BEHAVIOURAL ADAPTATIONS OF MALARIA VECTORS: EVALUATING VECTOR AND HUMAN INTERACTIONS ON MALARIA VECTOR ABUNDANCE AND BITING DYNAMICS POST LLINs INTERVENTION IN ATATAM, SOUTHERN GHANA

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THIS THESIS IS SUBMITTED TO THE UNIVERSITY OF GHANA, LEGON IN PARTIAL

FULFILLMENT OF THE REQUIREMENTS FOR THE AWARD OF MPHIL

IN ENTOMOLOGY DEGREE

AFRICAN REGIONAL POSTGRADUATE PROGRAMME IN INSECT SCIENCE

(*ARPPIS)

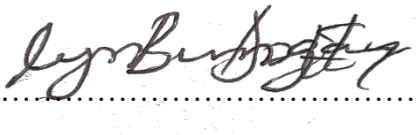
UNIVERSITY OF GHANA, LEGON, GHANA

NOVEMBER, 2024

*A JOINT INTER-SCHOOL INTERNATIONAL PROGRAMME FOR THE TRAINING OF ENTOMOLOGISTS IN WEST AFRICA [COLLABORATING DEPARTMENTS: CROP SCIENCE AND ANIMAL BIOLOGY AND CONSERVATION SCIENCE]

DECLARATION

I hereby certify that this thesis is the outcome of a research undertaken by me, Linus Dottey towards the award of Master of Philosophy in Entomology in the African Regional Postgraduate Program in Insect Science (ARPPIS), University of Ghana, Legon. This thesis has not been submitted either in part or full, for any other degree and all references to work of other people has been duly acknowledged.



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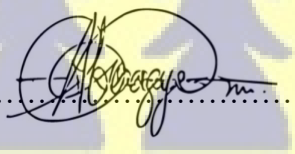

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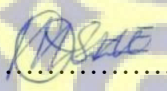

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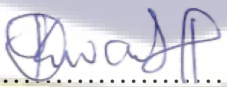

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ABSTRACT

Deployment of malaria control interventions including long-lasting insecticidal nets (LLINs), and indoor residual spraying (IRS) remain the mainstay of current vector control efforts and provide protection against indoor biting and resting mosquitos. This study investigated the abundance, biting activity and human behavioural influence on malaria transmission after the implementation of LLINs in Atatam, a small farming community in the Adansi-Asokwa District of the Ashanti region.

The standard Human Landing Catches (HLC) technique was used to collect 7,109 anophelines, which were used to estimate outdoor and indoor vector abundance and peak biting time activity of malaria vectors. A cross-sectional study surveyed thirty (30) households via questionnaire and interviews to assess human activity and sleeping patterns in the community.

The study found *Anopheles gambiae* s.l 83.9 % (5,963) and *Anopheles funestus* s.l 16.1 % (1,146) to be the predominant vector species biting humans in Atatam. The seasonal abundance of *Anopheles gambiae* s.l. was significantly higher than *Anopheles funestus* s.l. ($p < 0.05$). Similar to *Anopheles funestus* s.l. the abundance of *Anopheles gambiae s.l.* outdoors was higher than their abundance indoors. However, this was not statistically significant ($p > 0.05$). Late night (22 – 04 h) peak biting activity indoor and outdoor which is the historical biting time was exhibited by *Anopheles gambiae* s.l with early morning (04 – 06 h) peak biting activity also observed for *An. funestus* s.l. The survey showed 93.3 % LLINs coverage/ownership and 100 % usage amongst households in Atatam. The shift in biting time from the historical late night to early morning indoor and outdoor biting of *An. funestus* could be due to pressure from LLINs or people spending more time outside unprotected.

The shift in behaviour may lead to residual malaria transmission, necessitating intervention tools targeting vector populations biting individuals, particularly in rural settings, to address the gap in protection.

DEDICATION

I hereby dedicate this research work to God Almighty, my family and my friends.

ACKNOWLEDGEMENT

My sincere gratitude and appreciation first and foremost are directed to God Almighty whose love, mercy, and guidance have made it possible for this work to be successfully completed.

My intense thanks also go to my supervisors Prof. Michael Osae and Dr. Fred Aboagye Antwi for their splendid guidance and encouragement towards the success of this work.

I am highly indebted to Messrs; Gabriel Akosah-Brempong and Ekene Nwaefuna for the technical support they offered and Stanley Okyere you deserve a special recognition and appreciation for the statistical analysis. Much thanks also go to the faculty at African Regional Postgraduate Programme in Insect Science (ARPPIS), University of Ghana for their constructive comments and advice during the study. I am again grateful to Target Malaria at the University of Ghana, the Biotechnology and Nuclear Agriculture Research Institute of the Ghana Atomic Energy Commission; without you I wouldn't have come this far. Lastly, I am thankful to my colleagues for their support especially Samuel Essuaman and Jeffrey Baah. I am most grateful to all of you.

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LIST OF ABBREVIATION

NMEP……National Malaria Elimination Program

LLINs……Long-lasting insecticidal nets

IRS……Indoor residual spraying

NMCP.....National Malaria Control Program

WHO……World Health Organization

DNA……Deoxyribonucleic Acid

rDNA……ribosomal DNA

PMI……President's Malaria Initiative

ITN……Insecticide Treated Net

IRAC……Insecticide Resistance Action Committee

GSTs……Glutathione S-transferases

DDT……Dichlorodiphenyltrichloroethane

AChE……Acetylcholinesterase

KDR……Knockdown Resistance

VGSCs……Voltage-gated sodium channels

CDC-LT……Centre for Disease Control Light Trap

HLC……Human landing catches

RAMSRI……..Radiological and Medical Sciences Research Institute

HBR……Human Biting Rates

s.l……sensu lacto

s.s……sensu stricto

Pf……*Plasmodium falciparum*

Po……*Plasmodium ovale*

Pm……*Plasmodium malarie*

CHAPTER ONE

1.0 INTRODUCTION

Malaria continues to be a major public health problem globally especially in Sub-Saharan Africa. An estimated 249 million malaria cases were recorded globally in 2022, with the African region accounting for about 94% of all cases (World Malaria Report, 2023). According to the annual malaria report, malaria accounts for approximately 20% of all outpatient visits and 22% of all hospital admissions in Ghana (NMEP, 2022). Moreso in Ghana, according to the National Malaria Elimination Program (NMEP) in 2022, 5.2 million confirmed cases of malaria were recorded with 151 deaths, a decline from the previous 2,799 deaths in 2012 (Ministry of Health, 2023). Malaria is still a public health and socioeconomic challenge in spite of these gains.

Primarily, malaria in humans is known to be caused mostly by five species of *Plasmodium: P. vivax*, *P. malaraie*, *P. ovale*, *P. falciparum* and *P. knowlesi*. *Plasmodium falciparum* however is the predominant and most virulent malaria parasite accounting for morbidity and mortality in the African sub region. The two major vectors of malaria in sub-Saharan Africa (*Anopheles gambiae* and *Anopheles funestus* complex) all bite mainly indoors at night (Killeen, 2006). Sleeping under a treated net during this period can thus significantly lower the risk of contracting malaria.

Insecticide-based tools are the primary means of controlling diseases transmitted by mosquitoes. The widespread implementation of malaria control interventions like indoor residual spraying and long-lasting insecticidal nets, which provide protection against indoor biting and resting mosquitoes, has been responsible for the remarkable improvements in malaria control over the past ten years (Okumu *et al.,* 2012). Malaria control strategies typically

target either parasites or vectors. Malaria therapy uses medications to target parasites, while vector control uses LLINs and IRS (Bhatt *et al*., 2015).

There are reports indicating these anti-vector intervention strategies leading to a significant decrease in vector populations and malaria transmission (Aikpon *et al.,* 2014 & Coleman *et al.,* 2017). In Ghana, antimalarial medicines, indoor residual spraying of insecticide, and the distribution of long-lasting insecticidal nets (LLINs) have all been used in the fight against malaria. Over the years, Ghana has achieved great strides in the control of malaria.

Malaria incidence has decreased from 27.5% in 2011 to 8.6% in 2022, and confirmed cases per 1000 people have decreased from 192 in 2019 to 159 in 2020 (NMEP, 2023). Despite progress, malaria still remains a significant public health issue in Ghana (NMEP, 2023).

However, it is currently yet to be determined whether interventions that are deployed against malaria vectors could increase residual transmission intensity through changes in behaviour or how changes in behaviour may impact prevalence of malaria transmission (Carnevale & Manguin, 2021). Temporal avoidance has been observed in *Anopheles funestus* in Tanzania and *Anopheles funestus* and *Anopheles arabiensis* in Kenya, as evidenced by a behavioural shift from nighttime to evening aggression (Ototo, 2015). In Benin, peak biting activity in *An. funestus* was shifted to the early morning (Moiroux *et al.,* 2012a). In regions of Kenya with high bed net coverage, trophic avoidance was observed in *An. funestus* and in *An. gambiae* s.s., along with a shift towards increased zoophilic behavioural patterns (Ndenga *et al.*, 2016). Also, human activities and patterns of sleep may also play a significant role in malaria transmission, as getting exposed to malaria vector bites occurs when unprotected individuals and vector biting activity overlap in time and space (Edwards *et al*., 2019; Finda *et al*., 2019; Monroe *et al*., 2019). Indoor interventions are dependent on vector human biting behaviour during the night. *Anopheles gambiae* and *Anopheles funestus* complexes, the main malaria vectors,

exhibits behavioural plasticity with some species such as *Anopheles arabiensis* exhibiting more zoophagy and exophagy. But since the scale up of prevention and control tools in sub-Saharan Africa, there is mounting proof that malaria vectors are changing their biting patterns to times and in locations where individuals are not protected (Briët & Chitnis 2013).

Nevertheless, human activities and sleeping patterns in various eco-epidemiological contexts have received little to no consideration in these investigations, with majority of them concentrating on vector behaviour. The efficacy of LLINs becomes ineffective in the evenings when people are outdoor or early morning when they are out of bed (Monroe *et al.,* 2020). Due to the overlap between unprotected individuals and vector biting activity, outside activities such as farming, selling, watching television, and working in security also raise the risk of malaria transmission (Doucoure *et al*., 2020 & Monroe *et al.,* 2020).

Understanding geographic variations in vector biting behaviour and determining the times and locations of human exposure to vectors are essential for achieving elimination. This information is essential for assessing the likelihood that the existing indoor mosquito control methods will be successful and for creating effective interventions that take the local eco-epidemiological setting into account.

1.1 Rationale

Despite progress made towards malaria vector control through IRS and LLINs, mosquitos are reported to have developed resistance to the insecticides used in these interventions which undermines the continuous effectiveness of these tools for malaria control (Hemingway *et al*., 2016, WHO, 2023). Moreso, WHO in 2023 reports that, global malaria case incidence was 58 per 1000 population at risk, and with 608,000 estimated malaria deaths.

Anopheles funestus has been reported to be among the main vectors transmitting malaria in Atatam (Riveron *et al*., 2016 & Mugenzi *et al.,* 2022). The populations of *An. gambiae* and *An.*

funestus alternate with *An. gambiae* being predominant in the rainy season and *An. funestus* dominating during dry season (Riveron *et al*., 2016 & Mugenzi *et al.,* 2022). However, preliminary data on the deployment of LLINs in 'Atatam' has shown a remarkable reduction in populations of *An. funestus* and change in species composition to predominantly *An. gambaie* populations over a period (Akosah-Brempong *et al*., unpublished data).

Furthermore, preliminary asymptomatic malaria screening in the community indicates that malaria positivity remains high (48.6%) post intervention (Akosah-Brempong *et al*., unpublished). Malaria vector interventions may lead to suppression of vector populations, cause changes in vector species composition and behaviour making the post-intervention vector community less likely to encounter insecticide. Behavioural modifications could include a change in feeding time, shifting from human to feed on animals, or an increase in exophilic behaviour (Thomsen *et al*., 2017).

In 2021, the Ghana National Malaria Elimination Program vector control efforts distributed LLINs including synegist-based nets and insecticides combination nests nationwide that are effective against pyrethriod resistant vector populations. The selection pressure brought on by LLINs may have led to behavioural modifications, giving early-biting and/or outdoor-biting vectors a selective advantage, causing a behavioural shift in the population. These behavioural modifications may lead to residual malaria transmission which might be observed in the vector population.

In view of the increasing prevalence of malaria in the Atatam community (Akosah-Brempong *et al*., unpublished data) which is becoming more concerning, there is a need to enhance control intervention strategies through a deeper understanding of vector resting and feeding behaviour as this is essential to the success of existing vector control tools. This could also provide a guide for improved efforts for the control of malaria in endemic regions.

To better understand how current malaria control interventions affect malaria vector populations, this study aims to investigate the feeding/resting patterns as well as species composition of malaria vectors in the Atatam community.

1.2 Main Objective

To characterize the species composition, human interaction and behaviour of major malaria vectors in a malaria endemic community, Atatam in Southern Ghana.

1.3 Specific Objectives:

1. To estimate the comparative abundance/density of outdoor and indoor malaria vectors in Atatam, Southern Ghana.
2. To investigate the dynamics of outdoor and indoor biting pattern for malaria vectors in Atatam, Southern Ghana.
3. To investigate the coverage and use of LLINs and human behaviour influence on malaria transmission in Atatam.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 The Burden of Malaria in Africa

One of the biggest public health issues in the world, particularly in Sub-Saharan Africa, is malaria. Despite decades of efforts, it continues to exact a heavy toll on health, economic development, and well-being, particularly in regions with limited healthcare infrastructure (Okumu *et al*., 2022; WHO 2023). The WHO reports an estimated 249 million cases in 2022 compared to 244 million in 2021, 244 million in 2020 and 233 million in the pre-pandemic year of 2019. Children under the age of 5 are particularly vulnerable, representing around 80% of malaria deaths in high-burden areas, especially in Africa. Pregnant women are also at heightened risk of severe malaria, which can lead to maternal and infant mortality, as well as complications like low birth weight (World Malaria Report 2023).

The socioeconomic impacts of malaria are profound and multi-dimensional, affecting productivity, education, healthcare costs, and long-term development prospects (Okorosobo, 2011). According to WHO in 2023, with international spending per capita of US$ 2.00 and domestic spending per capita of US$ 1.10, which has more than doubled from US$ 0.50 in 2010, the WHO African Region had the most financing per person at risk in 2022. Patients with malaria and their households may pay for necessary expenses out of pocket, which are covered by loans, savings, and income. This covers payment made informally or formally at the time of using any health service, provided by any kind of provider, and in any sort of environment.

Absenteeism from work may result from malaria, especially in the informal and agricultural sectors where many African workers are engaged. Adults with frequent malaria episodes are less able to work, which affects household income and productivity (Sarma *et al*., 2019).

Post-malaria weakness and weariness can cause weeks of decreased labour production, even after individuals recover. Since malaria transmission frequently occurs during planting or harvesting seasons, impacting food production and local economies, the agriculture sector is disproportionately affected (McDermott and Grace, 2012). Even though malaria transmission and mortality have significantly decreased, the disease still poses a serious threat to sustainable development and the alleviation of poverty.

2.1.1 Malaria Situation in Ghana

Malaria is endemic throughout Ghana, meaning the disease is continuously present across all regions. Transmission occurs year-round, with a more intense transmission during the rainy season (Quartey, 2016). In the rain forest zone, malaria transmission is severe and persistent with only minor changes; however, the peak of transmission is soon after the main rainy season. Due to year-round favourable conditions for disease transmission, malaria is stable and endemicity is high in the forest zone (Obacha, 2016).

In 2022, the nation reported 151 malaria-related deaths and over 5.2 million confirmed cases of malaria. The in-patient malaria mortality rate during that period was 0.48 per 100,000 people, with 0.05 per 100,000 people in children under the age of five (Ministry of Health, 2023). While malaria cases have declined due to concerted efforts, the disease remains one of the leading causes of morbidity and mortality (World Malaria Report 2023).

2.1.2 Major Malaria Vectors in Africa

The *Anopheles gambiae* complex consists of multiple closely related species, some of which are major vectors of malaria, while others play a lesser or no role in transmission. These species are morphologically indistinguishable but differ genetically, behaviourally, and ecologically (Coetzee *et al.,* 2000 and Coluzzi *et al*., 2002). Members of this complex consists of *Anopheles gambiae sensu stricto* s.s, *Anopheles arabiensis*, *Anopheles coluzzii*, *Anopheles*

quadriannulatus, *Anopheles melas*, *Anopheles merus*, and *Anopheles bwambae.* The *Anopheles funestus* complex consists of several species, with *Anopheles funestus* s.s. being one of the most important malaria vectors in Africa, second only to the *Anopheles gambiae* complex in importance. Members of this complex are made of *Anopheles funestus* s.s, *Anopheles vaneedeni*, *Anopheles rivulorum*, *Anopheles parensis*, *Anopheles leesoni*, *Anopheles fuscivenosus,* and *Anopheles aruni* (Coetzee and Fontenille, 2004). *An. gambiae s.s., An. arabiensis*, of the *An. gambiae* complex and *An. funestus* s.s. of the *An. funestus* subgroup are the primary mosquito species linked to malaria transmission in Africa; they are all widely spread throughout tropical and subtropical regions of the continent. The two most effective malaria vectors in Africa are *An. gambiae s.s.* and *An. funestus s.s.* (Sinka *et al*., 2010, Sinka, 2013) due to their strong anthropophily, which means they feed on human blood (Dabire *et al*., 2008, Seyoum *et al*., 2012, Dadzie *et al*., 2013).

According to Sinka *et al*., (2010), *An. arabiensis* is classified as zoophilic, exophagic, and exophilic; nevertheless, its feeding and biting habits vary according to geographic region. Research however shows that, *An. gambiae s.s*. has two molecular forms, M and S, which can be recognized as *An. coluzzii* and *An. gambiae* respectively (Caputo *et al*., 2021). *Anopheles gambiae* s.s. identifies as one of the most effective malaria vectors in the world (Coetzee, 2005). It has the widest global distribution of the species and is a freshwater species. Its diverse ecology, large range, and a host of other characteristics, including lifespan, anthropophily, and a short larval development phase, enable it keep contributing as one of Sub-Saharan Africa's most effective malaria vectors (Killeen, 2006). Large permanent or semi-permanent freshwater bodies with emergent vegetation around their edges, such as ponds, swamps, weedy and grassy areas of rivers, streams, furrows, and ditches, are characteristic habitats for *Anopheles funestus* larvae. The presence of vegetation is important for their breeding because their aquatic stages have a strong preference for shaded habitats and use

emergent vegetation as a place to hide from predators; they can hardly survive in water bodies that are directly exposed to sunlight (Dia, 2013).

