavango East region, Northern Namibia during the 2018/2019 and 2019/2020 malaria seasons.

c) To determine which housing structure (traditional, zinc and modern) offered a high risk of mosquito bite exposure during the 2019/2020 malaria season.

d) To determine human social activities and behaviours that expose them to mosquitoes indoors and outdoors, hence malaria transmission in the 2018/2019 and 2019/2020 malaria seasons.

e) To determine the effect of climate variables (temperature, rainfall, and humidity) on species abundance on the major malaria vectors in the Kavango East region, Northern Namibia in the 2018/2019 and 2019/2020 malaria seasons.

1.4. Hypotheses of the study

a) There is no significant change in species composition, abundance and distribution of the major malaria vectors in the Kavango East region, Northern Namibia between the 2018/2019 and 2019/2020 malaria seasons.

b) There is no significant difference in the biting behaviour of the major malaria vectors between indoor and outdoor in the Kavango East region, Northern Namibia during the 2018/2019 and 2019/2020 malaria seasons.

c) There is no significant difference in the risk of mosquito bites in traditional, zinc or modern structured houses in the Kavango East region, Northern Namibia during the 2019/2020 malaria season.

d) There is no significant difference in the risk of human social activities and behaviours that expose them to mosquitoes indoors and outdoors in the 2018/2019 and 2019/2020 malaria seasons.

e) There is no significant effect of climate variables (temperature, rainfall, and humidity) on species composition, abundance and distribution of the major malaria vectors in the Kavango East region, Northern Namibia during the 2018/2019 and 2019/2020 malaria seasons.

1.5. Significance of the study

While Namibia is gearing towards malaria elimination by 2022, there is still very limited up to date data available on the spatiotemporal bionomics of malaria vectors. This study will fill this knowledge gap by describing the malaria vector species currently present in the Kavango East region in relation to climate variables, their biting behaviour (whether biting indoor or outdoor and the peak biting hour(s)) and abundance. It is also imperative to determine the peak mosquito biting time and see if this is associated with the period people are indoors or outdoors. The timing of the biting activity to overlap with human sleeping patterns is important in determining how relevant vector control strategies such as LLINs are in protecting vulnerable populations from vectors responsible for malaria transmission. A better understanding of the spatiotemporal bionomics of malaria vectors will help the National Vector-Borne Disease Control Programme to formulate evidence-based and effective malaria control strategies.

1.6. Limitations of the study

Differences in blood type (Shirai et al., 2004), gender and/or carbon dioxide emission may influence the attractiveness of humans to mosquitoes, thereby affecting the number of human landing catches obtained from different individuals. The use of sentinel sites to represent regions could give a biased overall regional outlook as mosquito distribution varies at a micro-geographical level. Additionally, the difference in sampling time and duration may have biased the comparison of species composition and abundance between the two seasons. Due to the delay in the procurement of materials and supplies, sampling was equally delayed. The 2019/2020 sampling was done from the end of April to the beginning of May which is the start of winter. On most nights, it was too windy which greatly affected the catches.

1.7. Delimitation of the study

The study focused on species of *An. gambiae* complex (*An. arabiensis, An. merus* and *An. gambiae*) and *An. funestus* group (*An. funestus, An. leesoni, An. rivulorum* and *An. vaneedeni*) as they are the main African malaria vectors. The study was also conducted in one region, Kavango East region. This region was chosen based on its malaria epidemiology (about 70% of malaria cases in Namibia originate from this region) and climatic conditions that favour mosquito breeding and malaria transmission. The climatic conditions are favourable for the survival of both the vector and parasite as a result malaria endemicity is highest as compared to other regions (Kamwi, 2005). Shadikongoro village was chosen as it is a malaria hotspot in the region.

CHAPTER TWO: LITERATURE REVIEW

2.1. Malaria vectors of Namibia

In a study that was done by La Grange (1988) in Namibia, the major malaria vectors were *An. gambiae* s.s. and *An. arabiensis* and to a lesser degree, *An. funestus* s.s. However, in a study done by Kamwi (2005), only *An. arabiensis* and *An. funestus* s.s. were found. This was attributed to the *An. gambiae* s.s. being missed from the 2005 study or that the *An. gambiae* s.s. found in 1988 could have been *An. arabiensis.* It was argued that the *An. gambiae* s.s. was not confirmed by molecular methods as that is the golden standard method for identifying members of *An. gambiae* complex.

At the time at which this study was conducted, there was no up to date data on the diversity of malaria vectors in Namibia because the latest study was conducted in 2005 (Kamwi, 2005). This may have resulted in malaria incidences significantly increasing from 2013 because species composition, abundance and biting behaviour were not known. These cases could have been reduced if the behaviour and composition of the vectors were understood better because vector control would have been administered based on the knowledge of vector bionomics.

2.2. Global malaria control and elimination strategies

The Global Technical Strategy for Malaria was developed by WHO to assist countries in eliminating malaria. The strategy is a provision for comprehensive and technical guidance to countries, with an emphasis on the importance of increasing malaria responses as a movement towards elimination (WHO, 2015). The operational requirements of the strategy include strengthening health systems, addressing the emergence of multi-drug

and insecticide resistance, and intensifying national, cross-border and regional efforts to amplify response for malaria outbreak to protect the vulnerable groups of people. It also urges countries to increase investments across all interventions which include preventative measures, diagnostic testing, treatment, and disease surveillance. One of the pillars of these strategies is vector control (Figure 2).

2.2.1. Vector control

This is an important intervention in malaria control and elimination. The ability of vectors to transmit parasites and their vulnerability to vector control measures differ across mosquito species and are dependent on local environmental factors. Vector control interventions should therefore be applied based on local epidemiological and entomological data. Presently, the most implemented vector control strategies are ITNs/LLINs, IRS and housing improvements:

2.1.1.1. Insecticide-treated Nets (ITNs)/Long Lasting Insecticide-treated Nets (LLINs)

Nets are used to target mosquitoes that feed indoors in the late evenings to reduce the human-biting rate of the mosquito and its daily survival rate (Tizifa et al., 2018). Nets are treated with insecticides such that if the mosquito picks up a lethal dose, the mosquito dies before it becomes infective, in turn disrupting the transmission of malaria. According to Kitau et al. (2012), pyrethroid treated nets effectively kill *An. gambiae, An. funestus* and *An. arabiensis.* Some studies have shown a negative effect of LLIN use while others showed no effect at all. For instance, some mosquitoes showed signs of resistance to the insecticides used in LLINs or they changed their biting behaviour (Steinhardt et al., 2017).

It has been reported that in the last decade, members of the *An. gambiae* complex in West Africa have become resistant to pyrethroids and the *kdr* mutations have been implicated as the resistance mechanism in these populations (Koumba et al., 2018). In Côte d'Ivoire where hut trials in adjacent resistant and susceptible populations were experimented on, the results showed no apparent difference in the effectiveness of LLINs on the two localities (N'Guessan et al., 2007). This is supported by studies done in Haiti that did not see any added protection by nets (Steinhardt et al., 2017). Additionally, because of the barrier that nets create, mosquitoes are forced to prefer biting outdoors in the early hours resulting in residual malaria transmission (Kenea et al., 2019). As a result of this, Pates and Curtis (2005) argued that insecticide-treated nets only reduce mosquito populations for a short period but then become ineffective. Despite all these conflicting results, the World Health Organization still recommends LLINs as a core intervention for malaria control (WHO, 2019).

In Namibia, the distribution of mosquito nets treated with pyrethroids is one of the main methods of preventing malaria transmission (MoHSS, 2019). The distribution of LLINs was introduced in 1993 targeting the most vulnerable groups (pregnant women and children under the age of five). However, the NVDCP set a goal in 2012 to achieve 95% LLIN coverage of the entire population and not merely vulnerable groups. Over 625 000 LLINs were distributed at health facilities, outreach sites, antenatal clinics and through mass campaigns (Gueye et al., 2014). In 2014, 87 900 LLINs were distributed in Zambezi, Kavango and Omusati regions as these regions showed to have the highest malaria caseloads in the country (Gueye et al., 2014). In 2015, approximately 800 000 LLINs were distributed to the vulnerable groups in Kavango East and West, Ohangwena and Kunene

regions (MoHSS, 2019), although it is not clear whether the recommended WHO coverage was attained. It was further reported that these LLINs did not offer any added protection, so they were discontinued.

2.1.1.2. Indoor Residual Spraying (IRS)

As a recommendation by WHO (2015), IRS should be used together with LLINs. However, an insecticide with a different action mode to that used on LLINs should be used for spraying. To avoid insecticide resistance, insecticides sprayed should be rotated periodically (Medzihradsky et al., 2018). Since the 1960s, the main malaria control intervention in Namibia has been IRS, primarily with Dichloro-diphenyl-trichloroethane (DDT). In recent years, DDT is mainly used on traditional structures such as huts and deltamethrin is used on cement block structures (Gueye et al., 2014). With this intervention, there is no direct prevention of mosquitoes from biting humans. However, it decreases the daily survival rate of mosquitoes that rest on sprayed walls thus interrupting the transmission of infection to other people. For this to be effective, 80% of the houses in an area need to be sprayed (MoHSS, 2019).

In 2008 in Namibia, only 48.9% of the population in at-risk areas (Northern regions) were covered. This was due to the delay in obtaining insecticides. The low coverage resulted in a heightened number of cases (62.2 per 1000 population) in 2008 as compared to 2011 (6.5 per 1000 population) (MoHSS, 2019), hence showing the importance of IRS. As discussed by Mumbengegwi et al. (2018), the recommended IRS coverage was not achieved in the western Zambezi region during the 2014/2015 malaria season. This was attributed to people being absent from their homes when spraying was done or sprayers not turning up as scheduled. People also refused to have their houses sprayed because the

insecticides leave an unappealing look on the wall. To overcome this shortcoming, there has been a development of an emulsion paint impregnated with insecticide which the Government of Namibia may adopt. This way, the composition of the paint serves both as a means of delivering an appealing look as well as chemical insecticides for the control and elimination of mosquitoes (Mosqueira et al., 2010).

The paint contains insecticides such as deltamethrin, permethrin and cypermethrin which are all pyrethroids. Apart from having insecticides, the paint also contains insect repellents. The study to determine the efficacy of this paint was done in Burkina Faso and the results showed an increase in the mortality of mosquitoes in houses coated with the insecticide paint as compared to those with LLINs (Mosqueira et al., 2010). The paint seems to be a good working tool against insects as another study done in Abidjan, Côte d'Ivoire reported similar results on the reduction of the apparent density of tsetse flies by around 90% within a little more than six months (Acapovi-Yao et al., 2014). However, this has never been done in Namibia as a vector control intervention.

2.1.1.3. Housing Improvements

According to Nguela et al. (2020), housing improvement may be associated with a reduction in Anopheline density and malaria transmission. A significant increase in malaria infection risks is associated with having open eaves, an earthen roof, living close to water reservoirs or deteriorating housing which are in turn linked to poverty (Ghebreyesus et al., 2000). The number of vectors and malaria incidences are reduced significantly when living in houses with the highest quality as compared to those living in lower quality structures (Ghebreyesus et al., 2000; Carter, 2014; Liu et al., 2014; Zhao et al., 2016; Tusting et al., 2017). Housing structure improvements such as house screening,

the closing of eaves and installation of the ceiling have resulted in the reduction of transmission by 80% in most areas (Tusting et al., 2015) and are known to be fairly inexpensive (Pega & Wilson, 2016).

In a low transmission country such as Namibia where there is a movement towards elimination, it would be cost-effective to improve houses with poor structures in malaria hotspots, thereby reducing malaria reception. Although this movement may have higher-up costs especially in areas of moderate and high transmission, it is permanent which makes it cost-effective (Mosqueira et al., 2010). This, when coupled with other interventions such as IRS and distribution of LLINs, may greatly reduce malaria transmission by mosquitoes that feed indoors (Lwetoijera et al., 2013).

2.1.1.4. Larval source management

Vector control interventions such as larval source management aim to reduce the number of mosquitoes reaching adulthood thereby reducing malaria transmission. This is done by permanently destroying mosquito breeding sites by filling them up or by pumping standing water out of swampy or marshy areas. Another way vectors are controlled is through larviciding which is the addition of chemical and biological insecticides to water bodies. There are different types of insecticides used in larval control and they differ in their mode of action. Surface films such as mineral oils and alcohol or silicon-based surface products block the filtration of air into the water which suffocates the larvae and pupae (Yapabandara and Curtis, 2002). The oils used today do not harm the environment as they are biodegradable. Synthetic organic chemicals such as organophosphates (temephos, pirimiphos-methyl) disrupt the nervous system of immature stages of mosquitoes. Other types of larvicides are biological. Microbials such as *Bacillus*

thuringiensis israeliensis and *B. sphaericus* are added to a body of water and when the toxins they produce are ingested, they cause the gut of mosquitoes to undergo lysis (Fillinger et al., 2003). Additionally, the introduction of fish to the breeding site reduces larval abundance as the fish feed on larvae. In Lake Victoria, Kenya, the density of *An. gambiae* s.l adults were reduced by 91.5% in July 2001 - September 2005 due to larviciding (Fillinger and Lindsay, 2011).

The effectiveness of the interventions targeting larval stages of the mosquito varies widely from species to species. In general, these interventions may be effective if the habitats are large and comply with environmental modification but are less effective if the habitats are small, widely dispersed and temporal (Castro et al., 2009). For instance, *An. gambiae* s.l breeds in numerous small pools of water that form due to rainfall. The larvae develop very fast and escape the water in a short time before the body of water dries out. This makes it difficult to pinpoint exactly where the breeding sites will develop in order to disrupt the development of adults (Beier et al., 2008). As a result, prevention of malaria through larval mosquito control on a large scale in Africa has not been attempted although, in areas such as desert fringe where habitats are more stable and predictable, it has proven extremely effective. While IRS and LLNs target adult mosquitoes, larval source reduction helps reduce the number of mosquitoes reaching adulthood thereby reducing malaria transmission.

Figure 2. Malaria prevention and control interventions (Jima et al., 2010).

2.2.2. Chemoprevention

The success of malaria control greatly rests on antimalarial drug treatment (Sharp & Freese, 1990) and vector control interventions. Anti-malarial treatment deals with the human reservoir of parasites while vector control interventions address the mosquito reservoir (Medzihradsky et al., 2018). Anti-malaria drugs being used currently are artemisinin-based combination therapy. The artemisinin compound acts to reduce the number of parasites in the first three days of treatment while the partner drug works on parasite elimination (cure) (Okell et al., 2014). The drug partnering the artemisinin derivative determines the efficacy and this usually exceeds 95% for artesunate-mefloquine, artemether-lumefantrine, and dihydroartemisinin-piperaquine.

