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INFLUENCE OF TEMPERATURE ON THE GROWTH, DEVELOPMENT AND SUSCEPTIBILITY OF *ANOPHELES GAMBIAE (S.L.)* MOSQUITOES TO PYRETHROIDS

BY

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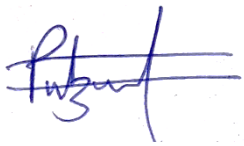
DECLARATION

I, Thomas Peprah Agyekum, declare that, except for references to other people's work, which have been duly acknowledged, this thesis, submitted to the School of Public Health, University of Ghana, is the result of my original research, and that the thesis has not been presented for any degree elsewhere. This thesis write-up was done under the joint supervision of Professor Julius Fobil, Dr. John Arko-Mensah, Professor Jonathan Hogarh and Dr. Paul Kingsley Botwe.



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DEDICATION

This work is dedicated to God Almighty for His guidance and protection through this academic journey. I also dedicate this work to my parents, Mr. Samuel Agyei Agyekum and Madam Comfort Boakyewaa, and my siblings for all the support. To my lovely wife, Abigail and our son, Nana Yaw, I appreciate all the sacrifices and understanding.

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TABLE OF CONTENT

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

AAEP	American Association of Equine Practitioners
AChE	Acetylcholinesterase
ANOVA	One-way analysis of variance
ARPPIS	African Regional Postgraduate Program in Insect Science
CDC	Centre for Disease Prevention and Control
CDNB	1-chloro-2,4-dinitrobenzene
CE	Carboxylesterases
CEC1	Cecropin
CI	Confidence interval
CO_2	Carbon Dioxide
CTAB	Cetyl Trimethyl Ammonium Bromide
DDT	Dichloro-diphenyl-trichloroethane
DEF1	Defensin
DFID	Department for International Development
DNA	Deoxyribonucleic acid
DTNB	Dithio-bis2-nitrobenzoic acid
ECDC	European Centre for Disease Prevention and Control
EFSA	European Food Safety Authority
EIP	Extrinsic Incubation Period

EPA	Environmental Protection Agency
FAO	Food and Agriculture Organization
GABA	Gamma-Amino Butyric Acid
GSTs	Glutathione-S-Transferases
H_2O_2	Hydrogen Peroxide
HD	High Definition
IGS	Intergenic spacer
IPCC	Intergovernmental Panel on Climate Change
IQR	Interquartile Range
IRAC	Insecticide Resistance Action Committee
IRS	Indoor Residual Spraying
ITNs	Insecticide Treated Nets
JEV	Japanese Encephalitis Virus
K_3PO_4	Potassium Phosphate
KDR	Knockdown Resistance
KDT	Knockdown time
KRR	Knockdown Resistance Ratio
LED	Light Emitting Diode
LLINs	Long-lasting insecticidal nets
MFO	Mixed-Function Oxidases

MGSTs	microsomal Glutathione-S-Transferases
NMIMR	Noguchi Memorial Institute for Medical Research
NOS	Nitric Oxide Synthase
NSE	Non-Specific Esterases
OC	Organochlorine
OD	Optical Density
OLS	Ordinary least square
OP	Organophosphorus
PBO	Piperonyl butoxide
PCR	Polymerase Chain Reaction
PMI	President's Malaria Initiative
POPs	Persistent Organic Pollutants
PRISMA	Preferred Reporting Items for Systematic Reviews and Meta-Analyses
RDL	Resistance to Dieldrin
rDNA	ribosomal DNA
RoB	Risk of Bias
ROS	Reactive Oxygen Species
SD	Standard Deviation
SDG	Sustainable Development Goal
SINV	Sindbis Virus

SYRCLE's	Systematic Review Center for Laboratory Animal Experimentation's
TMBZ	Tetramethylbenzidine
UG-IACUC	University of Ghana Institutional Animal Care and Use Committee
UNDP	United Nations Development Program
UNFCCC	United Nations Framework Convention on Climate Change
UNICEF	United Nations International Children's Emergency Fund
UV	Ultra Violet
VNVL	Vestergaard Noguchi Memorial Institute for Medical Research Vector Labs
WHO	World Health Organization
WMO	World Meteorological Organization
WNV	West Nile Virus
ZIKV	Zika Virus

OPERATIONAL DEFINITION OF TERMS

Biting rate: The daily feeding rate of a vector on a host (Paaijmans et al., 2013a).

Development time: It is the time (in days) from the hatching of mosquito eggs to adult emergence.

Extrinsic incubation period: It describes the time it takes for parasites to develop in the mosquito from the point of ingestion via an infected blood meal through to the point at which sporozoites enter the salivary glands and the mosquito becomes infectious (Ohm et al., 2018).

Fecundity: It is expressed as the number of eggs laid per female mosquito (Mamai et al., 2017).

Gonotrophic cycle length: The average number of days that gravid mosquitoes took to oviposit after taking a blood meal (Mala et al., 2014).

Immature mosquito: This consists of the egg, larval and pupal stages of mosquitoes.

Larval source management: the management of aquatic habitats (water bodies) that are potential larval habitats for mosquitoes to prevent the completion of development of the immature stages (WHO, 2013a).

Longevity: It is the number of days a mosquito lives after emergence.

Metabolic enzymes: They are enzyme systems that insects have developed to help them naturally detoxify insecticides and other foreign compounds (FAO, 2012; Gatton et al., 2013).

Number of adults produced: The number of adult mosquitoes emerged from the total number of larvae.

Pupation success: It is the proportion of larvae that pupated from the larval stage.

Resistant population: a population is considered resistant if its response to an insecticide in detection tests drops significantly below its normal response (Georghiou & Mellon, 1983).

Susceptible population: a population that has not been subjected to insecticidal pressure and in which resistant individuals are either absent or rare (WHO, 2016a).

Time to pupation: It is estimated as the time from egg hatching to the onset of pupation.

ABSTRACT

Background: *Anopheles* mosquitoes are responsible for transmitting malaria and lymphatic filariasis. They are among the notable vector species for their crucial role in transmitting malaria. The survival of the vector is of great interest as it affects its ability to transmit diseases. The biology and ecology of mosquitoes are strongly dependent on ambient temperature. The mosquito's life cycle includes four stages: egg, larva, pupa and adult. Indeed, the rearing temperature of the immature stages (egg, larva, and pupa) can significantly impact the completion of the life cycle, the overall fitness of the adult, and ability to transmit disease. In recent years, global warming and possible future warmer climate have prompted many studies to focus on the effects of elevated temperatures on both the morphology and the biology of various species, including vectors. Despite the importance of temperature variability on *An. gambiae (s.l.)* mosquito's development and survival, there is still the need to explore how and whether or not elevated temperatures associated with climate change is likely to reduce or increase the vector's population dynamics by modifying the life cycle, reduce the efficacy of insecticides, and increase the expression of metabolic enzymes in *An. gambiae (s.l.)* mosquitoes.

Objective: This study aimed to investigate the influence of elevated temperatures on the growth and development of *An. gambiae (s.l.)* mosquitoes, and the effectiveness of pyrethroid insecticides in such higher temperatures.

Methods: *Anopheles gambiae (s.l.)* eggs were obtained from colonies established in the laboratory and were incubated, hatched and reared under eight temperature regimes (25, 28, 30, 32, 34, 36, 38 and 40 °C) using climate-controlled incubators (RTOP-1000D, Zhejiang, China), with photoperiod of 12:12 (L:D) and 80 ± 10% relative humidity. Larvae were fed 10 mg of TetraFin goldfish flakes (Tetra Werke, Melle, Germany). All adults were fed with a 10%

sugar solution soaked in cotton wool. In addition, female mosquitoes used to estimate fecundity and longevity were blood-fed using a guinea pig on day four (4) post-emergence. Larvae were monitored daily for development to the next stage. The time to pupation, pupation success, number of adults produced, and sex ratio of the newly emerged adult was recorded. Molecular identification of *An. gambiae (s.l.)* mosquitoes was done using polymerase chain reaction (PCR) to identify the composition of sibling species in the *An. gambiae* complex. Larval survival and adult longevity were monitored every 24 hours, and data were analyzed using Kaplan-Meier survival analysis. Furthermore, analysis of variance (ANOVA) was used to assess the relationship between temperature and development time, time to pupation, length of the gonotrophic cycle, biting rate and fecundity. Kruskal-Wallis test was also used to assess the relationship between temperature and pupation success, pupal mortality, the number of adults produced, and sex ratio.

Digital images of larvae, pupae, adult wings and proboscis were captured using stereo microscope with inbuilt camera (Leica EZ4 HD, Leica Microsystems Limited, Switzerland) and body parts were measured using Leica Application Software, version 3.4.0 (Leica Microsystems Limited, Switzerland). Data on larval, and pupal weight and size, adult weight, size and proboscis length were log-transformed and analyzed using ordinary least square (OLS) regression with robust standard errors. In addition, three to five-day-old non-blood-fed *An. gambiae (s.l.)* mosquitoes were used for insecticide susceptibility test following the WHO bioassay protocol. Batches of 20 – 25 non-blood-fed female adult *An. gambiae (s.l.)* mosquitoes from each temperature regime (25 – 32 °C) were exposed to two pyrethroid insecticides (0.75% permethrin and 0.05% deltamethrin). The knockdown rate after 60 min and mortality at 24 h were recorded. The levels of four metabolic enzymes (MFO, GST, α-EST and β-EST) were examined in both mosquitoes that were not exposed and those exposed to pyrethroids.

Results: *An. gambiae (s.l.)* mosquitoes used in this study consisted of *An. gambiae (s.s.)* and *An. coluzzii*. Development time of immature mosquitoes significantly decreased ($F(5, 24) = 133.55$, $P < 0.001$) with increasing temperature. Log-rank test showed that larval survival ($X^2(6) = 5353.12$, $P < 0.001$) decreased with increasing temperature. In addition, Kruskal-Wallis test showed that the number of adults produced ($X^2(5) = 28.16$, $P < 0.001$) decreased with increasing temperature, with male mosquitoes disproportionately produced at higher temperatures than females. Larval ($\beta_{\text{larval size}} = 0.11$, 95% CI; 0.14, 0.09, $P < 0.001$) and pupal ($\beta_{\text{pupal size}} = 0.12$, 95% CI; 0.14, 0.10, $P < 0.001$) size significantly decreased with increasing temperature. Furthermore, longevity of both blood-fed (log-rank test; $X^2(4) = 904.15$, $P < 0.001$) and non-blood-fed (log-rank test; $X^2(4) = 1163.60$, $P < 0.001$) mosquitoes decreased with increasing temperature. The results further showed that the fecundity of mosquitoes significantly ($F(2,57) = 3.46$, $P = 0.038$) reduced with increasing temperature. Body size and proboscis length also decreased with increasing temperature. The mortality of *An. gambiae (s.l.)* mosquitoes to pyrethroids decreased at temperatures above 28 ºC. Mosquitoes reared at higher temperatures were more resistant to the insecticides tested (deltamethrin and permethrin) and had more elevated enzyme levels than those reared at low temperatures ($P < 0.05$).

Conclusion: Mosquitoes could not breed beyond temperatures at 36 ºC. Therefore, if the ambient environmental temperatures rise to 36 ºC, possibly as a consequence of climate change, it is likely to reduce or inhibit malaria transmission and perhaps its eradication in a future warmer temperature. In conclusion, warmer temperature is potentially hostile to a considerable proportion of emerging mosquitoes and may inhibit their survival such that the numbers of potential vectors may decrease. This study contributes to the knowledge on the relationship between temperature and growth and development of *An. gambiae (s.l.)*

mosquitoes and provides helpful information for modelling vector population dynamics in a future warmer climate.

PREFACE

Publications related to this work

Agyekum, T. P., Botwe, P. K., Arko-Mensah, J., Issah, I., Acquah, A. A., Hogarh, J. N., Dwomoh D., Robins T. G., & Fobil, J. N. (2021). A systematic review of the effects of temperature on *Anopheles* mosquito development and survival: implications for malaria control in a future warmer climate. *International Journal of Environmental Research and Public Health, 18*(14),7255. https://doi.org/10.3390/ijerph18147255.

Agyekum, T. P., Arko-Mensah, J., Botwe, P. K., Hogarh, J. N., Issah, I., Dwomoh, D., . . . Fobil, J. N. (2022). Effects of elevated temperatures on the development of immature stages of *Anopheles gambiae (s.l.)* mosquitoes. *Tropical medicine and international health, 27*, 338–346. https://doi.org/10.1111/tmi.13732.

Agyekum, T. P., Arko-Mensah, J., Botwe, P. K., Hogarh, J. N., Issah, I., Dwomoh, D., . . . Fobil, J. N. (2022). Effects of Elevated Temperatures on the Growth and Development of Adult *Anopheles gambiae (s.l.)* (Diptera: Culicidae) Mosquitoes. *Journal of Medical Entomology, 59*(4), 1413-1420. https://doi.org/10.1093/jme/tjac046.

Agyekum, T. P., Arko-Mensah, J., Botwe, P. K., Hogarh, J. N., Issah, I., Dadzie, S. K., . . . Fobil, J. N. (2022). Relationship between temperature and *Anopheles gambiae sensu lato* mosquitoes' susceptibility to pyrethroids and expression of metabolic enzymes. *Parasites & vectors, 15*(1), 163. https://doi.org/10.1186/s13071-022-05273-z.

CHAPTER ONE

INTRODUCTION

1.1 Background

Anopheles mosquitoes represent a major public health threat because of the diseases they transmit (Benelli, 2015), which overall, forms a significant part of all morbidity and mortality records (Brito et al., 2013). They are responsible for transmitting pathogens such as malaria parasites, arboviruses and filarial worms (Gendrin & Christophides, 2013). In Ghana, malaria is an endemic disease, and the prevalence of malaria still accounts for 38.0% of all outpatient visits with children under 5 years being the most vulnerable groups (Ejigu & Wencheko, 2021).

The control of diseases transmitted by *Anopheles* mosquito through infective bites rely strongly on chemical insecticides; use of impregnated treated nets (ITNs), outdoor spraying and indoor residual spraying (IRS) (Nnko et al., 2017). In an attempt to control the vector, four main classes of insecticides – pyrethroids, organochlorines, carbamates, and organophosphates have been used historically (Liu et al., 2011; Baffour-Awuah et al., 2016). Of all these insecticides, the pyrethroid class has been widely used to control *Anopheles* mosquitoes in recent years. However, there have been reports of increasing resistance of the vector to the insecticide (Hunt et al., 2011; Ranson et al., 2011; Dadzie et al., 2017; Mouhamadou et al., 2019).

Anopheles mosquitoes are poikilotherms (i.e. have to survive and adapt to environmental stress), therefore their growth and development characteristics rely on ambient temperatures (Reinhold et al., 2018). These characteristics comprise the length of gonotrophic cycles, biting rate, fecundity, survival and development of the immature mosquitoes (Vantaux et al., 2016). Consequently, any factor capable of modifying any of these characteristics can influence the

potential of mosquitoes to transmit diseases. Temperature is a typical example of the factors that could affect the ecology and biology of *Anopheles* mosquitoes and their potential to transmit diseases (Alto & Bettinardi, 2013; Ezeakacha & Yee, 2019).

Over the past two decades, atmospheric temperature has been increasing (UNFCCC, 2007), and this is projected to affect the development times and the vectorial capacity of *Anopheles* mosquitoes (Mohammed & Chadee, 2011). Climate parameters such as temperature, humidity, and rainfall not only can substantially affect the growth and development of mosquitoes, but also the sporogonic development of malaria parasites, *Plasmodium* spp. within their bodies (Guerra et al., 2010; Hay et al., 2010; Afrane et al., 2012). Regards to *Anopheles* mosquitoes, studies conducted on growth and development characteristics and temperature have focused mainly on the development of the immature (egg, larvae and pupae) stages (Christiansen-Jucht et al., 2014) with little attention paid to the adult.

Temperature variations can stress the adult mosquito, and the stress can make them more susceptible to external stressors leading to death (Lafferty & Mordecai, 2016). This may seem to imply that insecticide exposure (stressor) could induce high mortality or may go a long way to increase insecticide resistance. On the contrary, temperature has been shown to affect the efficacy of insecticides against mosquito species such as *An. arabiensis, An. funestus, An. gambiae (s.s.),* and *An. stephensi* (Glunt et al., 2014; Oxborough et al., 2015; Glunt et al., 2018). It is unclear how rearing temperature may affect the efficacy of insecticides and the susceptibility of or resistance of *Anopheles gambiae* mosquitoes (the predominant malaria vector in Ghana) to insecticides. In addition, temperature affects the mosquito's immune system (Murdock et al., 2012a; Murdock et al., 2013), decreases molecular stability while increasing enzyme function and membrane permeability (Lafferty & Mordecai, 2016). Yet, it is still

unclear how variations in rearing temperature affect the expression of metabolic enzymes in mosquitoes (Qin et al., 2014; Camara et al., 2018).

A growing body of literature recognizes the importance of how temperature affects the development and survival of *An. gambiae (s.l.)* mosquito since the parasite's development and disease transmission depend on the survival of the vector (Rajatileka et al., 2011). In sub-Saharan Africa, *An. gambiae (s.l.)* mosquito is one of the most predominant and important malaria vectors (Baffour-Awuah et al., 2016; Riveron et al., 2016). Elevated temperatures associated with climate change are likely to influence population dynamics of mosquitoes, affect the efficacy of insecticides and expression of metabolic enzymes.

1.2 Problem Statement

Temperature variations could directly affect the dynamics of vector-borne diseases (Polgreen & Polgreen, 2017; Roberts et al., 2018) by modifying the risk of disease transmission, especially where the extrinsic incubation period (EIP) gets closer to the lifespan of the vector (Alto & Bettinardi, 2013). A projected increase in temperature in Africa (Sylla et al., 2016) is anticipated to affect malaria transmission by altering key growth and development characteristics of *Anopheles* mosquitoes (Davies et al., 2016). However, studies that have examined either the net effects of rearing or ambient temperature on the growth and development of both the immature stages and adult *An. gambiae (s.l.)* mosquitoes are rare (Christiansen-Jucht et al., 2014, 2015; Shapiro et al., 2017).

Temperature affects the population growth rates of mosquitoes by altering traits that enable these adult insects to live successfully in their habitats (Lyons et al., 2013). As a result of the high thermal conductivity of rearing water coupled with the inability to escape adverse conditions, the immature stages of mosquitoes are mostly affected by temperature (Oliver & Brooke, 2017). This may affect the growth and development of the immature stages of *An.*

gambiae (s.l.) mosquitoes (Afrane et al., 2012; Davies et al., 2016) and the overall fitness of the adult *An. gambiae (s.l.)* mosquito by altering key growth and development characteristics such as fecundity, body size, adult longevity, blood-feeding behavior, gonotrophic cycle length, and biting rate (Davies et al., 2016; Shapiro et al., 2017; Ezeakacha & Yee, 2019). These characteristics can affect mosquito survival and parasite development and influence disease transmission (Christiansen-Jucht et al., 2014).

The control of mosquitoes have focused on mainly the adult mosquito; however, vector control measures have not achieved the expected results owing to challenges such as inadequate financing (Shretta et al., 2016; WHO, 2017), gaps in control management (Kokwaro, 2009), and vector resistance to insecticides (Badolo et al., 2012; Riveron et al., 2015; Yewhalaw & Kweka, 2016). The toxicity and bioaccumulation of insecticides are influenced by temperature (Lushchak et al., 2018) and an increase in temperature could worsen vector control measures by reducing the efficacy of the insecticides, further increasing resistance (Glunt et al., 2018). Furthermore, an increase in temperature could increase the expression of metabolic enzymes (Angilletta Jr et al., 2009). Increase in metabolic enzymes could increase the detoxification of insecticides in mosquitoes, reduce the effectiveness of the insecticide, and increase resistance of mosquitoes to insecticides (Nardini et al., 2012; Panini et al., 2016). This may present a significant threat to malaria control and affect the achievement of sustainable development goals, especially goal 3 (ensure healthy lives and promote wellbeing for all ages) (UNDP, 2015).

Due to the sensitivity of mosquitoes to temperature, several studies have focused on the effects of temperature on mosquito and their ability to transmit diseases (Paaijmans et al., 2013a; Ciota et al., 2014). However, there are still data gaps regarding the effects of elevated temperatures on adult *An. gambiae (s.l.)* mosquitoes' traits such as fecundity, gonotrophic cycle length and

biting. For instance, few studies have considered the impact of temperature on gonotrophic cycle length and biting rate (Shapiro et al., 2017). Only a few studies; if any at all, have evaluated the effects of different rearing temperatures on the sex ratio of emerged adult *An. gambiae (s.l.)* mosquitoes. This provides significant information on the population dynamics of mosquitoes and could inform control interventions. Furthermore, there is little information on the effects of rearing temperatures on the susceptibility of adult *An. gambiae (s.l.)* mosquitoes to pyrethroid insecticides and the expression of metabolic enzyme levels (Kristan et al., 2018).

1.3 Conceptual Framework

This study considered only the effects of temperature on *An. gambiae (s.l.)* mosquitoes. However, varying climate parameters such as temperature, rainfall and humidity could strongly affect the growth and development of mosquitoes (Abiodun et al., 2016). Temperature is considered one of the most significant factors that affects biological processes and physiological functions, including growth and reproduction in ectotherms such as mosquitoes (Ezeakacha & Yee, 2019). It also plays a vital role in the growth and development of mosquitoes (Oliver & Brooke, 2017) and could modify the completion of the entire life cycle.

Temperature could affect the growth and development of both the immature (egg, larvae and pupae) and adult stages of mosquitoes. The conditions experienced at the immature stages could affect the overall fitness of the adult mosquito by affecting the longevity, fecundity, body size, length of the gonotrophic cycle and biting rate (Figure 1).

Temperature could significantly affect the metabolism and survival rate of insects and the efficacy of insecticides (Jaleel et al., 2020). Expression of metabolic enzymes such as mixed-function oxidase (MFO), Glutathione-S-transferases (GSTs), acetylcholinesterase (AChE), and non-specific esterase (NSE) in mosquitoes could detoxify insecticides more rapidly and render

the insecticides ineffective. This could lead to the resistance of mosquitoes to insecticides. Overall, the effects of temperature on the growth and development of both immature and adult mosquitoes, expression of metabolic enzymes and susceptibility of mosquitoes to insecticides could alter the vector's population dynamics and ultimately affect mosquito control efforts.

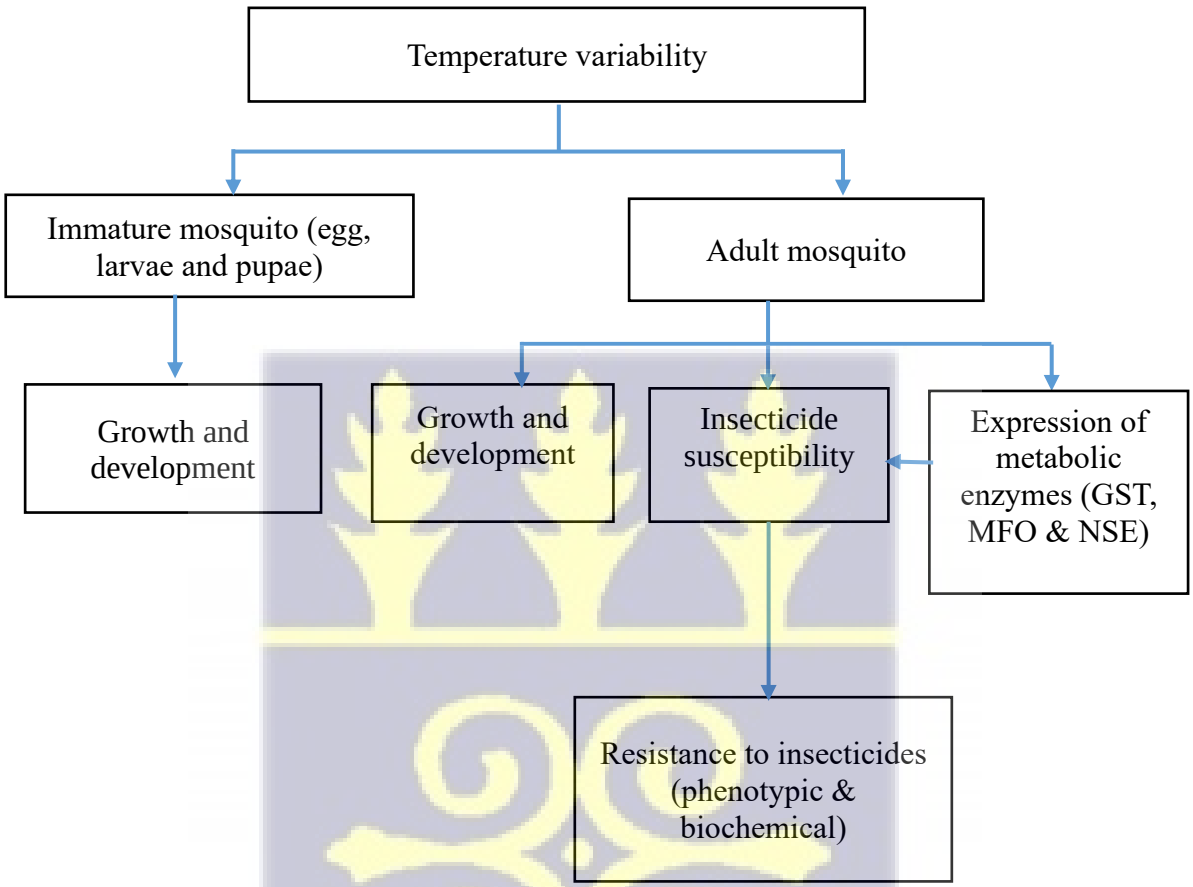

Figure 1: Conceptual framework showing the relationship between temperature and growth, development and susceptibility of mosquitoes to insecticides

1.4 Justification

Malaria is still a major public health concern in Ghana, and vector control has been a major intervention to reduce malaria burden. Thus, climate change or increasing temperature threatens the current control measures and studies that will increase our knowledge to be better prepared in developing policies for control is needed. It is not clear how temperature may affect the susceptibility of *An. gambiae (s.l.)* mosquitoes to insecticides or expression of metabolic enzymes. Therefore, understanding how temperature affects the growth and development, and the susceptibility of *An. gambiae (s.l.)* mosquitoes to insecticides in elevated temperatures is critical. This will help policymakers put measures to mitigate the effects of temperature on the mosquito population. In addition, findings could guide mosquito control interventions and safeguard public health.

1.5 General Objective

The general objective of the study was to investigate the influence of elevated temperatures on the growth and development, and susceptibility of *An. gambiae (s.l.)* mosquitoes to pyrethroid insecticides.

1.5.1 Specific Objectives

The specific objectives were to;

- Examine the influence of temperature on the developmental stages of *An. gambiae (s.l.)* mosquitoes.
- Assess the relationship between temperature and the growth and development of adult *An. gambiae (s.l.)* mosquitoes.
- Evaluate the effects of varying temperatures on the susceptibility of *An. gambiae (s.l.)* mosquitoes to pyrethroids and expression of metabolic enzymes.

1.6 Research Questions

This research sought to answer the under listed questions;

- Is there any relationship between temperature and the developmental stages of *An. gambiae (s.l.)* mosquitoes?
- Is there any relationship between temperature and growth and development of adult *An. gambiae (s.l.)* mosquitoes?
- What are the effects of temperature on *An. gambiae (s.l.)* mosquito's susceptibility to pyrethroids and expression of metabolic enzymes?

1.7 Research Hypotheses

The underlisted hypotheses guided the study.

- Increasing temperature will influence the developmental stages of *An. gambiae (s.l.)* mosquitoes.
- Increasing temperature will influence the growth and development of adult *An. gambiae (s.l.)* mosquitoes.
- Varying temperatures from 25 ^{o}C through to 40 ^{o}C will affect the susceptibility of *An. gambiae (s.l.)* mosquitoes to pyrethroids and expression of metabolic enzymes.

CHAPTER TWO

LITERATURE REVIEW

2.1 Introduction

This chapter provides an overview of key terms and concepts relevant to this thesis. The chapter presents biology of mosquitoes, control measures, insecticides used to control mosquitoes and resistance mechanisms. The existing gaps and limitations in the literature are also highlighted by assembling and evaluating the available evidence of the relationship between temperature and the development as well as survival of *Anopheles* mosquitoes. Research articles published up to March 2021 were systematically retrieved from PubMed, Science Direct, Scopus, ProQuest, Web of Science, and Google Scholar databases. The search strategies used keywords such as *Anopheles*, mosquito, malaria, temperature, temp*, season*, survival, insecticide resistance/susceptibility, metabolic enzyme, and longevity.

2.2 Biology of mosquitoes

Mosquitoes are regarded as the most important groups of arthropods, primarily because of their role in disease transmission (Do Nascimento et al., 2018). They belong to the family Culicidae and form the core of global entomological research because of their role as vectors in transmitting a wide array of debilitating parasitic and viral diseases that affect both humans and animals (Becker et al., 2010). Mosquitoes are slender insects with long legs, and are usually identified with scales on their bodies and their long proboscis (Harbach, 2007). About forty-one (41) genera of mosquitoes have been reported, and about 3500 species have already been reported from different parts of the world (Do Nascimento et al., 2018). These mosquitoes are grouped into two subfamilies of Anophelinae and Culicinae (Wilkerson et al., 2015; Foster et

al., 2017). There are three best-known genera of mosquitoes: *Anopheles*, *Aedes*, and *Culex* (Wilkerson et al., 2015).

Globally, mosquitoes are the most important insect pests that affect humans and animals (Gouge et al., 2016). They serve as vectors for several diseases, including malaria, dengue, lymphatic filariasis, and many other arboviral diseases, including Lassa and yellow fevers, which are accountable for hundreds of millions of morbidity and millions of deaths annually (WHO, 2019b). Projections by the World Health Organization (WHO) show that mosquito-borne diseases are the leading causes of morbidity and mortality in developing countries (WHO, 2005). However, not all mosquito species are of public health importance. From a medical perspective, the most important species belong to the following genera; *Aedes*, *Anopheles*, and *Culex* (ECDC, 2014). In addition to their impact on human health, mosquitoes play a crucial role in natural ecosystems. They serve as important pollinators and food sources for some animals such as amphibians, reptiles, birds, and mammals (Wong & Jim, 2016).