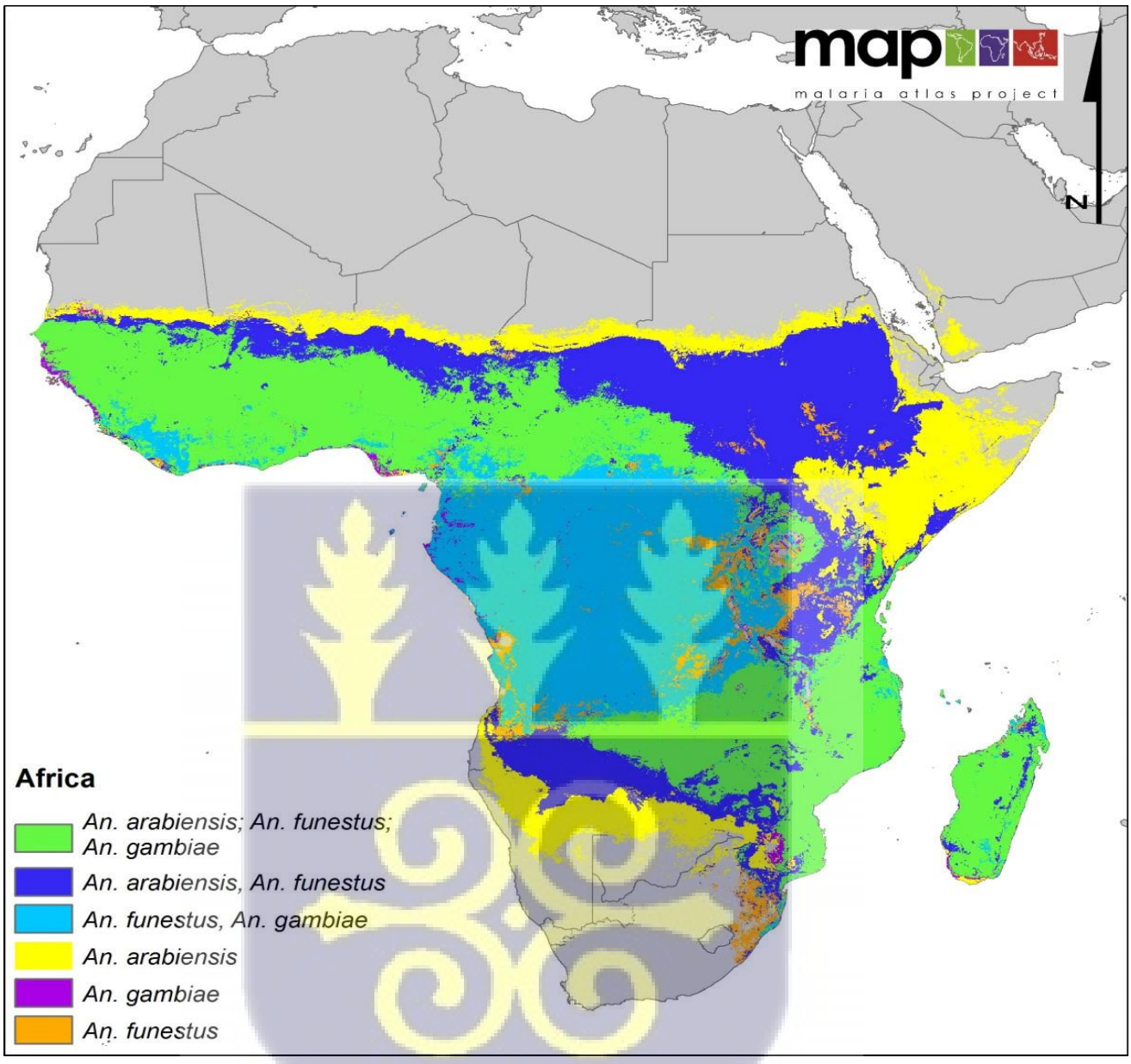

Figure 1; Map showing geographical distribution of malaria vectors in Africa (Sinka *et al*., 2012)

2.1.3 Distribution of Malaria Vectors in Ghana, their Feeding Preference and Resting Behaviours.

In Ghana, studies have shown that *Anopheles gambiae* s.l. and *Anopheles funestus* are the predominant malaria vectors and they coexist in sympatry over much of their range (Appawu *et al*., 2004 & Sinka *et al*., 2010). However, *Anopheles gambiae sensu stricto* (s.s.) of the *Anopheles gambiae* complex is the primary malaria vector and is found across the country since they account for about 95% of all Anopheles mosquitoes collected (Ministry of Health, 2022).

Due to its more versatile blood meal hosts, *Anopheles arabiensis* is thought to be a less effective vector than *Anopheles gambiae* s.s. and *Anopheles funestus* (Takken and Verhulst 2013). However, it continues to be the main vector of malaria in many parts of the continent, even since LLINs were introduced (Sinka, 2013). Although there have been reports of minor exophily (Mahande *et al*., 2007, PMI, 2016 & 2017), *Anopheles gambiae* s.s. is primarily endophilic (PMI, 2016 &2017).

Likewise, *An. funestus* is generally endophilic (Coetzee & Fontenille, 2004, PMI, 2016 & 2017) although exophily has been reported (Ndenga *et al.*, 2016, PMI, 2016- 2017). Also, An. melas as a vector is mainly anthropophilic, even in the presence of range of hosts. This species breed in brackish water even though they have tolerance for high salinity. They are revealed to be highly dominant in the western coast of the country (Tuno *et al*., 2010).

Anopheles stephensi, a species of mosquito traditionally found in parts of South Asia and the Middle East, has recently been reported in several parts of Africa and in Ghana recently (Mnzava *et al*., 2022, WHO 2023), raising concerns due to its potential to increase malaria transmission. *Anopheles stephensi* is a competent vector for malaria, capable of transmitting *Plasmodium falciparum* and *Plasmodium vivax*. Unlike other species, it thrives in both urban

and rural settings, making it especially dangerous in densely populated cities (Taylor *et al*., 2024).

2.2 Malaria Parasites

Human malaria is caused by *Plasmodium falciparum*, *Plasmodium ovale*, *Plasmodium vivax*, *Plasmodium malariae*, and *Plasmodium knowlesi* (Cox-Singh *et al*., 2008), which are spread by female *Anopheles* mosquito bites (Lee *et al*., 2009, Krief *et al*., 2010). The most prevalent and virulent species, especially in Sub-Saharan Africa, is *Plasmodium falciparum*, causing serious clinical problems (WHO, 2018). Southeast Asia has high prevalence of *Plasmodium vivax* and *Plasmodium knowlesi* (Cox-Singh *et al*., 2008, Lee *et al*., 2009) and the Western Pacific and the Asian mainland are also home to *Plasmodium ovale*, however Africa is where it is most prevalent.

Plasmodium malariae and *Plasmodium falciparum* are both found in the same geographic area; however, their prevalence varies (Collins & Jeffery 2005). *Plasmodium falciparum* (Pf) is the most common malaria parasite in Ghana, resulting in significant morbidity and death. In Ghana, *Plasmodium falciparum* mono-infection is the most common malaria infection type across all areas, with rates ranging from 95.8% (95% CI: 94.6 - 96.8) in the Ashanti region to 100% in the Western and Western North regions, with a countrywide prevalence of 98.2% (95% CI: 97.9 - 98.5) (Ministry of Health, 2022).

Mono-infection with *Plasmodium ovale* (Po) remained the second most common infection type (1.0%; 95% CI: 0.8-1.3). The national prevalence of *Plasmodium malariae* (Pm) is 0.4%, with 0.0% in the Eastern, Volta, Oti, Western, Western North, Northeast, and Savannah regions and 1.5% in the Ahafo Region. The prevalence of mixed Pf+Pm and Pf+Po infections is 0.1% and 0.3%, respectively. *Plasmodium vivax* has not been reported from health facilities or identified in any part of the country (Ministry of Health, 2022).

2.3 Vector Control and its Challenges

According to WHO, malaria has increased in some Sub-Saharan African countries, despite a significant decline in infections and clinical cases between 2000 and 2015. This can be attributed to the use of LLINs and IRS in areas with moderate to high transmission (Bhatt *et al*., 2015; WHO, 2022). However, LLINs and IRS are made to target indoor-feeding and indoor-resting malaria vectors, but some vectors also feed and rest outdoors, which reduces their effectiveness.

In locations with high usage of ITNs or indoor residual spraying (IRS), foraging may occur earlier in the evening or later in the morning, when human hosts are not protected by bednets or other indoor treatments (Ferreira *et al*., 2017). Mosquitoes may adapt to the usage of ITNs and IRS by increasing their outdoor host-seeking and zoophagy. Observations in the field after ITN and IRS implementation support the existence of behavioural alterations (Ndenga *et al.*, 2016).

Furthermore, insecticide exposure can cause indoor biting vectors to modify their behaviour by exiting homes early (Ranson *et al*., 2011, Strode *et al*., 2014, Hemingway *et al*., 2016), which could affect the efficiency of IRS and LLINs leading to a rise in malaria cases (Maxmen, 2012). Larval source management, a combination of methods aimed at preventing the development of larval mosquitoes into adults (Imbahale *et al*., 2012, Tusting *et al*., 2013), and biological control employing predatory fish like *Gambusia affinis* and *Tilapia nilotica* are additional vector control strategies (Howard *et al*., 2007) to reduce the number of malaria mosquitoes by feeding on their larvae and by using larviciding to decrease the number of malaria mosquito larvae (Fillinger and Lindsay 2006, Bukhari and Knols 2009, Mbare *et al*., 2014).

2.4 Mosquito Insecticide Resistance

Insecticide resistance in African malaria vectors presents a serious and growing challenge to malaria control efforts across sub-Saharan Africa, where vector control primarily depends on ITNs and IRS (https://allianceformalariaprevention.com/working-groups/net-mapping/). The effectiveness of these interventions is being progressively undermined as mosquito populations develop multiple resistance mechanisms, including target-site resistance through genetic mutations like *kdr* that prevent insecticide binding, metabolic resistance via enhanced detoxification enzymes such as P450s, cuticular resistance through thickened exoskeletons that reduce insecticide penetration, and behavioural resistance where mosquitoes alter their biting patterns to avoid coming into contact with treated surfaces (Riveron *et al*., 2018).

The major malaria vectors including *An. gambiae* s.s., *An. coluzzii*, *An. arabiensis*, and *An. funestus* s.s. now show widespread pyrethroid resistance across West, Central, East and Southern Africa, posing particular concern since pyrethroids are the primary insecticides used in long-lasting insecticidal nets (Sinka *et al*., 2010; Ranson *et al*., 2016 & https://www.intechopen.com/chapters/irmapper.com, 2017). Resistance patterns vary geographically, with DDT resistance common in West, Central and East Africa but rare in Southern Africa except for isolated cases in southern Malawi and among *An. arabiensis* populations in Madagascar, Mozambique and South Africa, while carbamate resistance predominates in West and Southern Africa and organophosphate resistance remains limited to certain areas of West and East Africa, though notably *An. funestus* remains fully susceptible to organophosphates continent-wide (Kawada *et al*., 2011; Bobanga *et al*., 2013 & Riveron *et al*., 2016).

The situation in Ghana exemplifies this growing crisis, where *An. funestus* s.s. populations demonstrated resistance to DDT, permethrin and bendiocarb by 2005, with mortality rates for

pyrethroids, carbamates and DDT dropping below 50% by 2014, while *An. gambiae* s.l. populations show resistance to all four major insecticide classes, particularly high-intensity resistance to pyrethroids and carbamates (Riveron *et al*., 2016). This resistance primarily stems from metabolic mechanisms involving upregulated enzymes like GSTe2, CYP6P9a, CYP6P9b and CYP6M7, without detectable target-site mutations, rendering current synergists like piperonyl butoxide ineffective against non-P450 mediated resistance (Riveron *et al*., 2016).

This escalating resistance threatens to reverse malaria control achievements and urgently requires innovative solutions including new insecticides, alternative vector control approaches, enhanced resistance management strategies, and continued surveillance to monitor resistance evolution across different regions and vector species. The development of non-chemical intervention methods and regionally tailored resistance mitigation strategies has become critical to sustain progress toward malaria elimination in Africa (Hemingway *et al*., 2016).

2.4.0 Mosquito Insecticide Resistance Mechanisms

According to the Insecticide Resistance Action Committee (IRAC), 2007: "Insecticide resistance is a heritable attribute in an insect population that results in a consistent failure of an insecticide product to give the level of control when utilized as recommended with all negative factors removed." Stated differently, it refers to a decrease in an insect population's vulnerability to an insecticide.

Data on resistance to insecticides have been reported to the WHO by 35 countries since 2021. Eighty-eight of the malaria endemic countries that submitted data for the years 2010 to 2020 had at least one collection site and one malaria vector that tested positive for at least one insecticide class; 29 had already shown evidence of resistance to pyrethroids, carbamates,

organochlorines, and organophosphates in multiple sites; and 19 had verified resistance to all four classes of insecticide in at least one site and one local vector (World Malaria Report 2023). Target site insensitivity, Cuticular Resistance (physiological), metabolic, and behavioural resistance are the four main categories of resistance mechanisms that have been described (Riveron *et al*., 2018).

2.4.1 Metabolic Resistance

Through enhanced activity of detoxifying enzymes, mosquitoes acquire the ability to more effectively breakdown or detoxify insecticides. Considering that it can impact several kinds of insecticides, this is one of the most prevalent and problematic types of resistance (IRAC, 2019). Three main metabolic detoxification gene families cytochrome P450s (P450s), esterases, and glutathione S-transferases (GSTs) are involved in the detoxification of insecticides in mosquitoes (Malik, 2016).

2.4.2 Target-Site Mutation

This occurs when mutations alter the binding sites of insecticides in the mosquito, reducing the efficacy of the chemicals. This occurs when mutations alter the binding sites of insecticides in the mosquito, reducing the efficacy of the chemicals (Casida *et al*., 2013). Insecticides such as dichlorodiphenyltrichloroethane (DDT) and pyrethroids target sodium channels. Knockdown Resistance (kdr), results from mutations in the voltage-gated sodium channels (VGSCs), where mosquitoes can survive normally lethal doses (Yawson *et al*., 2004). After binding to the sodium channels, they cause the insect's nervous system to repetitively discharge and its nerve membranes to depolarize (Yawson *et al*., 2004), resulting in death. Acetylcholinesterase (AChE) is a key enzyme in the nervous system, hydrolyzing acetylcholine neurotransmitters and terminating nerve impulses, and is the target for both OP and carbamate insecticides.

2.4.3 Cuticular Resistance

This involves modifications to the cuticle, or exterior layer, of the mosquito, which may thicken or become less porous, so decreasing the insecticide's ability to penetrate. Insecticide absorption is hindered by the thicker or more resilient cuticles that mosquitoes grow. Because of this mechanism, mosquitoes can withstand insecticide exposure for extended periods of time (Wood *et al*., 2010).

2.4.4 Behavioural Resistance

Through behavioural modifications, mosquitos reduce their exposure to insecticides, thereby lowering mortality rates. Mosquitoes may develop a tendency to avoid treated surfaces (e.g., walls sprayed with insecticides) or change their feeding patterns (Machani *et al*., 2022). For instance, mosquitoes might bite outdoors rather than indoors (exophily) or bite during periods when bed nets are not available to protect people (shifts in biting times). Also, mosquitoes may shift their resting behaviour to untreated surfaces, such as outdoors instead of indoors after feeding, to avoid contact with residual insecticides on walls or treated bed nets (Gatton *et al*., 2013).

2.5 Human Behavioural Influence on Biting Patterns of Malaria Vectors

The host-vector relationship, disease transmission dynamics, and developing malaria control methods requires knowledge of malaria mosquito blood-feeding habits, resting behaviour, and human activities or behaviour (Chaves *et al*., 2010). Historically, the primary malaria vectors *Anopheles gambiae* and *Anopheles funestus* have fed entirely indoors, with late-night peak biting activity (Mukisa *et al*., 2024). This behaviour coincides with the time most people are indoors and asleep.

However, following the upscaling of control tools in sub-Saharan Africa in recent times, there is growing evidence of malaria vectors shifting their biting behaviours toward times and places

where people are not protected (Cooke *et al*., 2015; Wamae & Githeko 2015; Meyers *et al*., 2016). The shift from classical late-night biting to either late morning biting (indoor and outdoor) and or early evening outdoor biting, together with high outdoor malaria transmission, could be due to pressure from the LLINs or humans spending more time unprotected outdoors (Nzioki *et al*., 2023).

For example, in Ghana, outdoor sleeping during the dry season is also a major factor contributing to peak outdoor biting in the late evenings. People sleep outside during the early nighttime because of high temperatures in the rooms and wait till about 02:00 h (Nalinya *et al*., 2022) when their rooms are cool enough to sleep indoors. During the dry season in the Sahel savannah areas, some people spend the entire night sleeping outdoors because their rooms become extremely warm (Akuoko *et al*., 2024).

2.6 Impact of LLINs Vector Control Implementation on Mosquito biting Behaviour

Long lasting are effective at reducing nighttime biting, which is the primary feeding time for many malaria vectors. According to Huho *et al*., 2013 and Bayoh *et al*., 2014, malaria vectors have been found to bite between midnight and late at night which is known as "historic biting times.". Both indoors and outdoors, *An. gambiae* s.s. biting activity in Tanzania followed the historical pattern. That of *An. arabiensis*, however, peaked indoors at 20:00 and outdoors at 22:00, respectively, when many individuals would still be active and not protected by LLINs. Furthermore, both species' outdoor biting activity was higher than their indoor biting activity.

(Geissbühler *et al*., 2007).

Additionally, in Ethiopia, *An. arabiensis's* early evening biting behaviour was consistent before (Yohannes *et al*., 2005) and after the implementation of LLINs (Yohannes & Boelee 2012). Also, *Anopheles funestus* and *Anopheles arabiensis* were more inclined to bite outdoors than

indoors with the maximum peaks occurring from 20:00 h to 22:00 h for both species (Kenea *et al.,* 2016). Research conducted between 1994 and 1996 in Mozambique, where the use of LLINs had just started, found that *An. funestus* was twice as likely to bite indoors as outdoors, whereas *An. arabiensis* was 1.5 times inclined to bite outdoors than indoors (Mendis *et al*., 2000).

In Zimbabwe, using CDC-LT, 69% of mosquitos were caught indoors and 31% outdoors. *Anopheles funestus* biting activity was nocturnal, with peak times ranging from 22:00pm to 23:00pm and 02:00am to 04:00am (Sande *et al*., 2016). Additionally, using rotator traps in western Kenya, the peak times for *An. gambiae* and *An. funestus* biting indoors and outdoors were from 18:00 to 22:00 and 04:00 to 6:00 (Ototo *et al*., 2015).

However, when bed nets were implemented in other regions, variations in biting behaviour were reported. For instance, Russell *et al*., (2011) reported a decline in the nocturnal activity of *An. gambiae* s.l after the distribution of LLINs and increased outdoor biting during the early evening hours in Solomon Islands. Additionally, In Equatorial Guinea, *An. gambiae* s.s. bites primarily during the early evening hours outdoors (Reddy *et al*., 2011, Overgaard *et al*., 2012). Furthermore, the peak of *An. funestus* biting in Senegal was observed in the early morning hours (from 7:00 to 11:00 h) (Sougoufara *et al*., 2014), whereas in Benin it shifted from 02:00 h to the early morning hours 05:00 h (Moiroux *et al*., 2012b).