2.2. The four-phase elimination-continuum

The malaria elimination continuum was developed by the WHO to assist control programs in malaria-endemic countries to determine their malaria status using malaria incidence

values (WHO, 2015). Namibia is one of the countries in Southern Africa that is transitioning towards malaria elimination which is achieved when there is no local malaria transmission in a defined geographical area. For a country to achieve a malaria-free status, a four-phase continuum needs to be implemented. The phases are control, pre-elimination, elimination, certification and prevention of reintroduction. Countries with a malaria blood-slide positivity rate among fever cases of >5 cases/1 000 population at risk are considered to be in the malaria control stage; <5 and >1 case/1 000 are in the pre-elimination stage and with rates of <1 case/1 000 are categorized as being in the elimination stage (Figure 3).

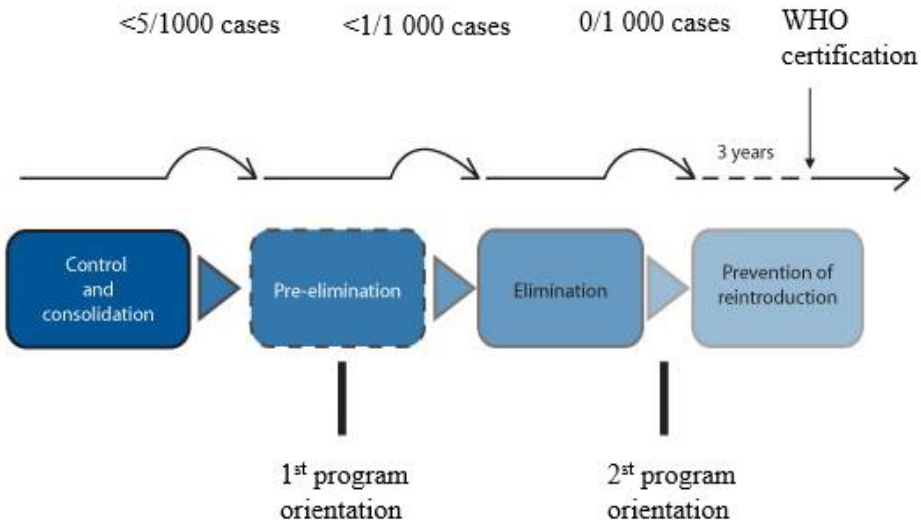

Figure 3. WHO malaria elimination continuum. The level of malaria incidence required before progressing onto the next phase (WHO, 2015).

2.2.1. Control

This phase involves the control of malaria vectors in a country to reduce morbidity and mortality and the burden of malaria. To successfully implement vector control strategies,

a few guiding aspects such as knowledge of micro-epidemiology of malaria including ecology and behaviour of the vector, social and cultural features of the human population and changes due to interventions or developments are to be followed (Hiwat et al., 2012). Distribution of insecticide-treated nets, indoor residual spraying, prompt and effective case management and the use of appropriate anti-malarial drugs are the main strategies employed. Once there is a reduction in transmission intensity and malaria incidences are brought down to less than five local cases per 1000 population per year, then a country is considered ready to move on to the pre-elimination phase of the continuum (Patouillard et al., 2011).

2.2.2. Pre-elimination

Upon bringing malaria incidences down, the implementation of strategies for pre-elimination can be made in target areas. This phase mainly involves strengthening malaria surveillance and health information system with cooperation from all health-care providers, identifying transmission foci and reducing onward transmission from existing cases (WHO, 2019). For pre-elimination to be successful, inter-country and cross border collaborations between countries with varying levels of transmission should be intensified (Yangzom et al., 2012) and access to private and/or public health care facilities to increase health coverage should be improved. Lastly, public and private health service staff need to be acquainted with the new goals of malaria elimination as well as providing free treatment and diagnostic services to further reduce malaria cases to less than one per 1000 population at risk per year.

2.2.3. Elimination

Transitioning from pre-elimination to elimination can only be achieved when the incidence of malaria in a certain area is reduced to less than one indigenous case per 1000 population in a year or to about 100 cases per district per year. In this phase, there should be a reduction of locally acquired cases to zero (Cohen et al., 2010). This involves the rapid identification, locating and elimination of any malaria transmission through a monitoring and surveillance strategy, reduction of human-vector contacts by intensifying vector control measures and improving personal protection (Kelly et al, 2012). Additionally, it is important to strengthen surveillance systems to be able to detect future malaria incidences.

2.2.4. Prevention of reintroduction and certification

A country can only be certified malaria-free if there has been no occurrence of three or more indigenous malaria cases of the same species per year in the same focus for three consecutive years. After elimination of malaria, prevention of re-establishment needs to be continued until malaria is eradicated which means that there should be a complete interruption of transmission of all forms of human malaria throughout the area (Cohen et al., 2010). To disrupt the transmission of malaria, there should be proper management of a high-performing health system to ensure early detection, mandatory notification and prompt treatment of all malaria cases, determination of all the possible causes of re-establishment and measurement of the risk for malaria re-establishment by monitoring of receptivity and vulnerability (WHO, 2015). After achieving zero malaria transmission in a country, political and financial commitment at national and subnational levels should be sustained.

2.3. Malaria burden in Africa

Malaria is most prevalent in sub-Saharan Africa as conditions are favourable for the survival of both the vector and the parasite (Onyango et al., 2016). Many factors make sub-Saharan Africa a very conducive environment for malaria transmission which include poverty, poor sanitation, weak public health systems, limited disease surveillance capabilities, natural disasters, armed conflict, migration, climate change, and the presence of counterfeit and/or sub-standard antimalarial drugs (Stresman, 2010). It is estimated that the rural population of sub-Saharan Africa will be outnumbered by the urban population by the year 2035. The increase in urban population may result in poorly monitored land use such as agriculture which has proven to be providing suitable breeding sites for mosquitoes. Additionally, poverty has resulted in poorly built houses that offer less protection against mosquito bites (De Silva & Marshall, 2012).

Another reason for the heavy malaria burden in Africa as stated by De Silva and Marshall (2012) is attributed to the availability of natural breeding sites that sustain vector populations in rural areas. Although natural breeding sites are present in urban areas, they are less preferred because they are temporal and do not provide ample time for the development of eggs and the emergence of adults. De Silva and Marshall (2012) further discussed that areas with high groundwater tables have conducive breeding sites as the soil is already saturated which allows for stagnant water to develop.

In 1990, Namibia received good rainfall resulting in the country experiencing a severe malaria epidemic. This led to the launch of the National Vector-borne Disease Control Program (NVDCP) by the Ministry of Health and Social Services (MoHSS). Of all hospital deaths in 2002, 8.6% were due to malaria. Malaria was responsible for 26.4% of

Outpatient Department (OPD) cases as well as 21.6% of admissions. The disease burden differed from region to region with Kavango and Zambezi regions having the highest rates of malaria morbidity and mortality (MoHSS, 2019). However, the malaria cases declined from 62.2 per 1000 population in 2008 to 6.5 per 1000 in 2011. The decline was influenced by the introduction of artemether-lumefantrine, improved IRS coverage as well as diagnosis with RDTs. These achievements paved the way for Namibia to be among eight countries in Southern Africa currently having the potential to eliminate malaria by 2030 (Gueye et al., 2014). However, since 2012 a fluctuating trend in malaria cases associated with the rainy seasons ranging from 1.4 cases per 1000 in 2012 to 10.3 per 1000 in 2016 had been observed (Mumbengegwi et al., 2018) (Figure 4).

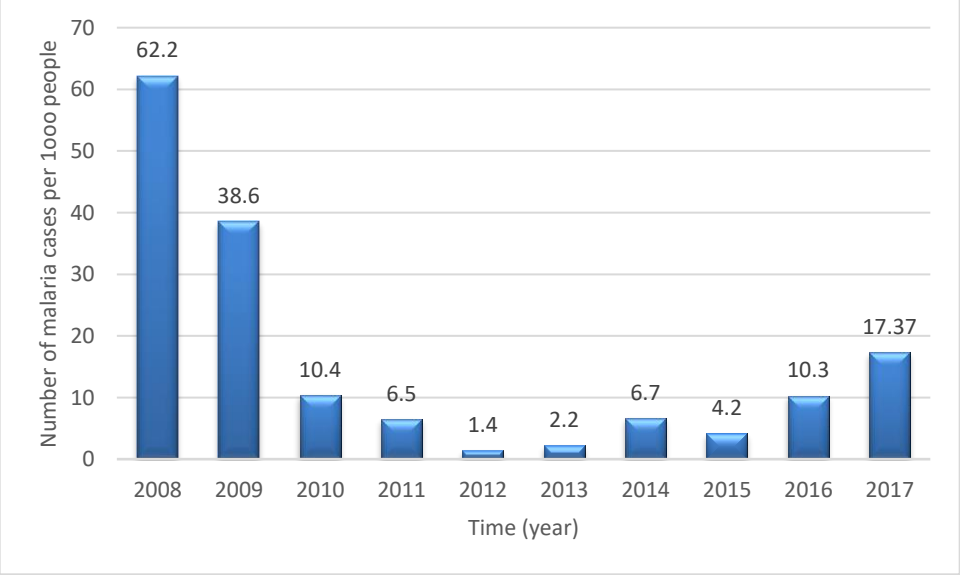

Figure 4. Malaria incidence per 1 000 population (Mumbengegwi et al, 2018).

2.3.1. Impact of environment on vector distribution and malaria transmission

The Northern Namibian environmental conditions are favourable enough to sustain a high abundance of Afro-Tropical malaria vectors. Temperature, rainfall and humidity are closely linked to the transmission of malaria in Namibia and therefore transmission varies

from year to year (Chanda et al., 2015). Malaria transmission is seasonal in the North-West and parts of the Central and South regions and follows the onset of rains which peak between April and May (De Langen et al., 2006). Due to low humidity, cold temperatures and dryness in these regions, the malaria transmission cycle is interrupted especially from August to October. On the other hand, Kavango and Zambezi regions have conditions (high average temperature, high rainfall, and high humidity [Figure 5]) that are favourable for mosquito breeding and parasite development.

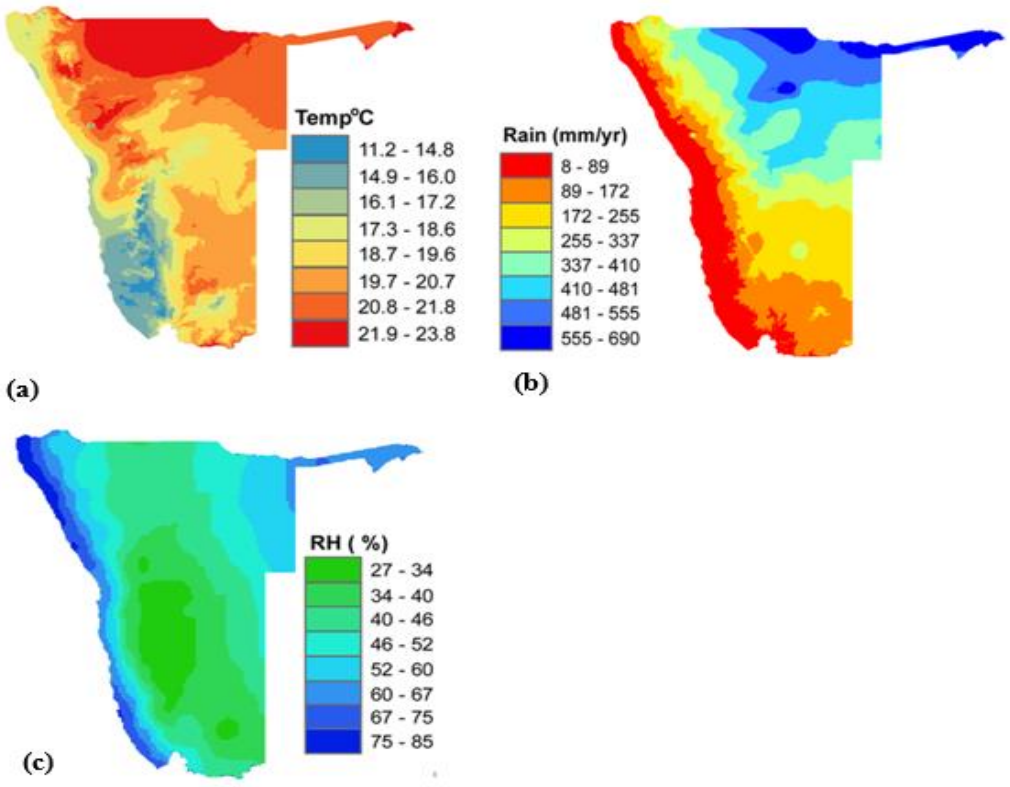

Figure 5. Average annual temperature (°C) (a), rainfall (mm) (b) and relative humidity (%) (c) in Namibia (https://images.app.goo.gl/LAVP5dHTNbWu8uTE7).

The seasonal predominance of malaria transmission does not allow individuals to acquire strong immunity to malaria. This allows the entire population to be exposed to severe infection in the same manner as visitors from nonmalaria endemic areas of the country

(MoHSS, 2019). From this information, it can be concluded that climate can either able or unable malaria transmission. However, determining the transmission of malaria based solely on environmental and climatic factors is limiting because there are other contributing factors such as human social activities and behaviours (Craig et al., 1999) but that is poorly linked to malaria transmission in Namibia.

2.3.2. Biting behaviour

According to a study done in Equatorial Guinea to determine the behaviour of the outdoor host-seeking *Anopheles gambiae* mosquitoes, it indicated that *An. gambiae* s.s. is both endophagic and exophagic whose biting peaks between 21:00 and 22:00 hours. Then after that biting decreases onwards (Reddy et al., 2011). In Cameroon, the same species bites mostly outdoors and in Ghana data shows that in the dry season, *An. gambiae* s.s. is more endophagic than in the wet season. *Anopheles melas'* indoor biting increases from 20:00 to 01:00 hours and peaks between 24:00 and 01:00 hours. Thereafter, biting only occurs outdoor. In South Africa, a newly discovered malaria vector *An. vaneedeni* is exophagic (Burke et al., 2017). This indicates that the biting behaviour of malaria vectors varies between species, regions, and seasons. Additionally, in Namibia, Kamwi (2005) observed that the anopheline species (*An. arabiensis* and *An. funestus* s.s.) caught in that study fed indoors and on human blood but no interest was paid to the peak biting hour(s) or whether there was a relationship between biting behaviour and human social activities.