Mosquitoes are very successful insects because they can acclimatize to a wide range of habitats (Becker et al., 2010). Aside the deserts and perpetually frozen areas, mosquitoes are ubiquitous, and found in humid tropics, subtropics, warm moist climates, and the temperate and cool regions (Do Nascimento et al., 2018). The activities of mosquitoes are, to some extent, species-specific. For instance, while some species are active at night or sunset, others are active during the daytime (Harbach, 2007). The flight habits of mosquitoes are also species dependent. Most local species linger around their breeding places whereas other species travel very far from their breeding habitats. Usually, the female mosquitoes cover a longer flight distance compared to the males. It has been reported that some mosquitoes move as far as 75 miles (about 121 km) from the breeding sites, though on the average they are mostly within a mile or 2 miles from their breeding habitat (AAEP, 2016).

Generally, mosquitoes are highly attracted to humans and are well adapted to breeding places created by human activities. The use of water storage containers in animal husbandry and other farming activities such as fish ponds and irrigation systems offer suitable breeding conditions for anthropophilic mosquitoes (Egbuche et al., 2016). With regards to feeding, both the male and female mosquitoes depend on plant nectar and fruit sap for energy. However, the female counterparts require blood meal as an additional dietary requirement and source of protein for the development of their eggs (Silver, 2008). When it comes to blood meal sources, different species prefer different host animals. Whereas some feed on humans and birds, others prefer other animal hosts (Gouge et al., 2016).

The geography of the mosquito reveals a wide global diversity, and in Ghana, the following species; *Culex*, *Aedes*, *Anopheles*, and *Mansonia* mosquitoes, have been reported across the country (Ughasi et al., 2012; Kudom, 2015; Kudom et al., 2015a; Owusu-Asenso, 2018).

2.3 Life cycle of mosquitoes

The life cycle of mosquito (Figure 2) is a complete metamorphosis (Becker et al., 2010) which comprises four different stages: egg, larvae, pupae and adult, and the whole cycle requires almost two weeks (Tokachil et al., 2017). The adult stage is free-flying, while the first three stages are aquatic (Jackman & Olson, 2002). In addition, the first three stages are called the aquatic or immature stages. Only the adults are involved in disease transmission, although the dynamics of the immature stages (larvae and pupae) play a significant role in determining the fitness of the adult mosquito for disease transmission (Li, 2009).

2.3.1 Egg stage

The eggs of mosquitoes differ significantly among the major groups of species and individual species (Eldridge, 2008). In order to find potential oviposition sites, gravid mosquitoes depend on olfactory and visual signals. As these mosquitoes come closer to a site, they use olfactory, visual, and tactile alerts to appraise the quality of the site for oviposition (Day, 2016). Mosquitoes oviposit one at a time, and they float on the surface of the water. The eggs are laid either singly (e.g., *Anopheles* and *Aedes* species) or stuck together in floating rafts (e.g. *Culex* species). Some species (e.g. *Aedes*) oviposit just above the water line or on wet mud (these eggs hatch only when inundated with water) while *Culex* and *Anopheles* species lay their eggs on water (Osman, 2010). Other species like the *Mansonia* lay eggs as submerged clusters attached to roots, stems, and leaves of aquatic vegetation (Day, 2016). In addition, adult females oviposit in numerous different ways depending on the species. Females lay between 100 to 300 eggs (Genoud et al., 2019) after a blood meal. The eggs are vulnerable to desiccation and hatch within the second or third day; however, hatching may take up to two to three weeks in colder climates (Coleman, 2009). Many mosquito species usually oviposit during the dawn and twilight periods (Day, 2016). Generally, when the mosquitoes oviposit, the eggs are white but grow dark within few hours (Farnesi et al., 2017).

In general, mosquitoes can be grouped into two categories per their egg-laying behavior as well as whether or not their embryos undergo diapause (innately dogged resting period) or dormancy period (externally triggered resting period) (Becker et al., 2010). In the first category, called rapid hatch (Day, 2016), the embryos do not undergo diapause or dormancy and hatch after the embryonic development is completed. However, in the second category (delayed hatch), the eggs do not hatch right after oviposition (Becker et al., 2010) but later when conditions are favorable for the eggs to hatch. Under this category, the eggs are usually drought-resistant, stay alive for long periods outside the water, and hatch shortly after being re-flooded (Day, 2016).

2.3.2 Larval stage

Mosquito larvae inhabit different water bodies, including temporary or permanent, extremely polluted or clean, stagnant or flowing, small or large (Becker et al., 2010). The larva has a well-developed head and a mouth with brushes to feed, a large thorax, and a fragmented abdomen (Pwalia, 2014). The larval stage is the longest of the three immature stages (Beck-Johnson et al., 2013) and the only stage where feeding occurs, making it vital for nutritional reserves accumulation to develop the adult in the pupal stage. The larval stage responses to fluctuating conditions are crucial in population size regulation and mosquito control (Owusu et al., 2017).

Larvae progress into four instar stages before reaching the pupal stage (Madzlan et al., 2016). At each molt, the head capsule is amplified to the full-size features of the subsequent instar, though the body continues to grow. Thus, one can use how big the head capsule is as a correct morphometric pointer for the larval instar (Becker et al., 2010). The larvae feed on dissolved foods in the breeding sites during the first two instars. During the third and fourth instar, they mainly survive on bacteria, algae, and other microorganisms to hoard sufficient energy for the transformation and further developments that take place during the pupal phase (Barfi, 2015).

In terms of appearance and morphology, there is a significant difference between the larvae and the adults. The larval stage survives in water, and their feeding behavior and breathing structures clearly show this. In general, it is easy to classify mosquitoes to species at the larval stage than using the adults (Eldridge, 2008). Except for *Anopheles* larvae that lay parallel to the water surface because of lack of respiratory siphon, most mosquito larvae have a respiratory siphon, which dangles from the water surface (Ponlawat & Harrington, 2009; Osman, 2010). The larvae (e.g., *Ae. vexans*) occasionally come together in particular places at the breeding sites to reduce the chance of predation of any single larva (Becker et al., 2010).

2.3.3 Pupal stage

The pupal stage comes after the larval stage and it is the third stage of the mosquito's life cycle and also the final stage of the aquatic or immature stages (Kauffman et al., 2017). The mosquito pupae are also known as tumblers and present fewer characters beneficial for identification (Eldridge, 2008). The pupa looks like a comma shape with the head and thorax merged into a cephalothorax and the abdomen located below it. At this stage, key transformations occur, resulting in the metamorphosis of larval tissues into adult tissues (Coleman, 2009).

Naturally, the pupal head and thorax are joined into a protuberant cephalothorax which possesses anterolaterally two respiratory trumpets. These are linked to the mesothoracic spiracles of the emerging adults to supply oxygen (Becker et al., 2010). In addition, the pupae have two big structures known as paddles that project from the tip of the abdomen (Eldridge, 2008). The pupal phase, a resting and non-feeding stage, is when the mosquito turns into an adult and usually takes about two days (Osman, 2010).

2.3.4 Adult stage

This is the final stage of the mosquito's life cycle and the only stage which is not aquatic. This stage is finalized when gas is pushed through the pupal and the pharate adult cuticle and into its midgut. The newly emerged adult mosquito moves carefully to avoid dropping onto the water surface as its appendages linger partially in the exuvia (Becker et al., 2010). Upon its emergence, the adult mosquito seeks a safe hideout in the neighboring vegetation to enable its wings to develop fully (Agyekum, 2017). Like other insects, the adult mosquito has three different body regions: head, thorax, and abdomen (Eldridge, 2008).

At emergence, the male mosquitoes are not sexually developed, as they have to spin their hypopygium via 180^{o} before they are ready to mate with the female mosquitoes (usually takes about a day) (Becker et al., 2010). Because of this, the male mosquitoes emerge earlier (usually

1 – 2 days) than the females to reach sexual maturity at the same time as the incipient females (Genoud et al., 2019). The male mosquitoes mate with the females as soon as they emerge (Diabate & Tripet, 2015). After mating, the females hoard more sperm in their spermathecae (a receptacle in which sperm is stored after mating) to inseminate several egg batches without copulation (Becker et al., 2010).

In terms of morphology, there are differences between male and female mosquitoes. The female mosquitoes possess short palpi and an extended stiletto-like proboscis that in most species has structures called stylets adapted for piercing the skin of their host for a blood meal. However, the male mosquitoes have long hairy palpi in addition to a long fleshy proboscis adapted for sucking plant nectar and other fluids from fruits and flowers (Eldridge, 2008).

Mosquitoes feed on plant nectar and other fluids from fruits and flowers, but the female, in addition to this, requires a blood meal to develop its eggs (Nyasembe & Torto, 2014). In most mosquito species, the female needs a blood meal to complete oogenesis (production or development of an ovum). This has caused mosquitoes to develop an intricate host-seeking behavior to identify and feed on a potential host. Generally, this host-seeking distribution is dependent on the species, host accessibility, and season (Verdonschot & Besse-Lototskaya, 2014). Most species are non-autogenous, implying that after copulation, the female mosquitoes require a blood meal to complete the egg development (Becker et al., 2010). After the adult mosquitoes have emerged, they are ready to commence their life cycle all over again by feeding, mating, and laying eggs.

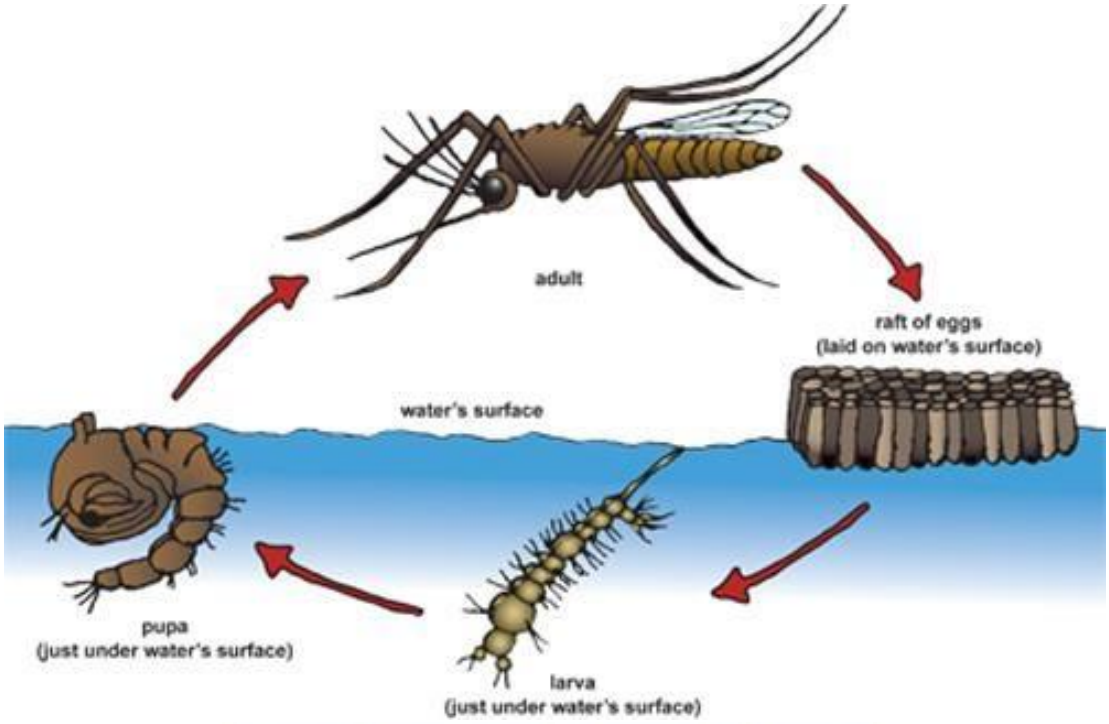

Figure 2: Life cycle of a mosquito

2.4 Common genera of mosquitoes

The well-known and medically essential genera of mosquitoes include *Anopheles*, *Aedes*, and *Culex* mosquitoes (Lebl et al., 2015).

2.4.1 *Anopheles* mosquitoes

Anopheles mosquitoes belong to the order Diptera, sub-order Nematocera, family Culicidae and sub-family Anophelinae (Agyepong et al., 2012). *Anopheles* mosquitoes usually breed in transparent, sunny, temporal water bodies like swampy areas, mining sites, foot and hoof print, roadside puddles, drainage trenches, and edges of boreholes (Baffour-Awuah, 2012). In addition, *Anopheles* mosquitoes are not limited only to these habitats; they look out for different habitats for breeding. According to Mattah et al. (2017), these mosquitoes breed within the environs of deteriorating infrastructure like poorly maintained drains, culverts, broken water pipes, car tire imprints on unpaved roads, market gardens/urban agricultural sites, open tins/cans, pools at construction sites, low lying areas that are prone to flooding, among others. However, some *Anopheles* species breed even in polluted or contaminated water bodies. For

instance, *An. gambiae sensu lato (s.l.)*, also referred to as *An. gambiae* complex, has been found in swamp extremely polluted with organic matter (Sattler et al., 2005). This contradicts the conservative view that *Anopheles* mosquitoes breed only in clear or clean water habitats (Baffour-Awuah, 2012).

Anopheles mosquitoes are the primary vectors responsible for the transmission of malaria in the world (Sokhna et al., 2013; Chabi et al., 2016; Huestis et al., 2017), and the vectors are continuously evolving (Sokhna et al., 2013). About 500 species of *Anopheles* has been described; however, only sixty (60) are reported to cause malaria (Sokhna et al., 2013). In addition to malaria-endemic areas, *Anopheles* mosquitoes that transmit malaria can also be found even in areas where malaria has been eradicated – these areas are always at risk of resurgence of the disease (Obacha, 2016). Many *Anopheles* species have been reported in the literature, and examples include but not limited to the *An. gambiae* complex, *An. funestus* group, *An. nili* group (Ossè et al., 2019), *An. moucheti*, *An. vinckei* (Paupy et al., 2013), *An. minimus* complex, *An. dirus* complex, and *An. subpictus* complex (Morgan et al., 2013).

Most important malaria vectors in Africa belong to a species complex, and these species are sometimes difficult to differentiate morphologically. Previously, due to the complexities regarding this, sibling species in the complex have often been considered as a single unit, notwithstanding the significant differences among these sibling species (Wiebe et al., 2017). In sub-Saharan Africa, the *An. gambiae* complex and *An. funestus* groups are the main vectors responsible for causing malaria (Nnko et al., 2017; Gouignard et al., 2019). The *An. gambiae* complex, which is the focus of this study, was documented in the 1960s, and it has been reported to include the most important malaria vectors in sub-Saharan Africa, chiefly of the dangerous malaria parasite – *Plasmodium falciparum* (Bashir et al., 2018). The complex consists of nine (9) morphologically indistinguishable sibling species. They are *An. gambiae*

sensu stricto (s.s.), *An. arabiensis*, *An. quadriannulatus*, *An. melas*, *An. merus*, *An. bwambae*, *An. amharicus* (Bass et al., 2007; Coetzee et al., 2013), *An. coluzzii* (Coetzee et al., 2013; Barron et al., 2018; Camara et al., 2018) and *An. fontenillei* (Barrón et al., 2019).

In Ghana, some of the *Anopheles* species reported include *An. gambiae (s.s.)*, *An. funestus*, *An. coluzzii*, *An. pharaoensis*, *An. rufipes*, *An. melas* and many more. The *Anopheles* species reported in the country are distributed based on ecological settings (Baffour-Awuah, 2012). For instance, *An. gambiae (s.s.)* and *An. funestus* – the most common species of *Anopheles* in the country, and *An. coluzzii* are found throughout the country (Baffour-Awuah et al., 2016). In most locations, *An. coluzzii* and *An. gambiae (s.s.)* sympatrically co-exist (Kudom, 2015). On the other hand, *An. arabiensis* predominates in the coastal savannah and northern regions. *Anopheles rufipes* and *An. melas* are also limited to the northern and coastal areas of the country, respectively (Baffoe-Wilmot et al., 2001; Yawson et al., 2004; De Souza et al., 2010).

Figure 3: Adult *Anopheles* mosquito

2.4.2 *Aedes* mosquitoes

Aedes is the most prominent tribe of mosquitoes with about 1256 species categorized into ten (10) genera (in descending order); *Aedes sensu, Verrallina, Armigeres, Psorophora, Eretmapodites, Heizmannia, Haemagogus, Zeugnomyia, Udaya*, and *Opifex* (Wilkerson et al., 2015). Many species of *Aedes* exist, and they include but not limited to *A. albopictus, A. aegypti, A. polynesiensis, A. scutellaris* complex (WHO, 2009a), *A. taeniorhynchus, A. vexans, A. sollicitans, A. togoi, A. atropalpus, A. triseriatus*, and *A. hendersoni* (Day, 2016). However, *A. aegypti*, and *A. albopictus* are of significant public health importance because of their role in transmitting diseases (Kraemer et al., 2015). Some notable diseases they transmit include dengue, yellow fever, chikungunya and Zika viruses (Kweka et al., 2018).

Aedes species can be found in natural and artificial receptacles capable of holding clear and clean water (Dom et al., 2013a; Dom et al., 2013b; Madzlan et al., 2016). Among the preferred breeding sites are earthen jars, ant traps, flower pots, drums, coconut shells, concrete tanks, and discarded tires (Simard et al., 2005; García-Rivera & Rigau-Pérez, 2006; Paupy et al., 2009). In addition, larvae can be found in natural sites like tree holes and bromeliads (García-Rivera & Rigau-Pérez, 2006). According to Dom et al. (2013b), *Aedes aegypti* usually prefers indoor artificial containers, and *A. albopictus* are more used to natural water receptacles found outdoors.

The feeding behavior of *Aedes* differs among species. For instance, while *A. albopictus* is opportunistic and has zoophilic feeding behavior, *A. aegypti* is (except in African populations) highly anthropophilic (Paupy et al., 2009). In addition, *A. albopictus* is primarily a daytime and exophagic (feeds outdoors) mosquito and prefers to bite in the early morning and late afternoon. However, several exemptions have been documented based on the region, human habitat, season, and host availability (Paupy et al., 2009). Though the *A. aegypti* mosquito is also a

daytime feeder and bites mainly in the morning or late afternoon, they generally rest in dark, indoor places like under beds, closets (García-Rivera & Rigau-Pérez, 2006) and behind curtains.

In Ghana, studies have reported *Aedes* species in all the ecological zones (Appawu et al., 2006; Ughasi et al., 2012; Owusu-Asenso, 2018). Some of them include *A. aegypti*, *A. africanus*, and *A. luteocephalus*.

Figure 4: Adult *Aedes* mosquito

2.4.3 *Culex* mosquitoes

Culex mosquitoes consist of more than a thousand species distributed globally (Mullen & Durden, 2009). They are also the most prevalent mosquito species across the African continent (Nchoutpouen et al., 2019), and are vectors of many important disease causing viruses, including; West Nile virus (WNV), Sindbis virus (SINV), Japanese encephalitis virus (JEV), and a range of nematodes (Mullen & Durden, 2009; Weaver & Lecuit, 2015; Gould et al., 2017).

Culex species are highly opportunistic and feed on both humans and animals. This behavior enhances their ability to transmit zoonotic diseases, making them a significant threat to public

health (Weissenböck et al., 2010). Unlike *Anopheles*, *Culex* mosquitoes usually breed in turbid water (Mahgoub et al., 2017); however, there are instances where *Anopheles* and *Culex* larvae have been found together in breeding sites even though their breeding ecology differs (Emidi et al., 2017).

There are so many species of *Culex,* but *Culex pipiens* complex is the most important species. It comprises six members: *Cx. pipiens* Linneaus, *Cx. australicus* Dobrotworsky and Drummond, *Cx. quinquefasciatus* Say, *Cx. globocoxitus* Dobrotworsky, *Cx. pallens* Coquillet, and *Cx. molestus* Forskll (Nchoutpouen et al., 2019). In Ghana, *Culex* species such as *Cx. quinquefasciatus* Say, *Cx. thallassius*, *Cx. decens*, *Cx. fuscocephala*, and *Cx. perexiguus* have been identified in different parts of the country (Opoku & Ansa-Asare, 2007; Kudom et al., 2015a, 2015b).

Figure 5: Adult *Culex* mosquito

2.5 *Anopheles* and malaria transmission

Malaria remains a disease of public health concern in terms of morbidity and mortality caused by four species of the genus *Plasmodium* (*P. falciparum, P. vivax, P. ovale* and *P. malariae*). Malaria is a protozoan infection that attacks the red blood cells in the human body through the bite of an infected female anopheline mosquito (Wang et al., 2013). The incidence of malaria is approximately 300 – 500 million clinical cases, resulting in 1 million deaths each year, where children under 5 are the most affected (Traoré et al., 2020). In 2017, approximately 219 million cases of malaria were recorded, with 435 thousand deaths worldwide (WHO, 2019b). Vector control such as long-lasting insecticidal nets (LLINs) and indoor residual spraying (IRS) remain key elements in reducing the transmission and burden of malaria in Africa (Otten et al., 2009).

There have been increased global efforts to control and eliminate malaria over the past two decades and this has prevented about 1.5 billion cases and 7.6 million deaths (WHO, 2021). Notwithstanding, malaria remains a major public health issue in Ghana. The country is one of the eleven high-burden countries accounting for over 70% of the global malaria cases and deaths (Ghosh & Rahi, 2019), and part of the two highest-burden countries in Africa reporting the highest absolute increase in malaria cases in 2018 (WHO, 2019a). Over the years, Ghana has made some progress in the prevention and control of malaria as there has been a significant decline (28 % in 2011 to 14 % in 2019) of nationwide parasite prevalence among children under five years (based on microscopy) (President's Malaria Initiative, 2022).

In Ghana, the dominant species responsible for the transmission of the disease are *An. gambiae (s.s), An. coluzzii, An. funestus*, and *An. arabiensis* (Chabi et al., 2016). The key feature that makes these species efficient malaria vectors are their resting behavior and blood source preference. These behavioral differences affect the vectorial capacity, suitability, and

effectiveness of vector control interventions (Akuamoah-Boateng et al., 2021). Additionally, environmental factors (temperature, relative humidity, vegetation, rainfall, etc) influence the survival of mosquitoes (Kristan et al., 2018; Agyemang-Badu et al., 2023). For instance, the larval stages of all species of *Anopheles* require water, thus the presence of a water source is related to increased distribution and density of larvae and an increased incidence of adult mosquitoes and consequently malaria (Ma et al., 2016).

Malaria is transmitted through the bite of an infectious female *Anopheles* mosquitoes (WHO, 2019b). When a female *Anopheles* mosquito feeds on a *Plasmodium*-infected individual, gametocytes are taken up with the blood meal into the midgut. Gametocytes produce gametes that, once fertilized, rapidly develop into motile ookinetes, which cross the mosquito midgut epithelium and settle on its basal side (within 16 – 30 h post-blood-feeding) (Kirchner & Waters, 2019). Ookinetes then transform into sessile oocysts, producing thousands of individual sporozoites within 10 – 14 days. After oocyst rupture, sporozoites migrate to the salivary glands where they reside, ready to start a new infection when the mosquito feeds on a host again (Kirchner & Waters, 2019).

Despite the efforts made to eliminate malaria, the transmission of malaria continues to occur and remains a critical public health issue, especially in Africa (Degefa et al., 2021). Factors such as increased insecticide resistance due to target site mutations, enhanced metabolic detoxification (Nwankwo, 2021), and behavioral resistance due to the preference of malaria vectors to bite outdoors and in the early evenings when people are indoors but unprotected (Degefa et al., 2021) contributes to the continual transmission of malaria globally.

2.6 Control of mosquitoes

Malaria vector control is the primary intervention for the global reduction and eradication of malaria (Kgoroebutswe et al., 2020). Vector control is measures of any kind against malaria-transmitting mosquitoes planned to limit the ability of mosquitoes to transmit disease (WHO, 2016b). The most common vector control measures include the use of insecticide-treated nets (ITN or LLIN) and indoor residual spraying (IRS) (WHO, 2017; Williams et al., 2018), with larval source management (LSM) as an additional control measure (Kgoroebutswe et al., 2020; McCann et al., 2021).

2.6.1 Use of mosquito bed nets

One of the important tools of the Roll Back Malaria (RBM) strategy is the use of mosquito bed nets (insecticide-treated net (ITN) or long-lasting insecticidal nets (LLINs)) (Toé et al., 2009; Zöllner et al., 2015). An insecticide-treated net (ITN) is a bed net intended to provide a physical barrier against mosquitoes and also processed with residual insecticide to repel or kill mosquito vectors (Birget & Koella, 2015; Lindblade et al., 2015). On the other hand, long-lasting insecticidal nets (LLINs) - an effective alternative to insecticide-treated nets (ITNs), last longer than insecticide-treated bed nets (ITNs) and maintain their biological efficacy for about three (3) years (Sriwichai et al., 2016; Yang et al., 2018). The LLINs have insecticides coated around or incorporated into their fibers (Yang et al., 2018).

The use of treated mosquito nets has had a great impact on reducing mosquito bites and in reducing malaria transmission (Mohammed, 2013; Castellanos et al., 2021). Policies aimed to promote universal access to bed nets (ITNs or LLINs) are developed in many malaria-endemic countries; however, the percentage of the people who slept under bed nets in 2015 in sub-Saharan Africa was estimated around 55 %, with most of them (about 68%) being children under-5 years old (WHO, 2015a). The usage of ITNs reduced malaria mortality rates in

children under-5 years old by 55 % (Eisele et al., 2010; Admasie et al., 2018). Unfortunately, many households who received bed nets for free or subsidized prices do not use them (Baume & Franca-Koh, 2011). The low usage of bed nets has been attributed to fixing the bed net above the mat, house design, the feeling of suffocation and discomfort because of the relatively high temperatures in rooms (Toé et al., 2009). These reasons could grind down the achievements made in ITN use and reduce the effectiveness of malaria control programs.

2.6.2 Indoor Residual Spraying (IRS)

Indoor residual spraying (IRS) of insecticides is a key method of reducing malaria vector transmission and has contributed to the decline in malaria prevalence globally (Tangena et al., 2020; Coleman et al., 2021). Indoor residual spraying (IRS) consists of applying a long-term, residual insecticide to potential mosquito hidden surfaces such as ceilings and interior walls of houses where mosquitoes might come into contact with the insecticide (WHO, 2013b).

Indoor Residual Spraying (IRS) programs remain the most extensively used technique for controlling mosquitoes (Choi et al., 2019b) and are highly effective and can also significantly reduce malaria incidence and mortality (Gogue et al., 2020) on the condition that mosquito hide-outs in targeted communities are identified and sprayed (Agyekum, 2017). However, in recent years, IRS programs face challenges due to the increasing vector resistance to insecticides and the overall cost implications of the program implementation (WHO, 2020). The increased cost of IRS products has been linked to a reduction in IRS coverage throughout sub-Saharan Africa (Chaccour et al., 2021).

This vector control method was introduced during the late 1940s when Dichloro-diphenyl-trichloroethane (DDT) was available and used to control mosquito vectors of malaria that entered houses (Van Den Berg et al., 2012). The success of house spraying for controlling

malaria depends on applying an adequate and uniform dosage of insecticide on all potential resting places of the adult female mosquito (WHO, 2015b).

Many sub-Saharan African countries have included IRS in their extensive malaria control plan in agreement with the Global Malaria Action Plan (GMAP) introduced by the WHO and Roll Back Malaria (RBM) Partnership. Globally, 185 million people (6 % of the global population at risk) were protected from malaria through the use of IRS in 2010 (WHO, 2011). However, in 2014, only 116 million (3.4 %) people were protected by IRS. There has been a reduction in the proportion of vulnerable population IRS protected (Cibulskis et al., 2016).

2.6.3 Larval source management (LSM)

Larval source management (LSM) refers to managing mosquito breeding habitats to prevent the development of immature stages (eggs, larvae and pupae) or reduce the number of immature mosquitoes (WHO, 2013a; McCann et al., 2017). The goal of LSM is to reduce the number of adult mosquitoes that bite to prevent malaria transmission (Choi et al., 2019a). Four types of LSM exist, they include habitat modification (a permanent modification to the environment such as surface water drainage), habitat manipulation (a recurrent activity such as flushing of streams and water-level manipulation); larviciding (regular application of biological or chemical insecticides to water bodies); and biological control (introduction of natural predators into water bodies) (Fillinger & Lindsay, 2011; WHO, 2013a).

It must be emphasized that larval source management (LSM) should not be considered a stand-alone intervention or replace core vector control interventions (using bed nets and IRS) but as an additional vector control measure (Fillinger & Lindsay, 2011; WHO, 2013a). Larval source management (LSM) provides the double benefits of reducing the numbers of house-entering mosquitoes and, importantly, those that bite outdoors (Fillinger & Lindsay, 2011).

2.7 Targets of stages of mosquito with insecticides

2.7.1 Larviciding

Larviciding denotes the process of killing the larvae of an insect (Baffour-Awuah, 2012). Many compounds comprising surface and oil films, bacteria larvicides, insect growth regulators, synthetic organic chemicals, and spinosyns can be used as larvicides (WHO, 2013a). Microbial larvicides such as *Bacillus sphaericus* or *Bacillus thuringiensis* or a combination of the two have proven to be effective in the control of mosquito larvae in different areas (Fillinger et al., 2009; Geissbühler et al., 2009; Fillinger & Lindsay, 2011; Baffour-Awuah, 2012; Maheu-Giroux & Castro, 2013; Afrane et al., 2016). These larvicides present numerous modes of actions against mosquito larvae. For instance, insect growth regulators thwart the development of the larvae, monolayers cause suffocation of mosquito larvae, and botanical or synthetic toxins directly interfere with the metabolic activities of insects (Fillinger & Lindsay, 2011).