Numerous research on African Anophelines' biting behaviour of have shown that changes in biting behaviour are widespread, regardless of location (indoors/outdoors) or time, even before the adoption of LLINs. In some regions, the implementation of LLINs had little effect on biting behaviour, whilst in others, mosquitos changed their biting hours to the early evening or late morning. Nevertheless, some research evaluated the biting behaviour of malaria vectors before and after the introduction of LLINs, with inconsistent findings (Mburu *et al*., 2019).

2.7 Estimating the Biting Behaviour of Malaria Vectors using Human Landing Catches.

Evaluating the biting behaviour of malaria vectors, indoors and outdoors, is crucial for understanding the dynamics of malaria transmission and designing effective vector control strategies. The human landing catches (HLC) has been one of the primary tools for evaluating malaria vector biting behaviour. Mosquitos are collected from human volunteers when they land on the skin to take a blood meal. The technique estimates vectors' peak biting hours, either indoor or outdoor. These biting preferences can also be used to determine the number of infectious bites that a single individual can receive in a given amount of time.

Many research have employed the HLC approach to analyse the biting behaviour of malaria vectors, both indoors and outdoors (Seyoum *et al*., 2012, Kabbale *et al*., 2013, Bayoh *et al*., 2014, Meyers *et al*., 2016).

A disadvantage of the HLC is that it is laborious and requires collectors to stay vigilant during the sample time. Standardizing HLC across sample places is challenging due to individual variability in mosquito attraction and collection abilities. The HLC raises ethical problems as it increases the danger of malaria among human collectors. However, administering malaria prophylaxis minimises this risk (Gimnig *et al*., 2013).

2.8 Assessing the Human Biting Patterns of Malaria Vectors

Estimating human exposure to mosquito bites can be done most directly using the human landing catch (HLC) (Kline 2006, Govella *et al*., 2010) since mosquitos are captured when they land on humans to take a blood meal. Different mosquito species and geographical locations have different biting patterns and preferences for indoor or outdoor bites. Additionally, these behaviours could change over time in reaction to vector control strategies like IRS and LLINs (Reddy *et al*., 2011, Russell *et al*., 2011, Moiroux *et al*., 2012a). As a result, evaluating the

biting behaviour of malaria vectors and the areas where most biting occurs is essential since information on these characteristics can reveal the sites and times when alternative interventions might be beneficial for vector control.

Additionally, entomological surveillance is crucial to public health. While entomological surveillance technologies are successful, they may only target specific parts of a mosquito population, such as females seeking host or resting mosquitos. To decide if a sample approach is suitable for answering a given issue concerning malaria vector behaviour, it's crucial to understand its strengths and disadvantages. (Mboera, 2005).

2.9 Factors Influencing Abundance and Species Composition of Major Malaria Vectors Distribution.

Geographical differences exist in malaria transmission. The distribution of the major malaria vectors could be the cause of the variation (Herrel *et al*., 2004). The primary characteristics of the various vector species include land use, seasonality, and geography (Afrane *et al*., 2012, Moiroux *et al*., 2012b, Bashar and Tuno 2014).

For instance, although the two species are sympatric in many areas, *An. gambiae* and *An. arabiensis* can be distinguished at relatively large scales by their degrees of aridity and their capacity to endure altitudes, temperatures, and humidity levels (Coetzee *et al.,* 2000). Temperature and rainfall patterns play a significant role in determining the abundance of mosquitoes. High temperatures accelerate mosquito development, while rainfall provides breeding sites. This supports *An. gambiae* and *An. funestus* abundance (Minakawa *et al*., 2002, Siraj *et al*., 2014).

Urban areas with poor sanitation and drainage systems may provide ample breeding sites for mosquitoes. High incidences of malaria have been associated at comparatively finer scales (e.g., within a village), to housing characteristics including exposed eaves, grass-thatched roofs, nearby irrigated area, and tethering livestock inside buildings, all of which presumably increase the number of malaria vectors (Yé *et al*., 2006, Mutuku *et al*., 2011, Temu *et al*., 2012, Animut *et al*., 2013).

2.9.1 Impact of Housing Structure on Malaria Transmission

The structure of houses in malaria endemic regions significantly impacts transmission dynamics such that areas with proper housing have low transmission compared to poor hosing areas.

Poorly constructed houses with gaps and openings provide easy access for malaria mosquitoes to effect transmission (Lindsay *et al*., 2003, Mutuku *et al*., 2011, Wanzirah *et al*., 2015).

Most people who contract malaria do so indoors, when they are bitten by malaria mosquito vectors that have entered the house through open eaves, which are the mosquitos' favourite entry locations (Njie *et al*., 2009).

Mud walls and thatch roofs provide malaria mosquitoes more places to enter and better places to rest (Ghebreyesus *et al*., 2000, Kirby *et al*., 2008). Several researches have evaluated the effectiveness of mosquito-proof housing materials, including nets, ceilings (Atieli *et al*., 2009, Ogoma *et al*., 2009, Kampango *et al*., 2013) and sand, rubble and cement (Kirby *et al*., 2008). House improvements led to a considerable reduction in malaria risk due to fewer mosquitos coming indoors and resulting in fewer bites.

CHAPTER THREE

3.0 MATERIALS AND METHOD

3.1 Study Site

The study was conducted in Atatam, a small community in the Adansi Asokwa District of the Ashanti Region. The community lies within (06° 17.377″ N, 001° 27.545″ W) located in the forest ecological zone of Ghana. (Fig 2). The area experiences an average temperature of 27^0C and a yearly average rainfall ranging between 1250 and 1705 mm (Asare-Nuamah and Botchway 2019). The community is drained by three small streams and small sections of swampy land, which are favourable for breeding the primary malaria vectors *An. funestus* and *An. gambiae* s.l. The introduction of illegal mining activities, 'Galamsey' in the community has also created pockets of breeding sites favourable for the breeding of some of these malaria vectors.

Housing structures in the community is almost uniform, consisting of mud walls with either iron sheet or thatched roofs, and open eaves that allow easy entry to blood seeking malaria mosquitos. The community locals are mainly engaged in cocoa and subsistence farming and most recently the youth in illegal mining of gold.

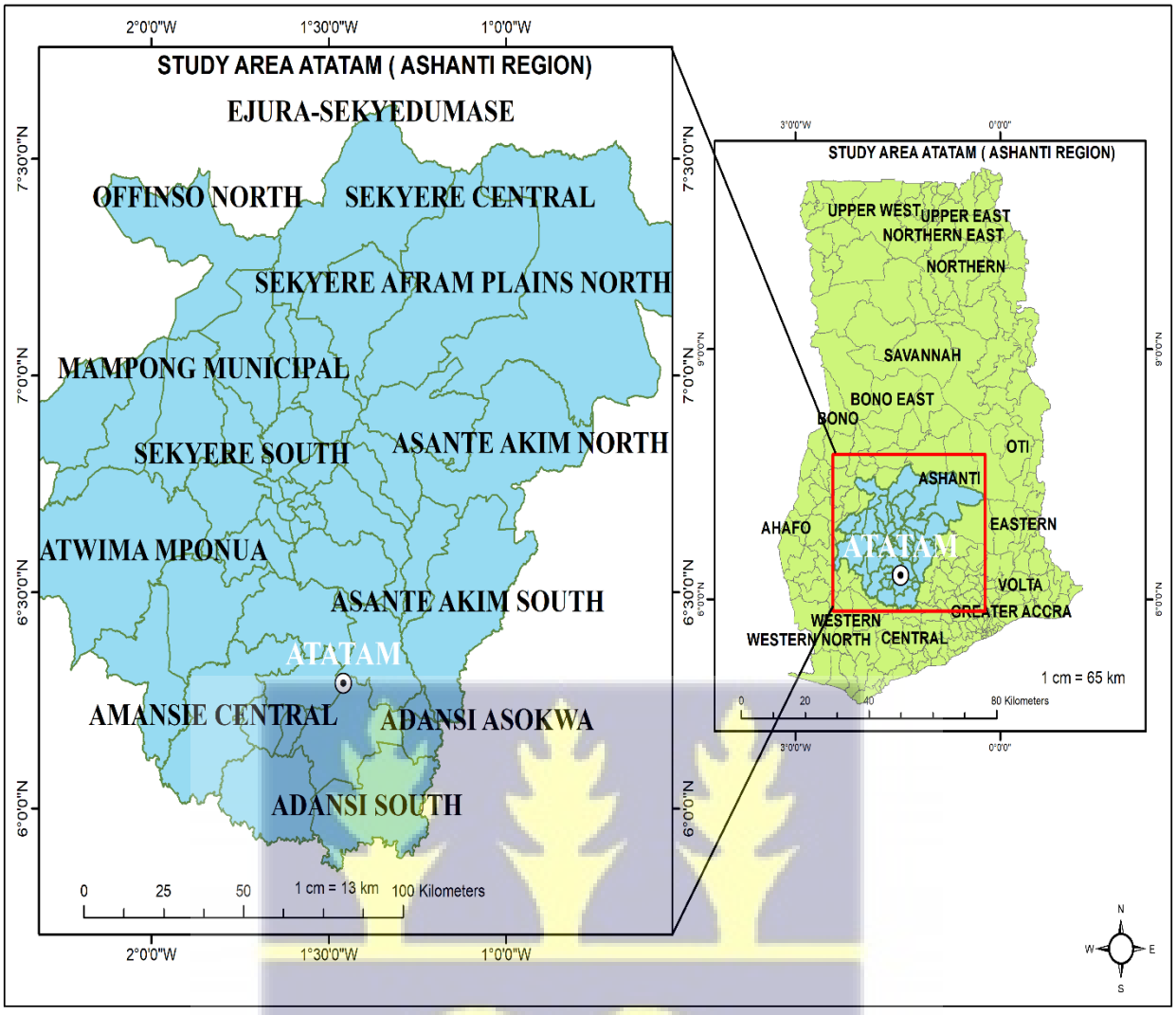

Figure 2: Map of Ghana showing the geographical location of the study site 'Atatam'

3.2 Experimental Design

This study adopted a cross-sectional experimental design to investigate the behavioural adaptations of major malaria vectors and their impact on malaria transmission in Atatam, a small rural community in Adansi Asokwa District in the Ashanti region. The study was conducted for twelve (12) nights, (four nights each in the minor rainy season, the dry season and the major rainy season) following the major seasons experienced in the study area. The minor rainy season spans from September to November and sometimes extends to December, the dry season from December to March and the major rainy season from March to July. The

minor rainy season collection was done in November 2023, the dry season collection in February 2024 and the major rainy season collection in May 2024.

3.3 Mosquito Sampling

The standard Human Landing Catches (HLC) technique was used to collect and estimate outdoor and indoor vector abundance and peak biting time activity of malaria vectors. (Kline, 2006; & Govella *et al*., 2010) The sampling was done for four (4) nights in four households, and this was repeated in each of the three seasons. Throughout the three research seasons, the same homes were used for the study. The selected houses were not less than 30 meters (30m) apart from each other. Human volunteers used for the study were in two groups of four individuals. For each night, four volunteers collect mosquitos indoors and four collect outdoors. Indoor and outdoor collectors were situated about ten meters from each other to avoid interference (Okello *et al*., 2006). Hourly collections were from 18:00 h to 06:00 h. Mosquito biting pattern was classified using three categories as follows: early evening (18:00 – 22:00 h), late night (22:00 – 4:00 h), and early morning (4:00 – 6:00 h) (Kenea *et al*., 2016).

Mosquitoes were caught when they attempted to bite a collector who sits with the lower part of their limbs exposed. Each collector was provided with a collection paper cup, torch light, mouth aspirator or hemolysis tube. Prior to the trial, all volunteers were trained on the HLC technique. The objectives of the study were explained to the volunteers, after which they were made to sign an informed consent form. The holding cups in which the mosquitoes were kept were already marked with the time and site of collection (indoors or outdoors). The volunteers worked fifty minutes collecting mosquitoes, taking ten-minute breaks every hour, and switching positions while they were seated to prevent bias based on their skills and attractiveness. All mosquitoes collected for each hour were placed in the same cup labelled with the time and site of collection.

Supervisors recruited from the community were assigned to coordinate the collection activity and carry out spot checks during the collection nights and address any challenges that arise in course of the study. Volunteers were screened for malaria parasites and given anti-malaria prophylactic drugs (Doxycycline hyclate) one week prior to the start of the study to protect volunteers during the collection period.

Plate 1. Indoor collection by volunteers

Plate 2. Outdoor collection by volunteers

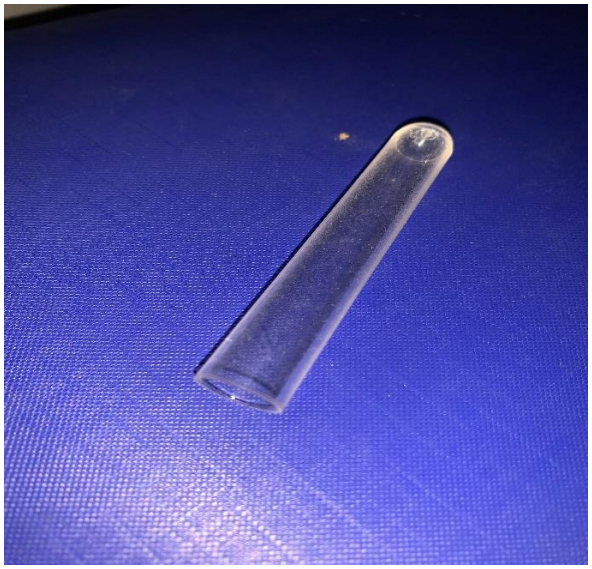

Plate 3. Haemolysis tube for mosquito collection

Plate 4. Mouth aspirator for mosquito collection

Plate 5. Cups holding mosquitos sampled from HLC

3.4 Anopheline Species Morphological Identification.

Mosquitos collected each day was identified morphologically using a dissecting microscope (GXM XTL3T Stereo 7-45x zoom Trinocular Microscope) according to the protocol by Gillies & Coetzee (1987). The distinct pale and black spots on the wings of the Anopheles species served as an indicator of identification with respect to other mosquito species.

The male has a plumose antenna, whereas the female has a pilose antenna; however, the female's palps typically lie close to the proboscis and are as long as the proboscis. The adult *Anopheles* rests at right angles to its resting surface. Additionally, they are characterized by spots in the middle of the tibia or white speckled tibia ornamentation (Gilles & Coetzee, 1987).

The keys used in identifying *Anopheles gambiae* includes; no tufts on the abdomen, legs are speckled, maxillary palp with three (3) pale bands, third main dark area on the costa and sub-costa with a pale interruption, maxillary palp with three pale bands, third main dark area of wing vein 1 with a pale interruption which is sometimes fused with the preceding pale spot.

The keys used in identifying *Anopheles funestus* includes; abdomen with no tufts, hind-tarsomere 4-5 not pale, and legs not speckled, wing with pale spots on the entire wing veins, wing with pale spot on basal 0.5 of costa, palp with apex pale and with less than 4 pale bands, 3rd main dark area without pale interruption, wing with 1 pale spot on the upper branch of vein 5, pre-accessory dark spot absent or if present, shorter or only slightly longer than adjoining pale spots, basal area of wing vein 1 entirely pale, sub-apical pale band on palp much shorter than apical dark band or 3rd main dark area longer than sub-apical pale spot, small species, wing less than 3.2mm, tip of wing vein 6 dark with no fringe spot.

3.5 Coverage, Usage of LLINs and Human Behaviour Survey

A cross-sectional study design was employed to understand human activity and sleeping patterns in the community. This behavioural study was carried out same period the vector

collection was done. A quantitative study was conducted in which thirty households in the community were interviewed. The inhabitants in each household were interviewed using questionnaire designed to collect data on demography, ownership, type off LLIN owned and usage, source of LLIN, how many use the LLIN, how often the LLIN is washed, whether they are still intact or holed and age of the LLIN, when people retire to bed, activities people are involved in when they are awake, whether people spend more time outdoors or indoors while awake, when people get out of bed and type of housing structure in the community.

Concurrently, field observations to ascertain responses from households was carried out during the period of the study. Amongst observations made were human behaviours across the three seasons (example: people tend to spend more time outdoors in the evenings during the dry season), what activities people stay up to do in the night and also how the LLINs were put to use. This information was used to understand vector and human behaviours across the seasons.

3.6 Statistical Analysis

All the data was summarized and analysed using R version 4.4.0 (R Core Team, 2024). A negative-binomial mixed model (estimated using REML and nlminb optimizer), which accounts for over-dispersion in count data, was fitted to predict the abundance of major malaria vectors identified in Atatam community with location inside households and species (formula: Abundance ~ Location inside households * Species) using the "*glmmTMB*" package (Magnusson *et al*., 2017). The model included season as random effect (formula: ~1 | Season). Similarly, a general linear mixed model (tweedie family with a log link) (estimated using REML and nlminb optimizer) was fitted to predict biting rate of major malaria vectors identified indoors in households in Atatam community with species and biting times (formula: Biting rate ~ Species * Biting times) using the "*glmmTMB*" package (Magnusson *et al*., 2017). The model included season as random effect (formula: ~1| Season). Furthermore, a zero-

inflated general linear mixed model (lognormal family with a log link) (estimated using REML and nlminb optimizer) was fitted to predict biting rate of major malaria vectors identified outdoors in households in Atatam community with species and biting times (formula: Biting rate ~ Species * Biting times) using the "*glmmTMB"* package (Magnusson *et al*., 2017). The model included Season as random effect (formula: ~1 | Season). Additionally, predictions from each model were computed on the response scale while conditioning it on the random effect and visualized using the "*ggeffects*" package respectively (Lüdecke *et al.,* 2020). Model dispersion parameters and assumptions were verified using the '*DHARMa*' package (Hartig, 2020). Both the vector behaviour data and the household survey data were summarised using descriptive statistics.

3.7 Ethics

Ethical approval for the study was obtained from The Radiological and Medical Sciences Research Institute (RAMSRI) ID No. *RAM/ERCP/SS/01/2021* of Ghana Atomic Energy Commission. The purpose of the study was explained to the study community and the volunteers. Volunteers were picked from the locals in the community to aid acceptance from residents. Informed approval was also sought from households where mosquitos were collected.

CHAPTER FOUR

4.0 RESULTS

4.1 Abundance of mosquitoes

4.1.1 Abundance of mosquitos across the three seasons

Over the three-season study period, a total of 7,304 mosquitos belonging to five genera were collected. A total of 7,109 (97.3 %) representing Anophelines were collected over the study period. 83.9 % (5,963) of the total Anophelines collected belonged to *Anopheles gambiae* group and 16.1 % (1,146) belonged to the *Anopheles funestus* group. The abundance of *Anopheles gambiae s.l.* was significantly higher than *Anopheles funestus s.l.* ($p < 0.05$) Table 1. A total of 159 (2.2 %) mosquitos belonging to the *Mansonia* species was also collected over the period, 28 (0.4 %) belonging to the *Anopheles pharoensis* group and 8 (0.1 %) of the total collected belonged to the *Aedes* group (Table 1).