2.3.3. Activities exposing humans to mosquito bites in a low transmission setting

People's needs to make ends meet have led to frequent short-duration movements into and out of favourable environments to the breeding sites of *Anopheles* species that carry

malaria (Markwardt et al., 2008). According to Martens and Hall (2000), population movement has been contributing to disease transmission since time immemorial. The movement of infected populations has led to the introduction of diseases into areas where the diseases were absent (Smith et al., 2017). People increase their chances of getting infected as they move into new areas and modify the environment (Guyant et al., 2015). For instance, some agricultural practices provide suitable habitats for *Anopheles* mosquitoes which increases the exposure of humans to mosquitoes. Occupations such as security guards, cattle herders and forest workers promote the spread of malaria as their living and work conditions are poor and they are mostly found outdoor (Martens & Hall, 2000; Marshall et al., 2016). Cross-border movements also contribute to malaria transmission as people unintentionally move infectious mosquitoes into malaria-free areas, reintroducing the disease, making it almost impossible to eliminate malaria (Pindolia et al., 2013).

In the 1950s and 1960s, malaria had been eliminated from most areas in the Amazon region in Brazil. However, due to population movement in search of greener pastures, there has been an increase in malaria cases in new territories (Wesolowski et al., 2018). In another study that was done in India, malaria had been eliminated from the rural areas, but the programs neglected the problem in urban areas. This led to the resurgence of malaria in the 1970s (Martens & Hall, 2000). Population movement does not only cause the reintroduction of malaria vectors but also drives the spread of drug-resistant parasite strains (Tatem et al., 2014).

In Namibia, Tessema et al., (2019) showed that malaria transmission was happening locally although it was seeded by the importation of cases. Parasite genetic analyses

confirmed that the infections were imported from across Angolan and Zambian borders (Pindolia et al., 2012). Another study was conducted to determine how human activities influence malaria transmission, but the focus was on how travel to and from a high-risk area (Angola) increases risk in a low transmission Namibia (Smith et al., 2017). These movements ensure a continuous flow of parasites and vectors into Namibia. However, some night-time human social behaviours such as cooking, eating routines and some common social activities such as drinking alcohol were not included. These are important because people are exposed to mosquito bites for long hours without protective items such as mosquito nets. Therefore, the transmission might be high during those times.

To control and eliminate malaria, strategic plans need to be built on an understanding of population movement, human night activities as well as parasite movement loads and routes (Le Menach et al., 2011). As observed, Namibia remains the perennial threat of imported infections within the country and across the border with Angola and Zambia which seems to be the largest challenge facing the elimination of malaria. Even though vector species may be eliminated from Namibia, if there are still asymptomatic infected individuals who travel to these regions and are not protected at night while socializing, there is still going to be a resurgence of malaria transmission as they will import new species and parasites.

To combat this cross-border transmission, Namibia has partnered with Angola and Zambia and established the Trans-Kunene Malaria Initiative (TKMI) and NAM-ZAM in 2010 and 2015, respectively. The NAM-ZAM involves Katima Mulilo (Namibia) while TKMI involves the Southern Angolan provinces stretching from Kunene to Kavango East and West region (MoHSS, 2019).

2.3.4. Malaria “hotpops”

“Hotpops” or hot populations are groups of people that are at risk of malaria infection (Sturrock et al., 2013). As echoed by McCreesh et al. (2018), the populations most at risk are agricultural field workers, cattle herders and the likes. These outdoor activities increase the mosquito to human contact because ITNs and IRS only offer protection from indoor biting (Zaw et al., 2017). Some “hotpops” are quite easy to reach but others such as undocumented migrants are more difficult to reach. To identify “hotpops”, border screening should be robust during peak travel periods such as holidays to target the highest movement of people into the country (Sturrock et al., 2013). Identification of hotspots and “hotpops” or geographic areas or sub-populations where malaria risk is concentrated and where interventions should be targeted can be aided by surveying household conditions. Doing so may also curb vectors for other infectious diseases other than malaria (Ogoma et al., 2010).

CHAPTER THREE: MATERIALS AND METHODS

3.1. Research design

This was a repeated cross-sectional study that involved the collection of mosquito species, determination of mosquito biting behaviour, determination of human behaviours that expose humans to mosquito bites. This was done during the 2018/2019 and 2019/2020 malaria seasons (March-May) in the Kavango East Region of Namibia. The study was also a part of the MoHSS's yearly operational vector surveillance.

3.2. Study area

The study was conducted in Shadikongoro village (Figure 6) in the Kavango East region of Namibia. The village was chosen as it is the epicentre of malaria in the region. The ecosystem of Shadikongoro is dominated by the Kavango River which lies about 1,000 metres above sea level and runs for 1,700 kilometres from central Angola to the Kalahari in northern Botswana. The average annual rainfall in the area is 565 millimetres, except in the 2010/2011 rainy season when the place received 757 millimetres which happened to be the highest ever recorded rainfall. Since there is high rainfall which encourages the cultivation of a variety of crops and expansion of marshes that act as mosquito breeding sites, there is a high malaria transmission which peaks during the rainy season (November – April) (Angula & Kaundjua, 2016).

Even though the area is characterised by a hot semi-arid climate, there is a variation in temperature during winter with the diurnal temperature being roughly 26 °C (79 °F) and as low as 6 °C (43 °F). Despite this being the case, the variation in temperature during summer is less pronounced.

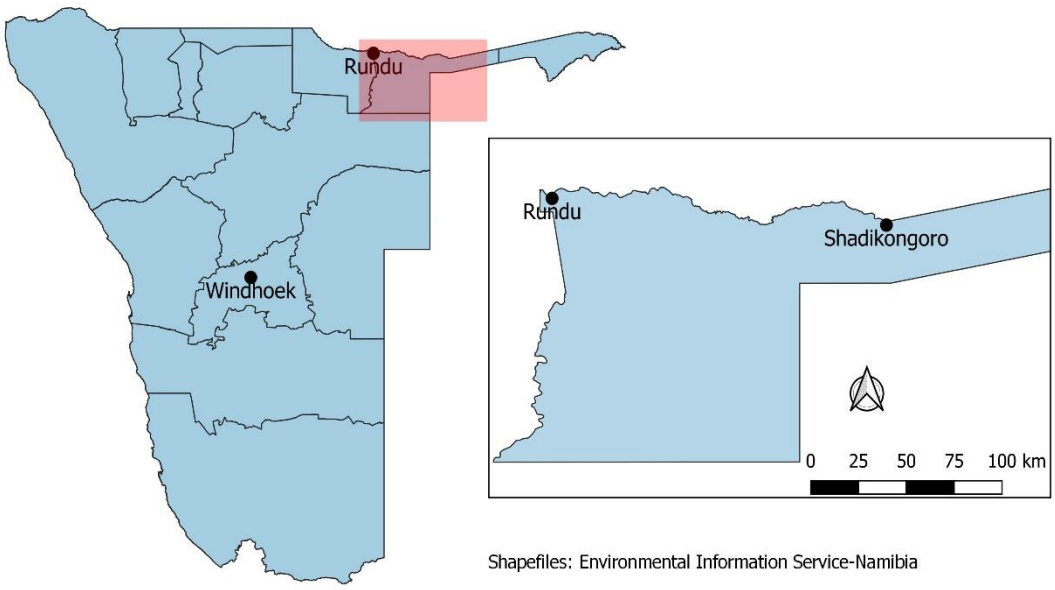

Figure 6. Study area showing the location in which mosquitoes were sampled during the 2018/2019 and 2019/2020 malaria seasons.

3.3. Mosquito collections

The study was conducted in March 2018 and from April to May 2019 for 8 consecutive days for each collection period. Every night, 4 houses were chosen to include different house structures, that is, traditional (mud), zinc and modern (cement). Individual houses were chosen based on the willingness of the owners to participate, whether the house was used by people and whether the houses were within 100 metres of each other. Adult mosquitoes were collected using the human landing catch method as shown in Figure 7 (Gimnig et al., 2013). Resting boxes, CDC light traps and tent traps were also used to capture mosquitoes. However, the yield was very low. This resulted in discarding the methods during the 2019/2020 sampling period.

Figure 7. Field research assistant collecting mosquitoes using the human landing catch method (Source: Google).

A pair of human landing collectors were used per evening. The collectors were rotated between locations to correct for possible variation in individual attractiveness. Each pair collected mosquitoes from residences at least 100 metres away from other collection houses. Indoor collectors were positioned in a central room within the house, often in the sleeping quarters with people sleeping in the rooms used as bait. Outdoor collectors were located 10 metres outside the perimeter of the same house. Collections were starting shortly after dusk (19:00 hours) and continued through the early morning hours (07:00 hours), with mosquitoes collected hourly stored in individual Styrofoam cups. The indoor and outdoor cups were stored in separate buckets (Appendix 6). In the morning, a form

(Appendix 1) containing the information on the number of mosquitoes collected per hour and the house structure type (Figure 8) was filled in after killing the mosquitoes with chloroform. Each mosquito was stored in an individual Eppendorf tube containing a small amount of silica drying agent separated from the mosquito by a thin layer of plain paper. The tube was accompanied by a label of the collection hour and the form serial number. These were then transported to the laboratory for morphological and molecular analysis.

Figure 8. Different house structure types from which mosquitoes were collected indoors and outdoors in the Kavango East region. (Traditional (a), zinc (b) and modern (c) house structures. Source: Tabeth Mwema).

Global Positioning System (GPS) coordinates for each collection house were recorded. These were used to develop a species distribution map using QGIS (version 3.4.5) compiled from shapefiles obtained from the Environmental Information Service Namibia database and map library. Climate data was collected from the meteorological centre in Windhoek (Namibia's Capital City).

3.4. Human behaviour data collection

The number of people observed indoors and outdoors where mosquito catches were being done was counted and the type of activities humans engaged in was equally noted on a form (Appendix 2) every hour. This was to determine where the risk of mosquito biting was occurring and to determine which human social behaviour exposed them to more mosquito bites. Any human outside the 10 metre radius was not included in the study and only household members were included.

3.5. Mosquito processing

In the laboratory, only female *Anopheles* mosquitoes were processed. Mosquitoes were identified morphologically using the key developed by Gillies & Coetzee (1987) (Appendix 5) and placed individually in Eppendorf tubes containing a small amount of silica drying agent separated from the mosquito by a thin layer of plain paper. Furthermore, molecular analysis was done using *An. gambiae* complex and *An. funestus* group species-specific Polymerase Chain Reaction (PCR) (Scott et al., 1993).

After PCR analysis on *An. gambiae* complex and *An. funestus* group, the samples that did not amplify from the first-round assay, were analysed using the *An. gambiae* complex/*An. funestus* group assay, just in case the samples were wrongly identified morphologically.

3.5.1. Polymerase chain reaction for *Anopheles gambiae* complex and *Anopheles funestus* group.

3.5.1.1. *Anopheles gambiae* complex

A mosquito leg was placed in a 0.2 ml micro-centrifuge tube. Each 12.5 µl PCR master mix contained 1.25 mM 10x reaction buffer, 1.25nM 10x dNTPs, 1.25 $MgCl_2$, 1.65 pmol *An. quadriannulatus* and 3.3 pmol of each primer (*An. gambiae*, *An. arabiensis, An. merus,* universal), 4.9 µl deionised water and 0.5 units of Thermostable DNA polymerase (Taq). The 12.5 µl master mix was then vortexed, centrifuged and added to each tube containing a leg. A leg of a positive control for *An. gambiae*, *An. arabiensis, An. merus* and *An. quadriannulatus* was included for amplification. The tubes were placed in a thermocycler PCR machine programmed for an initial denaturation of 92 ºC for two minutes, followed by 30 cycles of 94 ºC for 30 seconds, 50 ºC of annealing for 30 seconds, 72 ºC of extension for 30 seconds and final extension cycle at 72º C 5 seconds. The tubes were held at 8 ºC.

A 2.5% agarose gel was made by adding 10 g agarose to 400 ml of 1x TAE buffer and microwaved for 10 minutes. To this, 12 µl ethidium bromide was added, mixed well, and poured into a casting tray. For every 5 µl of PCR product, 1 µl of loading dye was added. Finally, the PCR products, Mw markers and one negative PCR control were loaded into the gel wells placed in an electrophoresis tank containing 1x TAE buffer. The gel was electrophoresed at 100V and visualized under a gel documenting system and sized against molecular weight and scored according to each species expected amplicon size (Table 1).

Table 1. The expected size of the amplicons of *An. gambiae* complex.

Species	Amplicon sizes (base pair)
An. merus	464
An. gambiae	390
An. arabiensis	315
An. quadriannulatus	153

3.5.1.2. *Anopheles funestus* group (Koekemoer et al., 2002)

To extract DNA from each specimen a leg of a mosquito was homogenized in a sodium-Tris-edetic acid (EDTA) buffer (0.1 M $NaCl_2$; 10 mM Tris, pH 8.6; 1 mM EDTA) and incubated at 94 °C for 12 minutes. Cell debris was precipitated by centrifuging for 1 minute and only 0.4 µl of DNA was used for PCR. The PCR in a 15 µl reaction contained 0.5 µM of each of the primers (universal, *funestus, leesoni, parensis, veneedeni, rivulorum, rivulorum*-like), 3µl of 5x Hot Firepol Blend master mix ready to load (Hot-Start, 1995) and 2 µl of DNA template. The PCR conditions were set as initial denaturation at 95 °C for 15 minutes, followed by 30 cycles of denaturation at 95 °C for 30 seconds, annealing at 46 °C for 30 seconds and extension at 72°C for 40 seconds, and a final extension at 72 °C for 10 minutes. The characteristics of each fragment of each species were viewed on a 1.5 % agarose gel stained with ethidium bromide against a 100 bp DNA ladder.

3.6. Data analysis

The main response variable collected in the study was the number of mosquitoes caught. The main predictor variables were climate factors (temperature, rainfall and humidity), human behaviour which was measured as types of human activities and structure types (Zinc, Mud and Modern). Before data analysis, diagnostic tests were done to test the

normality assumption of the data using the Shapiro-Wilk test (Razali & Wah 2011). Because the data was of count in nature, it violated the assumption of normality. No transformation of data was done as generalized linear models with a Poisson function can handle count data (Ahad et al., 2011). Descriptive statistics in terms of means (±SD) and medians (IQR) were used to summarise the data**.**

To determine the difference in species composition between the 2018/2019 and 2019/2020 malaria seasons and to determine the difference in biting behaviour between indoors and outdoors, the chi-square test of association was used. Mean biting density (which represents the average number of bites received per person per hour) was calculated as the total number of mosquitoes caught for each hour per species multiplied by the number of days data was collected. The proportion of people was calculated by dividing the number of people observed in each hour by the total number of people observed for the entire study in the indoor and outdoor compartments. Transmission exposure was obtained by multiplying the mean biting density of mosquito in each hour by the proportion of people present in the indoor and outdoor compartments. Data for biting density was collected between 7 pm and 7 am. This was done for both indoors and outdoors. The mean biting density for each species was determined on an hourly basis.