Larval control targets the aquatic stage of the mosquito by reducing mosquito larval sites, thus killing both indoor and outdoor biting mosquitoes (Fillinger & Lindsay, 2011). The suppression of mosquito larvae using larvicides serves as an excellent add-on to current methods such as the use of long-lasting insecticidal nets (LLINs) and indoor residual spraying (IRS) (Antonio-Nkondjio et al., 2018).

2.7.2 Adulticiding

Mosquito adulticide is a kind of insecticide used to eradicate adult mosquitoes (CDC, 2016). Adulticiding is just a word used to describe mosquito management activities targeted at adults. The control of the adult mosquito is the most common type of mosquito control (WHO, 2009b) and depend primarily on the use of long-lasting insecticidal nets (LLINs) and indoor residual spraying (IRS) (Steketee & Campbell, 2010; Alemayehu et al., 2017; Camara et al., 2018). These are the most powerful and primarily used interventions (WHO, 2009b). In addition, in

sub-Saharan Africa and other developing countries, some households use mosquito repellents such as mosquito coils to control adult mosquitoes (Agyekum, 2017).

2.8 Class of insecticides and their targets

One way of controlling malaria is to prevent the infective mosquito from biting individuals, and this is done using insecticides (bed nets and indoor residual spraying) (Agyekum, 2017). Efforts from international agencies such as the US President's Malaria Initiative (PMI), World Bank, United Nations International Children's Emergency Fund (UNICEF), and Department for International Development (DFID), among others, as well as home governments to reduce malaria burden in sub-Saharan Africa have resulted in scaling up vector control measures, and this has resulted in a decline in malaria cases (WHO, 2011).

The current control programs are primarily dependent on pyrethroid-based insecticides, which are the only recommended insecticides by the WHO for insecticide-treated nets (ITNs) (WHO, 2006). All the interventions aimed at the adult mosquito mainly depend on chemical insecticides. For instance, chemical insecticides such as pyrethroids are used for making long-lasting insecticidal nets (LLINs) (Essandoh et al., 2013), indoor residual spraying (IRS) (Da Cruz et al., 2019), and most mosquito repellents (Vences-Mejía et al., 2012; Hogarh et al., 2018). Pyrethroid insecticides are the only insecticide endorsed by the WHO to be used in most indoor residual sprays and treated bed nets (WHO, 2017). Other insecticides such as carbamates, organochlorines and organophosphates have been used to control mosquitoes (Cuervo-Parra et al., 2016). The efficacy of these insecticides has been challenged as mosquitoes have developed resistance to some classes of insecticides (Annan et al., 2014; Baffour-Awuah et al., 2016; Antonio-Nkondjio et al., 2017). Any modifications in mosquitoes could worsen the effectiveness of insecticides in the control of mosquitoes.

2.8.1 Carbamate insecticides

Carbamates are a class of insecticides structurally and mechanistically similar to organophosphate (OP) insecticides. Carbamates are N-methyl Carbamates derived from a carbamic acid and cause carboxylation of acetylcholinesterase at neuronal synapses and neuromuscular junctions (Silberman & Taylor, 2020). They are an important class of pesticides used worldwide in public health, among rural and urban settings. They include carboxyl (Sevin), aldicarb (Temik), and Propoxur (Baygon) (Adam & Lawson, 2010).

Carbamate insecticides are synaptic poisons and bind to an enzyme found in the synapse called acetylcholinesterase. This enzyme is designed to stop a nerve impulse after it has crossed the synapse (Adam & Lawson, 2010). Carbamate insecticides bind to and cause reversible inhibition of the acetylcholinesterase enzyme. Elevated acetylcholine levels because of acetylcholinesterase (AChE) inhibition leads to increased neurotransmitter signaling (Silberman & Taylor, 2020). Therefore, the poisoned synapses cannot stop the nerve impulse leading to paralysis due to energy exhaustion (Ishak, 2014).

2.8.2 Organochlorine (OC) insecticides

Organochlorines (OC) are a group of chlorinated compounds extensively used as pesticides. They belong to persistent organic pollutants (POPs) with high persistence in the environment (Jayaraj et al., 2016). Organochlorine (OC) insecticides have been used to control malaria and typhus; however, they are prohibited in most advanced countries because of their high persistence in the environment and their harmful effects (Karasali & Maragou, 2016). Organochlorine (OC) insecticides can be categorized into three groups: (a) Dichloro-diphenyl-trichloroethane (DDT) and its analogues; (b) lindane and the cyclodienes; and (c) mirex and chlordecone (Ray, 2010). Examples of OC insecticides include DDT, Aldrin, chlordane, toxaphene, lindane and dieldrin (Rhee & Aks, 2007).

The mechanism of action differs slightly based on the type of organochlorine. For instance, DDT-type insecticides act on the peripheral nervous system. At the axon's sodium channel, they prevent gate closure after activation and membrane depolarization. Sodium ions leak through the nerve membrane and create a destabilizing negative "after potential" with hyperexcitability of the nerve. This leakage causes repeated discharges in the neuron spontaneously or after a single stimulus (Coats, 1990). On the other hand, the chlorinated cyclodienes type binds at the picrotoxinin site in the gamma-aminobutyric acid (GABA) chloride ionophore complex, which inhibits chloride flow into the nerve (Coats, 1990).

2.8.3 Organophosphorus (OP) insecticides

Organophosphorus insecticides, often known as organophosphates (OPs), contain phosphorus/phosphoric acid esters or thiophosphoric acid esters (Adam & Lawson, 2010). Examples of organophosphates include malathion, diazinon, parathion, fenitrothion, dichlorvos, and chlorpyrifos (Ishak, 2014; Riches, 2015). Organophosphorus (OP) insecticides are slightly more toxic than carbamates, and this may be attributable to the fact that their binding to the receptor is irreversible, while that of carbamates is reversible (Sánchez-Bayo, 2012). Organophosphates kill insects by binding to and inhibiting the transmission of acetylcholinesterase (AChE) at the synaptic junction of the insect nervous system (Ishak, 2014). This results in the accumulation of acetylcholine in nerve tissue and effectors organs, with the main site of action being the peripheral nervous system (Adam & Lawson, 2010).

2.8.4 Pyrethroid insecticides

Pyrethroids are a synthetic derivate of pyrethrins, natural organic insecticides produced from the flowers of *Chrysanthemum cinerariaefolium* and *C. coccineum*. They are used in many synthetic insecticides and are highly specific against insects (Gajendiran & Abraham, 2018). It

is one of the major insecticides used in mosquito control programs (Nkya et al., 2013; Bowman et al., 2018).

Pyrethroids act on either the central nervous system or peripheral nerves, such as sensory axons or motor axons at low concentrations. The action on peripheral nerves is exceptional to this class of insecticide and complicates studies on the mode of action because it is difficult to determine the site of action (central or peripheral) that might be vital in toxicity (Coats, 2012).

Pyrethroids may be divided into two groups: type I and type II pyrethroids. Examples of type I pyrethroids include allethrin, permethrin, and lismethrin, while type II includes fenvarelate, deltamethrin, and cypermethrin (Adam & Lawson, 2010). Type I pyrethroids produce repetitive action potentials at the nerve function level due to the increase in depolarizing after-potential, but type II pyrethroids cause membrane depolarization resulting in discharges from sensory neurons (Narahashi, 2010). Pyrethroids are axonic poisons, which bind to a protein in nerves called the voltage-gated sodium channel. Usually, this protein opens, causing stimulation of the nerve and closes to terminate the nerve signal. Pyrethroids bind to this gate and prevent it from closing normally, which results in continuous nerve stimulation (Adam & Lawson, 2010).

2.9 Mechanisms used by mosquitoes to develop an insecticide resistance phenotype

The prolonged use of pyrethroids and other insecticides has resulted in resistance against malaria vectors (WHO, 2016a). It is projected that the swift expansion of insecticide resistance in malaria vectors due to the repeated use of insecticides could affect malaria control efforts (Antonio-Nkondjio et al., 2017). Many resistance mechanisms exist in individual mosquito species (Liu, 2015).

According to the Insecticide Resistance Action Committee (IRAC), resistance is "the selection of a heritable characteristic in an insect population resulting in the repeated failure of an insecticide product to provide the intended level of control when used as recommended" (IRAC, 2006). Resistance to insecticides arises when increased levels of an enzyme system cause it to detoxify the insecticide faster than usual, consequently averting it from reaching its site of action (WHO, 2012).

There are four types of insecticide resistance mechanisms in the malaria vector: target site, metabolic, cuticular, and behavioral resistance (Corbel & N'Guessan, 2013; Kabula et al., 2014a; Kabula et al., 2014b; Matowo et al., 2014). Out of these four, the first two (target site and metabolic resistance) are the most common and extensively studied (Matowo et al., 2010; Ranson et al., 2011; Corbel & N'Guessan, 2013; Liu, 2015). Usually, these two mechanisms combine, resulting in complex cross-resistance and high resistance levels (Labbé et al., 2011). This study focused on metabolic resistance in *An. gambiae (s.l.)* mosquitoes.

The four types of insecticide resistance mechanisms can be grouped into two major categories; (a) mechanisms that decrease the effective dose of the insecticides (metabolic, cuticular, and behavioral resistance) and (b) mechanisms that render the dose of insecticide ineffective (target site resistance) (Oliver, 2015). Figure 6 summarizes the mechanism of insecticide resistance.

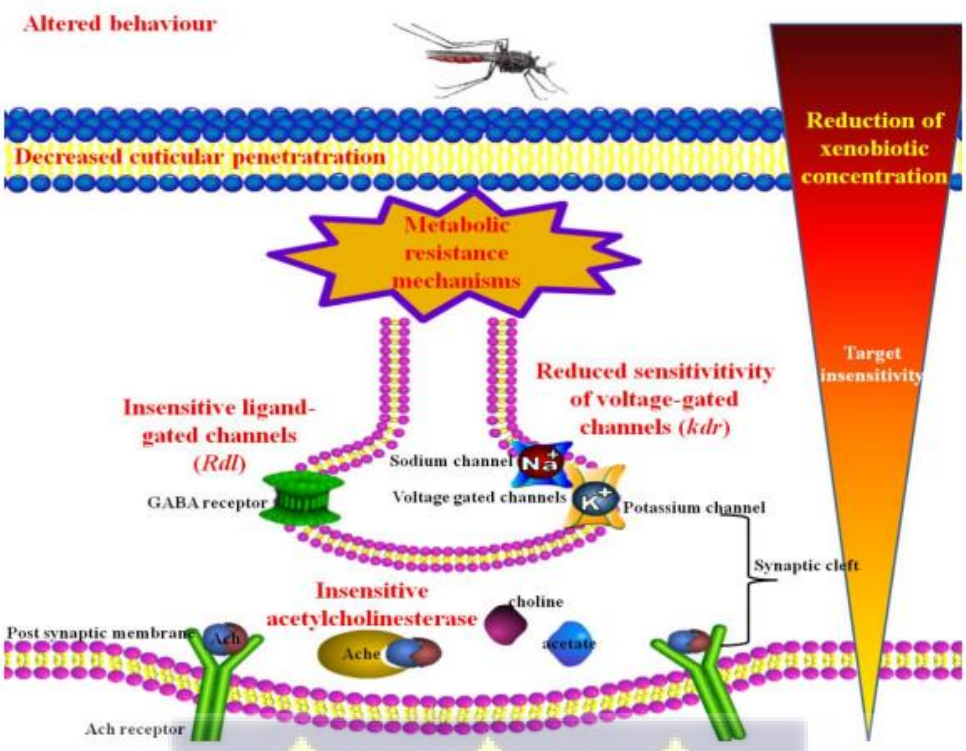

Figure 6: Summary of mechanisms used by mosquitoes to produce a resistance phenotype
Resistance mechanisms are highlighted in red text. The mechanisms corresponding to the red region on the concentration gradient (altered behavior, decreased cuticular penetration and metabolic mechanisms) aim to reduce the efficacy of the insecticide. The remaining mechanisms, all resulting in target site insensitivity, are all targeted at rendering the dose of the insecticide inactive (Oliver, 2015).

2.9.1 Target site resistance

Target site resistance results from modification in the structure or mutation (point mutation) of genes that encode target proteins interacting with insecticides (Casida & Durkin, 2013). It causes a mutation in the target that prevents the insecticide from binding to its target organ (Hardy, 2014), eliminating or significantly reducing the effectiveness of the insecticide (FAO, 2012). The main targets of insecticides are receptors or enzymes of the nervous system: acetylcholinesterase (AChE), the voltage-dependent sodium channel (CNaVdp), and the receptor of c-aminobutyric acid (Sokhna et al., 2013). For instance, after insecticides bind to the sodium channels, they cause the nervous system of the insect to repeatedly discharge and depolarize the insect's nerve membranes (Narahashi, 2002), which eventually leads to the death of the insect (Liu, 2015). According to Sokhna et al. (2013), mutation of these targets is a very effective resistance mechanism, inducing cross-resistance to all insecticides acting on the same

target. Numerous insecticide target modifications have been studied: the voltage-dependent sodium channel encoded by the *kdr* gene, the synaptic acetylcholinesterase (AChE1) encoded by the ace-1 gene, as well as the gamma-aminobutyric acid (GABA Receptors) encoded by the RDL gene (Labbé et al., 2011; Ranson et al., 2011).

2.9.2 Cuticular resistance

Cuticle thickening is a gene-regulated process that an insect goes through as it ages. The cuticle is placed in a circadian pattern, leading to growth rings of diurnal non-lamellate and nocturnal lamellate structure (Wood et al., 2010). The insect's body is shielded by a cuticle, a hard exoskeleton providing support and protection from ultraviolet radiation, dehydration, and fungal and bacterial pathogens. The cuticle comprises many layers, each having different physicochemical properties. The outermost waxy layer includes saturated and unsaturated hydrocarbons, free alcohols, free fatty acids, wax esters, sterol esters, glycerides, and aldehydes (Suarez et al., 2011).

When insects are exposed to insecticides, the insecticides have to cross the insect's cuticle before reaching the target site to induce the effect. In this case, the cuticle functions as the first line of defense against insecticides. Cuticular barriers are developed in resistant insects by forming a thicker cuticle or changing the cuticular structure to lessen insecticide penetration into their body (Dang et al., 2017). Cuticular resistance has been linked with pyrethroid resistance in *An. funestus* and *Triatoma infestans* (Pedrini et al., 2009; Wood et al., 2010).

2.9.3 Behavioral resistance

Behavioral resistance is any avoidance behavior that results in an increased chance of survival for an insect or its offspring (Pittendrigh et al., 2014). The definition of behavioral resistance has been contested as most cases of behavioral resistance to insecticides are just antipathy behaviors that are either learned or based on avoidance or simple repellency (Zalucki &

Furlong, 2017). Behavioral avoidance is more preferred to behavioral resistance as "it is an innate and involuntary response to external stimuli rather than a permanent, genetically-based shift in behavior as the development of apparent fixed behavioral changes because of insecticide selective pressure has not been sufficiently documented to occur under natural conditions" (Chareonviriyaphap et al., 2013). Numerous types of behavioral resistance have been described in insects; some are connected with the mobility and others immobility of the insect. Both mechanisms allow the insect to evade contact with the toxic product or reduce the duration of the contact (Sokhna et al., 2013).

Behavioral modifications have been reported in mosquitoes exposed to insecticides. These modifications include a shift from endophilic (i.e. resting in houses after blood meals) to exophilic (i.e. resting outdoors after blood meals) behavior, changes in the time of feeding, and host choice preference (Sokhna et al., 2013; Wamae et al., 2015). Behavioral resistance is less frequently observed than physiological resistance in which the insect comes into contact with the insecticide but is not killed (Russell et al., 2013).

2.9.4 Metabolic resistance

Metabolic resistance is due to point mutations that affect protein activity (e.g. a change in binding affinity or a modified substrate specificity) or through modifications in the cis/trans-regulatory loci of the enzyme families (Ishak, 2014). It is most often found in insects and based on enzyme systems that the insects have developed to help them naturally detoxify insecticides and other foreign elements (FAO, 2012; Gatton et al., 2013). The common metabolic enzymes associated with resistance include; mixed-function oxidase (or monooxygenase), glutathione-S-transferases (GSTs), acetylcholinesterase (AChE), and non-specific esterase (NSE) (alpha- and beta-naphthyl acetate) (WHO, 2013b; Yahouédo et al., 2016; Camara et al., 2018; Leong et al., 2019).

Upregulation of enzyme levels or an upsurge in the metabolism of insecticides indicates the potential involvement of a metabolic enzyme in resistance (Liu, 2015). High levels of metabolic enzymes, in general, have been reported in insecticide-resistant mosquitoes of several *Anopheles* species in Africa (Liu, 2015). For instance, Camara et al. (2018) quantified the mean levels of non-specific esterase (NSE) (alpha- and beta-naphthyl acetate), glutathione S-transferases (GST), and mixed-function oxidases (MFO) in *An. gambiae (s.l.)* from Côte d'Ivoire. Likewise, in north-eastern Tanzania, the levels of non-specific esterase and mixed-function oxidase levels in *An. arabiensis* have been reported (Matowo et al., 2010).

2.9.4.1 Metabolic enzymes

2.9.4.1.1 Cytochrome P450 monooxygenases

Cytochrome P450, or CYP gene, is a major family of genes found in almost all living organisms such as animals, plants, bacteria, fungi, and protists. The role of the enzyme has been recorded in many physiological processes (Feyereisen, 2012). The Cytochrome P450 (CYP) superfamily comprises hemeproteins that play a crucial role in the breakdown of many endogenous (e.g. hormones) and xenobiotic compounds, including pyrethroids (Bebe & Panemangalore, 2005; Hardstone et al., 2007). Cytochrome P450 in insects is essential because of their role in both the bioactivation and detoxification of insecticides (Hardstone et al., 2007), and is the prominent enzyme family responsible for pyrethroid breakdown. Approximately, 111 P450 enzymes are found in *An. gambiae (s.s.)* mosquitoes (Ranson et al., 2011).

Naming a cytochrome P450 gene always start with the core symbol "CYP", followed by an Arabic numeral indicating the CYP family (e.g. CYP3, CYP6), a capital letter M, N, and P represents the subfamily (e.g. CYP3M, CYP6P), and another Arabic numeral which demonstrates the individual gene/isozyme/isoform/isoenzyme (e.g. CYP6M2, CYP6P3) (Badyal & Dadhich, 2001). Specifically, five 'candidate' P450 genes are explicitly overexpressed in pyrethroid-resistant adult mosquitoes; CYP6Z1, CYP6Z2, CYP6M2,

CYP325A3 (Müller et al., 2008), and CYP6P3 (Ranson et al., 2011). These genes encrypt enzymes that can cohere to pyrethroid insecticides; however, only two genes (i.e. CYP6P3 and CYP6M2) can break down the insecticide (Ranson et al., 2011). According to Yahouédo et al. (2016), there is a widespread overexpression of CYP6M2 and CYP6P3 in West Africa, especially in Ghana, Benin, and Nigeria.

2.9.4.1.2 Glutathione-S-transferases (GSTs)

Glutathione S-transferases (EC2.5.1.18) are ubiquitous in both plant and animal species (Alias, 2016) and are essential in the detoxification of xenobiotic (e.g. herbicides, and insecticides) and endogenous compounds (Samra et al., 2012). In insects, GSTs may serve as a binding protein that sequesters pyrethroids before breaking down by enzymes such as cytochrome P450 and carboxylesterases (Samra et al., 2012). Glutathione S-transferases (GSTs) are a superfamily of versatile isoenzymes implicated in several intertwined cellular metabolisms, comprising detoxification of xenobiotic compounds (Gunasekaran et al., 2011).

The nomenclature of the insect GSTs comprises three parts; the name of the species from which the enzyme was extracted, the class of the enzyme, and a number representing the specific genome organization or the order of discovery. For instance, AgGSTD5-5 is *An. gambiae* GST, letter D denotes the delta class of the enzyme, and the number "5-5" shows a homodimer enzyme (Che-Mendoza et al., 2009). In addition, GSTs are grouped based on their cellular localizations into three main families: cytosolic, microsomal, and mitochondrial/peroxisomal GSTs (Sookrung et al., 2018). The greater part of insects GSTs are cytosolic dimeric enzymes, which are grouped into six classes, namely; delta, sigma, epsilon, theta, zeta, and omega, depending on the specificity of the substrate, chromosomal location, and sequence similarity (Samra et al., 2012; Shou-Min, 2012; Sookrung et al., 2018). Glutathione S-transferases (GSTs) with more than 40% sequence identity are usually put in the same class (Samra et al.,

2012). In mosquitoes, three microsomal GSTs (MGSTs) have been reported, but only cytosolic GSTs have been associated with insecticide resistance (Che-Mendoza et al., 2009). High levels of GST levels have been linked to insecticide resistance in various insects (Che-Mendoza et al., 2009). GST levels have been reported in insecticide-resistant mosquitoes, including some *Anopheles* and *Aedes* species (Liu, 2015).

2.9.4.1.3 Acetylcholinesterase (AChE)

Acetylcholinesterase (AChE) (EC 3.1.1.7) is an important enzyme involved in the termination of nerve transmission at cholinergic synapses by hydrolyzing the neurotransmitter, acetylcholine (ACh) released from the presynaptic terminal, making it an effective target for organophosphate and carbamate insecticides (Shang et al., 2012). It belongs to the carboxylesterase family of enzymes and is responsible for hydrolyzing the acetylcholine at nerve synapses. In the absence of hydrolysis, there is a build-up of acetylcholine, resulting in the repeated firing of neurons and eventually death by enervation (Gunning & Moores, 2001).

In vertebrates, ACh serves as an excitatory transmitter for voluntary muscle in the somatic nervous system. It also acts as both preganglionic and postganglionic transmitter in the parasympathetic nervous system and preganglionic transmitter in the sympathetic nervous system (Fulton & Key, 2001). Acetylcholinesterase is a widespread resistance mechanism in vector mosquitoes such as *Aedes*, *Anopheles*, and *Culex* (Polson et al., 2011; Scott & McAllister, 2012; Akiner, 2014).

2.9.4.1.4 Non-specific esterase (NSE)

Non-specific esterase is one of the esterases' substrate specificity (Sood et al., 2016). Esterase is a major family of enzymes responsible for insecticide resistance in agricultural pests and disease vectors. Non-specific esterase plays a crucial role in the resistance of mosquitoes to carbamate, organophosphates (OPs), and pyrethroid insecticides (Prasad et al., 2017).

The esterase family comprises many enzymes that catalyze the hydrolysis of esters. Esterases are associated with important physiological roles of insects such as development, behavior, reproduction, and insecticide resistance. Esterases can be categorized according to alpha- or beta-esterase based on their ability to hydrolyze the substrates alpha- and beta-naphthyl acetate, respectively (Dahan-Moss & Koekemoer, 2016). Resistant insects overproduce non-specific esterase or carboxylesterases by gene up-regulation to rapidly sequester the insecticides. This mechanism has been reported in many insect species such as mosquitoes, aphids, cattle ticks, cockroaches, *Cimex hemipterus* and *Cimex lectularius* (Matowo et al., 2010; Dang et al., 2017).

2.10 Predominant insecticides in Ghana

In Ghana, the predominant insecticides for malaria control are pyrethroids, particularly the long-lasting insecticide-treated nets (LLINs) that are distributed for free to the public, and indoor residual spraying (IRS) (Kawada et al., 2014; Dadzie et al., 2017; Brake et al., 2022). Additionally, mosquito coils, though not part of the approved methods of mosquito control, are widely used in Ghana to control mosquitoes. Most of these mosquito coil products on the Ghanaian market are pyrethroid-based with active ingredients such as esbiothrin, dimefluthrin, allethrin, and meperfluthrin (Hogarh et al., 2018). Overall, the use of pyrethroids in both LLINs and IRS has been effective in reducing malaria transmission in Ghana, although there are concerns about the development of insecticide resistance and the need for continued vigilance in monitoring and adapting control strategies (Baffour-Awuah et al., 2016; Chabi et al., 2016; Amlalo et al., 2022; Owusu-Asenso et al., 2022).

Temperature is a critical factor underlying insecticide toxicity and has been reported to affect the efficacy of public health insecticides against some malaria vector species (*An. arabiensis* and *An. funestus*) (Glunt et al., 2018). The influence of temperature on toxicity can be either positive or negative, which depends on the mode of action of the insecticide and the insect species in question in addition to the route of exposure (Gordon, 2005). For instance, a study

conducted by Khan and Akram (2014) reported that the toxicity of deltamethrin decreased with increasing temperature with a negative temperature ratio (-2.42).

2.11 Effect of climate change and climate variability on insect vectors

Climate change and climate variability are often used interchangeably; however, there is a distinction between the two terminologies. According to the Intergovernmental Panel on Climate Change (IPCC), climate change is a statistically significant variation in either the mean state of the climate or its variability, persisting for extended periods (Elbers et al., 2015). On the other hand, according to the World Meteorological Organization (WMO), climate variability is defined as variations in the mean state and other statistics of the climate on all temporal and spatial scales, beyond individual weather events (WMO, 2019).

Climate change is no longer disputed; however, the controversy lies in the mechanisms liable for them (Beugnet & Chalvet-Monfray, 2013) and whether something should be done in response (Henderson et al., 2015). Climate change is projected to cause latitudinal and altitudinal temperature escalations. Projections by IPCC show that by the year 2100, global average surface temperature will rise by 1.1 – 6.4 °C. This increase is about 2 – 9 times higher than globally averaged warming within the past century (IPCC, 2007). In Africa, simulations of climate models under a range of possible emission scenarios propose that in all seasons, the median temperature upsurge lies within 3 and 4 °C, which is about one and half times the global mean (Herrero et al., 2010). Such a rise in temperature could modify the ecology and biology of many mosquito vectors and their transmission dynamics (Afrane et al., 2012).

Climate change or climate variability has direct effects on vectors (e.g. longevity, abundance, and distribution), hosts (e.g. distribution, abundance, and behavior), pathogens (e.g. lineage, incubation period, and replication), as well as their interactions (Tabachnick, 2010; Roiz et al., 2014). Due to the strong influence of temperature on insect's life cycle, they are expected to

react to climate change (Wilson & Maclean, 2011), and the variations in global climate will affect arthropod vectors such as mosquitoes by modifying their distribution patterns and ability to transmit diseases (Tabachnick, 2010).

In Africa, climate change or climate variability pose a significant threat to sustainable growth and development. The continent is the least contributor to climate change, yet to a larger extent, it is susceptible to climate change (Ampadu et al., 2018). In Ghana, climate change is experienced through decreased rainfall levels and increased temperatures (EPA, 2011; Mattah et al., 2018). Most climate studies in Ghana have focused on household's vulnerability (Antwi-Agyei et al., 2013; Antwi-Agyei et al., 2014), adaptation strategies (Fosu-Mensah et al., 2012; Antwi-Agyei et al., 2015), food systems/security (Armah et al., 2011; Codjoe & Owusu, 2011). Unfortunately, studies have not investigated the impacts of climate change or climate variability on mosquito's development, especially the *An. gambiae (s.l.)* species, which is the vector responsible for malaria.

According to WHO (2009a), climate change directly affects the biology of vectors, thus their abundance and distribution, which is a crucial factor of vector-borne disease outbreak. The climate parameter of interest in this research is temperature. It is considered as one of the essential abiotic environmental factors that affect physiological functions and biological processes as well as growth, reproduction, and locomotion in ectotherms (Ezeakacha & Yee, 2019). Furthermore, the temperature-dependencies are different for the four stages of the life cycle of the mosquito, and this leads to irregularities in population responses to temperature (Bayoh & Lindsay, 2003, 2004; Gilioli & Mariani, 2011; Lebl et al., 2013).

2.12 Summary of reviewed literature on temperature, *Anopheles* mosquito growth, development and survival

2.12.1 Introduction

Climate change is one of the most significant global challenges in the twenty-first century (Bulkeley, 2013). It is a global phenomenon (De Lima-Camara & Honorio, 2016; Sánchez García & Díez Sanz, 2018; Smith & Mayer, 2018), largely caused by anthropogenic activities and poses significant risks to a broad range of human and natural systems (National Research Council, 2011). Climate change is being experienced through an increase in global temperatures, sea-level rise, shrinking ice sheets, warming oceans, Arctic sea ice decline, glacial retreat, increasing extreme events, ocean acidification, and decreased snow cover (Dantas-Torres, 2015). Climate change may affect human health in many ways, including affecting livelihood and food security (Antwi-Agyei et al., 2014; Ampadu et al., 2018). In addition, climate change could directly influence the patterns of infectious diseases and vector-borne diseases (McIntyre et al., 2017) and modify vector distribution and the extension of geographical ranges of mosquitoes such as the malaria vector (Elbers et al., 2015).

Anopheles mosquitoes are poikilotherms with growth and development characteristics strongly dependent on the ambient temperature. These characteristics include the length of the gonotrophic cycle, fecundity, biting rate, longevity, and development of immature mosquitoes (Ciota et al., 2014). Thus, any factor that alters these characteristics can potentially affect the ability of mosquitoes to transmit diseases. Climate parameters such as temperature, humidity, and rainfall noticeably influence both the growth, development and survival as well as the sporogonic development of parasites in the host (Guerra et al., 2010; Hay et al., 2010; Afrane et al., 2012). Temperature also affects the immune system of mosquitoes (Murdock et al., 2012a; Murdock et al., 2012b; Murdock et al., 2013). Whereas the interventions aimed at controlling *Anopheles* mosquito employ insecticides, the efficacy of these insecticides is not only a function of the active ingredient, ambient temperatures also play a unique role in

determining the overall effectiveness of insecticide application for insect control (Glunt et al., 2014; Oxborough et al., 2015; Glunt et al., 2018).

Studies have considered the effects of temperature on different growth and development characteristics of mosquitoes, including development time, survival, body size, fecundity, and longevity (Mohammed & Chadee, 2011; Lyons et al., 2012; Carrington et al., 2013; Christiansen-Jucht et al., 2014; Ciota et al., 2014; Christiansen-Jucht et al., 2015; Oliver & Brooke, 2017; Ezeakacha & Yee, 2019). For instance, Ezeakacha and Yee (2019) investigated the role of temperature in affecting the carry-over effects and larval competition in *A. albopictus* mosquitoes and found that the development time of immature stages of mosquitoes and fecundity of adult mosquitoes decreased with increasing temperatures. The conditions at the immature stages of mosquitoes influence the quality of adult life (Mpho et al., 2002) as well as the determination of the age structure of the adult population (Beck-Johnson et al., 2013). No study has systematically synthesized the evidence linking temperature to the growth and development of *Anopheles* mosquitoes.