Overall, 54.3 % (n= 3,238) outdoor biting *Anopheles gambiae* and 45.7 % (n= 2725) indoor biting *Anopheles gambiae* mosquitos were collected during the study period. Of the 1146 *Anopheles funestus* collected, 53.9 % (n= 618) were collected outdoors and 46.1 % (n= 528) were collected indoors (Table 2). Similar to *Anopheles funestus s.l.* the abundance of *Anopheles gambiae s.l.* outdoors was higher than their abundance indoors. However, this was not statistically significant ($p > 0.05$).

The estimated mean abundance of mosquitoes indoors was higher (5.20, 95% CI: 1.31, 20.71) than the estimated mean abundance of mosquitoes outdoors (5.07, 95% CI: 1.27, 20.19) (Fig. 3). The estimated mean abundance of *Anopheles gambiae s. l.* was significantly higher (13.56, 95% CI: 3.44, 53.48) than *Anopheles funestus s. l.* (5.20, 95% CI: 1.31, 20.71) (Fig. 4).

The estimated mean abundance of *Anopheles funestus s. l.* indoors significantly varied from the estimated mean abundance of *Anopheles gambiae s.l.* outdoors ($p = <0.001$), but not from

the estimated mean abundance of *Anopheles funestus s. l.* outdoors (p = 0.314). Similarly, the estimated mean abundance of *Anopheles gambiae s.l.* indoors significantly varied from the estimated mean abundances of *Anopheles funestus s.l.* outdoors (p = <0.001) and *Anopheles gambiae s.l.* outdoors (p = <0.001) respectively. The estimated mean abundance of *Anopheles gambiae s. l.* was significantly higher than *Anopheles funestus s. l.* in both indoors and outdoors part of households in Atatam community. The estimated mean abundances for *Anopheles funestus s. l.* and *Anopheles gambiae s. l.* indoors was 5.20 (95% CI: 1.31, 20.71) and 13.56 (95% CI: 3.44, 53.48) respectively, and outdoors were 5.07 (95% CI: 1.27, 20.19) and 15.57 (95% CI: 3.95, 61.39) respectively (Fig. 5).

Table 1: Abundance of Mosquito species collected indoors and outdoors across the three seasons

Site	*Anopheles* species	Indoor				Outdoor						Total Overall	
		Minor Season	Dry Season	Major Season	Total Indoor	Minor Season	Dry Season	Major Season	Total Outdoor	95% CI(Indoor/Outdor)	*P-value*		*P-value*
Atatam	*An. gambiae* s.l	219	1249	1257	2725	252	1130	1856	3238	(3.44, 53.48) (3.95, 61.39)	$p > 0.05$	5963	
	An.funestus s.l	77	442	9	528	20	572	26	618	(1.31, 20.71) (1.27, 20.19)	$p > 0.05$	1146	$p < 0.05$
	An. pharoensis	0	0	10	10	5	0	13	18			28	
	Mansonia	9	2	40	51	22	3	83	108			159	
	Aedes	0	0	1	1	1	0	6	7			8	
	Total	305	1693	1317	3315	300	1705	1984	3989			7304	

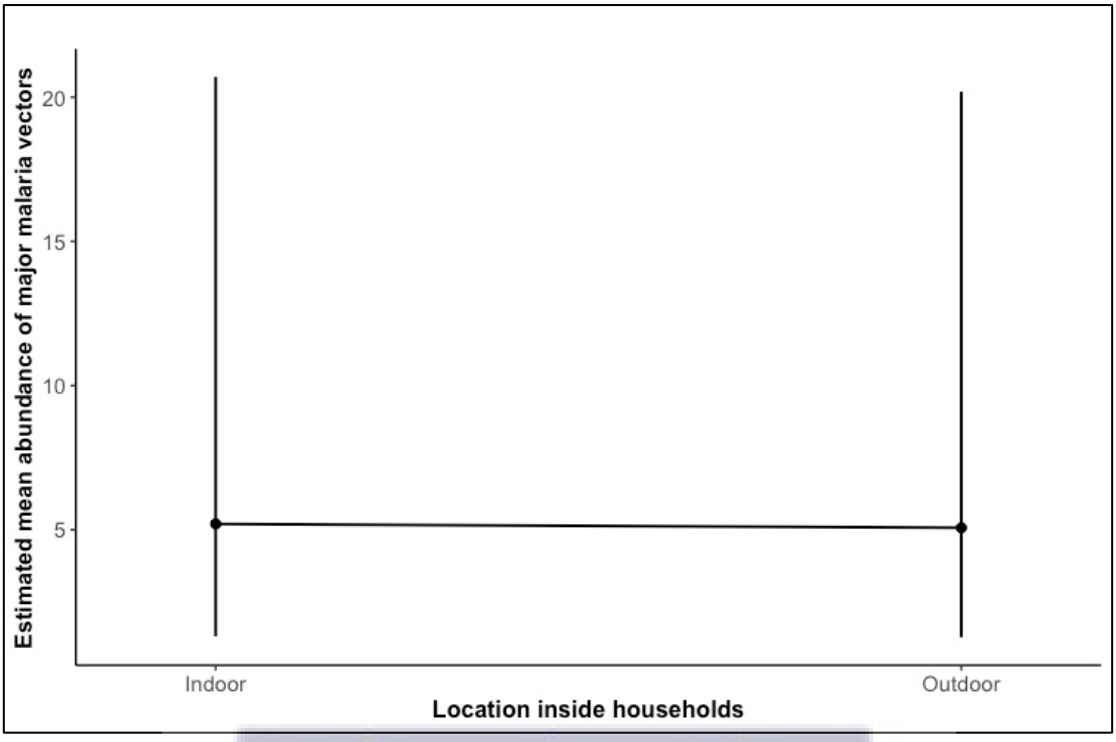

Figure 3: Estimated mean abundance of major malaria vectors identified indoors and outdoors in households at Atatam community.

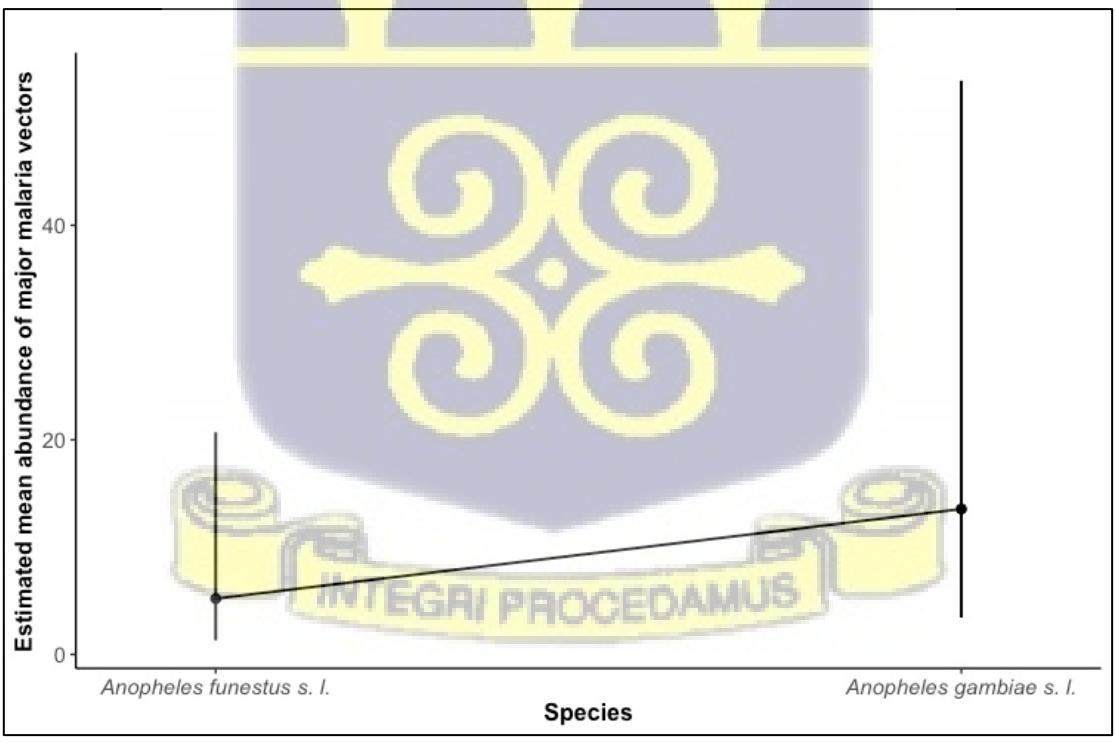

Figure 4: Estimated mean abundance of major malaria vectors identified in Atatam community.

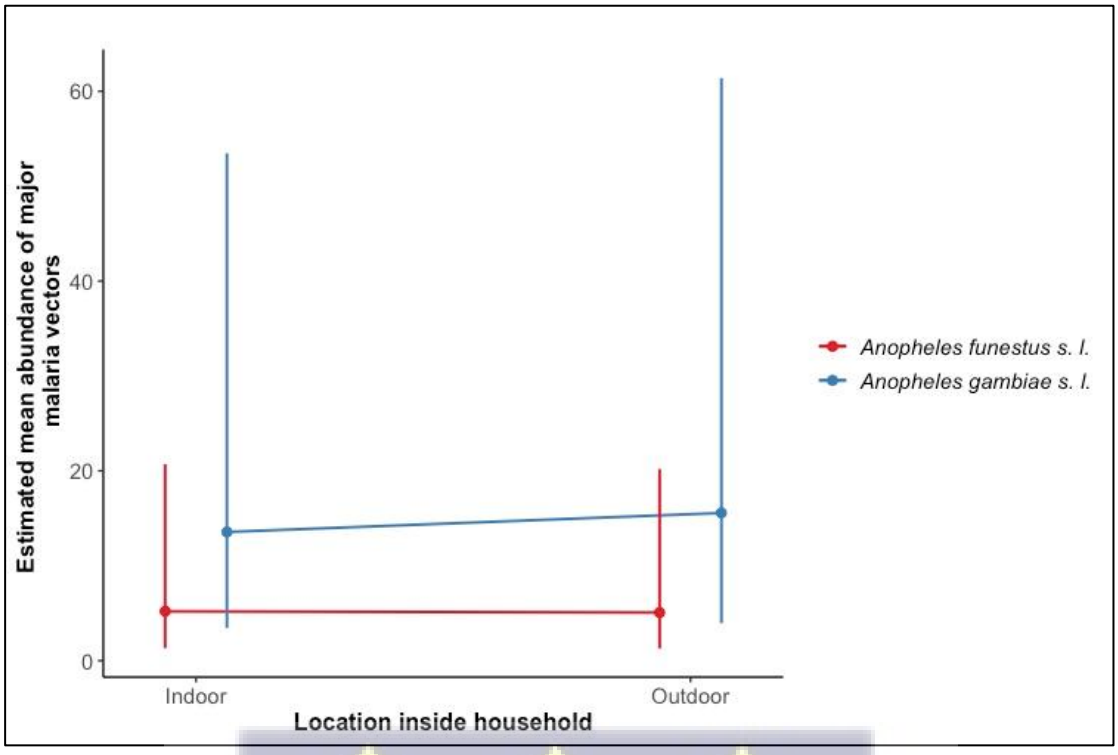

Figure 5: Estimated mean abundance of major malaria vectors in the indoor and outdoor part of households in Atatam community.

e4.1.2 Abundance of mosquitos in the minor rainy season

Overall, 617 mosquitos belonging to five genera were collected during the minor season. A total of 568 (92.1 %) Anophelines were collected over the period. Of these, 82.9 % (n=471) belonged to *Anopheles gambiae* group, 17.1 % (n=97) belonged to the *Anopheles funestus* group, 7 % (n=43) belonged to the Mansonia species, 0.6 % (n=4) belonged to the *Anopheles pharoensis* group and 0.3 (n= 2) belonged to the Aedes group. Of the 568 *Anopheles gambiae* s.l collected, 46.5 % (n= 219) were collected outdoors and 53.5 % (n=252) were collected indoors whereas of the 97 *Anopheles funestus* s.l collected, 79.4 % (n=77) were collected indoors and 20.6 % (n=20) outdoors. (Table 3).

Table 3: Summaries of Mosquito species collected indoors and outdoors at different times of the night (MINOR RAINY SEASON)

Site	*Anopheles* species	Indoor				Outdoor				Total Overall
		Early biting (18 - 22 h)	Late Biting (22 - 04 h)	Early morning (04- 06 h)	Total Indoor	Early biting (18 - 22 h)	Late Biting (22 - 03 h)	Early morning (04 - 06 h)	Total Outdoor	
Atatam	*An. gambiae* s.l	18	165	36	219	29	205	18	252	471
	An.funestus s.l	2	49	26	77	1	18	1	20	97
	An. pharoensis	0	0	0	0	1	1	3	5	4
	Mansonia	9	8	4	21	13	8	1	22	43
	Aedes	0	0	0	0	0	0	1	1	2
	Total	29	222	66	317	44	232	24	300	617

4.1.3 Abundance of mosquitoes in the dry season

An overall number of 3398 mosquitoes belonging to three genera were collected during the dry season. A total of 3393 (99.85 %) Anophelines were collected over the period. Of these, 70.1 % (n=2379) belonged to *Anopheles gambiae* group, 29.9 % (n=1014) belonged to the *Anopheles funestus* group, and 0.15 % (n=5) belonged to the Mansonia species. Of the 2379 *Anopheles gambiae* s.l collected, 47.5 % (n= 2379) were collected outdoors and 52.5 % (n=1249) were collected indoors whereas of the 1014 *Anopheles funestus* s.l collected, 43.6 % (n=442) were collected indoor and 56.4 % (n=572) outdoor. The *Anopheles gambiae* group was the most abundant species (70 %, n= 2379) collected during the dry season (Table 4).

Table 4: Summaries of Mosquito species collected indoors and outdoors at different times of the night (DRY SEASON)

Site	*Anopheles* species	Indoor				Outdoor				Total Overall
		Early biting (18 - 22 h)	Late Biting (22 - 04 h)	Early morning (04- 06 h)	Total Indoor	Early biting (18 - 22 h)	Late Biting (22 - 04 h)	Early morning (04 - 06 h)	Total Outdoor	
Atatam	*An. gambiae* s.l	181	826	242	1249	165	846	119	1130	2379
	An.funestus s.l	47	298	97	442	33	433	106	572	1014
	Mansonia	0	1	1	2	1	1	1	3	5
	Total	228	1125	339	1693	198	1279	226	1702	3398

4.1.4 Abundance of mosquitoes in the major rainy season

An overall number of 3301 mosquitoes belonging to five genera were collected during the major rainy season. A total of 3148 (95.4 %) Anophelines were collected over the period. Of these, 98.9 % (n=3113) belonged to *Anopheles gambiae* group, 1.1 % (n=35) belonged to the *Anopheles funestus* group. Of all mosquitoes collected, 3.7 % (n=123) belonged to the Mansonia group, 0.7 % (n=23) belonged to the *Anopheles pharoensis* group and 0.2 (n= 7) belonged to the Aedes group. Of the 3113 *Anopheles gambiae* s.l collected, 59.6 % (n= 1856) were collected outdoors and 40.4 % (n=1257) were collected indoors whereas of the 35 *Anopheles funestus* s.l collected, 25.7 % (n=9) were indoor biting mosquitoes and 74.3 % (n=26) outdoor biting mosquitoes. The *Anopheles gambiae* group was the most abundant species (94.3 %, n= 3113) collected during the minor rainy season (Table 5).

Table 5: Summaries of Mosquito species collected indoors and outdoors at different times of the night - (MAJOR RAINY SEASON)

Site	*Anopheles* species	Indoor				Outdoor				Total Overall
		Early biting (18 - 22 h)	Late Biting (22 - 04 h)	Early morning (04- 06 h)	Total Indoor	Early biting (18 - 22 h)	Late Biting (22 - 04 h)	Early morning (04 - 06 h)	Total Outdoor	
Atatam	*An. gambiae* s.l	209	735	313	1257	152	1356	348	1856	3113
	An.funestus s.l	2	4	3	9	4	10	12	26	35
	An. pharoensis	2	5	3	10	0	13	0	13	23
	Mansonia	8	27	5	40	23	55	5	83	123
	Aedes	0	1	0	1	4	2	0	6	7
	Total	221	772	324	1317	183	1436	365	1984	3301

4.2 Anopheles mosquito hourly biting activity

4.2.1 Hourly biting activity of anopheles in the minor rainy season.

The human hourly biting activity of *An. gambiae* s.l was observed from 18 – 06 h both indoors and outdoors. Two late night biting peaks and a small early morning biting peak were observed indoors for *An. gambiae* s.l biting activity. The indoor late-night small and first biting peak was observed from 22 - 11 h (mean 1.875 bites/person/hour) and a maximum late-night peak observed at 00 - 01 h (mean 2.438 bites/person/hour) when people were deep asleep. Also, the early morning indoor biting peak was observed at 04 – 05 h (mean 1.4 bites/person/hour). Late night outdoor biting peak was observed between 22 - 01 h with maximum biting peak at 22 – 23 h (mean 3.063 bites/person/hour) (Fig 6).

The human hourly biting activity of *An. funestus* s.l outdoor was observed from 21 – 05 h and 20 – 06 h for indoors. Outdoor biting activity had a small peak between 22 - 00 h (mean 0.188 bites/person/hour) and a maximum peak between 01 – 03 h (mean 0.25 bites/person/hour). More so, *An. funestus* s.l showed a steady increase in early morning biting activity indoors between 04 – 05 h (mean 1.063 bites/person/hour) when people were likely to be awake (Fig 7).

Overall, 79.4% (n=77) of *An. funestus* collected exhibited endophagic behaviour while 53.5% (n=252) of *An. gambiae* exhibited outdoor feeding preference.