To determine, the number of people doing mosquito biting risk activities at a particular hour between 7 pm and 7:00 am, data was summarized using descriptive statistics. A univariate and multivariate poisson analysis was carried out to independently determine whether human social activity was associated with mosquito bites indoors and outdoors.

The mean number of mosquitoes collected in each structure type was determined using descriptive statistics. For this analysis, the mosquito species of focus was *An. arabiensis*.

Furthermore, data on structure type and mosquito species of focus was only collected in the 2019/2020 malaria season. A univariate and multivariate logistic regression model following a negative binomial approach was used to determine if there was any statistical association between house structure type and mosquito density. To determine which structure offered a higher risk of bite exposure, mosquito densities were used as a response variable while house structure was a predictor variable. Relative risk (RR) and 95% confidence intervals were recorded.

A univariate and multivariate poisson model was also carried out to independently determine whether any of the prospectively defined independent factors (temperature, rainfall, and humidity) were significantly associated with species abundance. Throughout the analyses, confidence limits were set at 95%. The analysis was controlled for a year. In 2019, there was no data on humidity recorded. All this was analysed using STATA (Version 11, StataCorp LP, Texas, USA).

3.7. Research ethics

Prior to the commencement of fieldwork, local authorities and the chief of the village were informed of the study and its objectives. This information was distributed to the local household owners and consent (Appendix 3) was sought from them. The participants were recruited, consent was sought (Appendix 4) and necessary training was provided. Additionally, the participants were screened for malaria and offered malaria prophylaxis. Lastly, a timetable was handed out.

Figure 9. The order in which information was disseminated.

Ethical clearance and approval for the study was obtained from the University of Namibia. Research Ethics Committee. Because the study was conducted in collaboration with the Ministry of Health and Social Services as part of routine entomological surveillance for the malaria control programme, it covered the use of human subjects during the research.

CHAPTER FOUR: RESULTS

4.1. Species Composition and Abundance

In the 2018/2019 malaria season, a total of 1190 (Table 2) mosquitoes were collected both indoors (n = 406 [34.1%]) and outdoors (n = 784 [65.9%]) while in 2019/2020 only 768 mosquitoes were collected. The collected mosquitoes were identified both morphologically and molecularly (Figure 10) as belonging to *An. gambiae* complex and *An. funestus* group. In 2019/2020, only *An. arabiensis* was collected (Figure 11 [b]).

Table 2. Species distribution of *An. funestus* group and *An. gambiae* complex between the two collection malaria seasons.

Collection location	Species	Collection period		Total (%)
		2018/2019	**2019/2020**	
Indoor	*An. arabiensis*	334 (28.1)	120 (15.6)	454 (23.2)
	An. gambiae s.s	62 (5.2)	0 (0)	62 (3.2)
	An. funestus s.s	10 (0.8)	0 (0)	10 (0.5)
Sub Total		**406 (34.1)**	**120 (15.6)**	**526 (26.9)**
Outdoor	*An. arabiensis*	625 (52.5)	648 84.4)	1273 (65)
	An. gambiae s.s	158 (13.3)	0 (0)	158 (8.1)
	An. funestus s.s	1 (0.1)	0 (0)	1 (0.1)
Sub Total		**784 (65.9)**	**648 (84.4)**	**1432 (73.1)**
Grand Total (%)		**1190 (100)**	**768 (100)**	**1958 (100)**

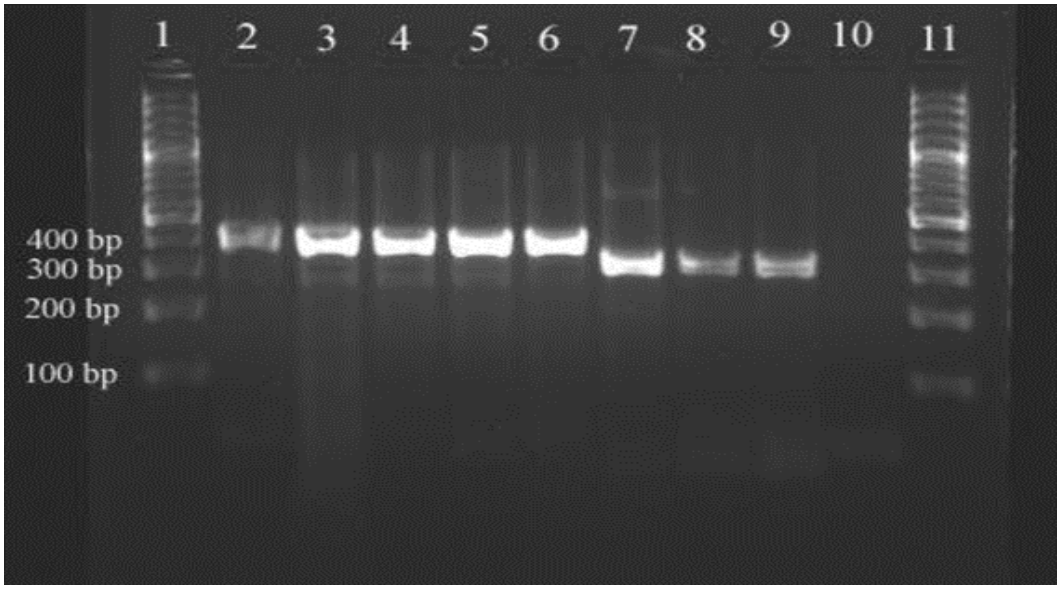

Figure 10. A gel showing amplified DNA bands of mosquitoes belonging to *An. gambiae* complex. Lane 2, positive control of *An. gambiae* s.s.; Lanes 3-6, 390 bp of *An. gambiae* s.s.; Lane 7, positive control for *An. Arabiensis*; Lanes 8 and 9, 315 bp of *An. arabiensis*; Lane 10, negative control.

From the *An. gambiae* complex, two species were identified; 80.6 % (n = 959) was *An. arabiensis* and 18.5 % (n = 220) was *An. gambiae* s.s. of the total samples collected, making *An. arabiensis* the most abundant species collected both indoors (n = 334 [28.1%]) and outdoors (n = 625 [52.5%]) (Figure 11). There were no *An. merus* or *An. quadriannulatus* identified in the collected samples. From the *An. funestus* group, only *An. funestus* s.s. (n = 11) was identified representing only 0.9 % of the total samples collected. *Anopheles leesoni*, *An. vaneedeni*, *An. parensis* and *An. rivulorum* were absent. Out of the Anopheles mosquitoes that were caught, 66.6 % were exophagic while 33.4 % were endophagic.

In 2019/2020, only one species, *An. arabiensis* was found (n = 768) of which 84.4 % were exophagic. Between 2018 and 2019, the relative proportion of *An. arabiensis* was reduced

by 9.8 %, *An. gambiae* s.s and *An. funestus* s.s. were both reduced by 100% which shows that species composition was significantly different between the two years ($X^2 = 24.0$, $p < 0.008$).

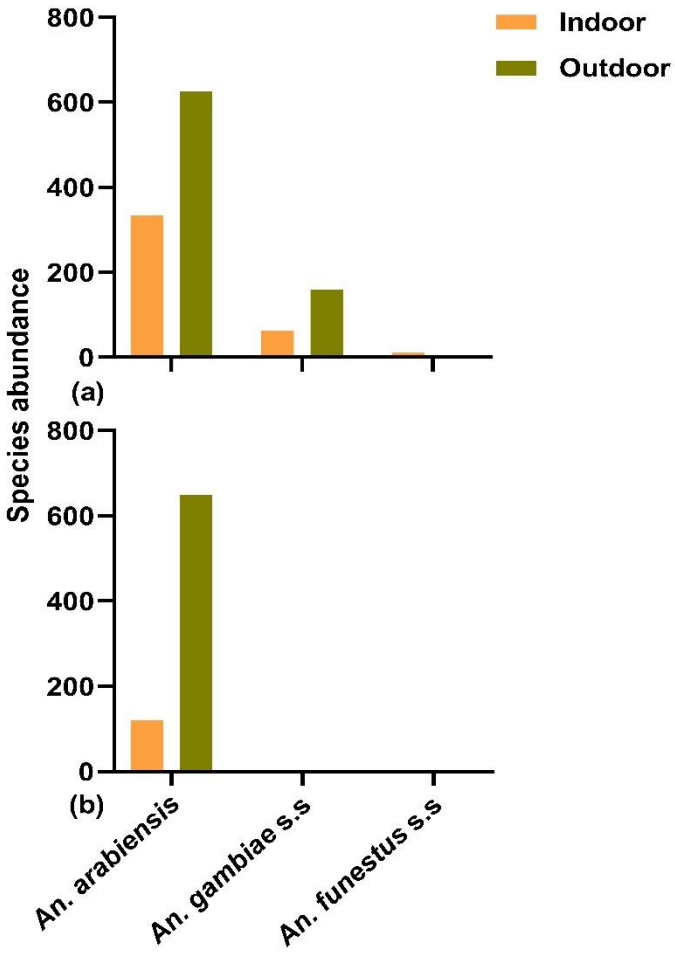

Figure 11. Species composition and abundance of the major malaria vectors caught indoors and outdoors in Kavango East region in 2018/2019 (a) and 2019/2020 (b) malaria seasons.

4.2. Biting behaviour of mosquitoes in relation to human behaviour in the 2018/2019 malaria season

Figures 12 to 14 show the biting profiles of *Anopheles* mosquitoes. They show the estimates of hourly biting/transmission exposure. The biting profiles of *An. arabiensis* (Figure 12) and *An. gambiae* s.s. (Figure 13) are similar in the sense that both species preferred biting outdoor, despite not having people outdoor. Despite high levels of outdoor biting, more biting exposure occurred mainly indoors because most people were indoors at the peak biting hours. Lastly, *An. funestus* s.s. preferred biting indoors (Figure 14).

4.2.1. *Anopheles arabiensis* biting profile

The highest proportion (65.2 %) of *An. arabiensis* was caught outdoors. Indoor biting sharply increased from 21:00 hours to 23:00 hours and from there onwards there was a steep decline. Biting increased again from 02:00 hours to 04:00 hours and thereafter, decreased. The peak biting hours were between 22:00 and 23:00 and between 04:00 and 05:00. The red graph shows the proportion of mosquito and human contact which peaked in the early evening hours as the number of people indoors increased but also decreased with the number of people in the early morning hours. Outdoor (Figure 12(b)), biting increased steadily in the early evening until 22:00 hours and stabilized until 24:00 hours of which after there was a sharp decline as the number of people decreased. Despite having the peak biting hours between 02:00 and 03:00 and between 04:00 and 05:00 hours outdoor, there were no people present outdoor hence the proportion of mosquito and human contact is zero. Even though *An. arabiensis* preferred biting outdoor (X^2 = 1532.7, $p < 0.001$), if the mosquitoes were carrying parasites, the risk of infection was indoor as the vast bulk of human contact to bites occurred when people were indoors.

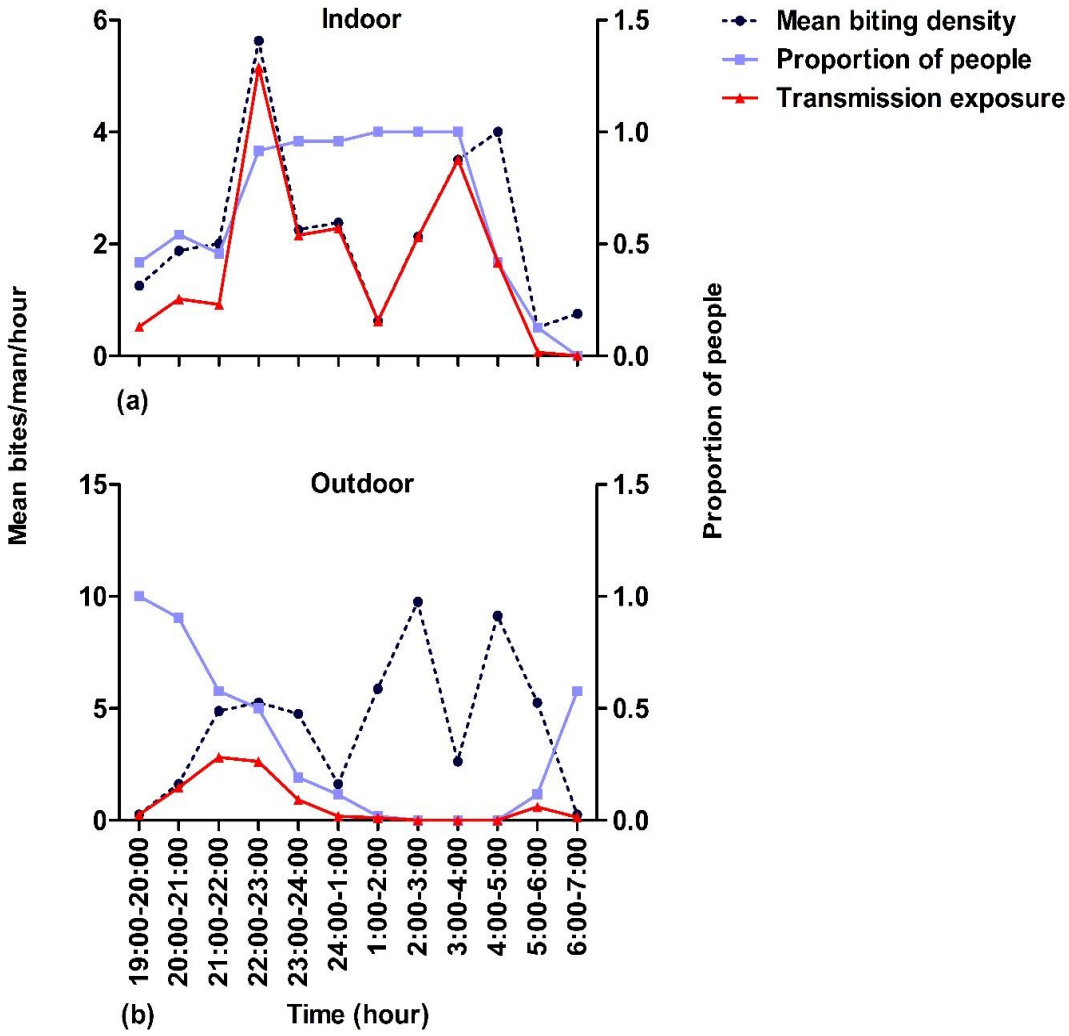

Figure 12. The hourly indoor (a) and outdoor (b) biting profile of *Anopheles arabiensis* from hourly mosquito sampling done in Kavango East during the 2018/2019 malaria season.