2.12.2 Methods

The findings of the systematic review were reported following the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines (Liberati et al., 2009). The systematic review has also been registered with PROSPERO (https://www.crd.york.ac.uk/prospero/display_record.php?ID=CRD42020196407 (accessed on 24 June 2021)) and had the registration number CRD42020196407 assigned to it.

2.12.2.1 Eligibility criteria

To assess the effects of temperature on *Anopheles* mosquito development and survival, original studies that considered either the immature or the adult *Anopheles* mosquitoes irrespective of the complex were included. In addition, the review included studies that considered either field

studies, laboratory studies, or both. Studies that evaluated any of the following outcomes: development rate, longevity, fecundity, length of the gonotrophic cycle, biting rate, susceptibility to insecticides, and the expression of enzymes and genes were also included. However, studies that did not focus on *Anopheles* mosquitoes and any of the listed outcomes were excluded. Studies not written in English were also excluded. In addition, review papers, books, opinions, scientific reports and perspectives, and duplicate records were all excluded.

2.12.2.2 Search Strategy and Selection Criteria

An initial search was conducted to identify keywords and synonyms. Research articles published up to March 2021 were systematically retrieved from PubMed, Science Direct, Scopus, ProQuest, Web of Science, and Google Scholar databases. This search was conducted in September 2020 and updated in March 2021 to retrieve any current articles. A detailed search strategy **(Appendix I)** was developed and used in the article searching stage of the systematic review. The search strategies used terms such as *Anopheles* mosquito, malaria, temperature, temp*, season*, survival, longevity, among others. Combinations of different search strings and search terms were employed for each electronic database to enhance the search's sensitivity and specificity. Articles were exported into EndNote reference manager (version X9). Three independent reviewers screened the titles and abstract of search results to assess potentially eligible studies. Full-text articles were then retrieved and reviewed to obtain the final set of articles included in the review. Disagreements in the screening and selection of articles were resolved by dialogue, and a consensus was reached at all stages.

2.12.2.3 Data Extraction

A data-extraction form was pretested and later revised to include author details, study type, study location, *Anopheles* species considered, the rearing conditions, and the outcome of interest. Data from the included studies were first extracted and reviewed by three reviewers

independently and later jointly to resolve disagreements. Where it became necessary, corresponding authors of some of the included studies were contacted for further information.

2.12.2.4 Risk of Bias Assessment

Three reviewers independently assessed the risk of bias of included studies. Disagreements were resolved through discussion and involvement of a fourth person where necessary. The risk of bias was assessed using the Systematic Review Center for Laboratory Animal Experimentation's (SYRCLE's) tool for animal studies (Hooijmans et al., 2014). The tool comprises ten (10) domains with six (6) types of bias: selection bias, performance bias, detection bias, attrition bias, reporting bias, and others. The ten (10) items are structured in sub-sections in question forms that require a "Yes (low risk)," "No (high risk)," or "Unclear (unclear risk)" answer.

2.12.2.5 Data Analysis

A narrative synthesis of all the included studies was performed based on the outcome of interest, and the findings were reported in tabular form for easy interpretation and understanding. All the included studies were quantitative; however, this review did not include a meta-analysis.

2.12.3 Synthesis of evidence and gaps in current literature on temperature, growth, development, and survival of *Anopheles* mosquito

2.12.3.1 Search results

From the search, 8130, 5926, 1403, 1156, 850, and 17 records were retrieved from Google Scholar, Scopus, Science Direct, PubMed, ProQuest, and Web of Science databases, respectively **(Appendix I)**. Four (4) additional articles were obtained through contacts with experts in the field and screening the reference lists of included studies. After removing duplicates and screening titles and abstracts, 65 records were included for full-text assessment. Thirty-six (36) articles were excluded with reasons **(Appendix II)**, while 29 articles (Wallace

& Merritt, 1999; Bayoh & Lindsay, 2003, 2004; Rúa et al., 2005; Impoinvil et al., 2007; Aytekin et al., 2009; Kirby & Lindsay, 2009; Olayemi et al., 2011; Phasomkusolsil et al., 2011; Charlwood & Bragança, 2012; Lyons et al., 2012; Murdock et al., 2012a; Lyons et al., 2013; Murdock et al., 2013; Paaijmans et al., 2013a; Paaijmans et al., 2013b; Christiansen-Jucht et al., 2014; Glunt et al., 2014; Mala et al., 2014; Murdock et al., 2014; Christiansen-Jucht et al., 2015; Davies et al., 2016; Barreaux et al., 2016b; Faiman et al., 2017; Oliver & Brooke, 2017; Shapiro et al., 2017; Barreaux et al., 2018; Glunt et al., 2018; Mamai et al., 2018) fully met the inclusion criteria (Figure 7).

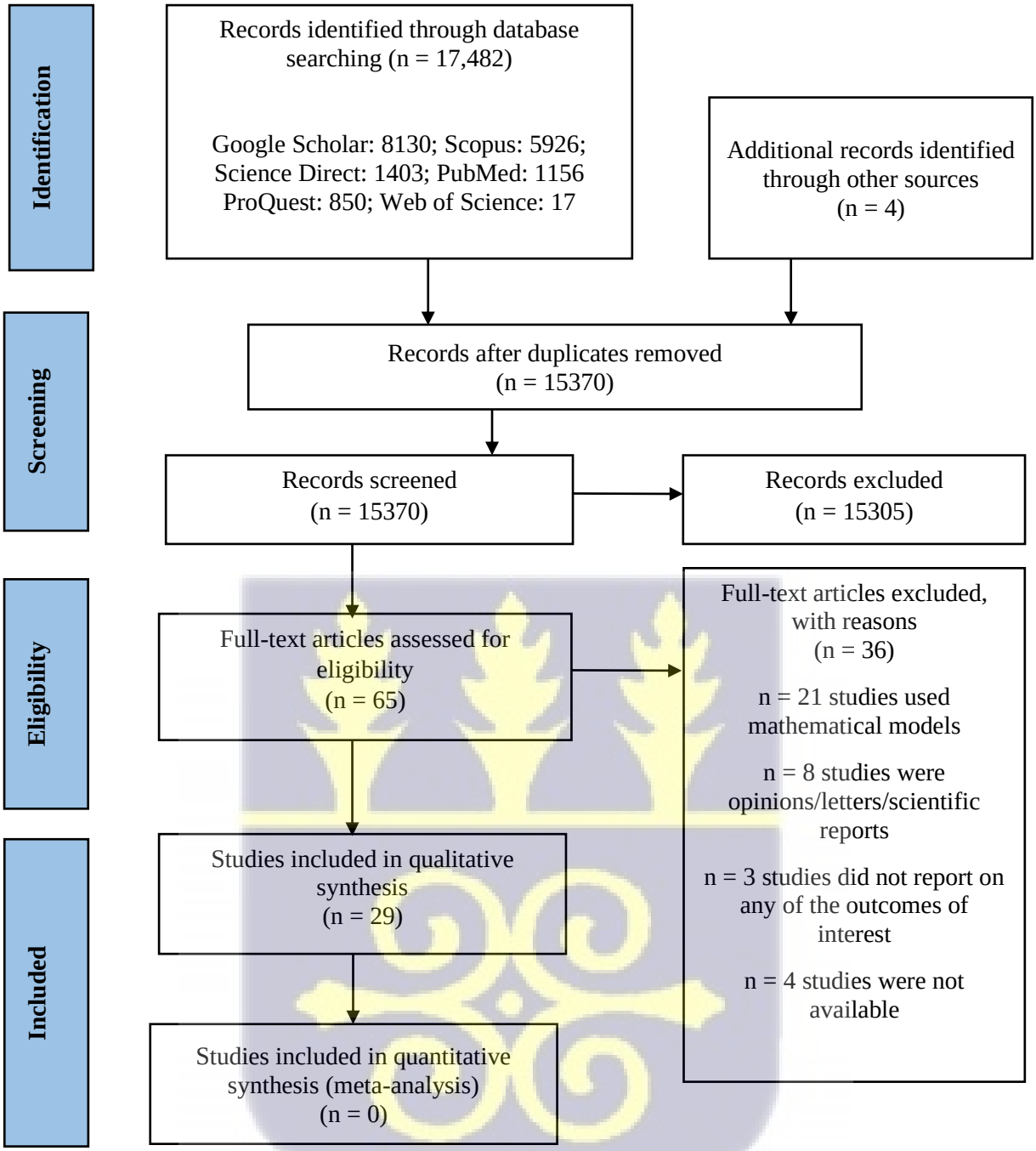

Figure 7: PRISMA flow diagram of search phases with numbers of studies included/excluded at each stage

2.12.3.2 Study Characteristics

The included studies consisted of twenty-six (26) laboratory-based studies, two (2) field-based studies, and one (1) study that employed both study designs. Different species of *Anopheles* mosquitoes were reported in the included studies. Most of the included studies were conducted in Africa (9), North America (9), Europe (8), and Asia (2). However, one study did not indicate the study location. About 12 different *Anopheles* species were reported in the 29 studies, and the majority of these species were *An. gambiae (s.s.)* (9), *An. arabiensis* (8), *An. stephensi* (7), and *An. funestus* (5) (Table 1).

Table 1: Characteristics of included studies

Study Characteristics	Frequency (N = 29)	Percentage (%)
Study design employed		
Laboratory-based	26	89.66
Field-based	2	6.90
Laboratory- & Field-based	1	3.45
Study location		
North America	9	31.03
Africa	9	31.03
Europe	8	27.59
Asia	2	6.90
***Anopheles* species studied**		
An. gambiae (s.s)	9	31.03
An. arabiensis	8	27.59
An. stephensi	7	24.14
An. funestus	5	17.24
An. quadrinnulatus	2	6.90
An. superpictus	1	3.45
An. coluzzii	1	3.45
An. pharaoensis	1	3.45
An. coustani	1	3.45
An. dirus	1	3.45
An. sawadwongporni	1	3.45
An. albimanus	1	3.45

2.12.3.3 Risk of bias (RoB) assessment

2.12.3.3.1 Selection bias

Except for one study (Mala et al., 2014), which was at low risk, all 28 studies reviewed were at high risk of sequence generation. With baseline characteristics, only two studies (Olayemi et al., 2011; Charlwood & Bragança, 2012) had unclear risk. Additionally, the remaining 27 had low risk. Concerning allocation concealment, the risk was unclear in twelve (12) studies (Bayoh & Lindsay, 2003, 2004; Impoinvil et al., 2007; Aytekin et al., 2009; Kirby & Lindsay, 2009; Charlwood & Bragança, 2012; Christiansen-Jucht et al., 2014, 2015; Davies et al., 2016; Barreaux et al., 2016b; Faiman et al., 2017; Barreaux et al., 2018), while the remaining fifteen (17) studies were at high risk. However, the absence of sequence generation and allocation concealment is unlikely to influence the findings **(Appendix III)**.

2.12.3.3.2 Blinding and randomization (Performance and Detection Bias)

Unlike drug trials, where it is easy to blind investigators from the intervention being administered, the investigator is not usually blinded to the treatments in most insect studies. Blinding and randomization were not applicable to the systematic review.

2.12.3.3.3 Bias (Attrition and Reporting)

All the 29 studies had a low risk of attrition and reporting bias. The studies presented a detailed and consistent reporting of all outcomes pre-specified in the methods section **(Appendix III)**.

2.12.3.3.4 Other Sources of Bias (Funding Source and Rearing of Mosquitoes)

Except for eight (8) studies (Rúa et al., 2005; Aytekin et al., 2009; Kirby & Lindsay, 2009; Olayemi et al., 2011; Phasomkusolsil et al., 2011; Charlwood & Bragança, 2012; Paaijmans et al., 2013a; Barreaux et al., 2016b) that failed to disclose funding sources, the majority of the studies (20) declared the source of funding and funders did not influence the results. However, one study (Shapiro et al., 2017) had an unclear risk. Although the study indicated that funding was acquired, it did not state or provide enough information to judge funding sources.

In assessing how temperature affects *Anopheles* mosquitoes, most of the studies reared the mosquitoes in incubators from either the egg or larval stage to adult. Rearing mosquitoes in incubators from the egg or larval to the adult stages may better assess the effect of temperature on the mosquito. Nine (9) studies (Olayemi et al., 2011; Charlwood & Bragança, 2012; Lyons et al., 2012; Murdock et al., 2012a; Murdock et al., 2013; Glunt et al., 2014; Mala et al., 2014; Murdock et al., 2014; Glunt et al., 2018) were at high risk of bias based on mosquito rearing conditions **(Appendix III)**. In some of these studies, adult mosquitoes were only exposed to the selected temperature regimes before outcome assessment, which may affect the study's outcome.

2.11.3.4 Effects of temperature on immature stages of mosquitoes

Sixteen (16) studies assessed the effects of temperature on different *Anopheles* species (Table 2). These studies considered larval and pupal development and survival, as well as egg hatchability. The way temperature affected the immature stages of mosquitoes differed from species to species, even among the same complex. The immature stages of *An. arabiensis* were more tolerant (in terms of survival) to a higher temperature than *An. funestus* (Lyons et al., 2012), and *An. quadriannulatus* (Davies et al., 2016). In addition, *An. arabiensis* showed faster development rates (in days) compared to *An. funestus* (Lyons et al., 2013) and *An. quadriannulatus* (Davies et al., 2016).

The minimum and maximum temperatures from these studies were 10 and 40 °C, respectively. One study (Christiansen-Jucht et al., 2015) indicated that higher temperatures (23 to 31 °C) resulted in smaller larval sizes and slowed the development from hatching to adult emergence. However, most studies (Bayoh & Lindsay, 2003; Aytekin et al., 2009; Kirby & Lindsay, 2009; Phasomkusolsil et al., 2011; Paaijmans et al., 2013b; Oliver & Brooke, 2017) observed that increasing temperature reduced the development time (in days) of the immature stages. For instance, Phasomkusolsil et al. (2011) observed that *An. dirus* and *An. sawadwongporni* larvae

reared at 30 °C displayed a significantly shorter developmental time (approximately 7 – 8 days) than those reared at 23 °C (12 – 14 days) ($P < 0.05$). Higher temperatures (30 and 35 °C) significantly increased larval development rates in two *An. arabiensis* strains–SENN DDT (one-way ANOVA: F = 15.1; $P < 0.01$) and SENN (one-way ANOVA: F = 12.4; $P < 0.01$) relative to their respective 25 °C control cohorts (Oliver & Brooke, 2017).

An increase in temperature significantly decreased the time to pupation of *An. gambiae (s.s.)* larvae from 9.2 ± 0.05 days at 21 °C to 8.3 ± 0.04 days at 25 °C and 7.8 ± 0.05 days at 29 °C (Barreaux et al., 2018), and increased larval mortality (Bayoh & Lindsay, 2004; Christiansen-Jucht et al., 2014). Christiansen-Jucht et al. (2014) reported that, an increase in temperature at varying intervals of 4 °C (from 23 °C to 27 °C, $P < 0.001$), 8 °C (from 27 °C to 35 °C, $P < 0.001$), and 12 °C (from 23 °C to 35 °C, $P < 0.001$) significantly decreased larval survival.

Increasing temperature decreased the time to hatch but not the hatching rate of *Anopheles* eggs. For instance, hatching of *An. arabiensis* eggs were fastest at 27 °C and slowest at 22 °C; nevertheless, most of the eggs hatched within two days irrespective of the water temperature (Mamai et al., 2018). There was no significant difference ($P > 0.05$) between the mean hatching rate of *An. dirus* and *An. sawadwongporni* eggs reared at 23 °C and 30 °C (Phasomkusolsil et al., 2011). However, extremely high temperatures can affect the hatchability of eggs. Impoinvil et al. (2007) observed that incubating eggs at 42 °C for a day resulted in a low mean hatching count relative to the other temperatures. There was no hatching of eggs when the incubation period was extended to 3, 7, and 10 days.

Table 2: Effects of temperature on immature stages of *Anopheles* mosquitoes

Author, Year	Study Type	Study Location	Species Considered	Conditions	Outcome Considered
Christiansen-Jucht et al. (2015)	Laboratory-based	United Kingdom	*An. gambiae (s.s.)*	23, 27, 31, and 35 ± 1 °C 12:12 (L:D) photoperiod RH 75% ± 5%	• Egg hatching time ** • Development time * • Larval size **
Davies et al. (2016)	Laboratory-based	South Africa	*An. arabiensis* *An. quadriannulatus*	25, 20–30, and 18–35 °C 12:12 (L:D) photoperiod RH 80%	• Egg hatching time ** • Development time ** • Larval survival **
Impoinvil et al. (2007)	Laboratory-based	Kenya	*An. gambiae (s.s.)*	Immature: 30–35 °C Adult: 22–27 °C RH 80–90%	• Egg hatching count *
Mamai et al. (2018)	Laboratory-based	Austria	*An. arabiensis*	22 ± 1 °C, 22–27 ± 1 °C, 27 ± 1 °C 12:12 (L:D) photoperiod RH 80%	• Egg hatching time ** • Pupation success
Phasomkusolsil et al. (2011)	Laboratory-based	Thailand	*An. dirus* *An. sawadwongporni*	23 and 30 °C	• Egg hatching time • Development time *
Aytekin et al. (2009)	Laboratory-based	Turkey	*An. superpictus*	15, 20, 25, 27, 30, and 35 °C, 12:12 (L:D) photoperiod RH 65% ± 5%	• Egg hatching count • Development time ** • Larval survival
Bayoh and Lindsay (2003)	Laboratory-based	United Kingdom	*An. gambiae (s.s.)*	10 to 40 °C (±1 °C), with 2 °C increments 12:12 (L:D) photoperiod RH 80% ± 10%	• Development time ** • Adult emergence **
Kirby and Lindsay (2009)	Laboratory-based	United Kingdom	*An. gambiae (s.s.)* *An. arabiensis*	25, 30, or 35 °C	• Development time ** • Larval survival **
Lyons et al. (2013)	Laboratory-based	South Africa	*An. arabiensis* *An. funestus*	15, 18, 20, 22, 25, 28, 30, 32 35, 15 °C–35, and 20–30 °C 12:12 (L:D) photoperiod RH 80%	• Development time ** • Survival of immature stages

Outcomes with a single asterisk (*) indicate that higher temperatures generally increased the outcomes; Outcomes with a double asterisk (**) indicate that higher temperatures generally decreased those outcomes; Outcomes with no asterisk indicate no significant effect of temperature.

Table 2 continued

Author, Year	Study Type	Study Location	Species Considered	Conditions	Outcome Considered
Oliver and Brooke (2017)	Laboratory-based	South Africa	*An. arabiensis*	25, 30, and 35 °C RH 80% ± 5%	• Development time **
Paaijmans et al. (2013b)	Laboratory-based	United States of America	*An. stephensi*	16 to 36 °C, with 2 °C increments	• Development time ** • Larval survival **
Wallace and Merritt (1999)	Field and Laboratory-based	United States of America	*An. quadrimaculatus*	18, 23, and 28 °C	• Larval survival **
Lyons et al. (2012)	Laboratory-based	South Africa	*An. funestus* *An. arabiensis*	20, 25, and 30 °C 12:12 (L:D) photoperiod RH 80%	• Larval survival **
Bayoh and Lindsay (2004)	Laboratory-based	United Kingdom	*An. gambiae (s.s.)*	10 to 40 °C (±1 °C), with 2 °C increments 12:12 (L:D) photoperiod RH 80 ± 10%	• Larval survival **
Christiansen-Jucht et al. (2014)	Laboratory-based	United Kingdom	*An. gambiae (s.s.)*	23, 27, 31, and 35 ± 1 °C 12:12 (L:D) photoperiod RH 75% ± 5%	• Larval survival **
Barreaux et al. (2018)	Laboratory-based	Switzerland	*An. gambiae (s.s.)*	21, 25, and 29 °C 12:12 (L:D) photoperiod RH 70% ± 5%	• Time to pupation**

Outcomes with a single asterisk (*) indicate that higher temperatures generally increased the outcomes; Outcomes with a double asterisk (**) indicate that higher temperatures generally decreased those outcomes; Outcomes with no asterisk indicate no significant effect of temperature.

2.11.3.5 Effects of temperature on the growth and development of adult mosquitoes

2.11.3.5.1 Longevity

Five (5) studies (Aytekin et al., 2009; Olayemi et al., 2011; Faiman et al., 2017; Oliver & Brooke, 2017; Barreaux et al., 2018) assessed the longevity of different *Anopheles* mosquitoes from either field or laboratory populations (Table 3). Olayemi et al. (2011) reported that the longevity and survival rate of *An. gambiae (s.l.)* mosquitoes were higher in the rainy season (17.48 ± 2.92 days and 84.5 % ± 10.46 %, respectively) than in the dry season (7.29 ± 2.82 days and 57.47 % ± 14.9 %, respectively). The rainy season is associated with cooler temperatures and the dry season with hotter temperatures. In addition, Faiman et al. (2017) observed that the longevity of *An. coluzzii* increased at a lower temperature; however, the main effect of temperature was not statistically significant ($P = 0.072$). They detected higher longevity at a lower temperature in each experiment and between 22 °C and 23.5 °C ($P < 0.001$) but not between experiments at 27 °C ($P = 0.072$). Similar trends were reported by Aytekin et al. (2009) and Barreaux et al. (2018). More adult *An. gambiae (s.s.)* died with every increase in temperature compared to the baseline temperature (i.e., 23 °C). All the p-values were statistically significant ($P < 0.001$) for comparisons of 27 °C vs. 23 °C, 31 °C vs. 27 °C, and 31 °C vs. 23 °C (Christiansen-Jucht et al., 2014).

Table 3: Effects of temperature on the longevity of *Anopheles* mosquitoes

Author, Year	Study Type	Study Location	Species Considered	Conditions	Outcome Considered
Aytekin et al. (2009)	Laboratory-based	Turkey	*An. superpictus*	15, 20, 25, 27, 30, and 35 °C, 12:12 (L:D) photoperiod RH 65% ± 5%	• Longevity **
Barreaux et al. (2018)	Laboratory-based	Switzerland	*An. gambiae (s.s.)*	21, 25, and 29 °C 12:12 (L:D) photoperiod RH 70% ± 5%	• Longevity **
Faiman et al. (2017)	Laboratory-based	United States of America	*An. coluzzii*	22, 23.5, and 27 °C, 2:12 or 11:13 L:D photoperiod RH 85% and 50%	• Longevity **
Olayemi et al. (2011)	Field and Laboratory-based	Nigeria	*An. gambiae (s.l.)*	**Seasons** Dry: 31.12 ± 1.09 °C, RH 44.01 ± 7.02% Rainy: 27.67 ± 1.27 °C, RH 69.51% ± 12.44%	• Longevity **
Oliver and Brooke (2017)	Laboratory-based	South Africa	*An. arabiensis*	25, 30, and 35 °C RH 80% ± 5%	• Longevity **

Outcomes with a single asterisk (*) indicate that higher temperatures generally increased the outcomes; Outcomes with a double asterisk (**) indicate that higher temperatures generally decreased those outcomes; Outcomes with no asterisk indicate no significant effect of temperature.

2.11.3.5.2 Body size

In most mosquito studies, wing length has been used as a proxy to measure mosquito body size. All the seven (7) studies (Aytekin et al., 2009; Kirby & Lindsay, 2009; Phasomkusolsil et al., 2011; Charlwood & Bragança, 2012; Christiansen-Jucht et al., 2015; Barreaux et al., 2016b; Barreaux et al., 2018) reported on body size showed a decrease in wing length and body weight with increasing temperature (Table 4). For instance, *An. dirus* and *An. sawadwongporni* mosquitoes reared at 23 °C were significantly heavier and longer than those reared at 30 °C ($P < 0.05$) (Phasomkusolsil et al., 2011). Barreaux et al. (2018) also observed that the wing length of *An. gambiae (s.s.)* mosquitoes decreased significantly ($F(2, 181) = 35.7$, $P < 0.0001$) with

increasing temperature from 3.27 mm at 21 °C to 3.23 mm at 25 °C and 3.02 mm at 29 °C. Except for Charlwood and Bragança (2012), who measured body sizes of field-collected mosquitoes; all the remaining studies measured the body size of adult mosquitoes reared from the egg stage through to adult. Only Christiansen-Jucht et al. (2015) measured the size of the larvae in addition to the adult mosquitoes.

Table 4: Effects of temperature on the body size of *Anopheles* mosquitoes

Author, Year	Study Type	Study Location	Species Considered	Conditions	Outcome Considered
Aytekin et al. (2009)	Laboratory-based	Turkey	*An. superpictus*	15, 20, 25, 27, 30, and 35 °C, 12:12 (L:D) photoperiod RH 65% ± 5%	• Body size **
Barreaux et al. (2016b)	Laboratory-based	Switzerland	*An. gambiae (s.s.)*	21 °C, 25 °C, and 29 °C	• Body size **
Barreaux et al. (2018)	Laboratory-based	Switzerland	*An. gambiae (s.s.)*	21, 25, and 29 °C 12:12 (L:D) photoperiod RH 70% ± 5%	• Body size **
Charlwood and Bragança (2012)	Field-based	Mozambique	*An. funestus*	17 to 33 °C	• Body size **
	Laboratory-based	United Kingdom	*An. gambiae (s.s.)*	23, 27, 31, and 35 ± 1°C 12:12 (L:D) photoperiod RH 75% ± 5%	• Body size **
Kirby and Lindsay (2009)	Laboratory-based	United Kingdom	*An. gambiae (s.s.)* *An. arabiensis*	25, 30 or 35 °C	• Body size **
Phasomkusolsil et al. (2011)	Laboratory-based	Thailand	*An. dirus* *An. sawadwongporni*	23 and 30 °C	• Body size **

The double asterisk (**) indicates that higher temperatures generally decreased those outcomes.

2.11.3.5.3 Fecundity, length of the gonotrophic cycle, and biting rate

Four (4) studies (Aytekin et al., 2009; Phasomkusolsil et al., 2011; Mala et al., 2014; Christiansen-Jucht et al., 2015) assessed the effects of temperature on fecundity. Similarly, four studies (Rúa et al., 2005; Paaijmans et al., 2013a; Mala et al., 2014; Shapiro et al., 2017) also assessed the effects of temperature on gonotrophic cycle length, with only one study (Shapiro

et al., 2017) considering biting rate (Table 5). Three studies reported on fecundity (Aytekin et al., 2009; Phasomkusolsil et al., 2011; Christiansen-Jucht et al., 2015) showed a decrease in fecundity with increasing temperature. For example, the mean number of eggs laid by *An. dirus* and *An. sawadwongporni* mosquitoes reared at 23 °C was significantly higher than those reared at 30 °C ($P < 0.05$) (Phasomkusolsil et al., 2011). However, according to Mala et al. (2014), significantly fewer *Anopheles* mosquitoes laid eggs during the dry season (38.2 %) than during the wet season (61.8 %) ($t = 8.85$, $df = 1$, $P < 0.05$). In addition, none of the adult mosquitoes emerged from a larval temperature of 20, 30, and 35 °C laid eggs (Aytekin et al., 2009).

All the studies reported on the gonotrophic cycle showed a decrease in gonotrophic cycle length with increasing temperature. The duration of the gonotrophic cycle was significantly different ($X^2(2) = 96.68$, $P < 0.001$) between the two seasons, as the duration of the first and second cycles was longer in the wet season (4.1 and 2.9 days, respectively) than in the dry season (3.0 and 2.2 days, respectively) (Mala et al., 2014). In contrast, the temperature of the adult environment did not influence the probability of *An. gambiae (s.s.)* female mosquitoes laying eggs after their first or third blood meal. However, after the second blood meal, an increase from 23 to 31 °C, and 27 to 31 °C led to a significantly lower possibility of laying eggs (0.72 vs. 0.46, $P = 0.002$, and 0.65 vs. 0.46, $P = 0.022$, respectively) (Christiansen-Jucht et al., 2015). Shapiro et al. (2017) also observed that the proportion of *An. stephensi* mosquitoes laying eggs was lower during the second gonotrophic cycle than the first; however, there was no noticeable effect of temperature on the probability of egg-laying in either cycle. Shapiro et al. (2017) discovered that the biting rates of *An. stephensi* increased with increasing temperature. From their results, biting rates almost doubled when the temperature increased from 21 to 32 °C. The biting rate was estimated in their study as the inverse of the length of the gonotrophic cycle.

Table 5: Effects of temperature on fecundity, length of the gonotrophic cycle, and biting rate of *Anopheles* mosquitoes

Author, Year	Study Type	Study Location	Species Considered	Conditions	Outcome Considered
Aytekin et al. (2009)	Laboratory-based	Turkey	*An. superpictus*	15, 20, 25, 27, 30, and 35 °C, 12:12 (L:D) photoperiod RH 65% ± 5%	• Fecundity **
Christiansen-Jucht et al. (2015)	Laboratory-based	United Kingdom	*An. gambiae (s.s.)*	23, 27, 31, and 35 ± 1 °C 12:12 (L:D) photoperiod RH 75% ± 5%	• Fecundity **
Phasomkusolsil et al. (2011)	Laboratory-based	Thailand	*An. dirus* *An. sawadwongporni*	23 and 30 °C	• Fecundity **
Mala et al. (2014)	Field-based	Kenya	*An. arabiensis* *An. pharaoensis* *An. coustani* *An. funestus*	Indoor Temp Dry season (28.22 ± 1.1 °C) Rainy season (27.12 ± 1.2 °C) Outdoor Temp Dry season (26.32 ± 0.33 °C) Rainy season (24.82 ± 0.33 °C)	• Fecundity * • Gonotrophic cycle length **
Paaijmans et al. (2013a)	Laboratory-based	United States of America	*An. stephensi*	22, 24, and 26 °C 12:12 (L:D) photoperiod RH 90% ± 5%	• Gonotrophic cycle length **
Rúa et al. (2005)	Laboratory-based		*An. albimanus*	24, 27, and 30 °C	• Gonotrophic cycle length **
Shapiro et al. (2017)	Laboratory-based	United States of America	*An. stephensi*	21, 24, 27, 30, 32, and 34 °C	• Gonotrophic cycle length ** • Biting rate *

Single asterisk (*) indicates that higher temperatures generally increased the outcomes; Double asterisk (**) indicates that higher temperatures generally decreased those outcomes.