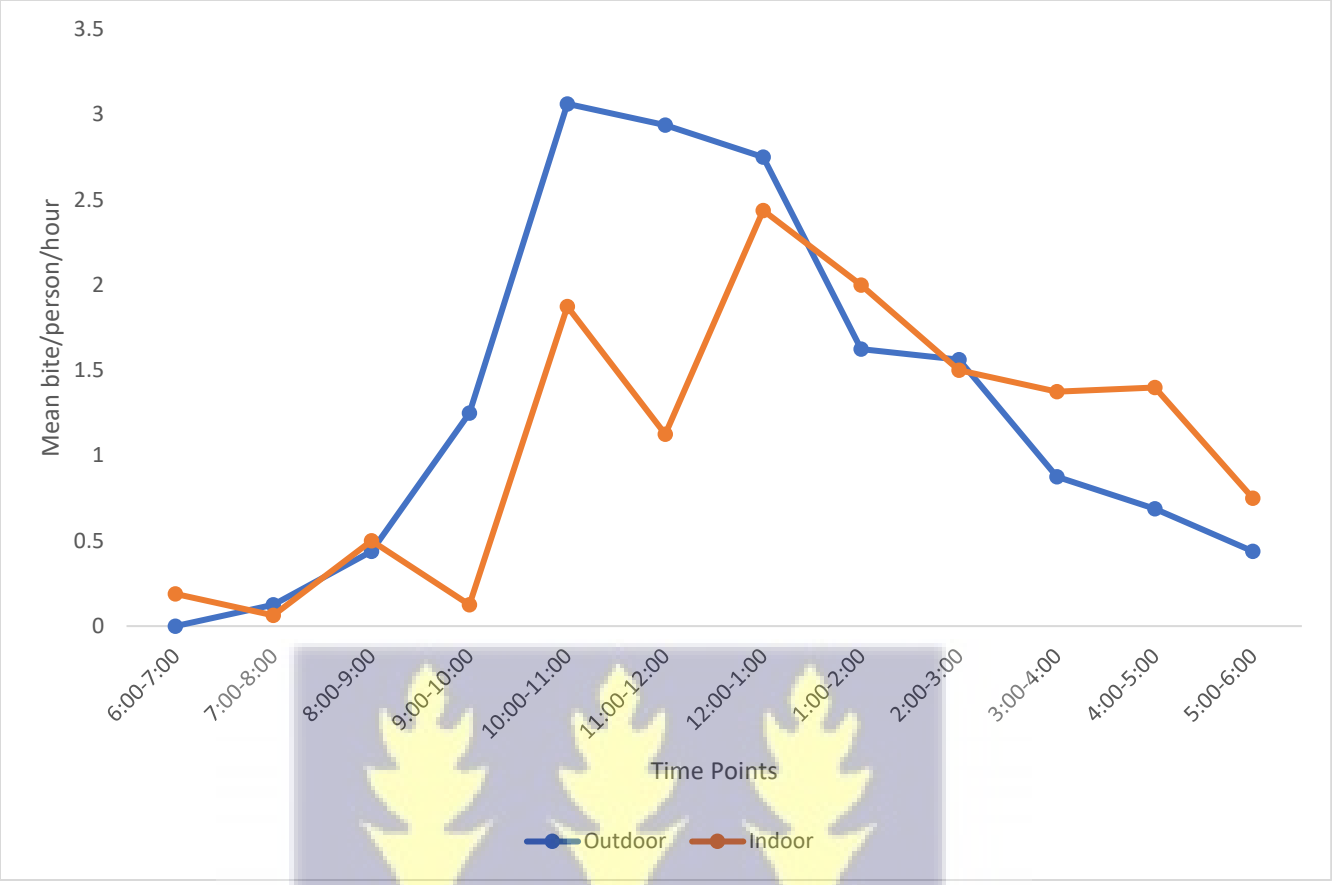

Figure 6: *An. gambiae* s.l bitting activity in Atatam - Minor rainy Season

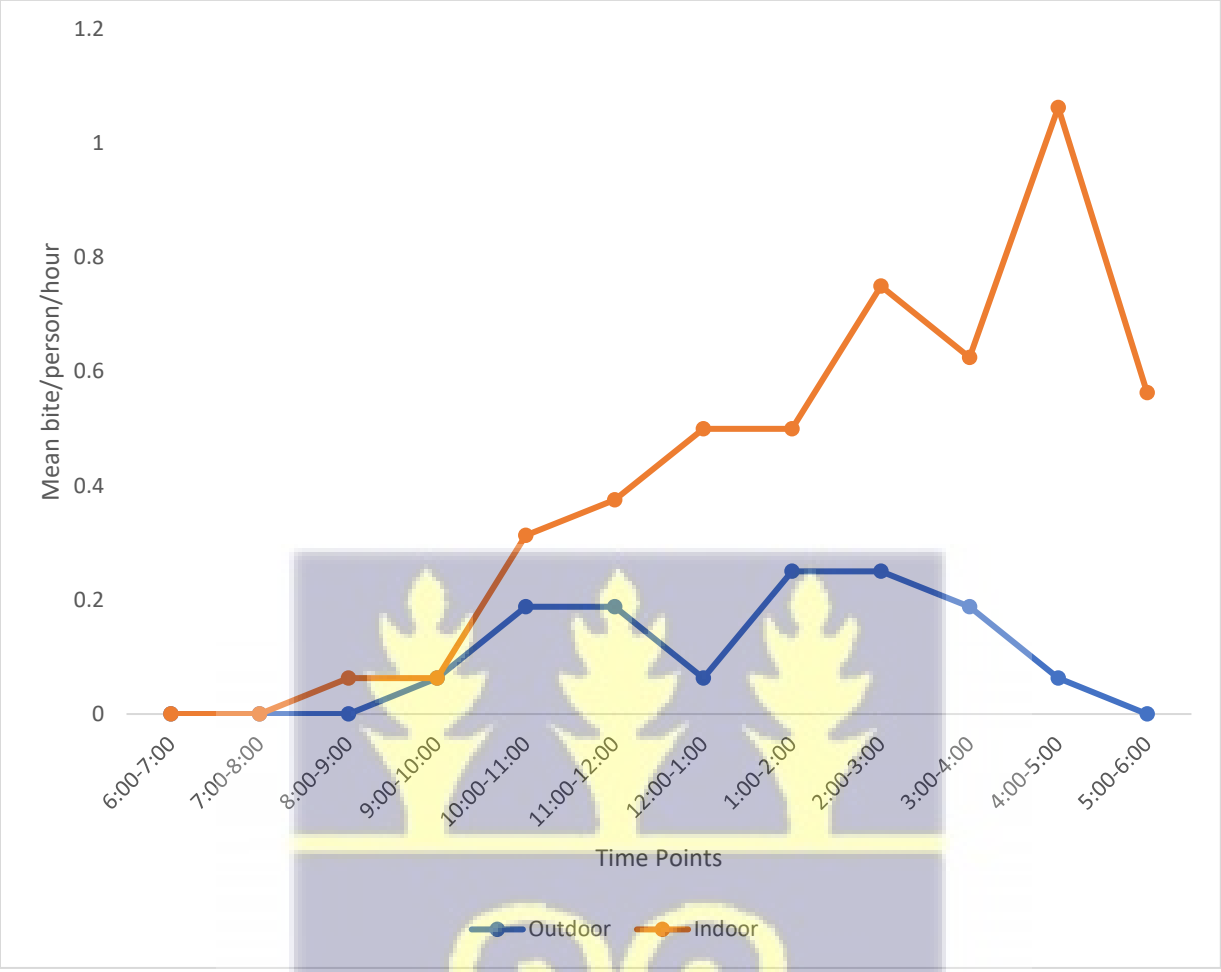

Figure 7: *An. funestus* s.l bitting activity in Atatam - Minor rainy Season

4.2.2 Hourly biting activity of Anopheles in the dry season.

The human hourly biting activity of *An. gambiae* s.l was observed from 18 – 06 h both indoors and outdoors, with two peak biting activity observed at late night and early morning for both indoor and outdoor. The indoor late-night maximum biting peak was observed from 23 - 04 h (mean 9.1 bites/person/hour) and a maximum early morning peak observed between 04 - 05 h (mean 9.625 bites/person/hour when people were less protected by bed nets. Outdoor biting peak was at its maximum from 01 – 02 h (mean 13.25 bites/person/hour) and a small early morning peak observed at 04 – 05 h (mean 5.313 bites/person/hour) (Fig 8).

The human hourly biting activity of *An. funestus* s.l was observed from 18 – 06 h both indoors and outdoors, while exhibiting late night outdoor biting activity which peaked from 03 - 04 h (mean 7.688 bites/person/hour) when people were moving between locations (ie individuals sleeping outdoors during the dry season receive bites at the time they decide to move indoors which leaves them less protected). Also, indoor early morning and late-night biting activity was seen in *An. funestus* s.l which was observed from 05 – 06 h (mean 3.313 bites/person/hour) and 00 – 05 h (mean 3.325 bites/person/hour) respectively (Fig 9).

Overall, 52.5 % (n=1249) of *An. gambiae* s.l collected exhibited endophagic behaviour while 56.4 % (n=572) of *An. funestus* exhibited outdoor feeding preference.

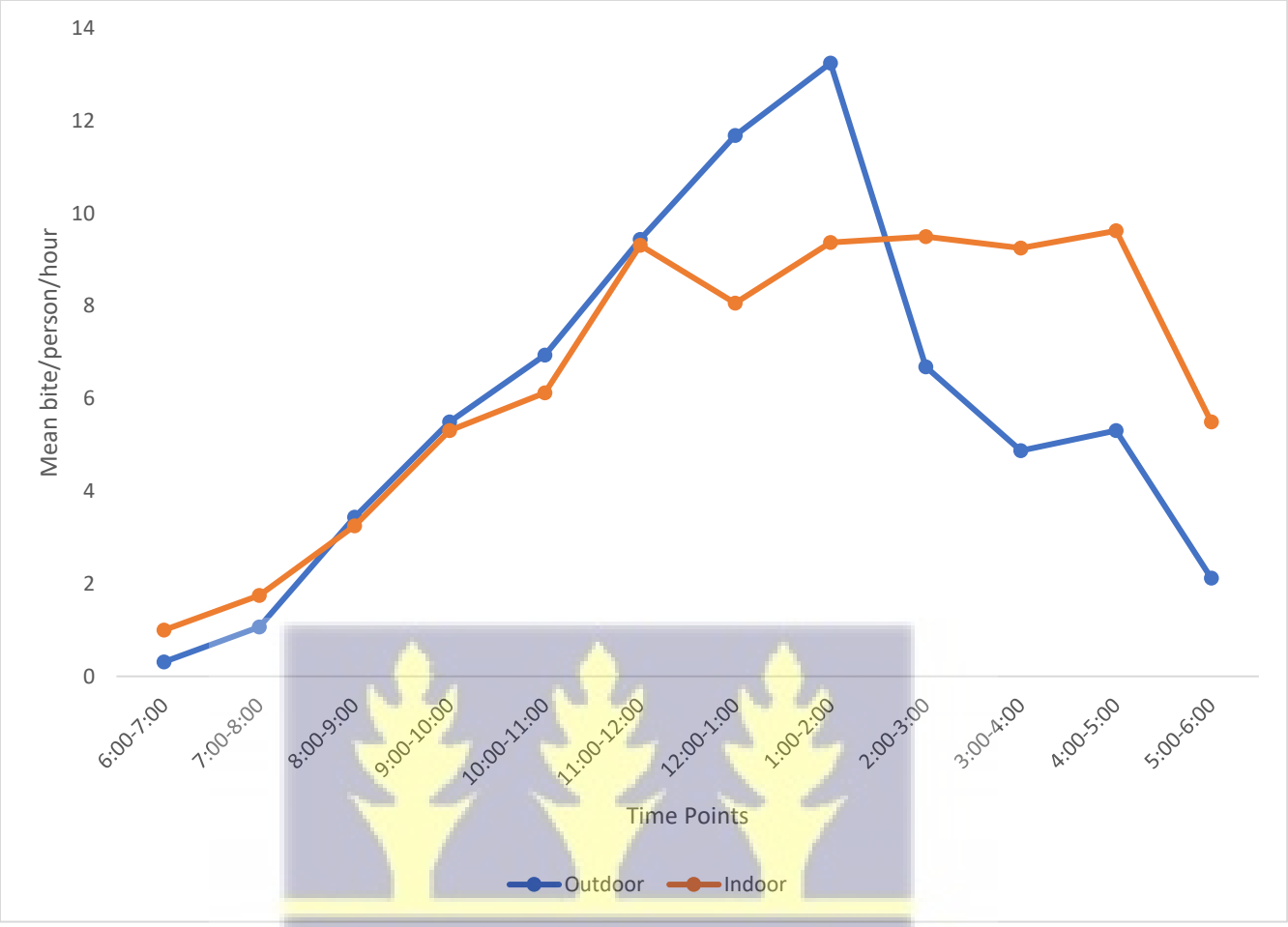

Figure 8: *An. gambiae* sl. biting activity in Atatam - Dry Season

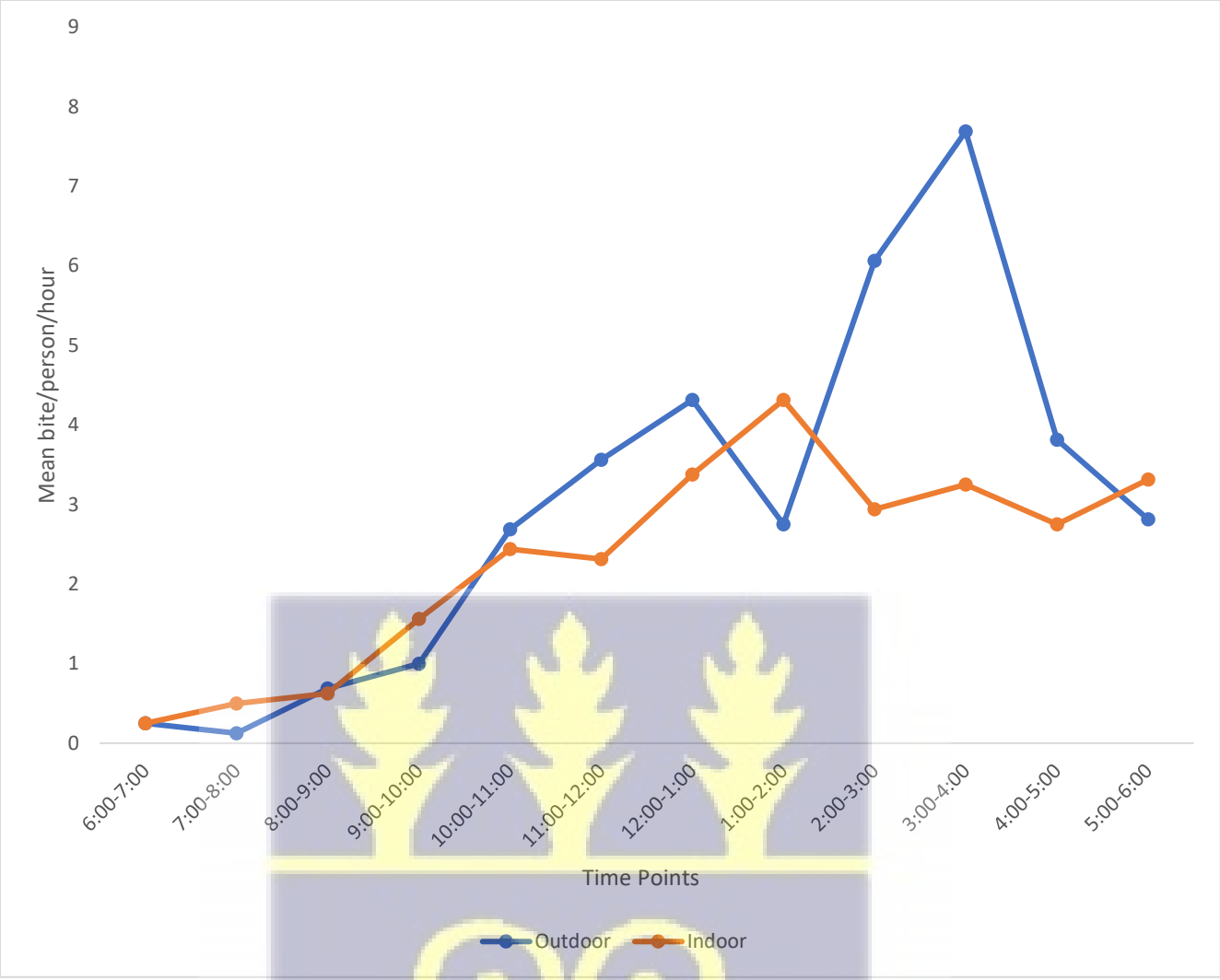

Figure 9: *An. funestus* sl. biting activity in Atatam - Dry Season

4.2.3 Hourly biting activity of Anopheles in the major rainy season.

The human hourly biting activity of *An. gambiae* s.l was observed from 18 – 06 h both indoors and outdoors, with peak biting activity observed at late night for outdoor with both late night and early morning peak biting activity observed indoors. The outdoor late-night peak biting was observed from 01 - 04 h (mean 16.229 bites/person/hour). The indoor late-night biting showed two biting peaks with a small peak observed at 23 – 00 h (mean 8.875 bites/person/hour) and a maximum late-night peak observed at 01 - 02 h (mean 11.3125 bites/person/hour). The early morning indoor biting peak was observed from 04 - 05 h (mean 11.6875 bites/person/hour) when people were likely to be awake (Fig 10).

The human hourly biting activity of *An. funestus* s.l was observed from 21 – 06 h both indoors and outdoors with no biting activity indoors between 22 – 02 h. The hourly biting activity both indoors and outdoors showed peak biting activity early evening, late night and early morning. The outdoor early evening biting activity was observed from 21 – 22 h (mean 0.25 bites/person/hour), late night observed at 00 – 01 h (mean 0.1875 bites/person/hour) and early morning peak observed from 05 – 06 h (mean 0.375 bites/person/hour). The indoor early evening biting activity was observed from 21 - 22 h (mean 0.125 bites/person/hour), late night observed from 02 – 04 h (mean 0.125 bites/person/hour) and early morning peak observed from 04 – 05 h (mean 0.1875 bites/person/hour) (Fig 11).

Overall, 59.6 % (n= 1856) of *An. gambiae* collected and 74.3 % (n=26) of *An. funestus* collected both exhibited exophagic behaviour.