4.2.2. *Anopheles gambiae* s.s. biting profile

The biting peak hours for *An. gambiae* s.s. indoors occurred between 19:00 and 20:00 hours, between 01:00 and 02:00 hours and between 05:00 and 06:00 hours. During the latter biting peak hour, the number of people had decreased, hence the reduced

transmission exposure. There was a steep decline in the number of mosquitoes biting in the early evening hours indoors which then peaks at 21:00 and 22:00 hours. The peak biting hours outdoor were between 23:00 and 24:00 hours and between 02:00 and 03:00 hours. Biting occurred throughout the night except between 01:00 and 02:00 hours. There was a decline in the number of people outdoor from 19:00 hours to 02:00 hours. *An. gambiae* s.s. showed a tendency to bite outdoor ($X^2 = 1532.719$, $p < 0.001$) but the human to mosquito contact at peak biting hours was 0.65 to zero. Despite having a high number (52) of people outdoor from 19:00 to 20:00 hours, zero mosquitoes were biting at that hour, hence the transmission exposure is equal to zero.

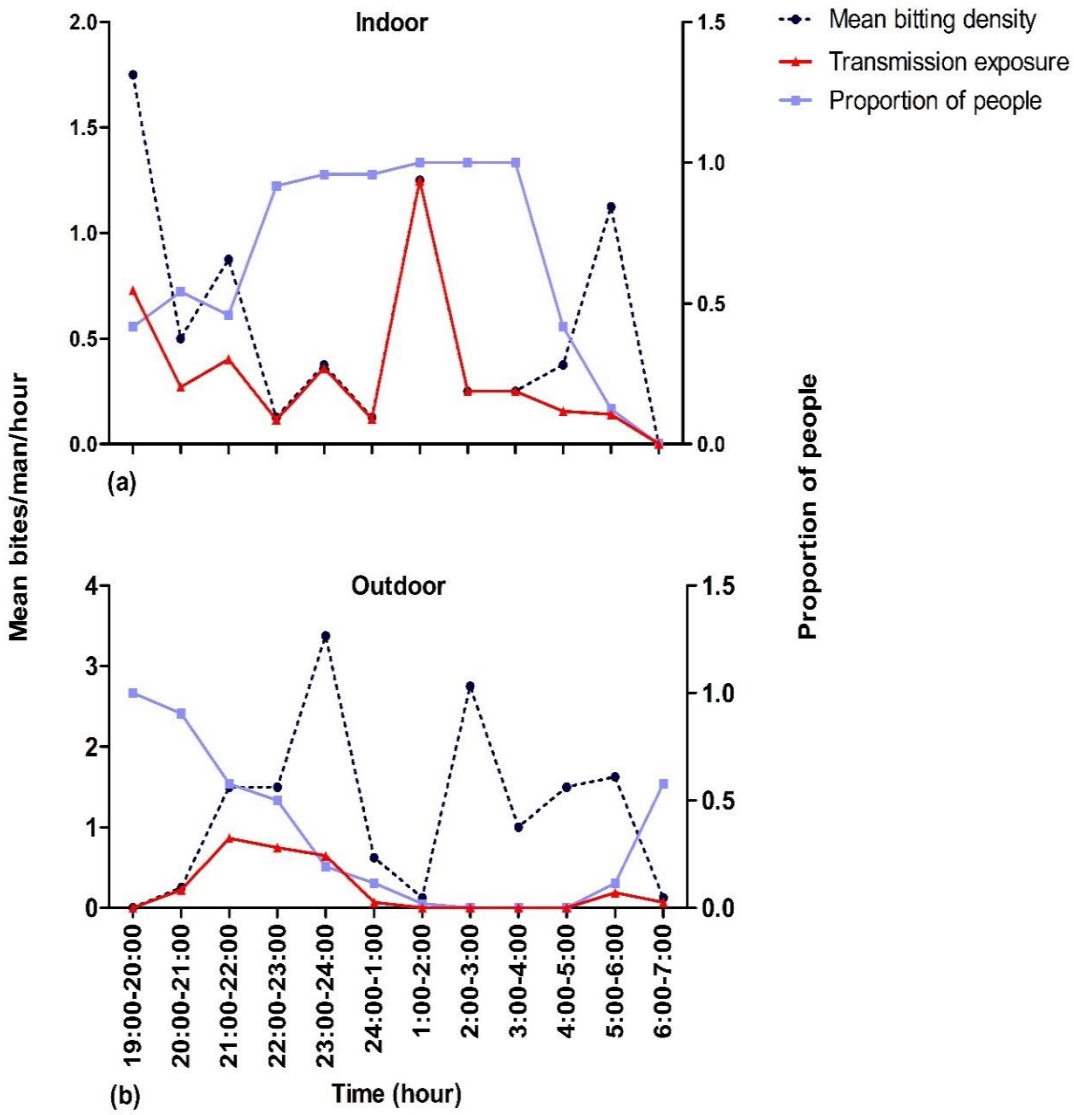

Figure 13. The hourly indoor (a) and outdoor (b) biting profile of *Anopheles gambiae* s.s. from hourly mosquito sampling done in Kavango East during the 2018/2019 malaria season.

4.2.3. *Anopheles funestus* s.s. biting profile

Anopheles funestus s.s. preferred biting indoors **(**X^2 = 1532.719, p < 0.001**)** but only beat from 19:00 to 22:00 hours despite having a large proportion of people indoor. The peak

biting hour occurred between 20:00 and 21:00 hours. Outdoor, biting only occurred between 01:00 and 02:00 hours but at that point, the transmission exposure was close to zero. However, this result needs to be considered with caution as there was only one individual mosquito caught and only one person observed outside.

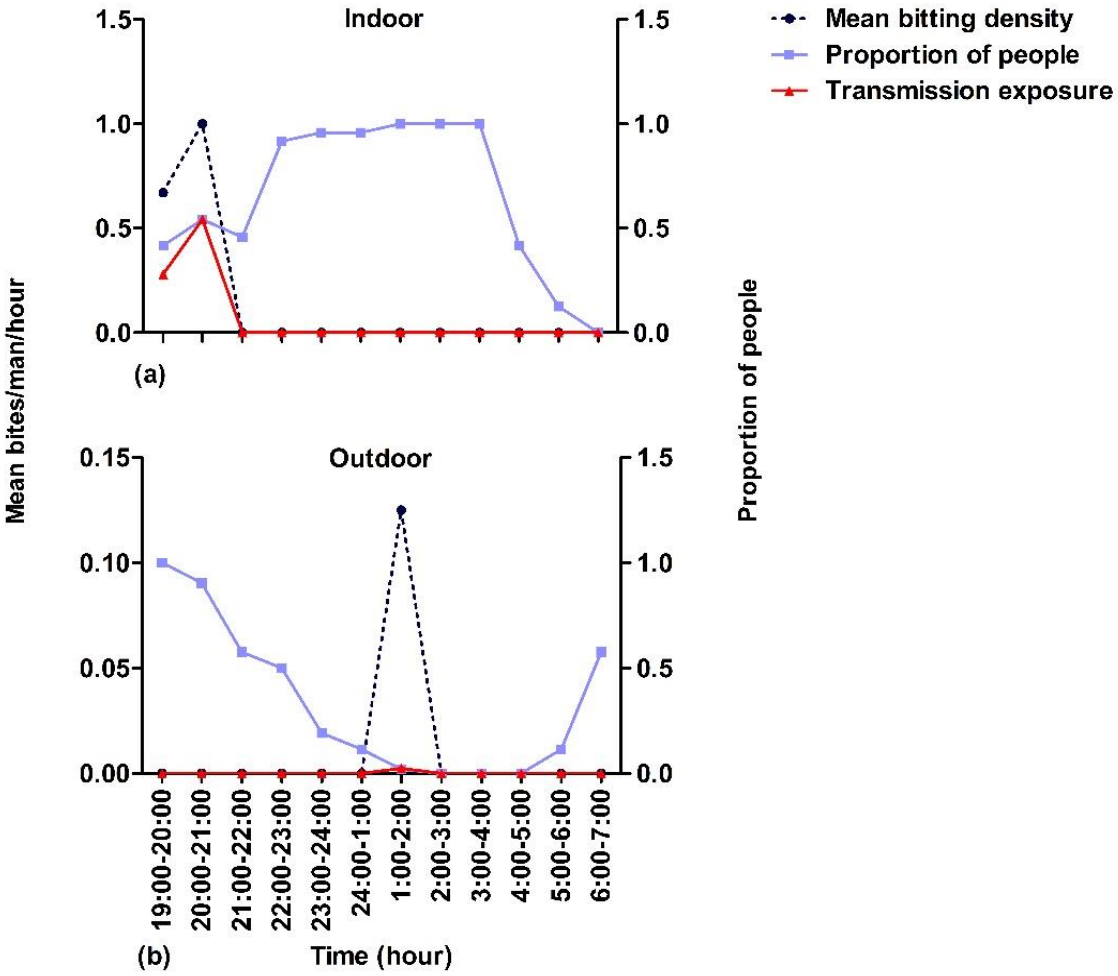

Figure 14. The hourly indoor (a) and outdoor (b) biting profile of *Anopheles funestus* s.s. from hourly mosquito sampling done in Kavango East during the 2018/2019 malaria season.

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4.3. Biting behaviour of mosquitoes in 2019/2020 malaria season in relation to human behaviour

The 2019/20 biting profile of *An. arabiensis* is similar to the one obtained during 2018/19. Mosquitoes were still biting outdoor. The human behaviour profiles between 2018/19 and 2019/20 did not change. People went to bed as early as 20:00 hours.

4.3.1. *Anopheles arabiensis* biting profile

During the 2019/2020 malaria season, *An. arabiensis* was observed to prefer biting outdoor ($X^2 = 22.1$, $p < 0.006$), this is similar to data obtained during the 2018/2019 malaria season. Despite biting throughout the night, there were no people observed outdoors. (Figure 15).

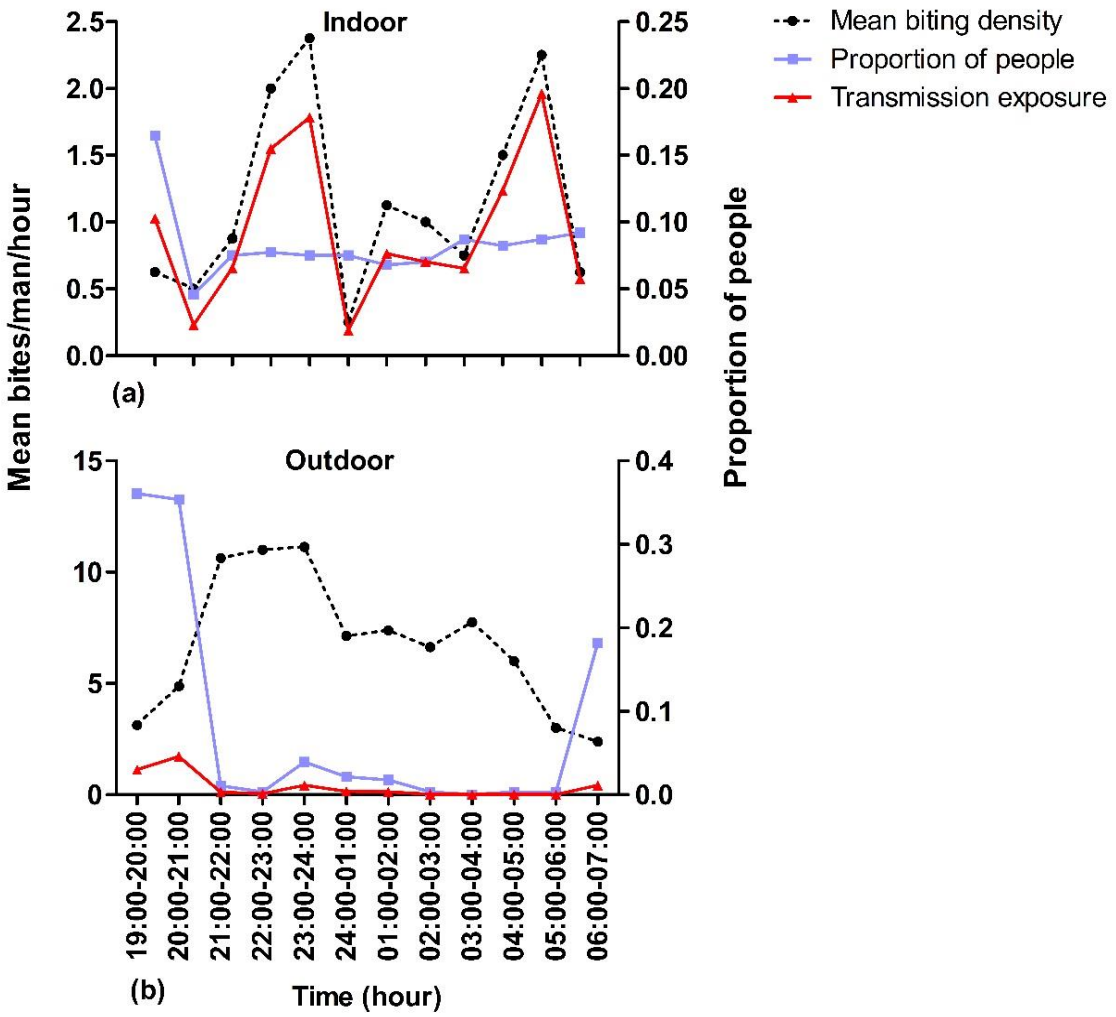

Figure 15. The hourly indoor (a) and outdoor (b) biting profile of *An. arabiensis* from hourly mosquito sampling done in Kavango East during the 2019/2020 malaria season.

4.4. House structure (traditional, zinc, modern) and biting behaviour of mosquitoes.

The risks of being bitten by mosquitoes in traditional houses was significantly higher (RR = 0.79, 95% CI: 01.23–6.56, p = 0.001) than in modern (RR = 0.48, 95% CI: 0.07–0.93,

p = 0.012) and zinc (RR = 0.15, 95% CI: 0.04–0.63, p<0.06) houses. Only *An. arabiensis* was collected in all three structure types.