2.11.3.6 Effects of temperature on the expression of enzymes and susceptibility to insecticides

Four (4) studies (Murdock et al., 2012a; Murdock et al., 2013; Murdock et al., 2014; Oliver & Brooke, 2017) assessed the effects of temperature on enzyme expression and immune responses in *Anopheles* mosquitoes (Table 6). Temperature significantly affected immune responses such as humoral melanization, defensin (DEF1), cecropin (CEC1), phagocytosis, and nitric oxide synthase (NOS) in *An. stephensi* mosquitoes. For instance, NOS expression peaked

at later sampling time points in mosquitoes kept at cooler temperatures (18 °C: 24 h; 22 °C: 18 h) compared to those held at optimal or warmer temperatures (26 – 34 °C: 12 h) (Murdock et al., 2012a). A study conducted by Murdock et al. (2014) also found that NOS significantly increased at warmer temperatures (28 °C) compared to colder temperatures (20 °C vs. 28 °C, $P = 0.002$; 24 °C vs. 28 °C, $P = 0.001$). Oliver and Brooke (2017) noted no significant increase in detoxification enzyme (cytochrome P450 and general esterases) systems of *An. arabiensis* mosquitoes at 25 and 37 °C.

Increasing temperature reduced the efficacy of insecticides in all three studies (Glunt et al., 2014; Oliver & Brooke, 2017; Glunt et al., 2018) that considered insecticide susceptibility (Table 6). Higher rearing temperatures and short-term exposure to 37 and 39 °C as adults increased pyrethroid resistance in adults of the *An. arabiensis* SENN DDT strain, and increased pyrethroid tolerance in the *An. arabiensis* SENN strain. There was a decrease in the toxicity of deltamethrin insecticide in the unselected SENN strain as the temperature increased. Likewise, increasing temperatures increased the resistance of the susceptible *An. arabiensis* strain to deltamethrin (Glunt et al., 2018). However, one study (Oliver & Brooke, 2017) observed no significant difference in mortality induced at either 37 or 39 °C for lambda-cyhalothrin (two-sample t-test: $t = 0.47$, $P = 0.64$) and permethrin (two-sample t-test: $t = -0.63$, $P = 0.55$).

Table 6: Effects of temperature on insecticide susceptibility, expression of enzymes and immune responses in *Anopheles* mosquitoes

Author, Year	Study Type	Study Location	Species Considered	Conditions	Outcome Considered
Glunt et al. (2018)	Laboratory-based	South Africa	*An. funestus* *An. arabiensis*	18 °C, 25 °C, and 30 °C RH 70% for 18 °C and 30 °C RH 80% for 25 °C	• Insecticide susceptibility (deltamethrin, bendiocarb, synergist PBO) **
Glunt et al. (2014)	Laboratory-based	United States of America	*An. stephensi*	12, 18, 22, and 26 °C	• Insecticide susceptibility (malathion, permethrin)
Oliver and Brooke (2017)	Laboratory-based	South Africa	*An. arabiensis*	25, 30, and 35 °C RH 80% ± 5%	• Insecticide susceptibility • Detoxification enzyme activity
Murdock et al. (2014)	Laboratory-based	United States of America	*An. stephensi*	20, 22, 24, 26, and 28 ± 0.5 °C 12:12 (L:D) photoperiod RH 80% ± 5%	• Nitric oxide synthase *
Murdock et al. (2013)	Laboratory-based	United States of America	*An. stephensi*	16, 26, 32 ± 0.5 °C; 16, 26, 32 ± 6 °C 12:12 (L:D) photoperiod RH 80% ± 5%	• Defensin • Cecropin • Nitric oxide synthase
Murdock et al. (2012a)	Laboratory-based	United States of America	*An. stephensi*	12, 18, 24, 28, and 34 + 0.5 °C 12:12 (L:D) photoperiod RH 80% ± 5%	• Humoral melanization • Cecropin • Phagocytosis** • Defensin • Nitric oxide synthase *

Outcomes with a single asterisk (*) indicate that higher temperatures generally increased the outcomes; Outcomes with a double asterisk (**) indicate that higher temperatures generally decreased those outcomes; Outcomes with no asterisk indicate no significant effect of temperature.

2.12.4 Discussion

This study reviewed and assessed literature for evidence of the effects of temperature on the growth and development of *Anopheles* mosquitoes, expression of enzymes and genes, and susceptibility to insecticides. The life cycle of mosquitoes is interdependent; thus, environmental conditions and individual characteristics in one life stage affect the other life stages (Green & McCormick, 2005; McCormick & Gagliano, 2008). An increase in temperature may have long-term repercussions on future generations (Green & McCormick, 2005). The sensitivities of adult mosquitoes to temperature differ from those of the juvenile stages (Beck-Johnson et al., 2013).

Some of the included studies reared mosquitoes at a constant temperature and only exposed them to different temperature regimes prior to outcome assessment. These studies might have missed the early effects of temperature on mosquitoes; hence, the outcome of interest could be affected. The effects of rearing temperature on the immature stages can affect the growth and development of the adult mosquito and its overall fitness (Carrington et al., 2013; Ukubuiwe et al., 2018; Ezeakacha & Yee, 2019).

2.12.3.1 Effects of temperature on immature stages of mosquitoes

The immature stages of mosquitoes play a critical role in determining vector-borne disease dynamics. For instance, the variations in mosquito population size are determined primarily by changes that occur during larval development and growth, directly affecting the transmission of vector-borne diseases. Moreover, the larval stage's carry-over effects can affect vectorial capacity traits such as fecundity, longevity, biting behavior, and vector competence (Barreaux et al., 2018).

From the review, the time to pupation *An. gambiae (s.s.)* mosquitoes was mentioned to significantly decrease with increased temperature (Barreaux et al., 2018). There is consistency

in the existing literature that the rate of development of the immature stages of mosquitoes is temperature-dependent (Protopopoff et al., 2009; Afrane et al., 2012). However, there were few inconsistencies in the effects of temperature on development times. It is unclear what could have accounted for differences in the results; further studies are needed to clarify these discrepancies. High temperatures are generally associated with faster development rates and have diverse effects on the insect's juvenile stages (Kingsolver & Huey, 2008; Kirby & Lindsay, 2009). However, extremely high (≥ 34 °C) temperatures delay larval development time and can induce high mortalities (Bayoh & Lindsay, 2003; Afrane et al., 2012). Some studies (Christiansen-Jucht et al., 2014, 2015) observed that no *Anopheles* larvae survived at 35 °C. The physiological explanation underlying this is unclear; however, one of the attributable reasons is that when fourth instar larvae are developing at a faster rate, they are unable to adjust to the associated nutrient consumption, metabolism, or accumulation, which is needed for the intricate physiological process in the change from larvae to pupa (Bayoh & Lindsay, 2003). In addition, thermal stress could affect the survival of immature mosquitoes (Ukubuiwe et al., 2018). The immature stages are sensitive to temperature because they usually live in small, isolated pools and cannot easily escape unfavorable environments (Amer et al., 2021). To overcome the thermal stress experienced, mosquitoes may have to increase their metabolic rates, resulting in higher energy expenditure (Shah et al., 2020). This could exceed oxygen supply from the environment leading to decreased performance, lowered tolerance to thermal stress (Pörtner et al., 2017), and the death of the mosquito.

The review showed that higher temperatures (23 to 31 °C) resulted in smaller larval sizes. This confirms the findings of Dodson et al. (2012), who reported that increasing temperature resulted in smaller body sizes for *Cx. tarsalis*. The mosquito's size, especially the female, influences many epidemiologically important physiognomies, such as longevity, gonotrophic cycle length, biting rate, immunocompetence, and intensity of infection (Christiansen-Jucht et

al., 2014). These physiognomies thus affect parasite development (Churcher et al., 2013) and mosquito survival (Dawes et al., 2009). This could explain why increasing temperature significantly increased larval mortality (Barreaux et al., 2018). It was noted that the way temperature affected the immature stages of mosquitoes differed from species to species, even among the same complex. However, the trend of increasing temperature with a small larval size did not change.

Only one study assessed the effects of temperature on the number of adults produced. The number of adults produced from the immature stages provides useful information in determining the population dynamics. Further studies are needed to assess how temperature influences the overall productivity (number of adults produced) of the immature stages. Furthermore, none of the studies evaluated the effects of temperature on the sex ratio of the emerged adults. The number of male and female mosquitoes emerging from the immature stages is critical in controlling mosquito populations as more males could increase the mosquito population due to increased mating probability (Mohammed & Chadee, 2011).

2.12.3.2 Effects of temperature on adult mosquitoes

2.12.3.2.1 Growth and development characteristics

The life expectancy of adult mosquitoes is sometimes shorter than the time required for the parasite to develop in the mosquito. Therefore, the longevity of the adult female mosquito is a significant factor in transmitting the parasite (Beck-Johnson et al., 2013). For example, malaria and other diseases such as dengue and filariasis require a minimum extrinsic incubation period (EIP) of 10 days before the female mosquito can be infective (Rajatileka et al., 2011). Before parasite transmission, the female mosquito must live longer to acquire the pathogen via a blood meal, survive beyond the extrinsic incubation period (EIP), and transmit the pathogen to a host during successive blood-feeding (Rajatileka et al., 2011). The review showed that increasing

temperature and seasonal temperature variations decreased the longevity and increased the mortality of *Anopheles* mosquitoes. In addition, newly emerged adult mosquitoes thrived better with elevated temperatures than older mosquitoes (Lyons et al., 2012). The longevity and survival rate of *An. gambiae (s.l.)* showed significant seasonal variations, with much higher values observed in the rainy season (low temperature) than in the dry season (high temperature) (Olayemi et al., 2011). Likewise, as temperatures increased from 15 to 35 °C, the longevity of *Anopheles* mosquitoes decreased. This is similar to other studies (Swain et al., 2008; Marinho et al., 2016; Bhuju et al., 2018) that reported that mosquito longevity and mortality are negatively affected at higher temperatures. Increasing temperature decreased the longevity of mosquitoes and increased mosquito mortalities (Marinho et al., 2016; Onyango et al., 2020). The relationship between temperature and longevity can be explained in two ways. First, higher temperatures may decrease the longevity by speeding the reaction rate of various metabolic processes that affect the growth and development of the mosquito. Second, higher temperatures might heighten the damage caused by the by-products of metabolism, such as reactive oxygen species (ROS) (Keil et al., 2015). This could make mosquitoes weak and induce high mortalities hence, decreasing the longevity of mosquitoes.

The review also revealed that increasing temperature reduced the body size of *Anopheles* mosquitoes. This is in agreement with the findings of Dodson et al. (2012), who reported that increasing larval rearing temperature resulted in smaller body size for *Cx. tarsalis*. The conditions in the larval environment can affect the size of the larvae and consequently the size of the adult mosquito (Lehmann et al., 2006). Generally, mosquitoes with large body sizes have more teneral reserves carried over from the juvenile stages; hence, they live longer than those with small body sizes (Barreaux et al., 2018). The size of mosquitoes affects many epidemiologically important traits, such as longevity, gonotrophic cycle length, biting rate, immunocompetence, and infection intensity (Christiansen-Jucht et al., 2014). Thus, these traits

affected parasite development (Churcher et al., 2013) and the vector's survival (Dawes et al., 2009). Furthermore, mosquito size may affect the flight range as larger mosquitoes may have a better flight range than smaller ones (Yeap et al., 2013). In this sense, increasing temperatures may reduce the spread of mosquitoes within a locality.

It was revealed that higher temperatures decreased the fecundity of *Anopheles* mosquitoes. This corroborates data in the literature, suggesting that higher temperatures reduce mosquito fecundity (Carrington et al., 2013; Marinho et al., 2016; Ezeakacha & Yee, 2019). However, one study (Mala et al., 2014) reported otherwise. The temperature difference between the two seasons reported in the study (Mala et al., 2014) was less than 2 °C (Table 5). Mala et al. (2014) findings may not only be attributed to seasonal variation as the mosquitoes used in their study might have come from a diverse population with different genetic compositions. Furthermore, the failure of adult mosquitoes emerged from a larval temperature of 20, 30, and 35 °C to lay eggs agrees with the findings of Ezeakacha and Yee (2019), who recorded no eggs laid by *A. albopictus* at the adult temperature of 20 °C in all the larval rearing temperatures used. The inability of mosquitoes to lay eggs at these temperatures could be that females were unmated, therefore, unable to produce mature eggs (Ezeakacha & Yee, 2019). These studies did not check the spermathecae of females or the mating status of mosquitoes. It is possible that mosquito mating may be affected by temperature. It would be of great interest for future studies to explore the effects of temperature on the mating success of *Anopheles* mosquitoes. This could provide useful information in controlling *Anopheles* mosquitoes in a future warmer climate.

Usually, higher temperatures may accelerate the digestion of blood meals, reduce the gonotrophic cycle's length, and modify mosquito fecundity (Afrane et al., 2006). The review supports this as increasing temperature reduced the length of the gonotrophic cycle of

Anopheles mosquitoes. An increase in temperature could fast-track blood meal digestion and lessen the gonotrophic cycle length (Mala et al., 2014). Lardeux et al. (2008) observed that an increase in temperature from 15 to 31 °C drastically reduced the length of the gonotrophic cycle of *An. pseudopunctipennis* from approximately nine to two days. Naturally, a relatively small number of female mosquitoes survive for quite a long period to complete more than two gonotrophic cycles (Sy et al., 2014). Therefore, any decrease in the gonotrophic cycle length can boost malaria incidence due to the increased frequency of egg-laying and biting rates of mosquitoes (Mala et al., 2014).

Only one study reported the relationship between temperature and biting rate (Shapiro et al., 2017). They observed that increasing the temperature from 21 to 32 °C increased the biting rates of *An. stephensi* mosquitoes. This may be attributed to the effects of temperature on a blood meal. Increasing temperature speeds blood meal digestion, leading to increased host biting rates (Afrane et al., 2012). The female mosquito bites its host to acquire a blood meal, which is needed to develop its eggs (Nature Education, 2014). Blood feeding and egg production are closely related, and blood-feeding is crucial for the female mosquito to acquire the malaria parasite and transfer it to its host (Shaw et al., 2021). Thus, any factor that affects the biting rate has a detrimental effect on mosquito's ability to produce eggs and transmit diseases. An increase in mosquito biting rate implies that the vector may feed more frequently on its host and increase its potential to transmit diseases (Afrane et al., 2012).

2.12.3.2.2 Expression of enzymes and susceptibility to insecticides

High temperatures modify biochemical processes, increase metabolic rates (Oliver & Brooke, 2017), and affect the mosquito's immune system (Murdock et al., 2012a; Murdock et al., 2012b; Murdock et al., 2013). It has been shown that temperature can have a striking and diverse qualitative and quantitative effect on mosquito's immune responses by affecting the immune

challenge time and nature (Murdock et al., 2012a). The review on the expression of immune responses suggested that there were complex interactions between time, temperature, and the type of immune challenge. Most of the immune responses studied by Murdock et al. (2012a) were more robust at low temperature (18 °C) than high temperature. This is consistent with the findings of Suwanchaichinda and Paskewitz (1998), who reported that the percentage of female *An. gambiae (s.l.)* heavily melanizing beads were highest when held at 24 °C compared to 27 and 30 °C. In addition to innate immunity, melanin production plays a crucial role in physiological processes such as cuticular tanning and egg hardening, explaining the fast rate of humoral melanization at lower or cooler temperatures (Murdock et al., 2012a). In addition, NOS expression significantly increased at warmer temperatures (i.e., 28 °C) relative to colder temperatures (Murdock et al., 2014), which is consistent with similar studies (Murdock et al., 2012a; Murdock et al., 2013). According to Shapiro et al. (2017), their model suggested 29 °C as the optimum temperature required for malaria transmission. Therefore, an increase in NOS expression at higher temperatures could be an essential mosquito defense that could hinder parasite development (Murdock et al., 2012a).

Only one of the studies reviewed (Oliver & Brooke, 2017) assessed the effects of temperature on detoxification enzyme activity (cytochrome P450 and general esterases). It showed that the detoxification enzyme systems of the mosquitoes were not significantly affected by an increase in temperature. It is unclear what could have accounted for the lack of significant effect of temperature on detoxification enzyme expression. Further studies are needed to investigate the effects of rearing temperatures on the expression of detoxification enzymes in *Anopheles* mosquitoes. Temperature affects mosquito nervous system sensitivity, immune responses, and metabolic activities, consequently influencing the efficacy of insecticides (Kristan et al., 2018). None of the studies considered the effects of temperature on target site resistance–one of the most common and well-studied forms of insecticide resistance (Matowo et al., 2010; Ranson

et al., 2011; Corbel & N'Guessan, 2013; Liu, 2015). Generally, metabolic and target site resistance can co-occur in the same population (Ochomo et al., 2013) and can lead to complex cross-resistance and high resistance levels (Labbé et al., 2011). It is unclear how higher or warmer temperatures will shift metabolic rates and target site insensitivity in mosquitoes, especially *Anopheles* species.

For susceptibility, it was revealed that higher temperatures reduced insecticide toxicity in *An. funestus* and *An. arabiensis* mosquitoes. The reduced toxicity at high temperatures might be due to higher enzymatic activities, which could increase detoxification of the insecticide (Matzrafi, 2019). In addition, how temperature affected the toxicity of deltamethrin differed from that of bendiocarb. However, the synergistic PBO completely restored pyrethroid susceptibility irrespective of the temperature. The difference in the toxicity of the two insecticides could be attributed to the differences in the mode of action. Bendiocarb, which belongs to carbamates, are nerve poisons that work by inhibiting acetylcholinesterase. On the other hand, deltamethrin belonging to pyrethroids alters the normal function of insect nerves by modifying the kinetics of voltage-sensitive sodium channels (Casida & Durkin, 2013).

This review further revealed that the mosquito strain played a critical role in how temperature affected the toxicity of deltamethrin, and its temperature coefficient was not always positive or negative (Glunt et al., 2018). This is consistent with the findings of Hodjati and Curtis (1999), who also found that the toxicity of 0.25% permethrin on resistant *An. stephensi* exhibited a slight negative temperature coefficient (between 16 °C and 28 °C) and a strongly positive temperature coefficient (between 28 °C and 37 °C). Many mechanisms have been ascribed to the reduced efficacy of insecticides at elevated temperatures. For instance, pyrethroid insecticides are axonic poisons and control sodium ions' movement during nerve impulse movement. Generally, neuron sensitivity declines between temperatures of 30 to 35 °C, which

influences the efficacy of insecticides. In addition, at low temperatures, neurons exposed to pyrethroid insecticides receive a high concentration of the insecticide due to reduced biotransformation. This makes the neuron more sensitive to the resulting stimulus because of a prolonged duration of steady-state resting potential (Khan & Akram, 2014).

It needs to be emphasized that mosquito rearing temperature is critical, as it may influence the quality of the adult mosquito (Mpho et al., 2002) and its susceptibility to insecticides. The rearing, exposure, and post exposure temperatures can influence mosquito susceptibility to insecticides (Glunt et al., 2014). Besides, the association between temperature and insecticide efficacy differs based on the mode of action of an insecticide, method of application, target species, and quantity of insecticide contacted or ingested by the target species (Amarasekare & Edelson, 2004).

2.12.3.3 Implications of findings for malaria control in a future warmer climate

Climate change is anticipated to shift the distribution of vector-borne diseases such as malaria (Ngarakana-Gwasira et al., 2016). Both the malaria vector and the parasite itself are sensitive to climate parameters, particularly temperature and rainfall (Ngarakana-Gwasira et al., 2016). Studies have reported that variations in climate parameters profoundly affect the development of malaria parasites and mosquito longevity, which ultimately affects malaria transmission (Hoshen & Morse, 2004).

Both extreme low and high temperatures affect mosquito development and survival (Lyons et al., 2013). Studies have reported the effects of extreme low and high temperatures on the development of the malaria parasite. For instance, Mordecai et al. (2013) indicated that both insect and parasite physiology limit malaria transmission to temperatures between 17 and 34 °C. At a temperature of 25 °C, the malaria parasite needs only 12 days to complete its development; however, over 30 days is required for the parasite to develop and become

infectious when the temperature is 20 °C (Stresman, 2010). This is very important for malaria control because if parasite development takes a longer time, then the likelihood that a mosquito will survive longer for the parasite to transmit the disease will decrease drastically (Ikemoto, 2008). On the other hand, the development of *An. gambiae (s.l.)* is greatly impeded when temperatures are low, and its larvae are unable to develop and die at temperatures below 16 and 14 °C, respectively (Afrane et al., 2012).

The fate of malaria control in a future warmer climate can be seen from two directions. First, in a future warmer climate, areas that are currently cold (below 17 °C) and do not support the survival of malaria vectors and parasites to complete their development could provide suitable conditions for their survival and development due to an increase in temperature. The second direction that may be considered as the great news is that if the mosquitoes and the parasite fail to adapt to increasing temperatures, especially in currently warmer areas (temperatures above 34 °C), such as sub-Saharan Africa, then these areas could start experiencing a reduction in malaria cases. Ultimately, these countries can eradicate the disease because mosquitoes may not survive long to complete the parasite incubation period at temperatures higher than 34 °C (Bayoh & Lindsay, 2003; Christiansen-Jucht et al., 2014). It is noteworthy that factors such as plasticity, adaptation, thermal regulation, and daily, monthly, and seasonal climatic variations, and microclimates (Paaijmans et al., 2013b; Lefevre et al., 2018) may influence malaria transmission. However, these factors were not included in this review.

2.12.5 Conclusion

The growth and development characteristics (development time, larval survival, and pupation success) of immature stages of *Anopheles* mosquitoes are adversely affected by increasing temperature. However, information on these characteristics alone may not be sufficient to predict the population dynamics of mosquitoes. Production capacity (number of adults

produced) which plays a vital role in estimating the number of adult populations produced from the immature stages is understudied. In addition, previous studies have not considered the impact of increasing temperature on the sex ratio (proportion of male to female) of *An. gambiae (s.l.)* mosquitoes. There is evidence that the availability of female mosquitoes plays a crucial role in determining population dynamics and disease transmission. Only three (3) studies have demonstrated a link between temperature and sex ratio; however, these studies were done on *Aedes* rather than *Anopheles gambiae (s.l.)* mosquitoes.

With regards to the adult mosquito, previous studies have established the effects of increasing temperature on the longevity, fecundity, gonotrophic cycle length, biting rate and body size of *Anopheles* mosquitoes. However, these studies focused more on species such as *An. stephensi*, *An. pharaoensis*, *An. coustani*, *An. albimanus*, *An. dirus*, *An. sawadwongporni*, and *An. superpictus* with less attention on species such as *An. coluzzii* (one of the malaria vectors in Ghana).

Finally, a search of the literature revealed three (3) studies which examined the effects of temperature on the susceptibility of *An. funestus*, *An. arabiensis*, and *An. stephensi* but not on *An. gambiae (s.l.)* mosquitoes (most predominant malaria vector in Ghana). In addition, only one study examined the levels of expression of metabolic enzymes (cytochrome P450, α-esterase, and β-esterase) in mosquitoes (*An. arabiensis*) that were exposed to deltamethrin and malathion insecticides and those that were not exposed to any insecticides. Even though these enzymes are useful in determining the metabolic resistance mechanism of mosquitoes to insecticides, they do not provide a holistic assessment of this mechanism. Available scientific evidence has reported high levels of other metabolic enzymes such as GST and AChE in mosquitoes exposed to insecticides. However, the effects of temperature on the levels of expression of GST and AChE in *An. gambiae (s.l.)* mosquitoes have not been investigated yet.

Therefore, I intend to conduct a primary study to investigate the influence of elevated temperatures on the growth, development and susceptibility of *An. gambiae (s.l.)* mosquitoes to pyrethroid insecticides.

CHAPTER THREE

METHODOLOGY

3.1 Introduction

This chapter presents the materials and methods used in this study. The chapter describes temperature selection and maintenance, rearing of mosquito colonies, species identification, assessment of growth and development characteristics, the procedure used to conduct insecticide susceptibility test and biochemical analysis. A succinct description of quality control measures, statistical analysis and ethical considerations is provided.

3.2 Study design, temperature selection and maintenance

This study is an experimental study design. The experiment was carried out at the insectary of the African Regional Postgraduate Program in Insect Science (ARPPIS), University of Ghana, under eight temperature regimes (25, 28, 30, 32, 34, 36, 38 and 40 °C) in climate-controlled incubators (RTOP-1000D, Zhejiang, China) under constant relative humidity and photoperiod (Figure 8). Ghana has an average annual temperature ranging from 25 to 30 °C (Asante & Amuakwa-Mensah, 2015), so three temperatures were initially selected within this range; 25, 28 and 30 °C. A considerable warming across Africa is anticipated to reach 1.5 °C and is amplified at the 2 °C world, surpassing the mean global warming rate (Nangombe et al., 2019). Thus, to evaluate the effects of elevated temperatures on immature *An. gambiae (s.l.)* mosquitoes, a 2 °C increment at interval (Bayoh & Lindsay, 2004) from 30 °C was added to the selected temperatures until 40 °C maximum temperature was reached.

The climate incubators, RTOP-1000D, were programmed to have a photoperiod of 12:12 (light:dark) hours and relative humidity of 80 ± 10%. A HOBO MX1102 CO_2 logger (Onset Computer Corp., Cape Cod, MA, USA) was placed in each incubator and the insectary to

monitor the daily temperature and relative humidity (Shapiro et al., 2017). In addition, the temperature of the rearing water in each incubator was monitored using HOBO Pendant Temperature (UA-001-08) loggers (Onset Computer Corporation, Pocasset, MA) (Chadee & Martinez, 2016). Data were downloaded daily between 10:00 and 11:00 am to ensure that temperature and humidity remained stable throughout the experiment. Ambient and rearing water conditions for each temperature regime are listed in **Appendix IV**.

Figure 8: Climate control incubators (RTOP-1000D, Zhejiang, China)

3.3 Maintenance of *Anopheles gambiae (s.l.)* mosquito colony

This study used two laboratory strains of *An. gambiae (s.l.)* mosquitoes; Tiassalé and Kisumu strains. The Tiassalé strain (a mixture of *An. gambiae (s.s.)* and *An. coluzzii*) is resistant to four classes of insecticides (pyrethroids, organochlorines, carbamates, organophosphates) available for malaria control (Edi et al., 2012). It initially originated from Tiassalé, Cote d'Ivoire (Edi et al., 2012) and maintained in the Vestergaard – Noguchi Memorial Institute for Medical

Research Vector Labs (VNVL) insectary since 2010 (Chabi et al., 2019). The Kisumu strain is a susceptible reference strain from Kisumu, Kenya and has no history of insecticide resistance (Machani et al., 2020; Osoro et al., 2021). For the immature and adult mosquito experiments, *An. gambiae (s.l.)* (Tiassalé strain) was used. Both Tiassalé (as test group) and Kisumu (as reference group) strains were used for the insecticide susceptibility test and biochemical analysis. The eggs of both strains were obtained from the insectary of Vestergaard – Noguchi Memorial Institute for Medical Research Vector Labs (VNVL). The eggs on wet filter paper were placed in round plastic bowls (28 cm top diameter, 18 cm bottom diameter, 9 cm height) with 1 liter of dechlorinated tap water and 1.5 ml of yeast to induce hatching (Farnesi et al., 2017). Yeast contains hatching inducing compound (HIC) which is able to trigger egg hatching (Ng et al., 2021). The bowls were lined with A4 white paper to prevent eggs from sticking to the walls of the bowls and drying out. Enough fresh dechlorinated water kept at the respective temperatures was added daily to each bowl to maintain a volume of 1 liter due to evaporation.

Upon hatching, larvae were fed daily on 10 mg of TetraFin goldfish flakes (Tetra Werke, Melle, Germany). All adults were provided with 10% sugar solution soaked in cotton wool. However, in addition to the sugar solution, some adult mosquitoes used to estimate fecundity and longevity of blood-fed mosquitoes were allowed to feed on a guinea pig for twenty (20) minutes on day four (4). The position of the cages was rotated daily to control for the effects of position within the chamber. Guinea pigs used for the test were not given any anaesthesia, however, they were housed at the Noguchi Memorial Institute for Medical Research Animal House and a Veterinary Officer assessed and treated them on weekly basis.

3.4 Molecular identification of *An. gambiae (s.l.)* mosquitoes (Tiassalé strain)

3.4.1 DNA extraction from adult mosquitoes

To ensure that mosquitoes used in this study were truly *An. gambiae (s.l.)*, one hundred adult female mosquitoes (3 – 5 days old non-blood-fed) were randomly selected for molecular analysis. DNA was extracted from the sampled mosquitoes using the Cetyl Trimethyl Ammonium Bromide (CTAB) method of extraction following the procedures described by Mouhamadou et al. (2019). Each mosquito was homogenized in 200 µl of 2% CTAB, and the mixture was incubated at 65 ^{o}C for 5 min. Next, 200 µl of chloroform was added to each sample and mixed by inversion, then centrifuged at 12,000 rpm for 5 min at room temperature. The aqueous phase was transferred to new vials (1.5 ml), 200µl of isopropanol was added and mixed by inversion. The samples were centrifuged at room temperature (27 ^{o}C) at 12,000 rpm for 15 min. The isopropanol was removed, 200 µl of 70 % ethanol was added, and centrifuged at 12000 rpm for 5 minutes at room temperature. The ethanol was then decanted, and the pellets containing the DNA were dried by inverting the tubes overnight. The extracted DNA was reconstituted in 20 µl DNase-free water before storage at room temperature.