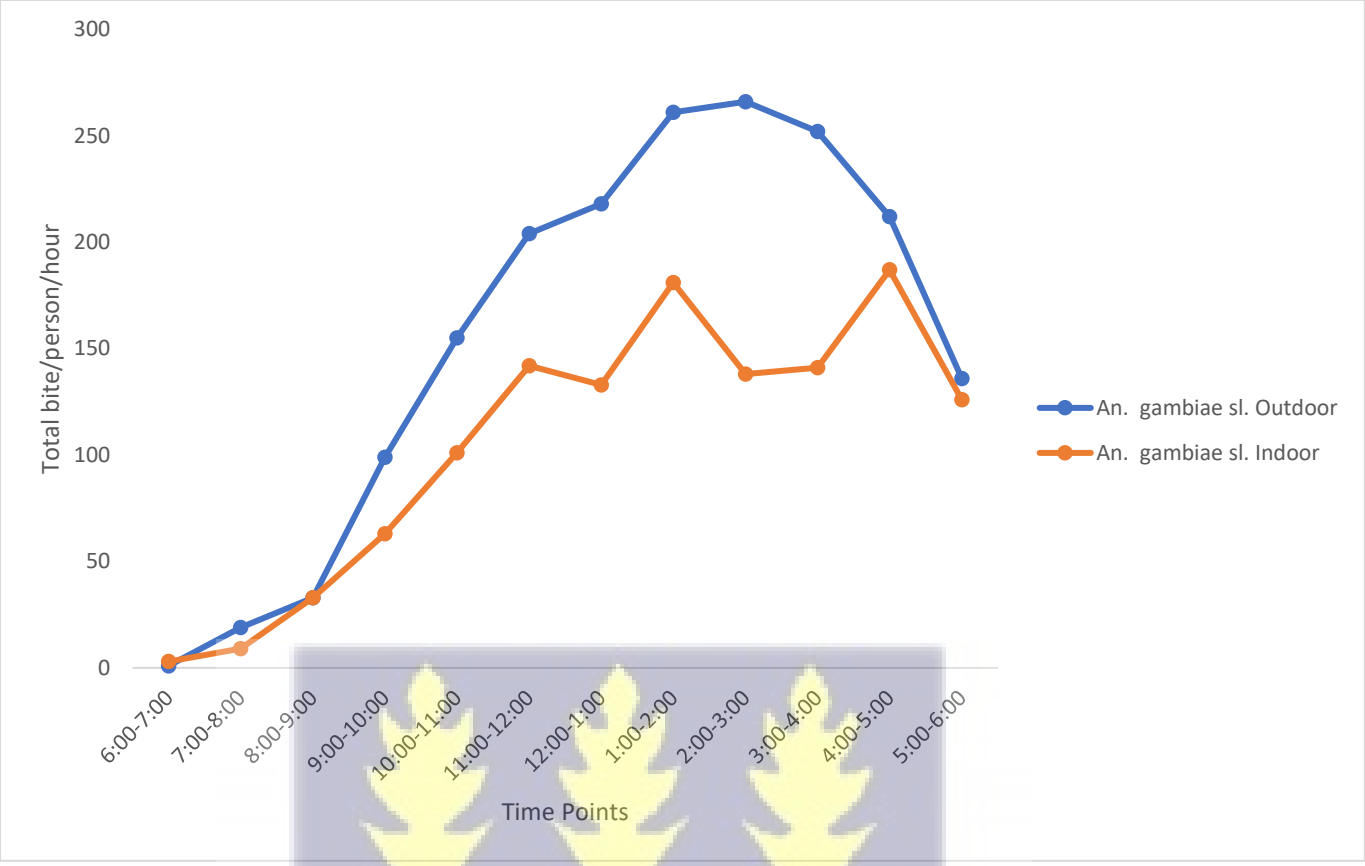

Figure 10: *An. gambiae* s.l bitting activity in Atatam - Major rainy Season

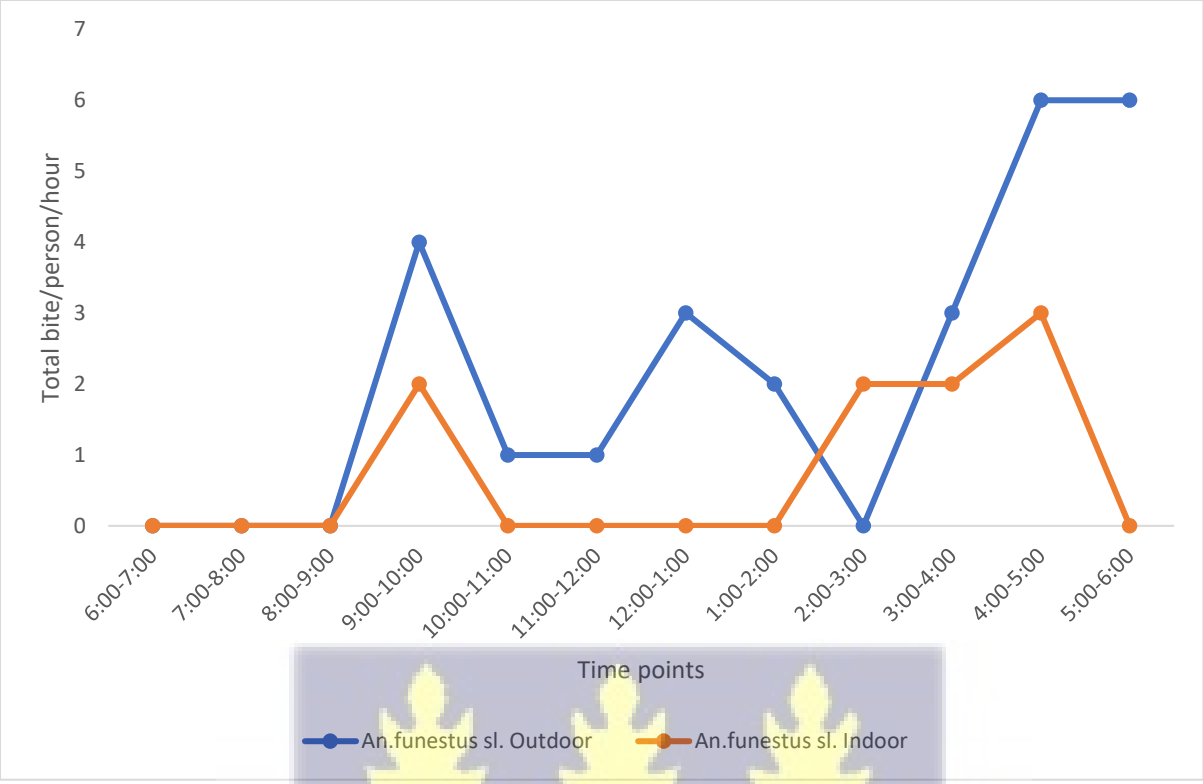

Figure 11: *An. funestus* s.l bitting activity in Atatam - Major rainy Season

4.3 Anopheline Human Biting Rates (HBR)

The overall mean (indoor and outdoor combined) HBR for *An. gambiae* s.l in the minor rainy season, dry season and major rainy season were (29.092 mosquitoes/person/night), (148.693 mosquitos/person/night), and (194.563 mosquitoes/person/night) respectively. Moreso, the HBR recorded for *An. gambiae* s.l in the individual seasons both indoor and outdoor were (13.339 vs 15.753 mosquitoes/person/night) in the minor rainy season, (78.064 vs 70.629 mosquitos/person/night) in the dry season and (78.563 vs 116 mosquitoes/person/night) in the major rainy season respectively.

The overall mean (indoor and outdoor combined) HBR for *An. funestus* s.l in the minor rainy season, dry season and major rainy season were (6.068 mosquitoes/person/night), (63.382 mosquitos/person/night), and (2.188 mosquitoes/person/night) respectively. Also, the HBR recorded for *An. funestus* s.l in the individual seasons both indoor and outdoor were (4.815 vs 1.253 mosquitoes/person/night) in the minor rainy season, (27.628 vs 35.754 mosquitoes/person/night) in the dry season and (0.563 vs 1.625 mosquitoes/person/night) in the major rainy season respectively.

4.3.1 Comparative estimate of the human biting rate of major malaria vectors collected both indoors and outdoors in Atatam community

4.3.1.1 Indoors human biting rate

The influence of biting times and species on the human biting rate of mosquitoes collected indoors in Atatam community has been summarized in Table 6. The human biting rate of *Anopheles gambiae s. l.* found indoors in households in Atatam community was significantly higher than *Anopheles funestus s. l.* Similarly, the biting rate of major malaria vectors identified indoors in households in the Atatam community was significantly lower during early night biting times (18 - 22 h) relative to early morning biting times (04 - 06 h) ($p < 0.05$). The human biting rate of major malaria vectors identified indoors in households in Atatam community during late night biting times (22 - 04 h) was also lower compared to the early morning biting times (04 - 06 h). However, this was statistically significant ($p > 0.05$).

The human biting rate of *Anopheles gambiae s. l.* found indoors in households in Atatam community was higher during early night biting times than in early morning biting times, relative to that of *Anopheles funestus s. l.* However, this was not statitiscally significant ($p> 0.05$). Similarly, the human biting rate of *Anopheles gambiae s. l.* was higher during late night biting times than in early morning biting times, relative to that of *Anopheles funestus s. l.* However, this was not statistically significant ($p> 0.05$). The random effect variance for season (3.92) was higher than the residual variance (0.25), suggesting a significantly high variability in the human biting rate of major malaria vectors across the three seasons (major, minor, and dry seasons).

The estimated mean human biting rate of *Anopheles gambiae s. l.* indoors was significantly higher than *Anopheles funestus s. l.* in Atatam community ($p = <0.001$). The estimated human biting rate of *Anopheles gambiae s. l.* indoors was 0.65 (95% CI: 0.07, 6.14) while that of *Anopheles funestus*

s. l. was 0.26 (95% CI: 0.03, 2.50) (Fig. 12). The estimated human biting rate of major malaria vectors identified indoors during the early night biting times (18 - 22 h) varied significantly from the estimated biting rate of major malaria vectors identified indoors during late night biting times (22 - 04 h) (p = <0.001) and early morning biting times (04 - 06 h) (p = <0.001) respectively. Likewise, the estimated human biting rate of major malaria vectors identified indoors during late night biting times (22 - 04 h) varied significantly from the estimated biting rate of major malaria vectors identified indoors during early morning biting times (04 - 06 h) (p = <0.001). The estimated human biting rate of major malaria vectors identified indoors in Atatam community was highest in the early morning biting times (04 - 06 h) (0.26, 95% CI: 0.03, 2.50) and lowest during the early night biting times (18 - 22 h) (0.09, 95% CI: 0.01, 0.84) (Fig. 13).

Furthermore, there was a significant variation in the estimated mean human biting rate of *Anopheles funestus s. l.* and *Anopheles gambiae s. l.* in the early morning (p = <0.001), early night (p = <0.001), and late-night biting times (p = <0.001) respectively. The estimated mean human biting rate of *Anopheles gambiae s. l.* was higher than *Anopheles funestus s. l.* in all the biting times respectively (Fig. 14). The estimated human biting rate of *Anopheles funestus s. l.* and *Anopheles gambiae s. l.* in the early morning (04 - 06 h), early night (18 - 22 h), and late night biting times (22 - 04 h) were: 0.26 (95% CI: 0.03, 2.50) and 0.65 (95% CI: 0.07, 6.14), 0.09 (95% CI: 0.01, 0.84) and 0.22 (95% CI: 0.02, 2.09), and 0.22 (95% CI: 0.02, 2.10) and 0.64 (95% CI: 0.07, 6.01) respectively.

Table 6: Influence of biting times and species on the human biting rate of major malaria vectors identified indoors in Atatam community.

	Human Biting rate of major malaria vectors				
Predictors	Estimates	SE	CI	Statistic	P-value
(Intercept)	-1.34	1.15	-3.60 – 0.91	-1.17	0.243
Anopheles gambiae s. l.	0.91	0.19	0.53 – 1.29	4.70	**<0.001**
Early night biting (18 - 22 h)	-1.09	0.22	-1.52 – -0.66	-4.98	**<0.001**
Late night biting (22 - 04 h)	-0.16	0.17	-0.49 – 0.17	-0.96	0.338
Anopheles gambiae s. l. × Early night biting (18 - 22 h)	0.02	0.27	-0.51 – 0.54	0.07	0.947
Anopheles gambiae s. l. × Late night biting (22 - 04 h)	0.15	0.21	-0.27 – 0.57	0.69	0.487
Random Effects					
σ2			0.25		
τ00 Season			3.92		

ICC	0.94
N Season	3
Observations	243
Marginal R2 / Conditional R2	0.084 / 0.944

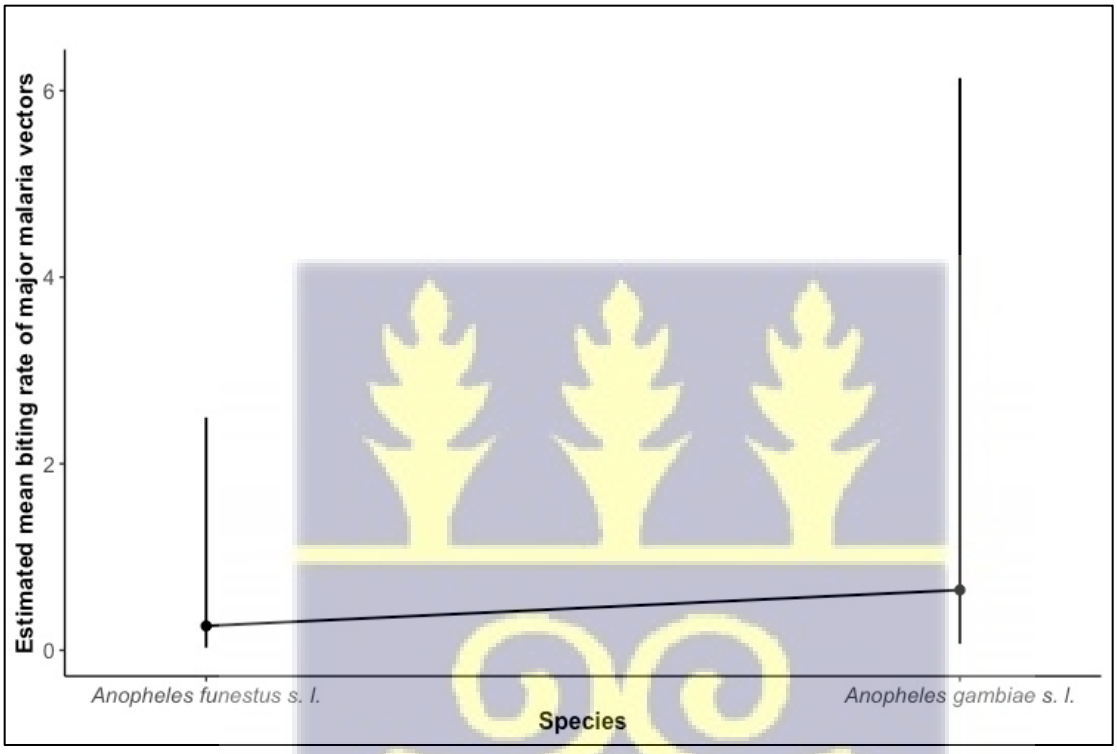

Figure 12: Estimated mean human biting rate of major malaria vectors found indoors in Atatam community

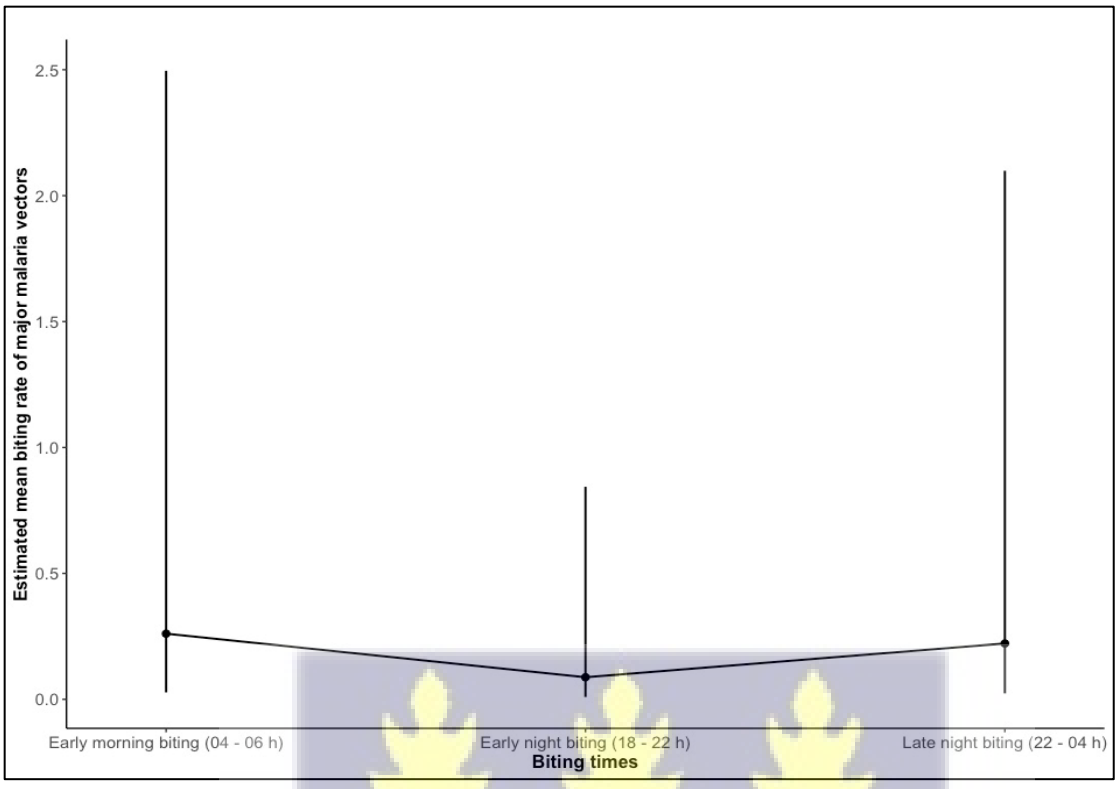

Figure 13: Estimated mean human biting rate of major malaria vectors found indoors at different biting times in Atatam community.

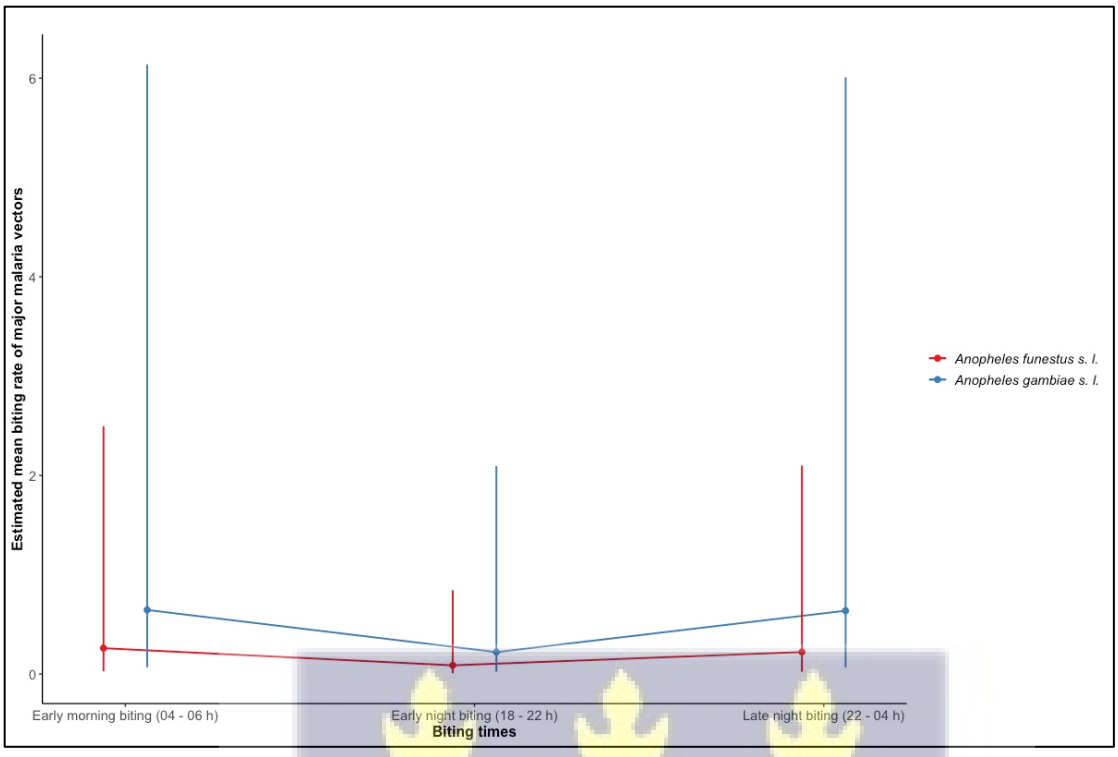

Figure 14: Estimated mean human biting rate of major malaria vectors found indoors at different biting times in Atatam community.