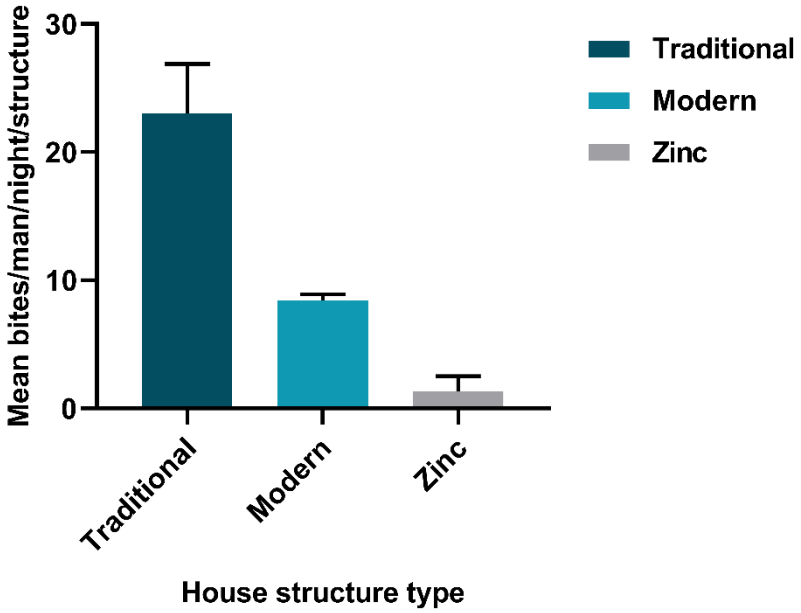

Figure 16. The mean number of mosquitoes biting per man different house structures per night in 2019/2020 malaria season.

4.5. Activities exposing humans to mosquito bites

A total of 13 activities were associated with the risk of mosquito bites. These sitting, sleeping, chatting, drinking, washing, cooking, digging, sweeping, bathing, praying, standing, and dancing. The study determined human activities that were associated with mosquito bites indoors and outdoors. In 2018/2019, a higher proportion of people were indoors sleeping in the late hours, resulting in higher exposures indoors than outdoors when few people were bathing. In the 2018/2019 and 2019/2020 malaria seasons, the interaction between mosquitoes and humans while sleeping indoors was associated with a higher risk of mosquito bites (2018/19: OR = 0.62, 95% CI: 0.17 – 0.91, p = 0.025;

2019/2020: OR = 0.32, 95% CI: 0.07 – 1.06, $p = 0.008$). Outdoor chatting (OR = 0.70, 95% CI: 0.31 – 1.58, $p = 0.01$) in 2019/2020 was a risk factors for mosquito bites.

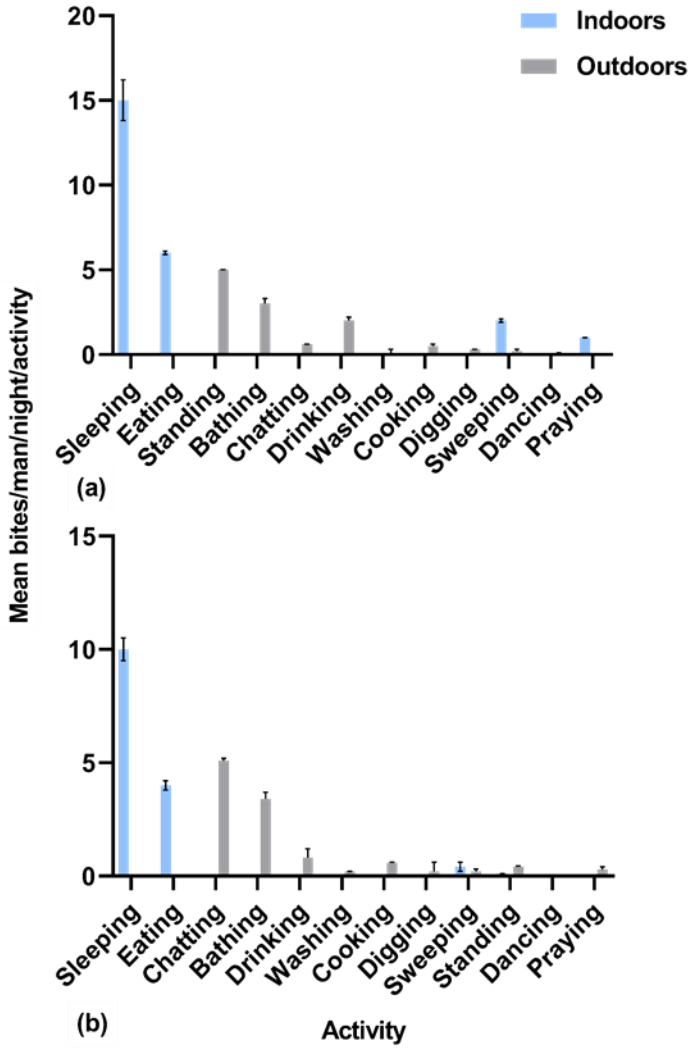

Figure 17. Indoor and outdoor activities exposing people to mosquito bites in the Kavango East region in 2018/2019 (a) and 2019/2020 (b) malaria seasons.

4.6. Species composition and abundance in relation to temperature, rainfall, and relative humidity in 2018/2019.

Although temperature and relative humidity had a positive correlation to species abundance (Coef.: 0.437; 95% CI. 0.397 – 0.477; Coef.: 0.452; 95% CI. 0.324 – 0.579, respectively), their effect on the abundance was not significant (Temperature: OR = 0.6, 95% CI: 0.10 – 1.58, $p = 0.067$; Relative humidity: Coeff = 0.23, 95% CI: 0.23 – 2.80, $p = 0.57$). Rainfall had a significant negative correlation (Coef.: -0.132; 95% CI. -0.182 – [-0.0809]) on abundance, but this could only explain 33% (Coeff = 0.33, 95% CI: 0.31 – 1.58, $p = 0.01$) of the differences in overall abundance. The general observation was an increase in abundance of all species with an increase in rainfall.

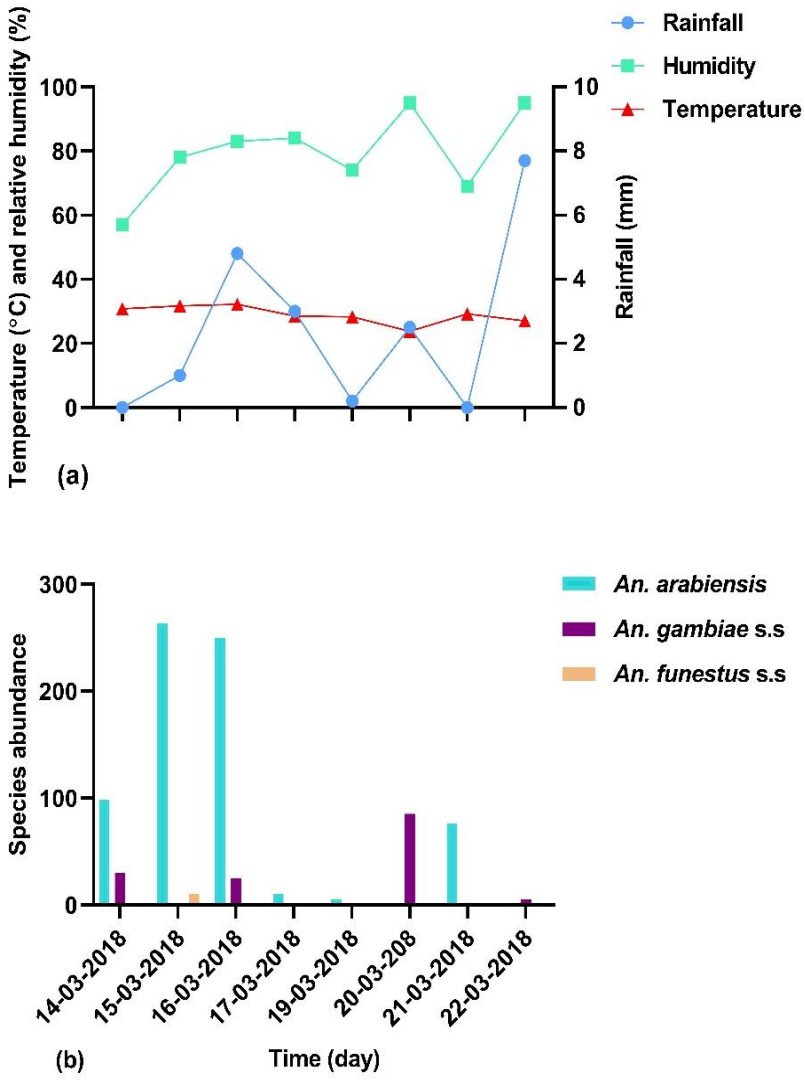

Figure 18. The figure is indicating daily climatic variables (a) and species composition and abundance (b) during the 2018/19 malaria season in Kavango East Region.

4.7. Species composition and abundance in relation to temperature, rainfall and relative humidity in 2019/2020.

In the 2019/2020 malaria season, temperature had a negative correlation (Coef: -0.246; 95% CI. -0.287 – [-0.206]) on the abundance of *An. arabiensis* while rainfall and relative

humidity had no correlation. Temperature had a significant effect on the abundance (Coeff = 0.6, 95% CI: 0.12 – 0.88, $p = 0.01$).

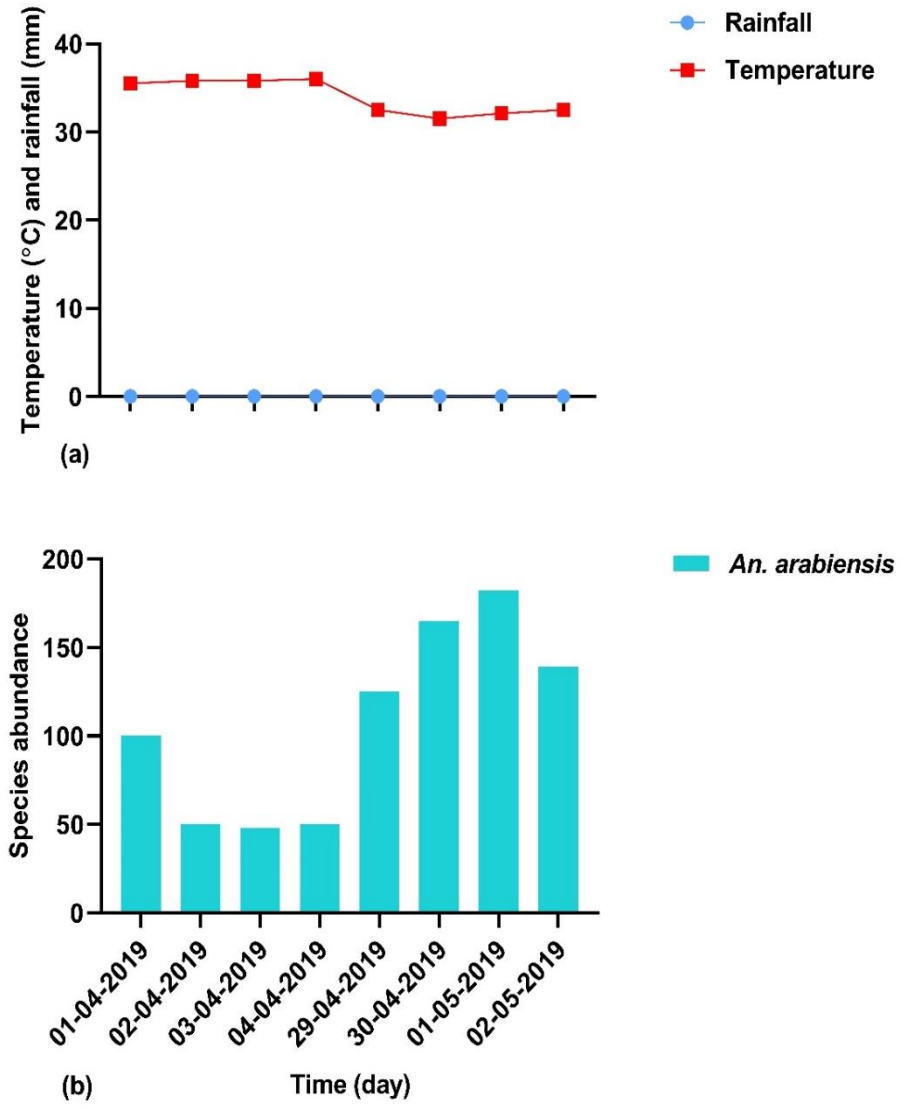

Figure 19. The effect of climate variables (a) on species composition and abundance (b) in the 2019/2020 malaria season in Kavango East Region.

4.8. Local species distribution maps

When all three species are included in mapping mosquito distribution (Figure 20), they appear to be randomly dispersed but when only one species (*An. arabiensis*) is used (Figure 21), it shows that there is clumping. Additionally, the closer to the water body the species are, the higher the species richness and abundance which reduce further inland.

Figure 20. *Anopheles* species distribution map of Kavango East region in the 2018/2019 malaria seasons.

Figure 21. *Anopheles* species distribution map of Kavango East region in the 2019/2020 malaria season.

CHAPTER FIVE: DISCUSSION

Successive control of malaria vectors largely depends on knowledge of vector behaviour. This study determined the current malaria vectors and confirmed the presence of *Anopheles gambiae* s.s., *An. funestus* s.s. and *An. arabiensis* by PCR method with *An. arabiensis* being the most abundant and *An. funestus* s.s. the least. The high abundance of *An. arabiensis* and *An. gambiae* s.s. as compared to *An. funestus* s.s. could be in their feeding plasticity. According to Wanji et al. (2003), *An. arabiensis* s.s. is anthropophilic but in the absence of a human blood meal due to barriers such as nets or protective clothing, its blood meal preference is dependent on the availability of potential hosts. According to Kitau et al. (2012), LLINs can control the species abundance and composition of mosquitoes. It was hypothesized that *An. arabiensis* can avoid contact with LLINs by feeding outdoors, unlike *An. gambiae* s.s. whose host feeding preference was restricted. These findings could explain the difference in species abundance and biting behaviour in this study as mosquito nets were distributed in Namibia from 2005 to 2011 (Gueye et al., 2014). Since *An. arabiensis* also prefers feeding on other hosts in the absence of humans (Killeen et al., 2017), it is less likely to persist in human-seeking when confronted with treated bed nets than *An. gambiae* s.s. and *An. funestus* s.s. that are more anthropophilic (Reddy et al., 2011; Govella et al., 2013). Therefore, the latter two species are more likely to pick up more proportion of the lethal dose of the insecticides which in turn reduces their abundance. Due to this diversity in the feeding behaviour of *An. arabiensis*, vector control interventions that target indoor resting vectors become less effective against this species (Gueye et al., 2014; Killeen et al., 2016).

Additionally, the lower abundance in the 2019/2020 season may have resulted from the delay in the procurement of materials and supplies which delayed sampling as well. The second phase of sampling was done from the end of April to the beginning of May which is the start of winter. On most nights, it was too windy which may have greatly affected the catches as the wind made it impossible for mosquitoes to land on a human long enough to be captured. Environmental and seasonal factors may affect the activity and attraction of anthropophilic Anophelines because the human landing catch uses the olfactory cues to lure mosquitoes (Lima et al., 2014). So, when it is too windy, the human odour is blown away making it difficult for the mosquito to locate the emitter.