3.4.2 Identification of *Anopheles gambiae (s.l.)* members by polymerase chain reaction (PCR)

Two different polymerase chain reaction (PCR) procedures were performed to identify the mosquito colony used in this study. The first method identified members of the *An. gambiae (s.l.)* using multiplex PCR. This is a PCR-based amplification of specific DNA nucleotide differences in the intergenic spacer (IGS) of the ribosomal DNA (rDNA). The PCR uses species-specific primers to amplify DNA sequences of interest, which are of a diagnostic size when visualized under UV light after agarose gel electrophoresis. This allows simultaneous detection of the two main malaria vectors (*An. gambiae (s.s.)* and *An. arabiensis*) from the secondary and non-vector sibling species (*An. quadriannulatus/merus/melas/bwambae*).

Amplification was conducted with primers targeting the rDNA gene (IGS). The primer pair used has been presented in Table 7. Reactions were made in 25 µl volume containing 0.5 µl DNA template, 2× GoTaq® Green Master Mix (Promega, Madison, WI, USA) 12.5 µl, 10 µM of each primer in 0.1 µl (Eurofins Genomic). The volume was adjusted with DNase-free water (11.6 µl). The temperature profile involved an initial denaturation step at 95 °C for 3 min, 35 cycles of 95 °C for 30 s, 54 °C for 30 s, and 72 °C for 1 min, followed by a final extension at 72 °C for 5 min. An aliquot of 5 µl of each PCR product was subjected to electrophoresis on a 2 % agarose gel stained with gel red and photographed with a BioDoc-it Imaging System (UVP, Upland, CA, USA). A 2 % agarose gel is commonly used for analyzing PCR products because it provides good resolution for fragments in the range of approximately 100 to 2000 base pairs (Thermo Fisher Scientific Inc, 2012). This study used 2% agarose gel because the 315 and 390 bp fragments are on the lower end of the size range for a 2% gel, while the 464 bp fragment is close to the middle of the range. The species was determined by the size of the PCR product (390 bp for *An. gambiae (s.s.)*; 315 bp for *An. arabiensis*; and 464bp for *An. melas*).

A second procedure using Sine PCR (Santolamazza et al., 2008) was used to identify *An. coluzzii* and *An. gambiae (s.s.)* mosquitoes. The PCR reactions were performed in a 25 µl reaction, which contained 0.4 µM of each of the following primer: F6.1a of sequence 5′-TCGCCTTAGACCTTGCGTTA-3′ used to identify *An. coluzzii* (*An. gambiae* M molecular form) and the R6.1b of sequence 5′-CGCTTCAAGAATTCGAGATAC-3′ for *An. gambiae (s.s.)* (*An. gambiae* S molecular form). The other reagents included 2× GoTaq® Green Master Mix (Promega, Madison, WI, USA) 12.5 µl, and 4 µl of DNA template extracted from individual mosquitoes. The volume was adjusted with DNase-free water (6.5 µl). The amplifications were done in a thermocycler (Alpha Cycler, UK) and programmed as follows: 94 °C for 5 min followed by 35 cycles of 94 °C for 30 s, 59 °C for 30 s, and 72 °C for 1min. At the end of amplification, the mixture was subjected to a final extension at 72 °C for 10 min.

The PCR products were allowed to migrate on 2 % agarose gels stained with gel red. The species expected band profile was 249 bp for *An. gambiae (s.s.)*, 479 bp for *An. coluzzii*, and 249, 479 bp for hybrid (*An. gambiae* M and S forms) after visualization with a BioDoc-it Imaging System (UVP, Upland, CA, USA). **Appendix V** shows gel photographs of the PCR performed.

Table 7: List of primers used for molecular identification of *Anopheles* mosquitoes

Primer name	Primer symbol	Primer sequence (5' – 3')
Universal	UN	GTGTGCCGCTTCCTCGATGT
An. gambiae	AG	CTGGTTTGGTCGGCACGTTT
An. arabiensis	AR/AA	AAGTGTCCTTCTCCATCCTA
An. melas	AM/ME	GTGACCAACCCACTCCCTTGA

NB: The UN is the forward primer for the *Anopheles gambiae* complex.

3.5 Temperature, growth and development of immature *An. gambiae (s.l.)* mosquitoes

This describes the procedures used to assess the effects of temperature on the following: development time, larval mortality and survival, larval weight and size, time to pupation, pupation success, pupal mortality, larval weight and size, number of adults produced, and sex ratio of emerged adults. All experiments were repeated five times.

3.5.1 Developmental time of immature mosquitoes, larval survival, and mortality

The eggs were collected on wet filter paper and placed in larval bowls with de-chlorinated tap water at each temperature regime. On hatching, one hundred and sixty (160) first instar larvae from each egg tray were distributed into larval bowls containing de-chlorinated tap water and observed from larval stage through to pupal stage to the adult stage. The same egg bowls were used as larval bowls, except that the paper lining was removed. The developing larvae were checked daily for mortality, and dead larvae were enumerated, and discarded. Larvae that did not move after being touched with pipette tip were recorded as dead. Larval mortality was

defined as death during the larval stage. The number of daily larval mortality was then used to estimate larval survival curves. In addition, the time from hatching to adult emergence was recorded as the development time (in days) of *An. gambiae (s.l.)* mosquitoes.

Figure 9: Assessment of the developmental stages of the immature *An. gambiae (s.l.)* mosquitoes

3.5.2 Time to pupation, pupation success, and mortality

Pupae were collected daily using a Pasteur pipette, enumerated, and placed into plastic cups with water (in mosquito cages) at various temperature regimes until the adults emerged. The plastic cups were labelled with the temperature at which the pupae were maintained in them. The number of larvae that pupated daily was enumerated. At the end of the development period, the time to pupation was estimated as the time of hatching to the onset of pupation. Pupation success (percentage of larvae that emerged as pupae) was also computed for each temperature regime as the number of larvae that pupated from the total number of larvae expressed as percentage. Pupal mortality was also assessed daily for each temperature regime. Any pupae that did not move when touched repeatedly with the pipette tip was considered dead, and any moribund pupae were added to the dead pupae to compute pupal mortality.

3.5.3 Larval and pupal weight and size

Fifty (50) of the fourth instar larvae and fifty (50) pupae were randomly selected from each temperature regime. The larvae and pupae were immobilized at a fridge temperature of 4 °C, blot dried on a tissue paper towel and weighed using an XS104 ultra-micro balance (Mettler Toledo Inc., Columbus, Ohio). In addition, a stereo microscope with LED and HD inbuilt camera (Leica EZ4 HD, Leica Microsystems Limited, Switzerland) connected to a laptop was used to capture digital images of the pupae and larvae at a magnification of 16X. Body parts were measured using the Leica Application Software, version 3.4.0 (Leica Microsystems Limited, Switzerland). Measurements included larval length (a proxy for larval size) from the distal tip of the head (without considering the feeding brush, antennae, and caudal hair) to the end of the anal segment (Manoukis et al., 2006), length of the cephalothorax of the pupae, and used as a proxy for pupal size (Koenraadt, 2008; Sasmita et al., 2019).

Figure 10: Measurement of larval and pupal size using Leica EZ4 HD microscope

3.5.4 The number of adults produced (production capacity) and sex ratio

The number of adults produced (production capacity) was calculated for each regime as the number of emerged adults divided by the total larvae (expressed as percentages). In addition, the sex ratio of adults from each temperature regime was determined as the ratio of adult males to adult females (Mohammed & Chadee, 2011; Ukubuiwe et al., 2016).

3.6 Temperature and the growth and development of adult *An. gambiae (s.l.)* mosquitoes

This section describes the methods used to investigate the effects of elevated temperature on adult longevity, length of gonotrophic cycle, biting rate, fecundity, body weight and size, and proboscis length.

3.6.1 Adult longevity

The longevity of mosquitoes was measured as the time of adult emergence to the time of death, which was assessed daily between 9:00 – 10:00 am. One hundred (50 males and 50 females) newly emerged mosquitoes were assigned to each temperature regime to monitor daily mortality and longevity. Any mosquito that had lost its wings and could no longer fly was considered moribund and recorded dead (WHO, 2016a; Agyekum, 2017; Ononamadu et al., 2020). Dead mosquitoes were removed, observed, identified for sex allocation, and enumerated. This experiment was repeated five times under all temperature regimes, and longevity was estimated for blood-fed and non-blood-fed mosquitoes. Taking blood meal is a realistic behavior of female mosquitoes. In addition, one of the important factors in malaria transmission is how long a mosquito can live after a potential infectious blood meal (Barreaux et al., 2018), hence, the rational for measuring longevity of blood-fed mosquitoes. Adult mosquitoes kept at 25 ^{o}C and 28 ^{o}C were blood-fed three times (day 4, 8 and 14); those kept at 30 ^{o}C were also blood-fed twice (day 4 and 8). However, mosquitoes kept at 32 ^{o}C and 34 ^{o}C were blood-fed only once (day 4) because of high mortalities that resulted in fewer females for

which reason the mosquitoes could not be fed 3 times as those reared under 25 and 28 ^{o}C temperature regimes.

3.6.2 Estimation of length of gonotrophic cycle, biting rate and fecundity

One hundred (3 – 5 days old) adult mosquitoes (50 males and females) were placed in a cage to estimate the gonotrophic cycle length. The mosquitoes were blood-fed on two (2) occasions. The number of days taken for mosquitoes to lay eggs after the first and second feeding with blood meal was recorded, and from the mean value, the length of the gonotrophic cycle was calculated. When the females failed to lay a second batch, the number of days to lay the first batch was considered to be the length of the gonotrophic cycle. The biting rate of mosquitoes was estimated as the reciprocal of the mean gonotrophic cycle for each temperature regime (Moller-Jacobs et al., 2014; Shapiro et al., 2017). These experiments were repeated five times for all colonies reared under the different temperature regimes.

In estimating fecundity, first day after the blood meal, twenty (20) fully engorged mosquitoes were randomly selected from each temperature regime and transferred individually into oviposition cups with 10% sugar solution. In each of the cup, five males were added to ensure that females were mated. Whatman filter paper on wet cotton wool was placed in each oviposition cup to provide oviposition substrate, and mosquitoes observed for egg-laying (a measure of fecundity). The filter paper was removed daily and checked for eggs, and the number of eggs on the filter paper under each temperature regime was enumerated. Fecundity was estimated from only the first batch of eggs laid by the mosquitoes, which, according to Costa et al. (2010), is a prognosis of total fecundity.

Figure 11: Oviposition cups for fecundity assessment

3.6.3 Body weight, size, and proboscis length

Fifty (50) of the four (4) days old adults (25 males, 25 females) that were not fed with a blood meal were randomly selected for each temperature regime, freeze-killed, and body weights were measured using an XS104 ultra-micro balance (Mettler Toledo Inc., Columbus, Ohio). Mosquitoes were weighed three times, and the average weight was used as the body weight of the mosquito. Measurement of body size and proboscis length were performed on the same mosquitoes that were used for weight measurements. A stereo microscope with LED and HD inbuilt camera (Leica EZ4 HD, Leica Microsystems Limited, Switzerland) connected to a laptop was used to capture digital images of wings and head of mosquitoes at a magnification of 20X. Mosquito wing length was used as a proxy for body size (Barreaux et al., 2018; Phasomkusolsil et al., 2018). The Leica Application Software, version 3.4.0 (Leica Microsystems Limited, Switzerland), was used to measure the proboscis length from the labial basal setae to the labella's apex (Harbach, 2010). Wings were also measured from the tip to the distal end of the alula (excluding the fringe) (Barreaux et al., 2016a). The mean length of the two wings was used in the analyses; however, only one was used when a wing was missing.

3.7 Insecticide susceptibility and metabolic enzyme level

3.7.1 Insecticide susceptibility

Two pyrethroid insecticide impregnated papers (0.75% permethrin and 0.05% deltamethrin) obtained from Universiti Sains Malaysia, Malaysia, were used to test the susceptibility of *An. gambiae (s.l.)* mosquitoes (Tiassalé strain) raised at 25, 28, 30, 32 and 34 °C. The quality of insecticide impregnated papers was tested against susceptible *An. gambiae (s.s.)* (Kisumu strain) (Pwalia et al., 2019). Permethrin and deltamethrin were selected because they were the most common insecticides used in malaria control programs (Monahan, 2017). Insecticide susceptibility tests were performed at a constant temperature of 27 ± 2 °C following the standard WHO protocol (WHO, 2016a).

For each mosquito strain, 20 – 25 female mosquitoes (aged 3 – 5 days), which were not fed with a blood meal were aspirated into six plain paper-lined WHO holding tubes; two tubes labelled as control replicates and four as insecticide-exposed replicates (WHO, 2016a) and observed under each temperature regime. Mosquitoes were gently put into exposure tubes and observed for 1 hour. The knockdown time (KDT) of females was recorded at 10-, 15-, 20-, 30-, 40-, 50- and 60-minutes' exposure period. After exposure, mosquitoes were transferred into holding tubes and provided with a 10% sugar solution soaked in cotton wool. Mortality was recorded 24 hours post-exposure (WHO, 2016a).

Figure 12: Insecticide susceptibility test following WHO protocols (WHO, 2016a**)**

3.7.2 Methods for measuring mosquito metabolic enzyme level

3.7.2.1 Biochemical assays

The levels of four metabolic enzymes (MFO, GST, alpha- and beta-esterase) were measured in mosquitoes reared at different temperature regimes (25, 28, 30, 32 and 34 ºC) and were not exposed to insecticides. In addition, the enzyme levels were measured in mosquitoes reared at temperature regimes of 25, 28, 30, and 32 ºC who survived exposure to pyrethroid insecticides (deltamethrin and permethrin). For measurement of enzyme levels of individual adult female *An. gambiae (s.l.)* (Tiassalé), biochemical assays were performed using the microplate enzyme system as described by Hemingway and Brogdon (1998) with minor modifications. Briefly, the mosquitoes were frozen in Eppendorf tubes at -80 °C in the laboratory until analysis. Mosquitoes reared under each temperature regime (25, 28, 30, 32 and 34 ºC) were individually homogenized in 1500 µl of potassium phosphate buffer (on ice). The homogenate was centrifuged at 14,000×rpm at 4 °C for 1 min, and the supernatant was used as an enzyme source for all the enzyme assays. All assays were done in duplicates using 96-well microplates. The absorbance values were measured using the SpectraMAX 340 PC (Molecular Devices, Sunnyvale, CA). The assays for the four enzymes are explained as described below.

3.7.2.1.1 Mixed-function oxidase (MFO)

Duplicate wells containing 100 µl of supernatant were prepared, and 200 µl of 3,3,5,5-tetramethylbenzidine (TMBZ) (with methanol as the solvent), and 25 µl of 3% hydrogen peroxide (H_2O_2) was added. The mixture was incubated for 5 min, and the OD value was measured at 620 nm. The mixed-function oxidase (MFO) level was estimated using the standard curve of absorbance for known cytochrome C concentrations. The enzyme level was expressed as equivalent units per mole of cytochrome P450/min/mg protein.

3.7.2.1.2 Glutathione S-Transferases (GSTs) assay

First, duplicate plates of 100 µl of supernatant were prepared, and 100 µl of 1-chloro-2,4-dinitrobenzene (cDNB) and reduced glutathione were added one after the other. The OD values were read immediately after adding the GST solution (T_o) at 340 nm. The plate was incubated for 5 min, and the OD values were measured again at the same wavelength. The Beer's law ($A = \varepsilon lc$) was used to calculate GST level, expressed as a mole of cDNB/min/mg protein. With the extinction coefficient (ε) of 4.39 $mM^{-1}cm^{-1}$, the OD values (absorbance) were converted into mole cDNB conjugates. The path length was 0.94 cm.

3.7.2.1.3 Non-specific esterase (NSE) assay

Mosquito homogenates were prepared, and supernatant poured into duplicate wells of 100 µl. To one set of the wells, 100 µl of 30 mM α-naphthyl acetate was added, while to the other, 100 µl of 30 mM β-naphthyl acetate was added. The plate was incubated at room temperature for 10 min. After incubation, 100 µl of dianisidine was added to each well, and the mixture was allowed to incubate for another 2 min, after which the OD values were measured at 620 nm. Depending on the standard curves of absorbance for known concentrations of α-naphthol or β-naphthol, the esterase level for each substrate was calculated. The enzyme level was expressed as mole of α-naphthol or β-naphthol/min/mg protein.

3.7.2.1.4 Protein assay

Owing to size differences in individual mosquitoes, correction in the analyses of all enzyme levels was done using the protein concentration as a standard correction factor. A commercial protein assay kit (Coomassie Plus, USA) was used to obtain the bovine serum albumin standard curve. After that, the protein concentration was transformed and calculated based on the same curve. The protein assay was conducted by mixing 200 µl of Coomassie plus dye reagent with 20 µl of mosquito homogenate and 80 µl of the potassium phosphate (K_3PO_4) buffer, and the plate was read at 620 nm.

Table 8: Number of *An. gambiae (s.l.)* mosquitoes from each rearing temperature regime used for biochemical analysis of metabolic enzyme level

Temperature regime (ºC)	Mosquitoes that were not exposed to pyrethroids	Mosquitoes that were exposed to pyrethroids
25	50	40
28	50	46
30	50	28
32	50	40
34	40	-

Note: Few mosquitoes survived after exposure to pyrethroids resulting in the unequal number of mosquitoes that were exposed to pyrethroids; all mosquitoes exposed to pyrethroids at 34 ºC died; hence there were no live mosquitoes to test for enzymes.

3.8 Quality Control

During the rearing of mosquitoes and laboratory analysis of samples, quality control measures were followed to ensure accurate results. Species identification was conducted to ascertain the composition of *An. gambiae (s.l.)* mosquitoes used in the study. A HOBO MX1102 CO_2 logger (Onset Computer Corp., Cape Cod, MA, USA) was placed in each incubator and the insectary to monitor the daily temperature and relative humidity to ensure that incubators were working efficiently. In addition, assessment of the growth and development of mosquitoes was repeated

5 times, and the instrument used for body weight measurement was calibrated to ensure they worked correctly. Measurement was repeated three (3) times, and the averages reported.

For DNA extraction, the negative control for the DNA extraction was an empty tube (no mosquito sample) which was taken through the extraction process alongside the main samples. This was tested during the PCR procedure to confirm its status. DNAse free water was used as a negative control for the PCR and a previously confirmed sample was used as a positive control. The test was conducted following the standard operating procedures for species identification and all equipment were calibrated.

The susceptibility test followed the WHO protocol, and the test was done in replicates of four (4) for each insecticide and two (2) control replicates (WHO, 2016a). In addition, the WHO susceptibility test was also conducted using a reference strain (*An. gambiae (s.l.)* Kisumu strain) to ensure that insecticides papers were effective. In cases where mortality in control treatments was between 5 – 20 %, mortalities were corrected using Abbott's formula.

Samples for biochemical analysis were stored in the -80 ^{o}C freezer to avoid the degradation of enzymes. During the test, mosquito samples were run in duplicates. Alpha naphthol and beta naphthol were used as controls for the alpha- and beta-esterase. Bovine Serum Albumin (BSA) was used as a positive control for protein assays and cytochrome C for the oxidase. Assays were run with serial dilutions of the positive controls (standard) following the protocol used for the main assay. The readings from this were used to draw curves/lines. The concentration range (best 7 consecutive concentrations) with at least R^2 of 0.8 (0.8 and above) was selected and used as controls for the main assay. The blank (a pair of empty wells) served as the negative control in both the standard and main assay.

3.9 Statistical Analysis

Data generated were entered into Microsoft Excel (Version 2016) and then exported into Stata version 15.1 (StataCorp LLC, Texas, USA) for analysis. GraphPad Prism v8.4.2 was used to generate some graphs. The assumptions of normality and homogeneity of variances were assessed using Shapiro-Wilk and Bartlett's tests, respectively. Continuous variables with normal distribution were presented as mean (standard deviation [SD]), and non-normally distributed variables were reported as median (interquartile range [IQR]). Unless otherwise stated, in all statistical analyses, a *p-value* of less than 0.05 was considered significant. Data were presented in tables and figures. The statistical methods employed in the analysis of each specific objective are elaborated below.

3.9.1 Temperature and the growth and development of immature *An. gambiae (s.l.)* mosquitoes

Data on development time and time to pupation were normally distributed. Therefore, a one-way analysis of variance (ANOVA) was used to explore the relationship between temperature and development time and time to pupation. In cases where the overall model showed statistically significant differences, the Tukey post hoc test was further used to determine where the differences existed.

Pupation success, pupal mortality, number of adults produced, sex ratio, larval and pupal measurements failed to meet the normality criteria. A Kruskal-Wallis test with Dunn's multiple range test was used to assess the effect of temperature on pupation success, pupal mortality, number of adults produced, and sex ratio of emerged mosquitoes.

Larval and pupal measurements were not normally distributed, hence log-transformed for the analysis. Ordinary least square (OLS) regression analysis with robust standard errors was used to determine whether any significant differences existed among the different temperature

regimes. Sensitivity analysis was further conducted using quantile and robust regression methods to determine how the regression coefficients and their respective standard errors change with respect to using different statistical models to assess the effects of temperature on larval and pupal weights and length.

With regards to larval survival data, survival analyses were performed using Kaplan-Meier survival analysis. Log-rank test and cox proportional hazard model were used to test the null hypothesis that larval survival did not change across the different rearing temperatures. The log-rank test compared the overall survival trend for the different temperature regimes, while the cox proportional hazard model was used for two-sample comparisons at one temperature against survival at the baseline temperature (25 ^{o}C). Median survival time with 95% confidence intervals was estimated for each of the temperature regimes.

3.9.2 Temperature and the growth and development of adult *An. gambiae (s.l.)* mosquitoes

Gonotrophic cycle length, biting rate, and fecundity data were normally distributed. Analysis of variance (ANOVA) was used to explore the relationship between temperature and gonotrophic cycle length, biting rate, and fecundity. In cases where the overall model showed statistically significant differences, a post hoc analysis using the Tukey test was further used to identify where the differences existed. On the other hand, adult weight, body size (wing length) and proboscis length were not normally distributed and were log-transformed. Ordinary least square (OLS) regression analysis with robust standard errors was used to determine whether any significant differences existed among the different temperature regimes. Sensitivity analysis was further conducted using quantile and robust regression methods to determine how the regression coefficients and their respective standard errors change with respect to using

different statistical models to assess the effects of temperature on adult weight, body size (wing length) and proboscis length.

Concerning adult longevity data, a non-parametric (Kaplan-Meier) survival analysis was performed to determine the effects of temperature and blood meal on the longevity of adult mosquitoes. The log-rank test compared the overall longevity trend for the five temperature regimes and blood meal effect on longevity. The cox proportional hazard model was used for two-sample comparisons at one temperature against the longevity at the baseline temperature (25 ^{o}C). Median longevity with 95% confidence intervals was estimated for each of the temperature regimes. In the results, P_{all} values represent p values for both blood-fed and non-blood-fed mosquitoes.

3.9.3 Insecticide susceptibility and expression of metabolic enzyme levels

3.9.3.1 Insecticide susceptibility

Insecticide susceptibility data were interpreted following WHO criteria: *An. gambiae (s.l.)* mosquitoes were defined as susceptible if mortality was greater than 98%; mortality between 90 – 98% indicates suspected resistance with more investigations needed; and mortality less than 90% suggests confirmation of the existence of resistant genes (WHO, 2016a). Mortalities in some of the control treatments exceeded 5 %: therefore, mortalities were corrected using Abbott's formula (Abbott, 1987) as follows;

$$\text{Corrected mortality (\%)} = \frac{Mortality\ in\ treatment\ (\%) - Mortality\ in\ control(\%)}{100 - Mortality\ in\ control\ (\%)} \times 100$$

Probit analysis was used to estimate KDT_{50} and mosquito's susceptibility status to permethrin and deltamethrin insecticides. The KDT_{50} is the time at which 50 % of the mosquitoes were knocked down. Using IBM SPSS Statistics software (version 23.0), the number of knocked-down mosquitoes was considered response frequency, the total number of mosquitoes per test

was regarded as the total number observed, and time was considered a covariate. Log base ten response was calculated from the data (Nnko et al., 2017).

The knockdown time 50 (KDT_{50}) was used to calculate the knockdown resistance ratio (KRR_{50}) by dividing the KDT_{50} of the test mosquitoes (*An. gambiae (s.l.)* Tiassalé strain) by that of the *An. gambiae (s.l.)* Kisumu reference susceptible strain in all four temperature regimes (Nwane et al., 2009; Dhiman et al., 2014). The KRR_{50} was scaled as follows: $KRR_{50} < 1$ = susceptible, 1 – 10 = low resistance, 11 – 30 = moderate resistance, 31 – 100 = high resistance, and $KRR_{50} > 100$ = very high resistance (Goindin et al., 2017).

3.9.3.2 Expression of metabolic enzyme level

The mean absorbance values of duplicate wells for each tested mosquito were converted into enzyme level and divided by the protein values. In addition, the median enzyme levels were estimated for each temperature regime. The quantities/levels of MFO, GSTs and non-specific esterase (alpha and beta) levels were not normally distributed and were analyzed using the non-parametric Kruskal-Wallis test. In cases where the overall model showed statistically significant differences, Dunn's multiple range test was further used to determine where the differences existed. A Mann-Whitney U test was used to compare the enzyme levels in mosquitoes that were exposed and those that were not exposed to pyrethroids.

3.10 Ethical Approval

The guinea pigs used in this study were housed at the Animal Experimentation Department, Noguchi Memorial Institute for Medical Research (NMIMR). Ethical approval for the use of guinea pigs to blood feed female *An. gambiae (s.l.)* mosquitoes was obtained from the University of Ghana Institutional Animal Care and Use Committee (UG-IACUC) with permit number UG-IACUC 001/20-21 **(Appendix VI)**.

CHAPTER FOUR

RESULTS

4.1 Introduction

This chapter is divided into three main sections, each of which presents the results relating to the specific objectives. The first section of this chapter presents the results of the effects of temperature on the growth and development of immature stages of *An. gambiae (s.l.)* mosquitoes, while the second section highlights the effects of temperature on the growth and development of the adult *An. gambiae (s.l.)* mosquito. In the last section, the result of insecticide susceptibility and expression of metabolic enzyme in *An. gambiae (s.l.)* mosquitoes are presented.

4.2 Composition of *An. gambiae (s.l.)* (Tiassalé strain) mosquitoes

Molecular analysis of mosquitoes was conducted to identify the composition of *An. gambiae (s.l.)* mosquitoes used in the study. The results showed that *An. gambiae (s.l.)* samples used in this study consisted of two species; *An. gambiae (s.s.)* (26.53 %) and *An. coluzzii* (23.47 %). A hybrid of the two species constituted 50.00 % (Table 9).

Table 9: Composition of *An. gambiae (s.l.)* mosquitoes

Anopheles gambiae (s.l.)	**Frequency (N)**	**Percentage (%)**
An. gambiae (s.s.) (S form)	26	26.53
An. coluzzii (M form)	23	23.47
M/S hybrids	49	50.00
Total	**98**	**100.00**

4.3 Effects of temperature on the growth and development of immature *An. gambiae (s.l.)* mosquitoes

4.3.1 Developmental time of the immature stages

To assess the relationship between temperature variability and development time of immature stages of mosquitoes, the mosquitoes were reared at different temperature regimes (25, 28, 30, 32, 34, 36, 38 and 40 ºC). Eggs incubated at 40 ºC failed to hatch even after seven (7) days, and those that hatched at 38 ºC died before pupation. Therefore, the development times at temperatures 38 and 40 ºC could not be calculated.

The results showed that mean development time (time it takes eggs to hatch into adults) of mosquitoes decreased with increasing temperatures; 36 ºC [9.6 (± 0.55) days], 34 ºC [13.01 (± 0.61) days], 32 ºC [14.54 (± 0.38) days], 30 ºC [16.08 (± 1.03) days], and 28 ºC [18.4 (± 0.89) days] compared to 25 °C [20.17 (± 0.75) days] (Table 10). When the temperature was increased from 25 to 36 °C, mean development time decreased by 10.57 days. One-way ANOVA showed a statistically significant difference ($F(5, 24) = 133.55$, $P < 0.001$) in development time of mosquitoes among the different temperature regimes. Further analysis using the Tukey post hoc test showed there were significant differences in the development time among the various temperature comparisons **(Appendix VII)**.

Table 10: Relationship between rearing temperature and development time of immature *An. gambiae (s.l.)* mosquitoes

Temperature regime (ºC)	Development time (in days) Mean (±SD)
25	20.17 (±0.75)
28	18.40 (±0.89)
30	16.08 (±1.03)
32	14.54 (±0.38)
34	13.01 (±0.61)
36	9.60 (±0.55)
38	-
40	-

NB: Larvae at 38 ºC died before pupating and eggs kept at 40 ºC did not hatch; SD = Standard deviation

4.3.2 Determination of larval mortality and survival under different temperature regimes

Eggs kept at 40 °C failed to hatch; therefore, larval mortality and survival were only estimated at seven (7) temperature regimes (25, 28, 30, 32, 34, 36, and 38 °C). Mortality was defined as death that occurred during the larval stage. Overall, larval mortality increased at temperatures above 28 °C. Larvae kept at 25 °C had the longest median survival time of 20 days, while those maintained at 38 °C recorded the least median survival time of 8 days (Table 11).