4.3.1.2 Outdoors human biting rate

The influence of biting times and species on the human biting rate of mosquitoes collected outdoors in Atatam community has been summarized in Table 7. The human biting rate of *Anopheles gambiae s. l.* found outdoors was significantly lower than *Anopheles funestus s. l.* ($p < 0.05$). Similarly, the human biting rate of major malaria vectors identified outdoors in early night biting times (18 -22 h) and late-night biting times (22 - 04 h) were both higher relative to early morning biting times (04 - 06 h) respectively. However, these differences were not statistically significant ($p > 0.05$).

The human biting rate of *Anopheles gambiae s. l.* outdoors was significantly higher during early night biting times than in early morning biting times, relative to that of *Anopheles funestus s. l.* ($p < 0.05$). Similarly, the human biting rate of *Anopheles gambiae s. l.* was higher during late night biting times than in early morning biting times, relative to that of *Anopheles funestus s. l.* However, this was not statistically significant ($p > 0.05$). The random effect variance for season (1.09) was quite high, suggesting a substantial variability in the biting rate of major malaria vectors across the three seasons (major, minor, and dry seasons).

The estimated mean biting rate of *Anopheles gambiae s. l.* outdoors was significantly higher than *Anopheles funestus s. l.* ($p = <0.001$). The estimated biting rate of *Anopheles gambiae s. l.* was 1.08 (95% CI: 0.32, 3.60) while that of *Anopheles funestus s. l.* was 0.40 (95% CI: 0.12, 1.39) (Fig. 15). The estimated biting rate of major malaria vectors identified outdoors during the early night biting times (18 - 22 h) varied significantly from the estimated biting rate of major malaria vectors identified outdoors during late night biting times (22 - 04 h) ($p = <0.001$) and early morning biting times (04 - 06 h) ($p = 0.014$) respectively. Likewise, the estimated biting rate of major malaria vectors identified outdoors during late night biting times (22 - 04 h) varied significantly from the

estimated biting rate of major malaria vectors identified outdoors during early morning biting times (04 - 06 h) (p = <0.001). The estimated biting rate of major malaria vectors identified outdoors was highest in the late-night biting times (22 - 04 h) (0.52, 95% CI: 0.15, 1.74) and lowest in the early morning biting times (04 - 06 h) (0.40, 95% CI: 0.12, 1.39) (Fig. 16).

Furthermore, there was a significant variation in the estimated mean biting rate of *Anopheles funestus s. l.* and *Anopheles gambiae s. l.* in the early morning (p = <0.001), early night (p = <0.001), and late-night biting times (p = <0.001) respectively. The estimated mean biting rate of *Anopheles gambiae s. l.* was higher than *Anopheles funestus s. l.* in all the biting times respectively (Fig. 17). The estimated biting rate of *Anopheles funestus s. l.* and *Anopheles gambiae s. l.* in the early morning (04 - 06 h), early night (18 - 22 h), and late night biting times (22 - 04 h) were: 0.40 (95% CI: 0.12, 1.39) and 1.08 (95% CI: 0.32, 3.60), 0.41 (95% CI: 0.12, 1.43) and 0.56 (95% CI: 0.17, 1.86), and 0.52 (95% CI: 0.15, 1.74) and 1.43 (95% CI: 0.44, 4.70) respectively.

Table 7: Influence of biting times and species on the biting rate of major malaria vectors identified outdoors in Atatam community.

	Human Biting rate of major malaria vectors				
Predictors	Estimates	SE	CI	Statistic	P-value
(Intercept)	-0.91	0.63	-2.15 – 0.33	-1.44	0.149
Anopheles gambiae s. l.	0.99	0.21	0.57 – 1.41	4.63	**<0.001**
Early night biting (18 - 22 h)	0.02	0.26	-0.49 – 0.53	0.07	0.941
Late night biting (22 - 04 h)	0.26	0.19	-0.12 – 0.63	1.34	0.179
Anopheles gambiae s. l. × Early night biting (18 - 22 h)	-0.68	0.31	-1.29 – -0.07	-2.19	**0.029**
Anopheles gambiae s. l. × Late night biting (22 - 04 h)	0.03	0.23	-0.41 – 0.47	0.12	0.907
Random Effects					
σ2			1.67		
τ00 Season			1.09		

ICC	0.39
N Season	3
Observations	239
Marginal R2 / Conditional R2	0.086 / 0.446

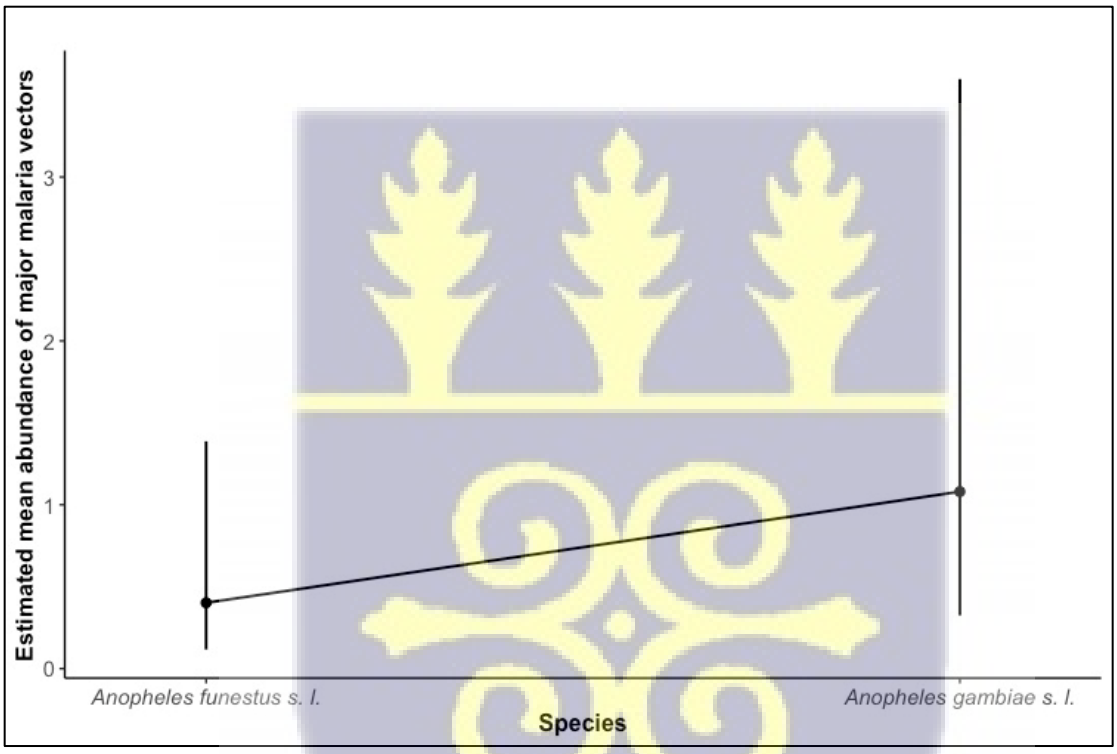

Figure 15: Estimated mean human biting rate of major malaria vectors found outdoors in Atatam community

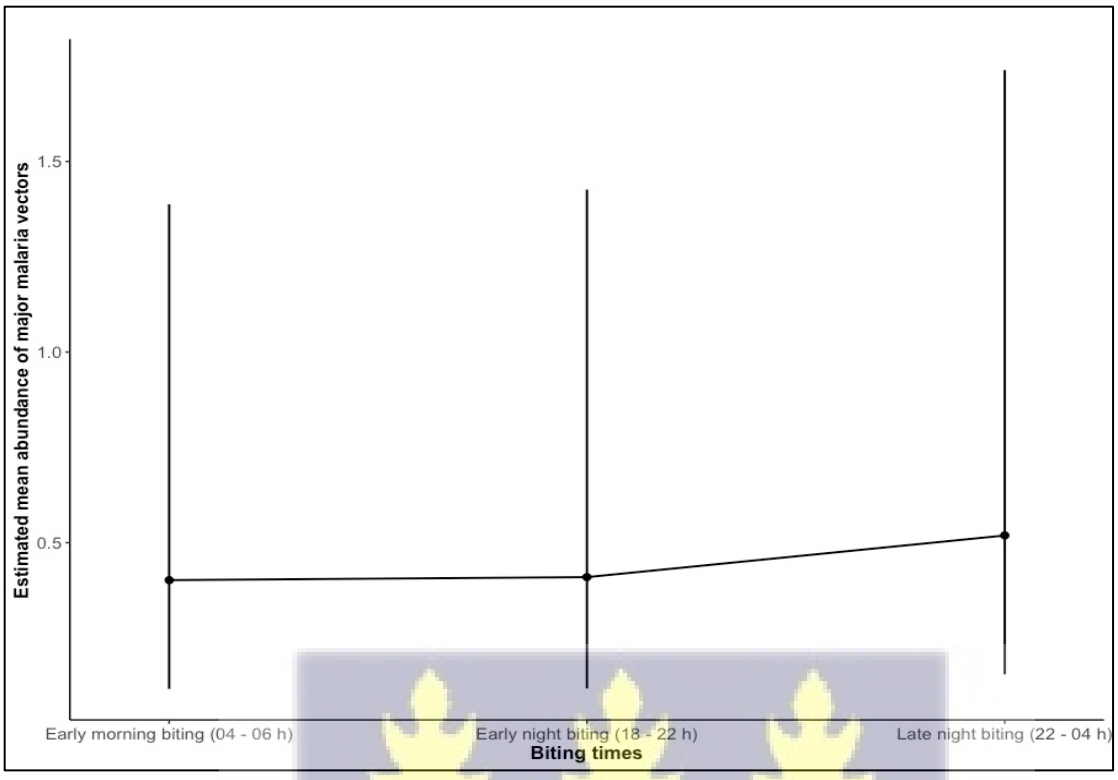

Figure 16: Estimated mean human biting rate of major malaria vectors found outdoors at different biting times in Atatam community.

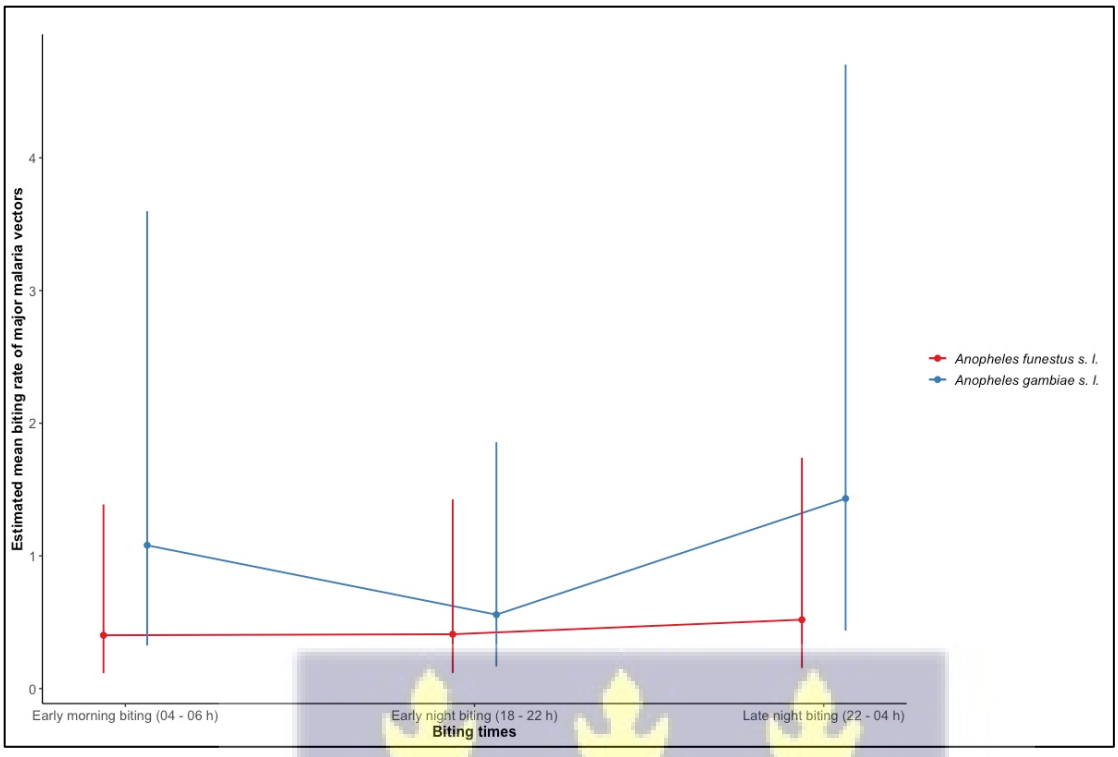

Figure 17: Estimated mean human biting rate of major malaria vectors found outdoors at different biting times in Atatam community.

4.4 Ownership and Usage of LLINs

The survey showed high (93.3 %, n=28) LLINs ownership among households in Atatam (Fig 18). The primary means of protection against malaria infection and mosquito bites is the presence of LLINs in almost every home in the community. Every household that owned LLINs make use of it every night and 92.9 % (n= 26 of 28) of the people that use it, wash only when it is dirty. Amongst some of the LLINs owned are Permanet 3.0 (n=18, 64.3 %), Permanet 2.0 (n=2, 7.1 %), MAGNet (n=4, 14.3 %) and Olyset (n=4, 14.3 %). The age distribution and sex ratio of individuals of households interviewed for the study consisted of 77 males (52 %) and 71 females (48 %). Of these, 24 (16.2 %) were aged 0-5 years, 43 (29.1 %) were aged 6-15 years, 16 (10.8 %) were aged 16-20 years and 65 (43.9 %) were aged 21 and above. When a high percentage of a community uses bed nets, mosquito populations and malaria transmission rates decline, indirectly protecting those who do not use nets (Polec *et al*., 2015).

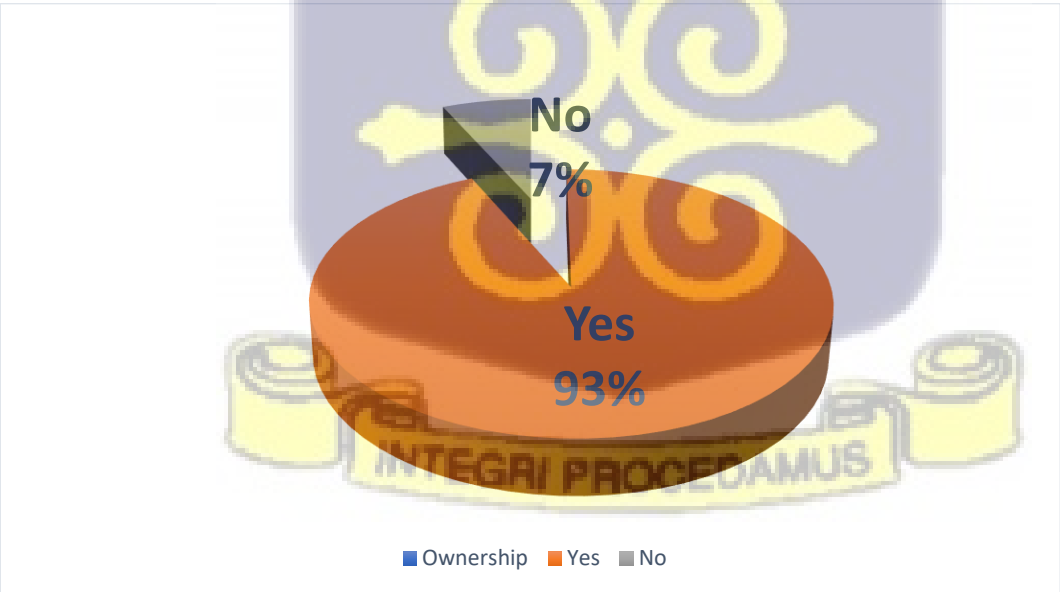

Figure 18: Frequency of LLIN ownership

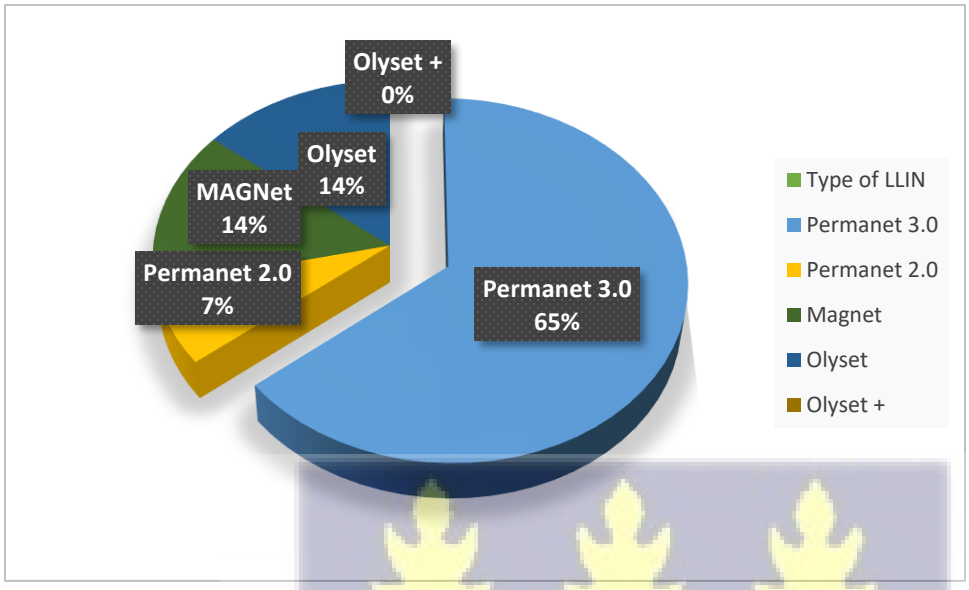

Figure 19: Types of LLIN owned by households

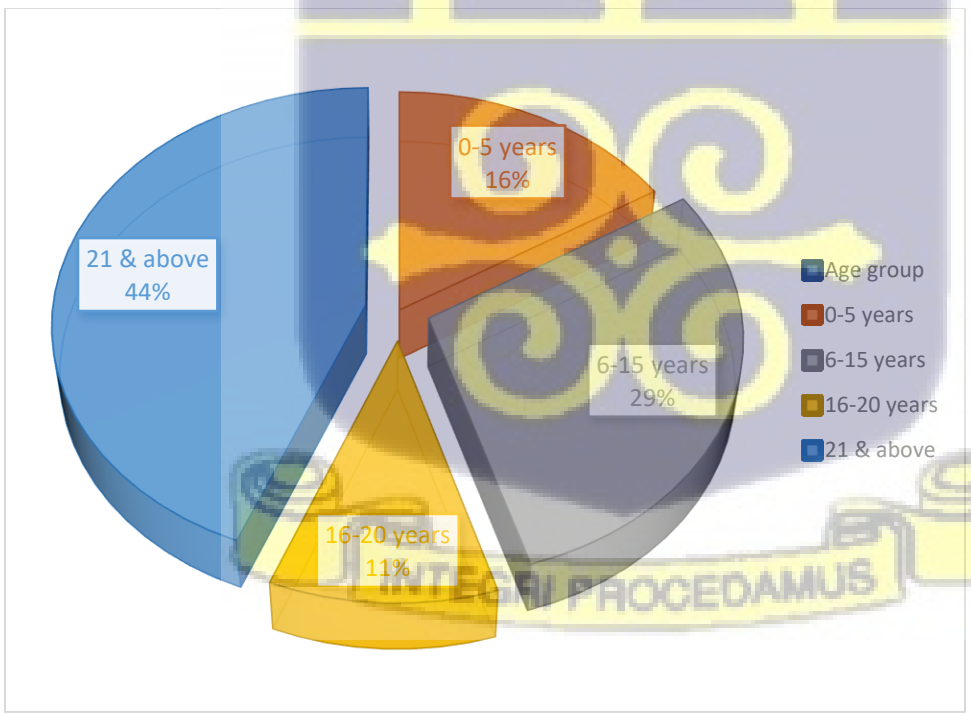

Figure 20: Age distribution among household individuals

4.5 Human Behaviour study and observation

From the study, most households 66.7 % were observed to retire to bed between 8 and 10 pm and woke up around 5 to 6 am 86.7 % of the time. People/households (n=25, 83.3 %) spent most of the time outdoors whiles awake. Some activities that people were engaged in whiles awake included domestic chores (40.8 % of the time), and most of the time (49 %) are engaged in socio-economic activities such as selling, watching television whiles a few individuals (10.2 %) were engaging in illegal mining. Whiles out of bed, people are all the time (100 %) outdoors and are involved in activities such as domestic chores (49 %), agricultural activities (39.2 %), religious activity (5.9 %) and illegal mining (5.9 %). Similar housing structures are seen in the community with most (83.3 %) of the houses made of mud with iron sheet roofing. Most households were also observed to spend time outdoors in the night-time during the dry season which spanned from December to March when the average temperature was relatively high.