In contrast to this study where *An. arabiensis*, *An. gambiae* s.s. and *An. funestus* s.s were present, a study by Kamwi (2005) did not find *An. gambiae* s.s. The absence of *An. gambiae* s.s. was attributed to the species being missed from the study. It was stated that the *An. gambiae* s.s. believed to have been found in Namibia by La Grange (1988) could have been *An. arabiensis* as there was no confirmation of the species by the cytogenetic method which was the golden standard then. Although there have been conflicting results as to what malaria vectors were present in the past, *An. gambiae* s.s has emerged as a vector which could be as a result of its importation from neighbouring countries (Nghipumbwa et al., 2018) and/or due to insecticide resistance. According to the 2018/2019 and 2019/2020 insecticide resistance studies, members of the *An. gambiae* complex and *An. funestus* group are resistant to IRS insecticides used in Namibia (personal observation).

Another reason for the absence of *An. gambiae* s.s. from Kamwi (2005) could be explained in the use of different trapping methods which may lead to heterogeneous and

incomparable results (Lima et al., 2014). The window exit traps (WETs) used in Kamwi's study mainly capture mosquitoes that escape from the house after feeding and/or resting indoors but do not specifically target host-seeking females. So, mosquitoes that sorely feed and rest outdoors could have been missed in that study. Additionally, window exit traps are more effective when there are no alternative exits (Wong et al., 2013), otherwise, species abundance might be underestimated. Govella et al., 2011 added that WETs reduce the entry points to the house which decreases the chance of mosquitoes entering the house in the first place. However, the HLC method in this study accounted for both indoor and outdoor feeding mosquitoes.

It was also observed that *An. arabiensis* and *An. gambiae* s.s preferred biting outdoors while *An. funestus* s.s preferred biting indoors. Although *An. arabiensis* and *An. gambiae* s.s. preferred biting outdoors, malaria transmission might be occurring indoors as the peak biting hours corresponded to when people were indoors sleeping. The transmission is even higher especially for people who might not be sleeping under mosquito nets. However, caution is given when dealing with the HLC because of its limitations. The method sometimes overestimates the human biting rate because no human being ever sits for that long with their limbs exposed just getting beaten by mosquitoes. It may also be biased in the sense that collections are dependent on the attractiveness and ability of the collector (Lima et al., 2014) and it can underestimate the abundance of mosquitoes when the numbers are low. Assessing when and where people are exposed to malaria vectors is important for targeting malaria prevention interventions.

Additionally, the results show that more anopheline mosquitoes were caught in traditional houses compared to modern and zinc structured houses. This may be due to the availability

of eaves and crevices in traditional houses. It is reported in the literature that eaves and crevices allow the formation of appropriate mosquito resting places and conducive humidity (Ngadjeu et al., 2020; Ng'ang'a et al, 2020). This is further supported by a study that was conducted in Cameroon which observed that houses that are poorly constructed are associated with an increase in entry and resting of mosquitoes and consequently increased malaria incidences (Nguela et al., 2020). It was also observed that mud houses are associated with low socioeconomic status with most households from these types of structures using fewer repellents against nuisance mosquitoes (Etang et al., 2011).

Another factor that could have contributed to high mosquito catches in traditional houses is the difference in insecticide absorption among the three different structures. Okumu et al. (2012) and Uragayala et al., (2018) discussed that the type of substrate onto which the insecticide is applied determines the efficacy of the active ingredients. In the Okumu et al. (2012) study, pirimiphos-methyl killed 100% of mosquitoes on thatched ceilings while DDT only killed 85% on the same structure. However, DDT sprayed on mud structures in the same period killed 97.5% of mosquitoes. In another study done to determine the effect of different substrates on pyrethroids, it was discovered that porous surfaces such as mud have variable insecticidal activities because they absorb insecticides but less porous surfaces such as wood would retain insecticidal activity for longer periods due to lower rates of absorption (Raeisi et al., 2010; Kassem et al., 2019; Rohani et al., 2020). Additionally, alkaline containing substrates such as mud reduce the effectiveness of the insecticide quicker than substrates without alkaline contents (Mutagahywa et al., 2015; Mandal et al, 2019). In another study that was done in Ghana, pirimiphos-methyl was effective on cement walls for up to fifteen weeks and continued to kill *An. gambiae* s.l

(Fuseini et al., 2011). However, according to Okumu et al. (2012), pirimiphos-methyl was almost fully degraded on walls by the third month in that study. Therefore, this suggests that when spraying insecticides, it is important to determine which insecticide is suitable for a particular structure/substrate, area, and length of the spray cycles.

The long-term efforts to curb malaria will be highly sensitive to changes in the world's climate, given the association between malaria transmission and climate (WHO, 2015). For malaria transmission to occur, a mosquito needs to survive long enough for the parasites to become infective. The length the parasite needs to be infective depends on temperature and the length of the vector's life depends mainly on relative humidity among other climatic factors. In the 2018/2019 malaria season, the results show that rainfall had a significant negative effect on the abundance of mosquitoes in the Kavango East region. There was a decrease in abundance with an increase in rainfall. Some studies have found that rainfall aids the availability of suitable breeding water pools. Since mosquitoes need water to breed, then their abundance should increase with rainfall. For instance, *An. gambiae* s.s. and *An. arabiensis* are highly associated with higher precipitation. However, torrential rain can also decrease mosquito abundance as it washes away the eggs and/or floods the ideal water pools needed for larval development (Paaijmans et al, 2007; LaPointe et al. 2012). Mosquitoes feed on microorganisms, so when a habitat is flooded it reduces the amount of food available, making the environment less habitable (Asigau and Parker, 2018).

To disrupt the transmission of malaria in Namibia, spraying of insecticides is initiated immediately before the onset of rains. The assumption is that suitable water pools for mosquitoes to lay eggs in are only available after the onset of rains (Soverow et al, 2009).

Unfortunately, this might not always be the case (Greenwood & Mutabingwa, 2002). Despite the rainfall being 0 mm throughout the two months in which sampling was done, there was a huge number of mosquitoes available, probably being supported by the Okavango River.

This is evident that IRS should include insecticides that last throughout the year. According to Okumu et al. (2012), compounds in IRS degrade significantly within the first few months after spraying, in many cases becoming ineffective earlier than the time when they would normally be due for re-spraying. It was further noted that DDT only lasts six months on the wall while pirimiphos-methyl lasts two to three months. So, if spraying is only done once a year, six to nine months of the remaining year people are not protected, resulting in malaria transmission year in and out.

In the 2019/2020 sampling season, there was no association between rainfall and abundance because it was 0 mm throughout the season. However, temperature had a significant negative effect on abundance. According to Asigau and Parker (2018), high temperatures speed up the growth of the aquatic stages of mosquitoes, but the same temperature is detrimental to the adult stages. It was further added that at 20.8 °C the pupal stage lasts for 61 hours while at 29 °C it only takes 37 hours. However, at 32 °C, the lifespan of adult mosquitoes significantly reduces. The ideal temperatures for adult survival and larval growth to adulthood is between 20 and 30 °C. Above 27 °C, the development of larvae is halted and above 30 °C, the abundance of adult mosquitoes is reduced (Beck-Johnson et al., 2017). This probably contributed to the observed variations in the collections in the 2019/2020 malaria season.

CHAPTER SIX: CONCLUSION AND RECOMMENDATIONS

The control of malaria largely depends on the use of the appropriate vector and parasite control measures. With regards to vector control, this requires an understanding of species composition and abundance, distribution, and biting behaviour in relation to human behaviour. Furthermore, the effectiveness of such interventions is also associated with understanding how climate variables affect species distribution, composition, and abundance. Although the Namibian government is dedicated to eliminating malaria by 2022, the current vector surveillance is not robust enough to get conclusive data on the role of climate variables on mosquito bionomics. As a result, vector surveillance should be done throughout the year to determine when exactly there is a peak in the abundance of mosquitoes.

As observed in this study, *An. arabiensis* and *An. gambiae* s.s. populations from Shadikongoro were observed feeding mainly outdoors. This could be the reason why there is still ongoing malaria transmission, more so in high-risk groups such as forest workers and security guards who are continuously exposed to outdoor biting mosquito populations. However, the mosquito-human interactions occurring indoors are overwhelmingly higher than those outdoors because people spend most of their hours indoors in the dark, implying a higher indoor transmission potential in the village.

The current interventions are meant to only target indoor feeding and resting mosquitoes and are not effective against outdoor transmitting vectors. However, more effort should be put into protecting outdoor workers if malaria transmission is to be disrupted. The above-mentioned groups should be encouraged to wear protective clothing during extended evening activities such as cooking, bathing, or sleeping. These should cover the

lower extremities as mosquitoes are believed to prefer biting those areas. Additionally, housing improvement needs to be done on poorly constructed structures and communities should be encouraged to have their houses sprayed. As observed, *Anopheles arabiensis* can aestivate which means that even when the rain has stopped, it can still survive and be highly infectious. Therefore, it is important to spray insecticides that last throughout the year and no compromise on quality through the poor implementation or use of substandard products should be made. Government reports of Namibia from 2014, 2015 and 2016 show that the IRS coverage was below 85% in those years, which is the recommended coverage by WHO. This needs to improve otherwise the gains made might never make an impact on malaria elimination.

The study identified when and where human-mosquito interactions are occurring and what activities expose humans to mosquito bites. This is important for identifying target context-appropriate vector control interventions. Identifying appropriate controls involves knowledge of vector behaviour and this can only be done by conducting regular bionomic studies.

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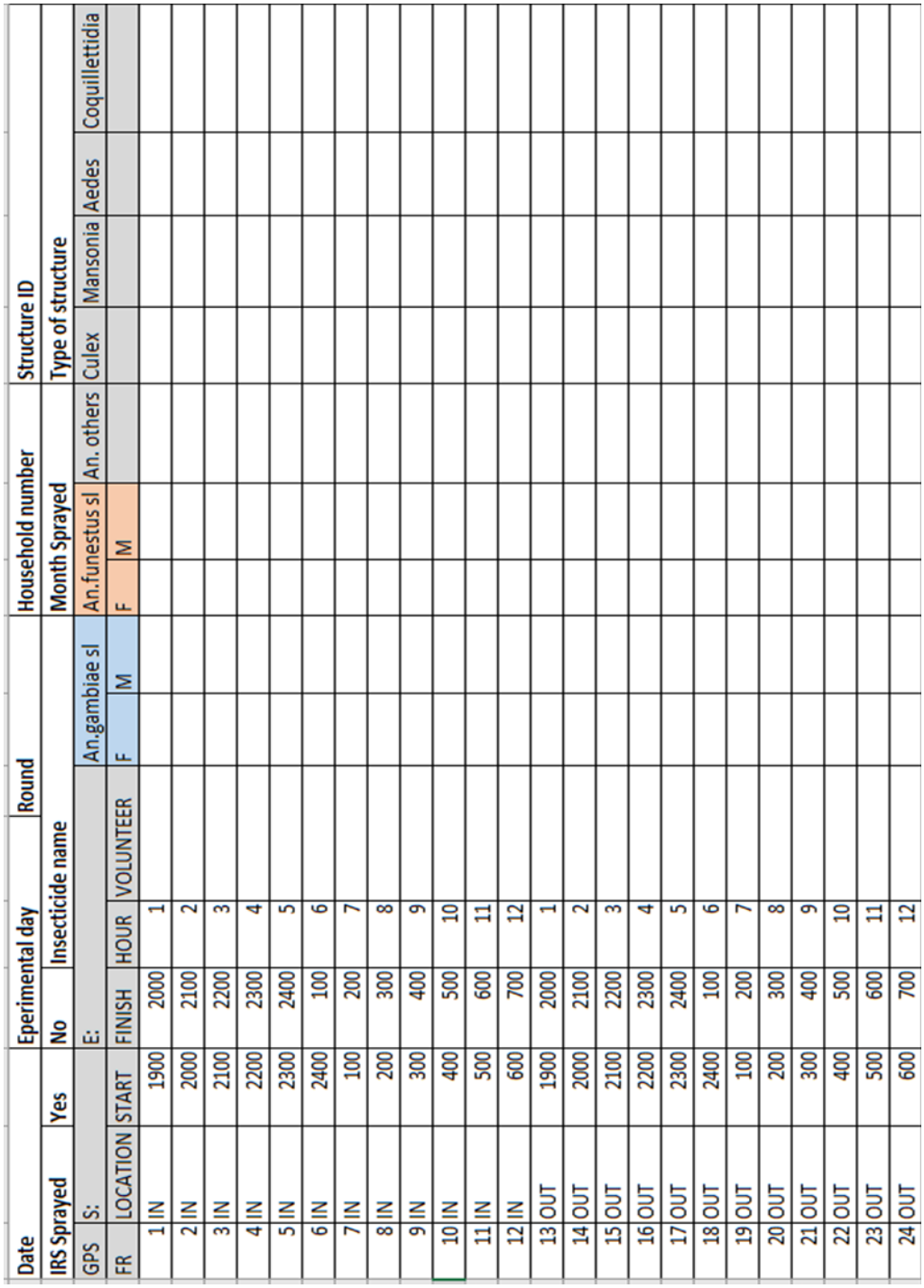
APPENDICES

Appendix 1. Human landing catch form



Date		Eperimental day		Round		Household number		Structure ID			
IRS Sprayed	Yes	No	Insecticide name			Month Sprayed		Type of structure			
GPS	S:	E:		An.gambiae sl		An.funestus sl		An. others	Culex	Mansonia	Aedes	Coquillettidia
FR	LOCATION	START	FINISH	HOUR	VOLUNTEER	F	M	F	M					
1	IN	1900	2000	1										
2	IN	2000	2100	2										
3	IN	2100	2200	3										
4	IN	2200	2300	4										
5	IN	2300	2400	5										
6	IN	2400	100	6										
7	IN	100	200	7										
8	IN	200	300	8										
9	IN	300	400	9										
10	IN	400	500	10										
11	IN	500	600	11										
12	IN	600	700	12										
13	OUT	1900	2000	1										
14	OUT	2000	2100	2										
15	OUT	2100	2200	3										
16	OUT	2200	2300	4										
17	OUT	2300	2400	5										
18	OUT	2400	100	6										
19	OUT	100	200	7										
20	OUT	200	300	8										
21	OUT	300	400	9										
22	OUT	400	500	10										
23	OUT	500	600	11										
24	OUT	600	700	12										

Appendix 2. Human behaviour characterization form

NAMIBIA ENTOMOLOGICAL SURVEILLANCE 2019
HUMAN BEHAVIOR CHARACTERIZATION

Sentinel Site_____ Village Name_____ Date_____ Household name_____ Structure ID_____