Table 11: Median survival times of *An. gambiae (s.l.)* larvae reared at different temperatures

Temperature regime (°C)	Number of mosquitoes	Number of deaths	Median survival (days) (95% CI)
25	800	373	20 (16, 22)
28	800	196	19 (16, 21)
30	800	527	17 (14, 19)
32	800	576	14 (12, 17)
34	800	606	13 (11, 14)
36	800	621	10 (9, 10)
38	800	800	8 (7, 8)
Total	**5600**	**3699**	

95% CI means 95% Confidence interval

Furthermore, survival curves were plotted and presented as Kaplan-Meier plots based on the number of larvae that died before pupating (n = 3699). From the results, larval survival decreased with increasing rearing temperature (Figure 13). Log-rank test showed that temperature regimes had significantly different survival functions ($X^2(6) = 5353.12$, $P < 0.001$). Cox proportional hazard model showed that larvae kept at 28°C had 35% increased risk of death compared to those kept at 25°C (HR = 1.35, 95% CI; 1.13, 1.61, $P = 0.001$). Similar trends were observed at 30 °C (HR = 2.63, 95% CI; 2.26, 3.06, $P < 0.001$), 32 °C (HR = 5.62, 95% CI; 4.79, 6.59, $P < 0.001$), 34 °C (HR = 11.37, 95% CI; 9.61, 13.45, $P < 0.001$), 36 °C (HR = 51.07, 95% CI; 42.33, 61.61, $P < 0.001$) and 38 °C (HR = 215.85, 95% CI; 175.45, 265.54, $P < 0.001$) **(Appendix VIII)**.

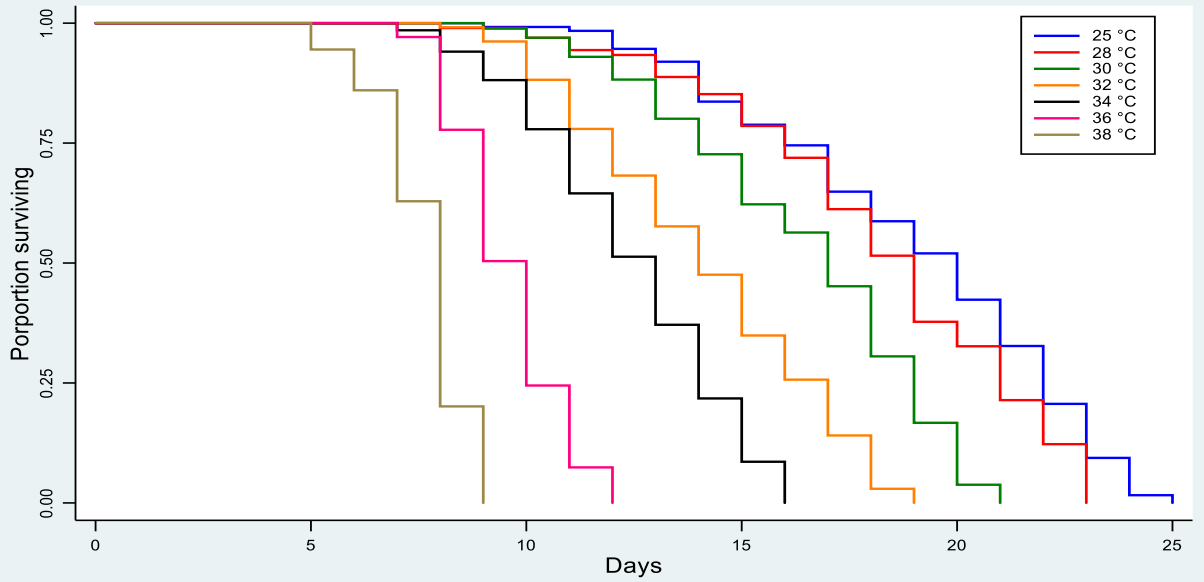

Figure 13: Kaplan-Meier survival plots of *An. gambiae (s.l.)* larvae reared at different temperatures

25 °C (blue) was set as baseline against which survival at other temperatures were compared; 28 °C (red), 30 °C (green), 32 °C (orange), 34 °C (black), 36 °C (pink), 38 °C (brown).

4.3.3 Measurement of time to pupation, pupation success, and mortality

The time to pupation, pupation success and mortality were estimated for larvae raised at 25, 28, 30, 32, 34 and 36 °C. Eggs incubated at 40 °C failed to hatch, and those that hatched at 38 °C died before pupation. The results showed that the mean time to pupation (time from hatching of eggs to the onset of pupation) decreased with increasing temperature, 25 to 36 °C. At 25 °C, larvae started pupating 12.80 (±1.30) days post-hatching and those in 28 °C pupated on day 11.80 (±1.10). Larvae raised at 30, 32, 34 and 36 °C pupated on day 11.00 (±0.71), 9.20 (±0.45), 8.80 (±0.84) and 7.40 (±0.55), respectively (Table 12). One-way ANOVA showed a statistically significant difference ($F(5, 24) = 27.07$, $P < 0.001$) in time to pupation among the temperature regimes. Further statistical tests using Tukey post hoc test revealed that except for 25 vs. 28 ($P = 0.481$), 28 vs. 30 ($P = 0.701$), and 32 vs. 34 ($P = 0.977$), there were significant differences in the time to pupation of mosquitoes in the remaining temperature comparisons **(Appendix VII)**.

The results further showed that the highest median pupation success was recorded at 28 °C [75 (IQR, 0.63) %] and the lowest at 36 °C [22.5 (IQR, 3.12) %] (Table 12). A Kruskal-Wallis test was conducted to determine if pupation success differed among the temperature regimes. It was revealed that, there was statistically significant difference ($X^2(5) = 27.23$, $P < 0.001$) in pupation success among the temperature regimes. Post hoc analysis using Dunn's multiple range test revealed statistically significant differences only between 25 vs. 34 °C ($P = 0.002$), 25 vs. 36 °C ($P < 0.001$), 28 vs. 32 °C ($P = 0.003$), 28 vs. 34 °C ($P < 0.001$), and 28 vs. 36 °C ($P < 0.001$) **(Appendix VII)**.

With regards to pupal mortality, the highest pupal mortality was recorded at 36 °C [59.52 (IQR, 14.04) %], followed by 25 °C [21.69 (IQR, 0.84) %], 34 °C [17.95 (IQR, 2.39) %], 32 °C [16.28 (IQR, 1.78) %] and 30 °C [15.09 (IQR, 2.36) %]. The lowest pupal mortality was recorded at 28 °C [13.56 (IQR, 2.73) %] (Table 12). Overall, a Kruskal-Wallis test showed a statistically significant difference ($X^2(5) = 23.15$, $P < 0.001$) in pupal mortality among the temperature regimes. Dunn's multiple range test showed statistically significant differences in only pupae kept at 25 vs. 28 °C ($P = 0.001$), 28 vs. 36 °C ($P < 0.001$), 30 vs. 36 °C ($P < 0.001$), and 32 vs. 36 °C ($P = 0.002$) **(Appendix VII)**.

Table 12: Time to pupation, pupation success, and pupal mortality of *An. gambiae (s.l.)* mosquitoes reared at different temperature regimes

Temperature regime (ºC)	Time to pupation (in days) Mean (±SD)	Pupation success (%) Median (IQR)	Pupal mortality Median (IQR)
25	12.80 (±1.30)	53.75 (3.12)	21.69 (0.84)
28	11.80 (±1.10)	75.00 (0.63)	13.56 (2.73)
30	11.00 (±0.71)	34.38 (1.87)	15.09 (2.36)
32	9.20 (±0.45)	28.13 (2.50)	16.28 (1.78)
34	8.80 (±0.84)	24.38 (1.88)	17.95 (2.39)
36	7.40 (±0.55)	22.50 (3.12)	59.52 (14.04)

SD = Standard deviation; IQR = Interquartile range.

4.3.4 Measurement of larval and pupal weight and size

Larval and pupal weight and size were calculated for *An. gambiae (s.l.)* mosquitoes reared at 25, 28, 30, 32, 34, and 36 °C. The results showed that the median larval weight decreased with increasing temperature from 25 °C [2.1 (IQR, 0.6) mg] to 36 °C [1.1 (IQR, 0.3) mg]. Similar trends were observed for larval size, with larger larvae being recorded at 25 °C [4.94 (IQR, 0.39) mm] (Table 13). In addition, the median pupal weight decreased with increasing temperature, from 25 °C to 36 °C; 2.1 (IQR, 0.7) to 1.5 (IQR, 0.1) mg respectively. Similarly, pupal size decreased with increasing temperature. At 25 °C, pupal size was 1.73 (IQR, 0.10) mm but decreased as temperature increased to 28°C [1.65 (IQR, 0.14) mm], 30 °C [1.68 (IQR, 0.08) mm], 32 °C [1.61 (IQR, 0.06) mm], and 34 °C [1.59 (IQR, 0.08) mm], and 36 °C [1.52 (IQR, 0.17) mm] (Table 13).

Further statistical test using ordinary least square regression with robust standard errors showed that a change in temperature from 25 to 28 ºC significantly decreased larval weight by 0.15 (95% CI; 0.26, 0.05, $P = 0.001$). Furthermore, increasing temperature from 25 to 36 ºC significantly decreased larval size ($\beta_{\text{larval size}} = 0.11$, 95% CI; 0.14, 0.09, $P < 0.001$), pupal weight ($\beta_{\text{pupal weight}} = 0.34$, 95% CI; 0.40, 0.28, $P < 0.001$) and pupal size ($\beta_{\text{pupal size}} = 0.12$, 95%

CI; 0.14, 0.10, *P < 0.001*). Overall, sensitivity analysis using quantile and robust regressions showed consistent coefficients with the ordinary least square regression (Table 14).

Table 13: *An. gambiae (s.l.)* larval and pupal weight and size at different temperature regimes

Temperature regime (°C)	Larval weight (mg) Median (IQR)	Larval size (mm) Median (IQR)	Pupal weight (mg) Median (IQR)	Pupal size (mm) Median (IQR)
25	2.10 (0.60)	4.94 (0.39)	2.10 (0.70)	1.73 (0.10)
28	1.60 (0.50)	4.59 (0.61)	2.00 (0.40)	1.65 (0.14)
30	1.50 (0.50)	4.72 (0.36)	1.80 (0.30)	1.68 (0.08)
32	1.50 (0.40)	4.71 (0.48)	1.70 (0.50)	1.61 (0.06)
34	1.30 (0.30)	4.63 (0.38)	1.70 (0.50)	1.59 (0.08)
36	1.10 (0.30)	4.42 (0.44)	1.50 (0.10)	1.52 (0.17)

IQR = Interquartile range.

Table 14: Relationship between temperature and *An. gambiae (s.l.)* larval and pupal measurements

Outcome	Temperature regime (ºC)	Ordinary Least Square regression with robust standard errors	Sensitivity Analysis	
			Quantile regression	Robust regression
		β [95% CI]	β [95% CI]	β [95% CI]
Larval weight	25	Ref	Ref	Ref
	28	-0.15 [-0.26, -0.05]**	-0.27 [-0.38, -0.16] ***	-0.21 [-0.31, -0.12] ***
	30	-0.26 [-0.36, -0.15] ***	-0.34 [-0.45, -0.22] ***	-0.31 [-0.40, -0.22] ***
	32	-0.27 [-0.36, -0.18] ***	-0.34 [-0.45, -0.22] ***	-0.32 [-0.41, -0.23] ***
	34	-0.35 [-0.45, -0.25] ***	-0.48 [-0.59, -0.37] ***	-0.41 [-0.50, -0.32] ***
	36	-0.52 [-0.61, -0.42] ***	-0.65 [-0.76, -0.54] ***	-0.57 [-0.66, -0.48] ***
Pupal weight	25	Ref	Ref	Ref
	28	-0.10 [-0.17, -0.03] **	-0.05 [-0.15, 0.05]	-0.09 [-0.17, -0.02] **
	30	-0.17 [-0.24, -0.10] ***	-0.15 [-0.25, -0.05] **	-0.17 [-0.24, -0.10] ***
	32	-0.20 [-0.27, -0.12] ***	-0.21 [-0.31, -0.11] ***	-0.19 [-0.26, -0.12] ***
	34	-0.22 [-0.30, -0.15] ***	-0.21 [-0.31, -0.11] ***	-0.23 [-0.30, -0.16] ***
	36	-0.34 [-0.40, -0.28] ***	-0.34 [-0.43, -0.24] ***	-0.34 [-0.40, -0.28] ***
Larval size	25	Ref	Ref	Ref
	28	-0.04 [-0.08, -0.01]*	-0.07 [-0.11, -0.04] ***	-0.06 [-0.09, -0.03] ***
	30	-0.04 [-0.07, -0.02] ***	-0.04 [-0.08, -0.01] *	-0.04 [-0.07, -0.01] **
	32	-0.05 [-0.08, -0.02] ***	-0.05 [-0.08, -0.01] *	-0.05 [-0.08, -0.02] **
	34	-0.07 [-0.09, -0.04] ***	-0.07 [-0.10, -0.03] ***	-0.07 [-0.10, -0.04] ***
	36	-0.11 [-0.14, -0.09] ***	-0.11 [-0.15, -0.07] ***	
Pupal size	25	Ref	Ref	Ref
	28	-0.02 [-0.04, -0.00] *	-0.04 [-0.06, -0.02] ***	-0.03 [-0.05, -0.02] ***
	30	-0.03 [-0.05, -0.02] ***	-0.03 [-0.05, -0.01] **	-0.03 [-0.05, -0.02] ***
	32	-0.06 [-0.08, -0.05] ***	-0.07 [-0.09, -0.05] ***	-0.07 [-0.09, -0.05] ***
	34	-0.08 [-0.10, -0.07] ***	-0.08 [-0.10, -0.06] ***	-0.09 [0-.10, -0.07] ***
	36	-0.12 [-0.14, -0.10] ***	-0.12 [-0.15, -0.10] ***	-0.13 [-0.15, -0.11] ***

Larval weight, pupal weight, larval size, and pupal size were log-transformed; single asterisk (*) represents significant difference at $P < 0.05$; double asterisk (**) means $P < 0.01$; triple asterisk (***) means $P < 0.001$; Ref means Reference. β means regression coefficients, 95% CI means 95% Confidence interval; *p-values* were generated using OLS with robust standard errors.

4.3.5 Temperature, number of adults produced, and sex ratio of *An. gambiae (s.l.)* mosquitoes

The number of adults produced (proportion of larvae that emerged as adults), and sex ratio of mosquitoes were estimated at 25, 28, 30, 32, 34 and 36 °C. Mosquitoes failed to develop at temperatures above 36 °C. The results showed that there was a decrease in the number of adults produced as temperature increased from 28 °C [66.25 (IQR, 3.13) %] to 30 °C [28.75 (IQR, 2.50) %], 32 °C [23.13 (IQR, 2.50) %], 34 °C [20.00 (IQR, 1.88) %] and 36 °C [10.63 (IQR, 3.13) %] (Table 15). A Kruskal-Wallis test revealed a statistically significant difference ($X^2(5) = 28.16$, $P < 0.001$) in the number of adults produced among the temperature regimes. Post hoc analysis using Dunn's test showed significant differences in 25 vs 36 °C ($P = 0.002$), 28 vs 32 °C ($P = 0.003$), 28 vs 34°C ($P < 0.001$) and 28 vs 36 °C ($P < 0.001$) **(Appendix VII)**. Furthermore, increasing temperature increased the number of male adult mosquitoes produced. The median sex ratio of male/female (M/F) ranged from 0.89 (IQR, 0.04) at 25 °C to 2.60 (IQR, 0.60) at 36 °C (Table 15). There was a statistically significant difference (Kruskal-Wallis test; $X^2(5) = 28.07$, $P < 0.001$) in the sex ratio of mosquitoes among the temperature regimes, with few females emerging at higher temperatures. Dunn's comparison test showed that sex ratio differed at 25 vs 34 °C ($P < 0.001$), 25 vs 36 °C ($P < 0.001$), and 28 vs 36 °C ($P < 0.001$) **(Appendix VII)**.

Table 15: Number of adults produced, and sex ratios of *An. gambiae (s.l.)* mosquitoes reared at different temperature regimes

Temperature regime (°C)	Adults produced (%) Median (IQR)	Sex ratio (M/F) Median (IQR)
25	41.88 (1.87)	0.89 (0.04)
28	66.25 (3.13)	1.08 (0.04)
30	28.75 (2.50)	1.88 (0.00)
32	23.13 (2.50)	2.08 (0.00)
34	20.00 (1.88)	2.20 (0.02)
36	10.63 (3.13)	2.60 (0.60)

IQR = Interquartile range.

4.4 Effects of temperature and the growth and development of adult *An. gambiae (s.l.)* mosquitoes

4.4.1 Adult longevity

This section of the study assessed the effects of temperature on the longevity of adult mosquitoes. Although adult mosquitoes emerged at 36 °C, they died within 24 hours. Therefore, longevity was estimated for only mosquitoes reared at 25, 28, 30, 32 and 34 °C. The results showed that the median adult longevity decreased with increasing temperature and differed between blood-fed and non-blood-fed mosquitoes. The difference in the median longevity of non-blood-fed and blood-fed mosquitoes was eight (8) days at 25 °C and two (2) days at 34 °C, respectively (Table 16).

Table 16: Median longevity of adult *An. gambiae (s.l.)* mosquitoes reared at different temperatures

Temperature regime (°C)	Median Longevity (days) (95% CI.)	
	Blood-fed mosquitoes	Non-blood-fed mosquitoes
25	10 (6, 15)	18 (15, 22)
28	10 (5, 14)	12 (8, 16)
30	6 (3, 10)	9 (6, 12)
32	4 (2, 6)	6 (3, 8)
34	2 (2, 4)	4 (2, 7)
Total	**5 (3, 10)**	**9 (5, 14)**

95% CI means 95% Confidence interval

Among blood-fed mosquitoes, Kaplan-Meier plots showed that longevity decreased with increasing temperature from 25 °C (22 days) to 28 °C (19 days), 30 °C (14 days), 32 °C (9 days) and 34 °C (5 days) (Figure 14A). A similar trend was observed in the longevity of non-blood-fed mosquitoes, although non-blood-fed mosquitoes lived longer than blood-fed mosquitoes (Figure 14B). Log-rank test showed that longevity of mosquitoes significantly differed among the different temperature regimes ($X^2(4) = 904.15$, $P < 0.001$ for blood-fed group, $X^2(4) = 1163.60$, $P < 0.001$; non-blood-fed group) **(Appendix IX)**.

The cox proportional hazard model was further used for two-sample comparisons at each of the temperature regimes (28, 30, 32, and 34 °C) against the longevity at the baseline temperature (25 °C). Cox proportional hazard model showed that non-blood-fed and blood-fed adult mosquitoes kept at 28°C had 189% (HR = 2.89, 95% CI; 2.43, 3.44, $P < 0.001$) and 26% (HR = 1.26, 95% CI; 1.07, 1.49, $P = 0.005$) increased risk of death, respectively, compared to those kept at 25°C. Similar trends were observed at 30 °C (HR = 6.86, 95% CI; 5.62, 8.37, $P < 0.001$ for non-blood-fed group, HR = 2.71, 95% CI; 2.28, 3.23, $P < 0.001$ for blood-fed group), 32 °C (HR = 16.46, 95% CI; 13.28, 20.40, $P < 0.001$ for non-blood-fed group, HR = 5.27, 95% CI; 4.37, 6.36, $P < 0.001$ for blood-fed group) and 34 °C (HR = 22.65, 95% CI;

18.15, 28.26, $P < 0.001$ for non-blood-fed group, HR = 11.16, 95% CI; 9.11, 13.67, $P < 0.001$ for blood-fed group). In addition, blood meal significantly (log-rank test; $X^2(1) = 217.63$, $P < 0.001$) reduced the longevity of adult mosquitoes in all the temperature regimes **(Appendix IX)**.

The longevity of female and male mosquitoes was also compared to determine if differences existed. Overall, the longevity of female mosquitoes was significantly higher (log-rank test; $X^2(9) = 925.98$, $P < 0.001$ for blood-fed group, $X^2(9) = 1198.52$, $P < 0.001$ for non-blood-fed group) than that of the male mosquitoes irrespective of whether the mosquitoes were blood-fed or not (Figure 14C & D, **Appendix X**). However, log-rank test showed no statistically significant difference in the longevity of male and female mosquitoes reared at 25 ºC ($X^2(1) = 3.36$ $P = 0.067$) and 32 ºC ($X^2(1) = 1.20$, $P = 0.274$) in the blood-fed group **(Appendix X)**.

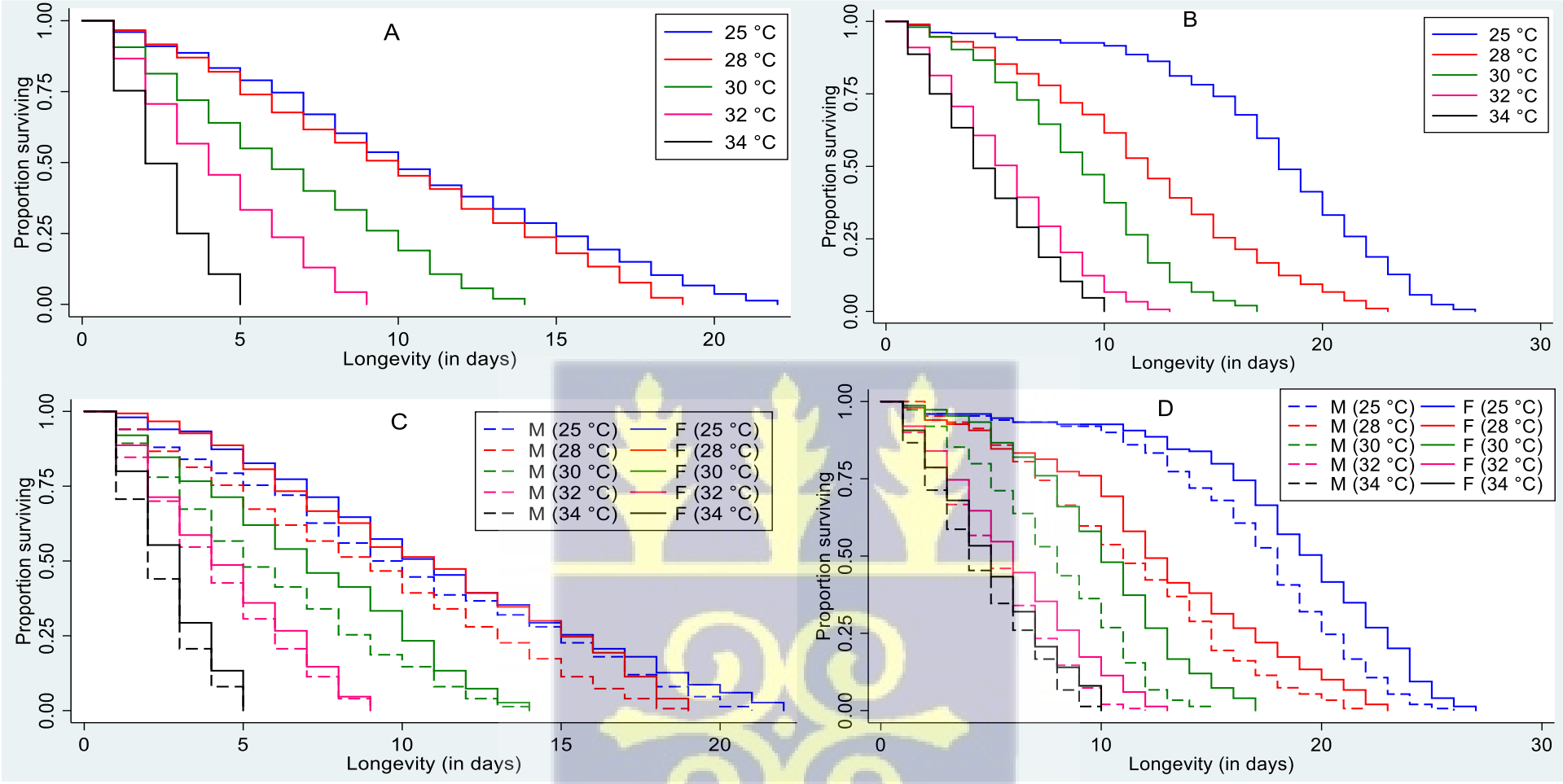

Figure 14: Longevity of adult *An. gambiae (s.l.)* mosquitoes reared under different temperature regimes.
A – longevity of blood-fed mosquitoes; B – longevity of non-blood-fed mosquitoes; C – longevity of male & female mosquitoes in the blood-fed group; D – longevity of male & female mosquitoes in the non-blood-fed group. The 25 °C temperature (blue) was set as the baseline against which longevity at different temperature was compared; 28 °C (red); 30 °C (green); 32 °C (pink); 34 °C (black); M & F represent Male & Female respectively.

4.4.2 Length of the gonotrophic cycle, biting rate and fecundity of *An. gambiae (s.l.)* mosquitoes

The length of gonotrophic cycle, biting rate and fecundity of mosquitoes were estimated at only three temperature regimes, 25, 28 and 30 °C because eggs kept at 40 °C failed to hatch, and larvae were unable to develop at 38 °C. In addition, adult mosquitoes that emerged at 36 °C died within the first day and adult mosquitoes that emerged from larvae at either 32 or 34 °C did not lay eggs.

The results showed that the gonotrophic cycle length was similar at 25 and 28 °C (both had 3.20 days) but reduced at 30 °C (2.90 days) (Table 17). One-way ANOVA showed no statistically significant difference ($F(2,12) = 1.00$, $P = 0.397$) in the gonotrophic cycle length among the various temperature regimes. With biting rate, increasing temperature from 25 to 30 °C increased the biting rate from 0.31 (±0.03) to 0.35 (±0.03) day^{-1} (Table 17). However, one-way ANOVA showed no statistically significant difference ($F(2,12) = 0.83$, $P = 0.460$) in biting rates among the various temperature regimes. In addition, the average number of eggs laid per female mosquito ranged from 75.68 at 25 °C to 55.43 at 30 °C (Table 17). One-way ANOVA showed that the mean fecundity significantly differed ($F(2,57) = 3.46$, $P = 0.038$) among the various temperature regimes. Further statistical test using Tukey post hoc test revealed a significant difference in fecundity only between 25 and 30 °C ($P = 0.027$) **(Appendix XI)**.

Table 17: Mean gonotrophic cycle length, biting rate, and fecundity of *An. gambiae (s.l.)* mosquitoes reared at different temperature regimes

Temperature regime (°C)	Gonotrophic cycle length (in days) Mean (±SD)	Biting rate (day^{-1}) Mean (±SD)	Fecundity Mean (±SD)
25	3.20 (±0.27)	0.31 (±0.03)	75.68 (±21.69)
28	3.20 (±0.57)	0.32 (±0.06)	65.57 (±28.61)
30	2.90 (±0.22)	0.35 (±0.03)	55.43 (±22.06)
32	-	-	-
34	-	-	-

SD = Standard Deviation; Mosquitoes at 32 and 34 did not lay eggs

4.4.3 Measurement of body weight, size, and proboscis length of adult mosquitoes

The body weight, size and proboscis length of adult mosquitoes were estimated at 25, 28, 30, 32 and 34 °C because eggs kept at 40 °C failed to hatch. Larvae were unable to develop at 38 °C and adult mosquitoes that emerged at 36 °C died within the first day. The results showed that body weight, size and proboscis length of adult mosquitoes decreased with increasing temperature. Adult weight decreased as temperature increased from 1.10 (IQR, 0.30) mg at 25 °C to 0.90 (IQR, 0.40) mg at 32 and 34 °C (Table 18). Further analysis using ordinary least square regression with robust standard errors showed that a change in temperature from 25 to 34 °C resulted in a statistically significant decrease in adult weight by 0.13 (95% CI, 0.24, 0.01, *P = 0.031*) (Table 19).

With regards to the body size of mosquitoes, it decreased with increasing rearing temperature from 25 °C [3.03 (IQR, 0.26) mm] to 34 °C [2.62 (IQR, 0.15) mm] (Table 18). Compared to 25 °C, body size significantly decreased with increasing temperature by 0.06 (95% CI, 0.08, 0.03, *P < 0.001*) at 28 °C, 0.07 (95% CI, 0.10, 0.05, *P < 0.001*) at 30 °C, 0.10 (95% CI, 0.12, 0.08, *P < 0.001*) at 32 °C, and 0.14 (95% CI, 0.16, 0.12, *P < 0.001*) at 34 °C (Table 19). On the other hand, proboscis length decreased with increasing temperature from 2.18 (IQR, 0.32)

mm at 25 ºC to 1.84 (IQR, 0.36) mm at 34 ºC (Table 18). Generally, proboscis length significantly decreased with an increase in temperature from the baseline (25 ºC) to 30, 32 and 34 ºC by 0.05 (95% CI, 0.09, 0.01, *P = 0.011*), 0.11 (95% CI, 0.15, 0.06, *P < 0.001*), and 0.13 (95% CI, 0.17, 0.09, *P < 0.001*), respectively. Sensitivity analysis using quantile and robust regressions showed consistent coefficients with the ordinary least square (OLS) regression (Table 19).

Table 18: *An. gambiae (s.l.)* mosquito weight, size and proboscis length at different temperature regimes

Temperature regime (ºC)	Adult weight (mg) Median (IQR)	Adult size (mm) Median (IQR)	Proboscis length (mm) Median (IQR)
25	1.10 (0.30)	3.03 (0.26)	2.18 (0.32)
28	1.00 (0.40)	2.85 (0.17)	2.09 (0.37)
30	0.95 (0.50)	2.79 (0.19)	2.03 (0.29)
32	0.90 (0.40)	2.73 (0.15)	1.92 (0.39)
34	0.90 (0.40)	2.62 (0.15)	1.84 (0.36)

IQR = Interquartile range.