CHAPTER FIVE

5.0 DISCUSSION

This study adopted a cross-sectional experimental approach for investigating the behavioural adaptations of major malaria vectors, their feeding behaviour, species composition and human behavioural influence on the malaria vectors and how this may have impacted malaria transmission dynamics in Atatam, a small rural community in the Adansi Asokwa District of the Ashanti region. It is evident from research that defeating malaria requires targeted interventions to manage the shifting behaviour of vectors (Okumu *et al.,* 2022). Combating malaria requires an understanding of the biting behaviour of malaria vectors, including the time, place, and frequency of human exposure to infectious mosquito bites in the environment. The current study's observations of outdoor human biting activity also suggest that it might contribute to the spread of malaria, highlighting the need for integrated vector management control strategies for more efficient vector management.

Numerous research (Braack *et al*., 2015; Govella *et al*., 2023; Milali *et al*., 2017) on the biting behaviour of African anophelines have shown that, even before the use of LLINs, variations in biting behaviour exist with respect to both locations (indoors/outdoors) and time frames. The implementation of LLINs had no effect on biting behaviour in certain regions, but in other areas, the biting time of mosquitoes changed, shifting to early evening or late morning. Only a few studies, with inconsistent findings, evaluated the biting behaviour of the vectors both before and after the implementation of LLINs (Moiroux *et al*., 2012a). This study however evaluated biting behaviour after implementation of LLINs.

The study found *Anopheles gambiae* s.l and *Anopheles funestus* s.l to be the predominant vector species biting humans in Atatam. This confirms reports from Riveron *et al*. (2016) and Mugenzi *et al.* (2022) indicating the major Anopheles species feeding on humans in the community. Previous studies in the Atatam community also showed populations of *Anopheles gambiae* s.l and *Anopheles funestus* s.l to alternate with *An*. *gambiae* s.l being predominant during the rainy season and *An*. *funestus* dominating in the dry season (Riveron *et al*.,2016 & Mugenzi *et al.,* 2022). However, findings from this study showed *Anopheles gambiae* s.l to be the most abundant vector species biting humans during minor rainy season, the dry season and the major rainy season. The population of *Anopheles funestus* s.l significantly reduced during the major rainy season. The change in the population abundance or density of *Anopheles funestus* overtime may be due to the recent nationwide distribution of LLINs including synergist-based nets and insecticide combination nests which are effective against pyrethriod resistant vector populations (Menze *et al*., 2020). Furthermore, illegal mining activities in the community which presents ecological threats including destroying preferred breeding sites of *Anopheles funestus* populations and favoured the breeding of *Anopheles gambiae* s.l populations due to their behavioural plasticity. *Anopheles funestus* s.l larvae are typically found in large, semi-permanent freshwater bodies with emergent vegetation surrounding their borders, such as ponds, swamps, weedy and grassy sections of rivers, streams, furrows, and ditches (Dia, 2013). Their aquatic stages can barely survive in water bodies that are directly exposed to sunlight; instead, they strongly favour shaded habitats and use emergent vegetation as a location to hide from predators. For this reason, the presence of vegetation is crucial for their survival (Dia, 2013).

The study further revealed that, late night peak biting activity indoor and outdoor which is the historical biting time (Mukisa *et al*., 2024) was exhibited by *Anopheles gambiae* s.l in the minor

rainy season, the dry season and during the major rainy season. This biting activity of *An. gambiae* s.l during the late-night hours coincides with times people are indoors and asleep. Because the peaks of *An. gambiae* late-night biting behaviour coincides with sleeping hours, adhering to LLIN usage could provide protection from infectious bites during this period.

Moreso, early morning (04 – 05 h) indoor peak biting activity was observed in the minor and major seasons with both indoor and outdoor early morning biting peaks observed for *An. gambiae* s.l in the dry season. This shift in behaviour is worrisome as this may lead to increase in human biting rate, resulting in residual malaria transmission. The shift in biting times for *An. gambiae* s.l coincides with times when people are usually out of bed and less protected by LLINs.

Early morning (04 – 06) peak biting activity across the three seasons was also observed for *An. funestus* with late night (01 – 02 h) outdoor peak biting activity observed during the dry season. It was observed during the dry season that individuals spend time outdoors during sleep hours due to high indoor temperatures, which made unprotected individuals and vector biting activity overlap in time and place. The high indoor biting despite this behaviour suggests these populations may be developing behavioural tolerance, continuing to bite indoors but potentially shifting to earlier or later biting times to avoid insecticide exposure, while still preferring the indoor environment. Indoor biting may provide better protection from desiccation and predators during the dry season, making indoor biting part of an overall survival strategy that increases during environmentally stressful periods as observed during the dry season.

Also, the selection pressure brought on by the LLINs including synergist-based nets and insecticide combination nests which is effective against *An. funestus* populations (Menze *et al*., 2020), may have led to behavioural modifications, giving early-biting vectors a selective advantage, causing a behavioural shift in the population. These behavioural modifications may lead to residual malaria

transmission, which undermines the effectiveness of interventions especially those that target indoor feeding human vectors. These findings however confirm works done by Otto, (2015) and Moiroux *et al.,* (2012a) where there is a behavioural shift from nighttime to early morning aggressiveness in *An. funestus* in Kenya and Benin respectively after high LLIN coverage.

Human biting rate (HBR) by *An. gambiae* s.l in the major rainy season was high as individuals were at a risk of receiving averagely 194.56 bites every night. Rainy season provides abundant breeding sites such as puddles, ditches, and stagnant water, which facilitate an explosion in mosquito populations (Siraj *et al*., 2014). A higher mosquito density, combined with increased outdoor human biting rates, leads to greater human exposure to mosquito bites and thus to malaria parasites. Also, high outdoor HBR by *An. funestus* in the dry season may be attributed to people spending long hours outdoors during sleep hours. This is an indication from the study that, there is a high rate of outdoor biting/feeding amongst the malaria vectors in the study community. This pattern corresponds to a study by Nalinya *et al*., 2022 where it was indicated that in Ghana, outdoor sleeping during the dry season is also a major factor contributing to peak outdoor biting in the late evenings. Akuoko *et al*., 2024 also stated that outdoor sleeping was common among individuals in the Sahel savannah areas during the dry season.

Out of all the Anopheles mosquitos collected, majority were exophagic (bite outdoors), with 54.3 % of *Anopheles gambiae* s.l and 53.9 % of *Anopheles funestus* s.l were outdoor biting mosquitos. Historically, many interventions such as IRS and LLINs have been designed to target mosquitoes that bite indoors (endophagic) and rest inside houses (endophilic) (Carnevale & Manguin, 2021). However, as mosquito behaviour changes due to various factors including insecticide resistance, environmental changes, and adaptation, more mosquitoes are biting outdoors, which undermines the effectiveness of conventional control methods such as IRS and LLINs (Sougoufara *et al*.,

2020). Similar patterns were reported from a study in Senegal on *An. gambiae* s.l and *An. funestus* (Sougoufara *et al*., 2020), and in Tanzania where *An. gambiae* and *An. funestus* exhibited outdoor biting after introduction of LLINs (Russell *et al*., 2011). The significance of the rise in outdoor biting activity could be attributed to the mosquitos' response to indoor-based interventions, such as the excito-repellence properties of pyrethroids used in LLINs (Moiroux *et al*., 2012a).

These show growing evidence of the importance of outdoor transmission, especially, since most current tools focus more on indoor mosquito populations which may no longer be adequate for transmission prevention due to fast changing composition and biting/feeding behaviour. This may lead to increased burden on vulnerable populations such as agricultural and labourer workers in rural communities, children and pregnant women involved in outdoor activities such as socializing, selling or domestic chores. This is especially problematic in regions where malaria transmission is high. The shift towards outdoor biting by malaria mosquitoes may make it harder to reach malaria elimination targets.

LLINs ownership (93.3 %) and usage (100 %) amongst households proves the extensive work of the National Malaria Elimination Program in the attempt to reduce the malaria burden. This was in line with the work that the National Malaria Elimination Programme (NMEP) and its partners have been doing since 2006 to encourage LLIN ownership and use in Ghana. These efforts were boosted by the work of other organizations such as the Malaria Awareness Campaign (MAC) organized in the community in 2021 and 2023. LLINs which act as barrier to reduce the number of mosquito bites people receive indoors during the nighttime has caused the mosquitos to change their biting behaviour. This study also revealed people only washed the net when it is dirty, which follows user recommendation to wash LLINs only when they become visibly dirty as it minimizes unnecessary washing and helps the insecticide last longer (Gnanguenon *et al*., 2014). Observing

to ascertain how people were putting the LLINs to use also revealed 46.4 % of households using the nets had more than two people sleeping under one net. Due to sleep behaviours, some people ended up outside the LLIN while sleeping which exposed them to mosquito bites. LLINs are typically designed to cover one or two people. If more than two people share the net, it may not provide full protection, increasing the risk of mosquito bites and malaria transmission.

When the net is overcrowded, people may touch the net while sleeping. Mosquitoes can bite through the net if skin is pressed directly against it, reducing the protective barrier that the LLIN provides. The more people sharing the net, the more strain it places on the fabric, increasing the likelihood of tears or holes, which was notable during the study as LLINs aged 11 months or more were the ones holed or torn. Damaged nets are less effective at preventing mosquito entry.

Human behavioural pattern such as outdoor presence during night hours overlapped with vector biting dynamics. The study revealed most human activities before and after bedtime were outdoors. This may have accounted for the vector outdoor abundance and biting. Moreso, mud with iron sheet roofing is the housing structure used in the community. Mud walls with open eaves are known to provide better resting surfaces and more entry points to malaria mosquitoes (Ghebreyesus *et al*., 2000, Kirby *et al*., 2008) and this favour entry for malaria-spreading mosquitoes.

CHAPTER SIX

6.0 CONCLUSION AND RECOMMENDATIONS

6.1 CONCLUSION

Anopheles gambiae s.l and *Anopheles funestus* s.l were found to be the predominant vector species responsible for malaria transmission in the community. By measuring a number of variables that define the dynamics of vector abundance and biting behaviour, including net ownership and use, human sleeping behaviour, seasonality, and mosquito biting rate, it was confirmed that shift in biting time from the historical late night to early morning biting (indoor and outdoor) of *An. funestus* and the early morning outdoor and indoor biting of *An. gambiae* could be caused by pressure from the LLINs or humans spending more time outdoors without protection.

Also, high *An. gambiae* density across the three seasons could be attributed to numerous breeding sites created by the activities of illegal mining. These findings have important implications for the epidemiology and strategies for the control of malaria in the study area. Additional control strategies are needed for ongoing interventions to better address the issue of outdoor malaria transmission and reduce indoor and outdoor biting vectors using a more diverse toolbox with integrated vector management strategies.

6.2 RECOMMENDATION

- It is recommended that; attention should be given to intervention tools which targets vector populations biting individuals during times that they are not protected as this behaviour presents a gap in protection given by LLINs and IRS especially in rural settings.
- The distribution of LLINs should be in synchronization with their life span. LLINs lose effectiveness over time due to wear, tear, and reduced insecticide potency. Distributing new nets before old ones expire insures continuous protection.

6.3 LIMITATIONS OF THE STUDY

- Sporozoite infectivity rates was not carried out to determine entomological inoculation rates in the community.
- Molecular species identification was not carried out to know the *Anopheles* vector populations which might be responsible for malaria transmission in the community.
- The duration of the collection was not enough to present trends that are conclusive.

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Appendix

Appendix 1:

Table 1: The influence of place of biting in a community (indoor and outdoor) and major malaria vectors on the abundance of mosquitoes, while accounting for seasonal variability.

	Abundance of major malaria vectors				
Predictors	Estimate	SE	CI	Statistic	P-value
(Intercept)	1.65	0.70	0.27 – 3.03	2.34	0.019
Outdoor	-0.03	0.14	-0.30 – 0.25	-0.19	0.852
Anopheles gambiae s. l.	0.96	0.12	0.72 – 1.20	7.87	**<0.001**
Outdoor × *Anopheles gambiae s. l.*	0.16	0.17	-0.16 – 0.49	0.99	0.322
Random Effects					
σ2			0.45		
τ00 Season			1.46		
ICC			0.76		
N Season			3		

Observations	482
Marginal R2 / Conditional R2	0.115 / 0.791

Appendix 2

Survey on the Ownership and Usage of Insecticide Treated Net and Human Behavioural Study in ATATAM community.

We will appreciate if you could help us complete this form. Any information obtained in connection with this study that can be identified with you will remain confidential as the research is for only academic purposes.

Questions	Responses	
1. Age group	0 – 5 years	
	6 – 15 years	
	16 – 20 years	
	21 years and above	
2. Gender	Male	
	Female	
3. Do you own an insecticide treated net?	Yes	No
4. Number of LLINs owned		
5. What type of net is it?		
6. Do you use it?	Yes	No
7. If yes, how often do you use it?	Always	Occasionally
8. If no, which other intervention tools do you use? Or what is the reason for not using at all		
9. Source of LLINs	Self-purchase	
	From health facility	
	From mass campaign	
10. How many people sleep under one net?	(1-2) persons	> 2
11. How often do you wash the net?	Every month	
	Whenever it's dirty	

	Every 4 months	
	never	
12. Age of LLIN currently being used		
13. Status of net	Net still intact	
	Has developed holes/torn	
14. What time do you normally sleep?	Before 7pm	
	Before 8pm	
	Before 9pm	
	Before 10pm	
	After 10pm	
15. What activities are you involved in whiles awake?	Domestic chores	
	Playing	
	Social-economic activities (ie selling, social gathering; watching television, night vigil)	
	Galamsey	
16. Where do you spend the most time whiles awake?	Indoor	
	Outdoor	
17. What time do you get out of bed?	Between 3 – 4 am	
	After 4 am	
	After 5 am	
	After 6 am	
18. What activities are you involved in when you are awake?	Domestic chores	
	Agricultural activity	
	Religious activity	
	Galamsey	
19. Where do you spend the most time whiles awake?	Indoor	
	Outdoor	
20. Type of Housing Structure	Mud with thatch roofing	

	Mud with iron sheet roofing
	Concrete with iron sheet roofing

Appendix 3: Ethical clearance letter

RADIOLOGICAL AND MEDICAL SCIENCES RESEARCH INSTITUTE

Ghana Atomic Energy Commission

P.O. Box LG 80

Legon – Accra

In case of reply, number and date of this letter
Our Ref.: RAMS/RPT/SF.43/350

Your Ref.: .....

Date: 15th March, 2022

ETHICAL CLEARANCE

ID No: **RAMS/ERCP/SS/01/2021**

The Ethical Review Committee of the Radiological and Medical Sciences Research Institute reviewed your amended proposed Study Protocol titled: **"Characterization of the Evolution of Insecticide Resistance, The Major Malaria Vector Anopheles Funestus and its Impact on Control Tools in Ghana" (RAMS/ERCP/SS/04/2021)**

Project Coordinator: Dr. Michael Yao Osae

GAEC/BNARI

P. O. Box LG 80,

Legon –Accra 0244 856 008

mosae5@yahoo.com

This approval requires that you submit the final full review to the Ethical Review Committee (ERC) at the completion of the study. The Ethical Review Committee may observe or cause to be observed procedures and records of the study during and after implementation.

Please note that any modification of the project must be submitted to the Ethical Review Committee for review and approval before its implementation.

You are also required to report all serious adverse events related to this study to the Ethical Review Committee within seven days verbally and fourteen days in writing.

You are requested to inform the Ethical Review Committee and your mother Organization before any publication of the research findings.

Please always quote the protocol identification number in all future correspondence in relation to this protocol.

Signed …… **Prof. George Obeng Adjei Chairman RAMSRI ERC**

Appendix 3: Data Sheet for human landing catches

HUMAN LANDING CATCHES - Species Identification (Morphological)

Community name:..... Community Code : ……. Sampling Round: ..... Place of collection: Indoor/Outdoor

Date of Collection: Date of Identification: Weather Conditions.

GPS: Latitide: Longitude: Elevation:

Time of Collection	Number Caught	SPECIES																							
		An. gambiae s. l.				*An. funestus s.l.*				An.:co, ga,ss				Culex (Specify):				*Aedes (Specify):*				*Mansonia*			
		UF	BF	HG	G	UF	BF	HG	G	ph	ph	HG	G	UF	BF	HG	G	UF	BF	HG	G	UF	BF	HG	G
6:00-7:00																									
7:00-8:00																									
8:00-9:00																									
9:00-10:00																									
10:00-11:00																									
11:00-12:00																									
12:00-1:00																									
1:00-2:00																									
2:00-3:00																									
3:00-4:00																									
4:00-5:00																									
5:00-6:00																									
Total																									

Keys for Anopheles: co = coustanii, ha = hargreaves, ph = pharoensis, ni = nili, ru = rufipees

Keys for Mansonia: af = africana, un = uniformis, me = metallicus