Household number_____ Round_____ Experimental day_____ GPS: S:_____ E:_____

Name of observer	Hour of observation	Location of observation (Inside/Outside)	Number of persons under bednet at END of Collection Hour	Number of persons not under bednet at END of Collection Hour	Activity (enter activity code; see activity codes below e.g. 1=sitting, 2=sleeping)	Number of people doing particular activity
	6:00-7:00 PM	Inside				
		Outside				
	7:00-8:00 PM	Inside				
		Outside				
	8:00-9:00 PM	Inside				
		Outside				
	9:00-10:00 PM	Inside				
		Outside				
	10:00-11:00 PM	Inside				
		Outside				
	11:00 PM - 12:00 AM	Inside				
		Outside				
	12:00 – 1:00 AM	Inside				
		Outside				
	1:00 – 2:00 AM	Inside				
		Outside				
	2:00 – 3:00 AM	Inside				
		Outside				
	3:00 – 4:00 AM	Inside				
		Outside				
	4:00 – 5:00 AM	Inside				
		Outside				
	5:00 – 6:00 AM	Inside				
		Outside				

Observations:_____

Activity: 1= sitting, 2= sleeping, 3= chatting, 4= drinking, 5= on phone, 6= washing, 7= cooking, 8= working, 9= digging, 10= sweeping, 11= bathing, 12= praying, 13= standing, 14= eating, 15= dancing, 16= grazing

Supervisors' Name and signature:_____

Appendix 3. Household participation consent form

HOUSEHOLD PARTICIPATION CONSENT

TITLE OF PROJECT: Evaluation of a tent trap for sampling human biting malaria vectors

IRB: Research Division, Ministry of Health and Social Services

PRINCIPAL INVESTIGATORS:

- Hans Angula, Vector Control focal point, National Vector-borne Diseases Control Programme
- Iitula Iitula, Insectary Manager, National Vector-borne Diseases Control Programme
- Deodatus Maliti, Medical Entomologist, Clinton Health Access Initiative

SPONSOR: National Vector-borne Diseases Control Programme

VERSION OF PROPOSAL: Version 2

DATE OF SUBMISSION: 8th February, 2018

Purpose of the Project

Malaria interventions are important in preventing malaria which may cause suffering and death to humans. These interventions include the annual indoor residual spraying, larviciding and bed nets. Assessment of the effectiveness of the interventions in protecting communities from malaria is important and should be conducted using appropriate sampling tools. This study aims to develop and evaluate tools for sampling mosquitoes that bite humans and transmit malaria. Sampling of mosquitoes will take place inside and outside of human houses and will help researchers to understand the type of mosquitoes transmitting malaria in Namibia, where they are found and their behavior.

The researchers have selected your household for this study, as it is a setting conducive to collect mosquitoes that transmit malaria. Therefore, we would like to invite you to participate in the study. You are not forced to take part and can choose whether or not you want to participate.

Explanation of Procedures

If you decide to participate, the project team will be trapping mosquitoes in your house using four different types of traps. The first type of trap is the human landing catch. Starting from 18:00 hours, a person will be sitting inside your house, it may not be necessary for the person to sit in your sleeping room; it may be a room that no one sleeps within your house. A person will do their work quietly without disturbing you and your family. The person will collect mosquitoes that enter your house and wanting to bite people. He/she will collect mosquitoes all the night to 06:00 hours in the morning. On the next night, a person will be using a tent trap to collect mosquitoes in the same room for the entire night. And on the third night a light trap will be placed about one meter close to a bed where people sleep. This trap uses light and fan to capture mosquitoes. The trap will be set at 18:00 hours and left to operate until at 06:00 in the morning. Another set of similar traps will be placed outside your house about 10m away. Every morning researchers will take out the traps and examine the trapped mosquitoes to record their number and type. Your house will participate in this study for period of four nights. You will not provide food or drinks to the trap operators; these will be provided by the project.

Risks

The risks involved in participating in this project are minimal. We will ensure that people trapping mosquitoes in your house behave in a descent manner and will not take or damage your property. If anything will be damaged by the trap volunteers, the project will compensate you.

Responsibilities of the project team

The project team will make sure the trap operators behave in a good manner and respect household members and your properties. Any damage proved to happen as a result of this study will be repaired by the project.

Questions

If you have questions about this project you may contact Mr. Hans Angula, 0811243435, litula litula, 0812191136 and Deodatus Maliti, 0814124900. Please feel free to ask any questions concerning this study before during and after participation.

Signatures

Household owner

I understand the procedures described above. My questions have been answered to my satisfaction, and I agree to participate in this project. I have been given a copy of this form.

Assigned Household Number of participant: _____

Name of participant: _____

Signature of Participant: _____ or thumb-print_____

Appendix 4. Trap operator consent form

CONSENT FORM

TRAP OPERATOR CONSENT

Title of proposal: Evaluation of a tent trap for sampling human biting malaria vectors

IRB: Research Division, Ministry of Health and Social Services

Principal investigators:

- Hans Angula NVDCP, 0811243435
- Iitula Iitula NVDCP, 0812191136
- Deodatus Maliti CHAI, 0814124900

Sponsor: National Vector-borne Diseases Control Program

Version of proposal: Version 2

This Informed Consent Form has two parts:
• Information Sheet (to share information about the research with you)
• Certificate of Consent (for signatures if you agree to take part)

You will be given a copy of the full Informed Consent Form

Date of submission: 8th February, 2018

PART I: Information Sheet

Introduction

We are from the National Vector-borne Disease Control Program (NVDCP) doing research on malaria, a disease that affects many people in Namibia. We are going to give you information and invite you to be part of the research that we will explain to you. You feel free to decide whether or not you will participate in the research. Before you decide, you can talk to anyone you feel comfortable with about the research.

There may be some words that you do not understand. Please ask us to stop as we go through the information and we will take time to explain. If you have questions to ask now or later, you can ask them to Mr. Hans Angula (0811243435), Mr. Iitula Iitula (0812191136) or Dr. Deodatus Maliti (0814124900).

Purpose of the research

Malaria is transmitted by mosquito bites. Knowing the type of mosquitoes that transmit malaria, their abundance and behavior is important in controlling mosquitoes in order to prevent malaria transmission to persons. Sampling of mosquitoes that transmit malaria is important for achieving good understanding of how mosquitoes transmit malaria. In this study we want to test a new trap for sampling mosquitoes that transmit malaria. This new trap is made up of a tent and a sanction fan which sucks mosquitoes into the trap. Other methods for collecting mosquitoes that will be used in this study are human landing catch and CDC light trap. We want to compare these three methods to see which one works best in collecting mosquitoes that transmit malaria. The way each of the traps works will be explained to you. You will also be taught how to use these traps to collect mosquitoes inside and outside of houses and any risks involved including their mitigation. If you want, you will also learn how to identify different types of mosquitoes.

Type of Research and procedures

This research will be about developing a new trap for sampling mosquitoes that cause malaria in Namibia. There will be three different kinds of mosquito traps that will be studied and compared to see which one works best in sampling mosquitoes that transmit malaria. To know if a trap is good in sampling mosquitoes, we will use that trap to collect mosquitoes inside and outside of households from early evening to early morning. If you participate in this research, you will have to use any of these traps during the night time when mosquitoes are flying around and looking for people to bite. Specific instructions and procedures for sampling mosquitoes using each of the tree traps will be given to you before you are enrolled into the study.

- As a participant you will be asked to collect mosquitoes landing on you before they bite you between 18:00 hrs and 06:00hrs. This involves collecting mosquitoes that land on your legs with a tube and a torch and placing these mosquitoes in cups that will be provided to you by the researchers.
- When using the tent trap, you will have to sleep in the tent and operate the trap which includes collecting all trapped mosquitoes every one hour for 12 hours starting from 18:00 hours to 06:00 hours.
- You will have to place a light trap inside and outside a household at 18:00hrs and remove the trap at 06:00hrs
- In the morning following a night catch, you will assist researchers in collecting and identifying trapped mosquitoes including taking a record of captured mosquitoes by filling data in special forms that will be provided
- You will be given instruction on how to use the traps and the discipline required during the study. You will have to adhere to these instructions.
- You will be asked to not smoke cigarettes or drink alcohol for the days or weeks that you are participating.

- You will be asked not to apply some kinds of lotion or soaps that repel mosquitoes, including any commercial or traditional mosquito repellents when participating in this research.
- We will provide you with Malarone medicine to prevent you from getting malaria if a mosquito with malaria bites you. You will need to take this medicine every week and sign a form to show that you have taken the medicine.
- You will need to take malaria test every week for all the period you will be participating in the study and sign a form to show that you have taken the test. The test and medicine will be paid for by the study.
- If you become sick we will provide you with the appropriate medical care and you will not participate in the study during this time. Medical costs will be paid by the study.
- You can leave the study at any time as you wish and you will not be obliged to state why you leave – it is your choice to take part.
- If you choose to participate in the study, you will have to comply with the instructions given by the researcher; otherwise the researcher will exclude you from the study.

Voluntary Participation

Your participation in this study is completely upon your wish. You can choose to participate or not to participate. You can withdraw from the study any time you want. When you leave the study, your payment for participation including any other benefits such as clinical care will cease.

Risks

When you participate in collecting mosquitoes, you may be bitten by mosquitoes. But we will provide you with a medicine every week for entire duration of the study to prevent you from getting malaria infection. If any harm occurs to you as a result of your participation to this study (e.g. snake bite, etc), we will provide you with the required care including taking you to hospital and paying for hospital charges for your treatment. Other risks include side effects due to the prophylaxis for malaria and discomfort from mosquito bites when you are working on a trap. You will take rest when such effects happen and we will seek medical help incase these side effects are high and need to be treated in hospital.

Benefits

If you participate in this study you will be paid…… for your work each night. You will learn how to collect mosquitoes in and outside houses. You will learn how to identify different kinds of mosquitoes and know which ones transmit malaria and those that do not transmit malaria. Upon good performance, you will be considered for recruitment to participate in similar study activities done by the NVDCP at your location every year. Your community will also benefit from the research on malaria which aims to find solution against the disease.

Right to Refuse or Withdraw

This is a reconfirmation that participation is voluntary and includes the right to withdraw. You don't have to participate in this study if you do not wish to do so. You may stop participating in the research at any time you choose and all you rights will be still be respected. Payment for your participation will cease when you withdraw and you will be paid only for the nights you participated.

Who to Contact

You will have contacts of persons in charge of this study to communicate any information or ask questions before, during and after the study. These persons are: Mr. Hans Angula (0811243435), Mr. Iitula Iitula (0812191136) or Dr. Deodatus Maliti (0814124900).

You can ask any more questions about any part of the research study, if you wish to. Do you have any questions? Please do not hesitate to ask.

PART II: Certificate of Consent

I, …… clearly understand the aims of the project entitled "*Evaluation of a tent trap for sampling human biting malaria vectors*", and I agree to participate in the study. During my participation in this study, I understand that mosquitoes can bite me and they may be carrying parasites. I understand that I may revoke my consent and leave the study at any stage.

Participant Name: _____

Participant Signature:

_____Date_____ (dd/mm/yyyy)

Witness Name: _____

Witness Signature: _____Date_____ (dd/mm/yyyy)

If participant is illiterate

A literate witness must sign (if possible, this person should be selected by the participant and should have no connection to the research team). Participants who are illiterate should include their thumb-print as well.

I have witnessed the accurate reading of the consent form to the potential participant, and the individual has had the opportunity to ask questions. I confirm that the individual has given consent to participate in the said study freely.

Name of witness_____

Signature of witness _____

Date _____(dd/mm/yyyy)

Thumb of the participant

_____

Statement by the researcher/person taking consent

I have accurately read out the information sheet to the potential participant, and to the best of my ability made sure that the participant understands that the following will be done:

1. Human landing catch will be conducted between 18:00hrs and 06:00 hrs
2. Tent trap collection will be conducted between 18:00 hrs and 06:00 hrs
3. CDC light trap collection will be conducted between 18:00 and 06:00 hrs
4. Participant has been requested to refrain from smoking, consuming alcohol and applying repellent or such substances on his/her body for the study duration
5. Participant will be provided with free malaria prophylaxis, screening and treatment for the duration of the study

6. Participant will be paid.....for work time when enrolled by the study.

I confirm that the participant was given an opportunity to ask questions about the study, and all the questions asked by the participant have been answered correctly and to the best of my ability. I confirm that the individual has not been coerced into giving consent, and the consent has been given freely and voluntarily.

A copy of this this form has been provided to the participant.

Name of Researcher/person taking consent_____

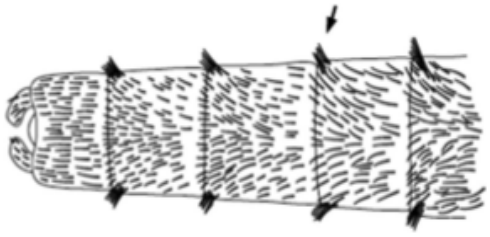
Signature of Researcher /person taking the consent_____

Date _____(dd/mm/yyyy)

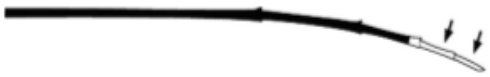
Appendix 5. Morphological identification key

Key to adult females

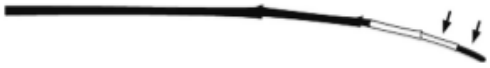
1. Abdominal segments with laterally projecting tufts of scales on segments II–VII ..... Section I
– Abdominal segments not so 2



2. Hindtarsus with at least last 2 hindtarsomeres entirely pale ..... Section II
– Hindtarsus not so 3



3. Hindtarsomere 5 mainly or entirely dark, hindtarsomere 4 white ..... Section III
– Hindtarsus not so 4



4. Legs speckled, sometimes sparsely ..... Section IV
– Legs not speckled 5

8. Maxillary palpus with 4 pale bands ..... Section VIII
– Maxillary palpus with less than 4 pale bands 9

9. Wing with pale interruption in 3rd main dark area (preapical dark spot) of vein 1, sometimes fused with preceding pale area ..... Section IX
– 3rd main dark area without pale interruption 10

10. Wing with 2 pale spots on upper branch of vein 5 ..... Section X
– Wing with I pale spot on upper branch of vein 5 ..... Section XI

Section I. Mosquitoes with laterally projecting tufts of abdominal scales

1. Wing almost entirely dark, costa without pale spots ..... *brumpti*
– Wing with abundant pale areas, costa with at least 4 pale spots 2

Appendix 6. Tabeth Mwema after 12 hours of mosquito collection with mosquitoes stored in different buckets and cups.