Table 19: Relationship between temperature and adult *An. gambiae (s.l.)* mosquito weight, body size and proboscis length

Outcome	Temperature regime (°C)	OLS regression with robust standard errors	Sensitivity Analysis	
			Quantile regression	Robust regression
		β [95% CI]	β [95% CI]	β [95% CI]
Adult weight	25	Ref	Ref	Ref
	28	-0.05 [-0.15, 0.05]	-0.10 [-0.24, 0.05]	-0.04 [-0.16, 0.07]
	30	-0.06 [-0.16, 0.03]	-0.10 [-0.24, 0.05]	-0.08 [-0.20, 0.03]
	32	-0.09 [-0.19, 0.01]	-0.20 [-0.35, -0.05]**	-0.10 [-0.21, 0.01]
	34	-0.13 [-0.24, -0.01]*	-0.20 [-0.35, -0.05]**	-0.08 [-0.19, 0.03]
Adult size	25	Ref	Ref	Ref
	28	-0.06 [-0.08, -0.03]***	-0.06 [-0.09, -0.04] ***	-0.06 [-0.08, -0.04] ***
	30	-0.07 [-0.10, -0.05] ***	-0.08 [-0.10, -0.05] ***	-0.07 [-0.09, -0.05] ***
	32	-0.10 [-0.12, -0.08] ***	-0.10 [-0.13, -0.08] ***	-0.10 [-0.12, -0.08] ***
	34	-0.14 [-0.16, -0.12] ***	-0.14 [-0.17, -0.12] ***	-0.14 [-0.16, -0.12] ***
Proboscis length	25	Ref	Ref	Ref
	28	-0.04 [-0.08, 0.00]	-0.03 [-0.11, 0.05]	-0.04 [-0.09, 0.00]
	30	-0.05 [-0.09, -0.01]*	-0.05 [-0.13, 0.02]	-0.05 [-0.10, -0.01]*
	32	-0.11 [-0.15, -0.06] ***	-0.10 [-0.18, -0.03] **	-0.11 [-0.15, -0.07] ***
	34	-0.13 [-0.17, -0.09] ***	-0.14 [-0.22, -0.06] ***	-0.13 [-0.17, -0.09] ***

Adult weight, adult size, and proboscis length were log-transformed; OLS means Ordinary Least Square; single asterisk (*) represents significant difference at $P < 0.05$; double asterisk (**) means $P < 0.01$; triple asterisk (***) means $P < 0.001$; Ref means Reference; β means regression coefficients, 95% CI means 95% Confidence interval; *p-values* were generated using OLS with robust standard errors.

4.5 Insecticide susceptibility and expression of metabolic enzyme levels

Susceptibility of mosquitoes to two pyrethroid insecticides (permethrin and deltamethrin) were assessed under different rearing temperature regimes (25, 28, 30, 32 and 34 ^{o}C). All mosquitoes reared at 34 ^{o}C died and mortality in control replicates exceeded 20 % (43.9 %) **(Appendix XII)**, therefore results were excluded in final analysis. Metabolic enzyme levels in live mosquitoes were measured in both female mosquitoes that were exposed and those that were not exposed to pyrethroids.

4.5.1 Mortality of *An. gambiae (s.l.)* mosquitoes after exposure to pyrethroid insecticides

The results of insecticide susceptibility of *An. gambiae (s.l.)* (Tiassalé) to deltamethrin and permethrin insecticides are presented in Figure 15. Mortality in the control replicates at 32 ^{o}C exceeded 5 %, therefore mortalities were corrected consistent with prescribed guidelines using Abbott's formula (Abbott, 1987).

Overall, deltamethrin insecticides induced higher mortality compared to permethrin insecticides irrespective of the temperature regime. Female mosquitoes showed high mortality (100 %) to deltamethrin at 28 ^{o}C and mortality decreased at temperature above 28 ^{o}C (Figure 15). Upon exposure to permethrin, mortality of *An. gambiae (s.l.)* (Tiassalé) mosquitoes decreased with increasing temperature from 28 °C (81.61 %) to 32 °C (43.06 %). All susceptible *An. gambiae (s.l.)* (Kisumu) mosquitoes exposed to permethrin and deltamethrin insecticides died irrespective of the rearing temperature **(Appendix XII)**.

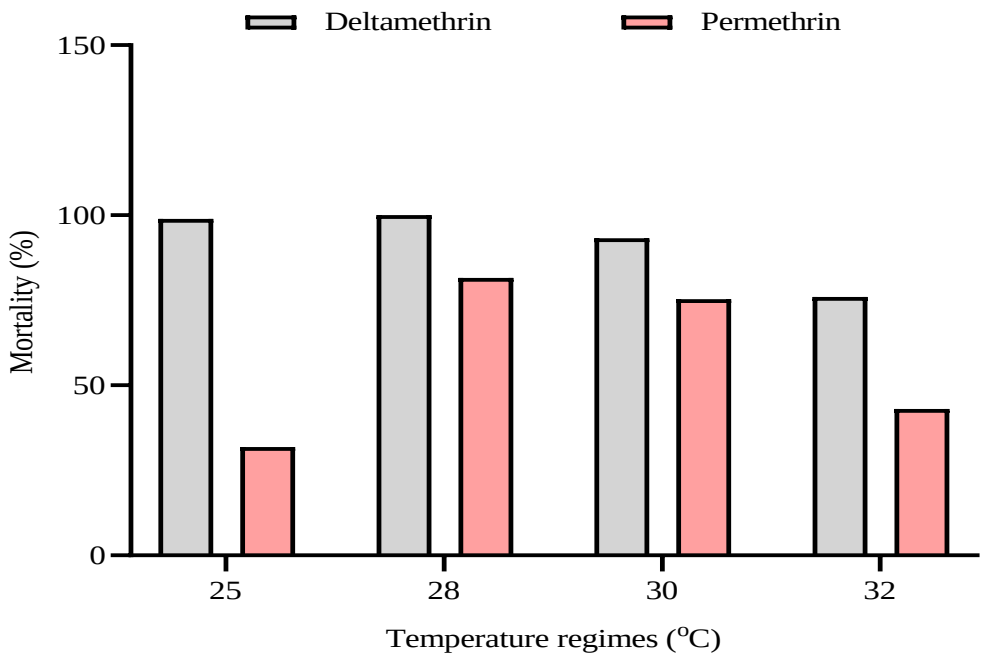

Figure 15: Insecticide susceptibility of *Anopheles gambiae (s.l.)* (Tiassalé strain) mosquitoes reared at different temperature regimes

4.5.2 Knockdown resistance ratio (KRR)

The time at which 50 % of the mosquitoes were knocked down (KDT_{50}) after exposure to insecticides was assessed under different temperature regimes, 25, 28, 30, 32 and 32 °C. The KDT_{50} of *An. gambiae (s.l.)* (Tiassalé) mosquitoes exposed to both permethrin and deltamethrin insecticides decreased with increasing temperature from 25 to 34 °C. For mosquitoes raised at 25 °C and exposed to permethrin insecticides, the KDT_{50} was higher (888.70 minutes) than the other temperature regimes (Table 20). Furthermore, the knockdown resistance ratio based on KDT_{50} for deltamethrin decreased with increasing temperature from 25 to 32 °C. With permethrin, the resistance ratio was highest at 25 °C (51.31), followed by 32 °C (4.12), 28 °C (3.87) and 30 °C (3.59) (Table 20). Generally, all the mosquitoes in this study had developed a certain level of resistance to permethrin and deltamethrin insecticides, which enabled them to survive the knockdown effect for some time.

Table 20: Knockdown resistance ratio of *An. gambiae (s.l.)* mosquitoes (Tiassalé strain) at different rearing temperature regimes

Insecticide	Temperature regime (ºC)	Test population (Tiassalé) KDT_{50} [95% CI]	Reference strain (Kisumu) KDT_{50} [95% CI]	KRR	Resistance status
Deltamethrin	25	40.18 [38.22, 42.23]	16.02 [15.12, 16.94]	2.51	low resistance
	28	32.33 [30.76, 33.88]	16.35 [15.45, 17.27]	1.98	low resistance
	30	28.02 [24.84, 31.36]	15.68 [14.63, 16.71]	1.79	low resistance
	32	22.85 [21.37, 24.36]	15.44 [14.33, 16.52]	1.48	low resistance
Permethrin	25	888.70 [224.30, 7113093.45]	17.32 [15.83, 17.92]	51.31	high resistance
	28	63.26 [56.06, 74.90]	16.34 [15.47, 17.23]	3.87	low resistance
	30	60.57 [55.03, 69.18]	16.88 [17.71, 20.26]	3.59	low resistance
	32	59.29 [52.81, 69.25]	14.39 [13.47, 15.29]	4.12	low resistance

KDT = Knockdown time; KRR = Knockdown resistance ratio, CI = Confidence Interval; KDT_{50} and 95% CI were obtained using probit analysis; KRR_{50} < 1 = susceptible, 1 – 10 = low resistance, 11 – 30 = moderate resistance, 31 – 100 = high resistance, and KRR_{50} > 100 = very high resistance

4.5.3 Influence of temperature and insecticide on the expression of metabolic enzyme

4.5.3.1 Mixed-function oxidase (MFO) level

The level of mixed-function oxidase was assessed in mosquitoes reared at 25, 28, 30, 32 and 34 ºC and were not exposed to pyrethroids. The results showed that MFO level was more elevated in mosquitoes reared at 32 ºC [4.55×10^{-9} (IQR, 4.13×10^{-9}) mol cytochrome P450/min/mg protein] compared to those reared at 34 ºC [1.94×10^{-9} (IQR, 3.80×10^{-10})], 30 ºC [1.49×10^{-9} (IQR, 2.26×10^{-9})], 28 ºC [8.85×10^{-10} (IQR, 1.50×10^{-10})], and 25 ºC [7.21×10^{-10} (IQR, 1.43×10^{-10}) mol cytochrome P450/min/mg protein] **(Appendix XIII)**. The levels decreased in the order of 32 > 34 > 30 > 28 > 25 (Figure 16). Generally, levels of oxidase differed significantly (Kruskal-Wallis test; $X^2(4) = 144.42$, $P < 0.001$) among the various temperature regimes. Dunn's multiple range test showed that all but 28 vs 30 °C ($P = 0.113$) and 30 vs 34 °C ($P = 0.010$) showed a statistically significant difference in oxidase level **(Appendix XIV)**.

The effect of rearing temperature on MFO level was also assessed in mosquitoes that survived after being exposed to pyrethroids. Mosquitoes reared at 30 ºC had higher [3.15×10^{-9} (IQR, 6.40×10^{-10}) mol cytochrome P450/min/mg protein] levels compared to those reared at 32 ºC [2.40×10^{-9} (IQR, 1.07×10^{-9})], 25 ºC [2.07×10^{-9} (IQR, 7.50×10^{-10})], and 28 ºC [1.34×10^{-9} (IQR, 2.50×10^{-10}) mol cytochrome P450/min/mg protein] (Figure 16). There was a significant difference (Kruskal-Wallis test; $X^2(3) = 68.18$, $P < 0.001$) in mixed-function oxidase level amongst the different temperature regimes. Further test using Dunn's multiple range test showed a significant difference ($P < 0.008$) in the various temperature regime comparisons **(Appendix XIV)**.

With the exception of mosquitoes reared at 32 ºC, mixed-function oxidase level in mosquitoes reared at 25, 28, and 30 ºC and exposed to pyrethroids were higher compared to those that were

not exposed to pyrethroids (Figure 16). A Mann-Whitney U test showed a statistically significant difference in mixed-function oxidase level observed among mosquitoes that were not exposed and those exposed to pyrethroids at 25 ºC (z = -7.72, *P < 0.001*), 28 ºC (z = -5.33, *P < 0.001*), 30 ºC (z = -4.68, *P < 0.001*), and 32 ºC (z = 5.12, *P < 0.001*) **(Appendix XV)**.

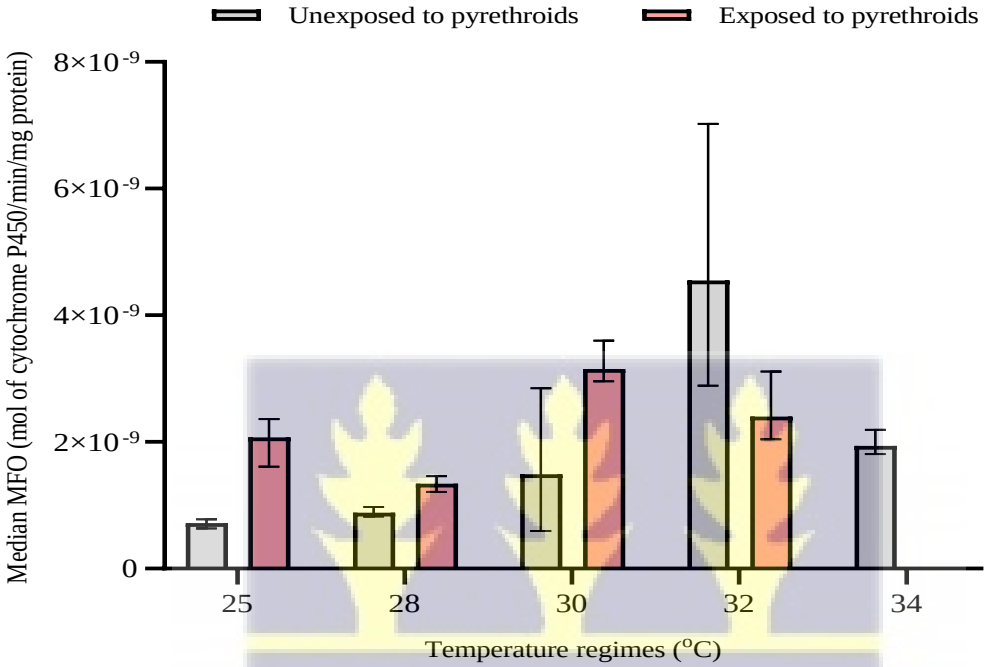

Figure 16: Median MFO level in *An. gambiae (s.l.)* mosquitoes reared at different temperature regimes. NB: No mosquito reared at 34 ºC survived after being exposed to pyrethroids, hence, no enzyme level was measured

4.5.3.2 Glutathione S-Transferases (GSTs) level

The level of GST enzyme was assessed in mosquitoes reared at 25, 28, 30, 32 and 34 ºC and were not exposed to pyrethroids. The results showed that GST enzyme level followed a trend (32 > 34 > 30 > 28 > 25) similar to that of MFO level (Figure 17). A Kruskal-Wallis test revealed a statistically significant difference ($X^2(4) = 89.06$, *P < 0.001*) in GST level of mosquitoes among the different temperature regimes. However, further analysis using Dunn's multiple range test showed statistically significant differences between 25 vs 32 °C (*P < 0.001*), 25 vs 34 °C (*P = 0.002*), 28 vs 32 °C (*P < 0.001*), 30 vs 32 °C (*P < 0.001*) and 32 vs 34 °C (*P < 0.001*) **(Appendix XIV)**.

With mosquitoes that were exposed to pyrethroids, the level of GST was highest at 32 °C [3.55×10^{-3} (IQR, 2.63×10^{-3})], followed by 25 °C [2.72×10^{-3} (IQR, 1.56×10^{-3})], 30 °C [2.62×10^{-3} (IQR, 1.69×10^{-3})] with the lowest recorded at 28 °C [1.35×10^{-3} (IQR, 6.59×10^{-4}) mol cDNB/min/mg protein] **(Appendix XIII)**. There was a statistically significant difference (Kruskal-Wallis test; $X^2(3) = 18.26$, $P < 0.001$) in GST level of mosquitoes among the different temperature regimes. Further statistical tests using Dunn's multiple range test showed statistically significant differences between 25 vs 28 °C ($P = 0.002$), 28 vs 32 °C ($P < 0.001$), and 30 vs 32 °C ($P = 0.005$) **(Appendix XIV)**. In addition, mosquitoes in the temperature regimes exposed to pyrethroids (except for those reared at 32 °C) had higher GST levels compared to those that were not exposed to pyrethroids (Figure 17). However, a Mann-Whitney U test showed that all but mosquitoes reared at 28 °C ($z = -0.52$, $P = 0.605$) showed a statistically significant difference ($P < 0.05$) in GST level **(Appendix XV)**.

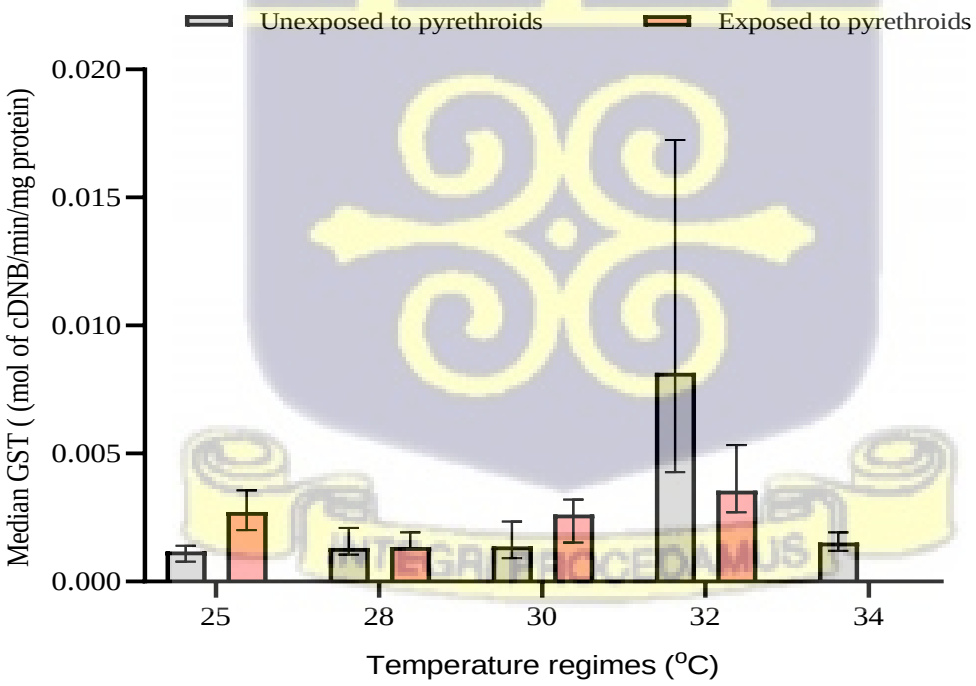

Figure 17: Median GST level in *An. gambiae (s.l.)* mosquitoes reared at different temperature regimes. NB: No mosquito reared at 34 °C survived after being exposed to pyrethroids, hence, no enzyme level was measured

4.5.3.3 Alpha-esterase level

The level of α-esterase enzyme was assessed in mosquitoes reared at 25, 28, 30, 32 and 34 ºC and were not exposed to pyrethroids. The highest median α-esterase level was recorded at 32 ºC [1.32×10^{-6} (IQR, 9.41×10^{-7})], followed by 34 ºC [4.04×10^{-7} (IQR, 1.56×10^{-7})], 28 ºC [2.83×10^{-7} (IQR, 4.32×10^{-7})], 25 ºC [2.52×10^{-7} (IQR, 7.30×10^{-8})] and 30 ºC [2.12×10^{-7} (IQR, 4.10×10^{-7}) mol α-naphthol/min/mg protein] **(Appendix XIII)**. Median α-esterase level did not increase with increasing rearing temperature; however, a Kruskal-Wallis test revealed a statistically significant difference ($X^2(4) = 99.46$, $P < 0.001$) in α-esterase level of mosquitoes among the different temperature regimes. Further statistical tests using Dunn's multiple range test showed a statistically significant difference between 25 vs 32 °C ($P < 0.001$), 25 vs 34 °C ($P < 0.001$), 28 vs 32 °C ($P < 0.001$), 30 vs 32 °C ($P < 0.001$), 30 vs 34 °C ($P < 0.001$), and 32 vs 34 °C ($P < 0.001$) **(Appendix XIV)**.

The level of α-esterase level in mosquitoes that were exposed to pyrethroids was also assessed. The results showed that α-esterase level was highest at 32 °C [3.13×10^{-7} (1.81×10^{-7})] and lowest at 28 °C [1.44×10^{-7} (2.40×10^{-7}) mol α-naphthol/min/mg protein] **(Appendix XIII)**. There was a significant difference (Kruskal-Wallis test; $X^2(3) = 11.31$, $P = 0.010$) in α-esterase level of mosquitoes among the different temperature regimes. According to Dunn's multiple range tests, a statistical difference existed only between 28 vs 30 °C ($P = 0.003$), and 28 vs 32 ($P = 0.001$) ºC **(Appendix XIV)**. Overall, mosquitoes that were not exposed to pyrethroids had higher α-esterase level than those exposed to pyrethroids (Figure 18). A Mann-Whitney U test showed that all but mosquitoes reared at 30 °C ($z = -1.53$, $P = 0.127$) showed a statistically significant difference ($P < 0.05$) in α-esterase level **(Appendix XV)**.

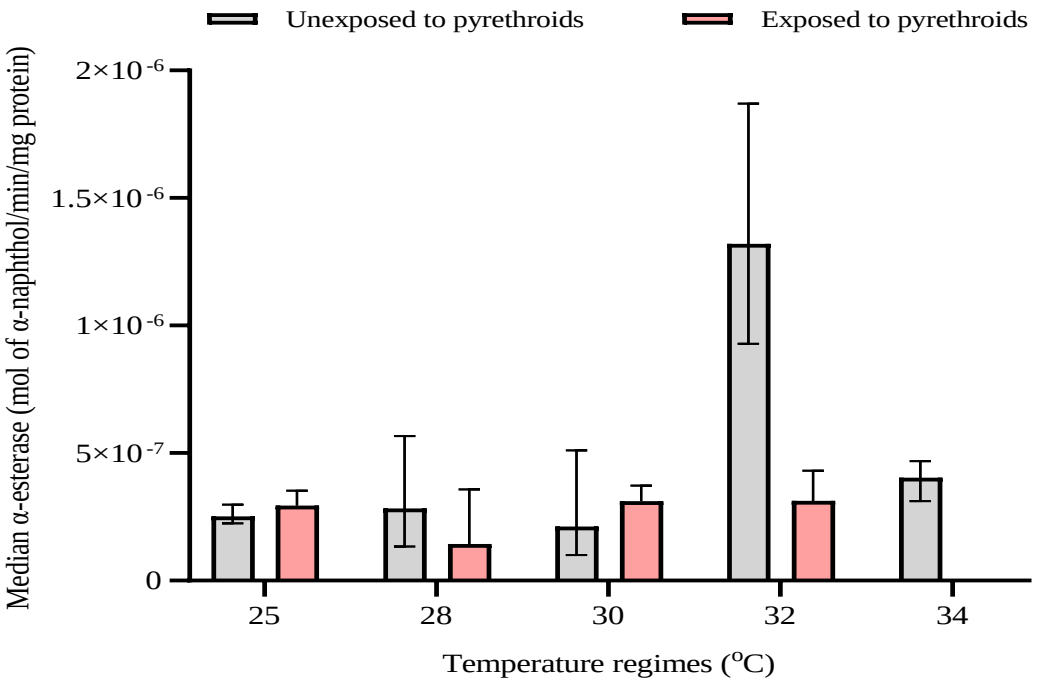

Figure 18: Median α-esterase level in *An. gambiae (s.l.)* mosquitoes reared at different temperature regimes. NB: No mosquito reared at 34 ºC survived after being exposed to pyrethroids, hence, no enzyme level was measured

4.5.3.4 Beta-esterase level

The level of β-esterase enzyme was assessed in mosquitoes reared at 25, 28, 30, 32 and 34 ºC and were not exposed to pyrethroids. The level decreased in the order of 34 ºC [3.56×10^{-7} (IQR, 1.54×10^{-7})] > 32 ºC [2.87×10^{-7} (IQR, 3.21×10^{-7})] > 28 ºC [1.38×10^{-7} (IQR, 1.82×10^{-7})] > 25 ºC [1.36×10^{-7} (IQR, 6.10×10^{-8})] > 30 ºC [1.30×10^{-7} (IQR, 2.93×10^{-7}) mol β-naphthol/min/mg protein] **(Appendix XIII)**. Generally, there was a statistically significant difference (Kruskal-Wallis test; $X^2(4) = 48.28$, $P < 0.001$) in β-esterase level in mosquitoes among the various temperature regimes. However, post hoc analysis using Dunn's multiple range test showed a statistically significant difference between 25 vs 32 °C ($P = 0.001$), 25 vs 34 °C ($P < 0.001$), 28 vs 34 °C ($P < 0.001$), 30 vs 34 °C ($P < 0.001$), and 32 vs 34 °C ($P = 0.003$) **(Appendix XIV)**.

With regards to mosquitoes that were exposed to pyrethroids, β-esterase level ranged from 1.11×10^{-7} (6.85×10^{-8}) at 28 °C to 2.64×10^{-7} (1.63×10^{-7}) mol β-naphthol/min/mg protein at 32 °C **(Appendix XIII)**. A Kruskal-Wallis test showed a statistically significant difference ($X^2(3) = 37.91$, $P = 0.001$) in β-esterase level in mosquitoes raised at different temperatures. Multiple comparison using Dunn's test showed statistically significant differences between 25 vs 28 °C ($P < 0.001$), 28 vs 30 °C ($P < 0.001$), 28 vs 32 °C ($P < 0.001$), and 30 vs 32 °C ($P = 0.007$) **(Appendix XIV)**. In addition, β-esterase level in mosquitoes reared at 25 and 30 °C and were exposed to pyrethroids was higher than in those that were not exposed to pyrethroids. On the other hand, β-esterase level in mosquitoes that were not exposed to pyrethroids was higher than in those that were raised at 28 and 32 °C and were exposed to pyrethroids (Figure 19). However, a Mann-Whitney U test showed that only mosquitoes reared at 25 °C showed a statistically significant difference ($z = -6.03$, $P < 0.001$) in β-esterase level **(Appendix XV)**.

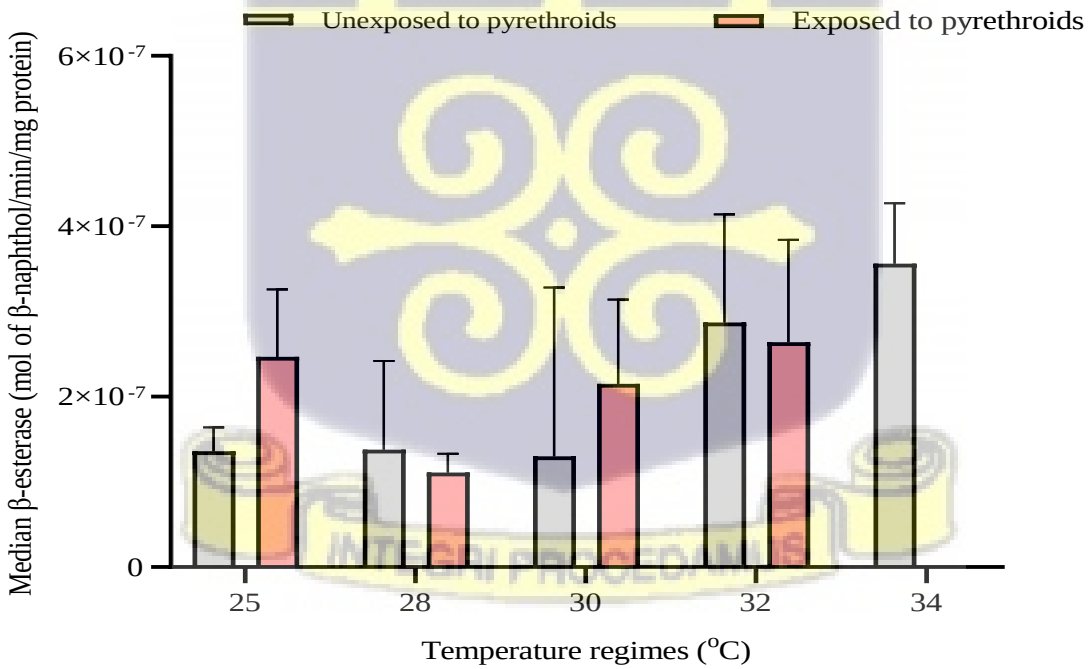

Figure 19: Median β-esterase level in *An. gambiae (s.l.)* mosquitoes reared at different temperature regimes. NB: No mosquito reared at 34 ºC survived after being exposed to pyrethroids, hence, no enzyme level was measured

CHAPTER FIVE

DISCUSSION

5.1 Introduction

The chapter is organized into three main sub-sections. The first section highlights the key findings on the relationship between temperature variability and growth and development of immature *An. gambiae (s.l.)* mosquitoes, while the second section focuses on the salient findings related to the growth and development of the adult mosquito. The final section focused on the effects of rearing temperature on *An. gambiae (s.l.)* mosquito's susceptibility to pyrethroid insecticides and expression of metabolic enzymes (MFO, GSTs and NSE).

5.2 Effects of temperature on the growth and development of immature *An. gambiae (s.l.)* mosquitoes

5.2.1 Hatching and development time of mosquitoes decreased with increasing temperature

The current study found that hatching time of eggs reduced with increasing temperature (30 – 38 ^{o}C). However, eggs did not hatch at the highest temperature of 40 ^{o}C. The failure of eggs to hatch at 40 ^{o}C could be attributed to the interruption of embryogenesis because of the physiological stress experienced by eggs at higher temperatures (Sukiato et al., 2019). Previous studies (Delatte et al., 2009; Sukiato et al., 2019) also reported similar findings of decreased hatching time with increasing temperature. The reduced hatching time of mosquitoes at higher temperatures is of great importance when exploring *An. gambiae (s.l.)* mosquito's success in breeding in temporary habitats where water availability is reduced by evaporation, e.g., stagnant water in footprints.

On the question of development time, this study found that development time of mosquitoes, like hatching time, decreased with increasing temperature. However, it was observed that larval

development differed among the four larval instars – the fourth instar had the longest developmental time. The observed decrease in development time with increasing temperature is probably due to a rise in mosquito body temperature, which increases respiration and metabolism rates and causes mosquitoes to develop faster, thereby shortening the development time (Sasmita et al., 2019). This observation is consistent with previous studies (Bayoh & Lindsay, 2003; Kirby & Lindsay, 2009; Ciota et al., 2014; Couret et al., 2014) where development times of immature mosquitoes decreased with increasing temperature. The fourth instar had the longest developmental time, and probably unsurprising, since this stage precedes the pupal stage, and likely possesses significant amount of nutrient reserves needed to transition to adulthood (Araújo et al., 2012). This observation is in agreement with findings of Loetti et al. (2011) where they observed differences in the development times for the immature stages of *Culex eduardoi*, with the fourth having the longest development time. Similar observations have been made with regards to development times for *Aedes albopictus*, where the fourth instar took the longest time to develop, accounting for 25 – 36% of total development time (Neven, 2000). The fact that temperatures are predicted to rise in future means time to completion of mosquito life cycle could reduce, as mosquitoes are likely to develop at a faster rate.

5.2.2 Survival time of *An. gambiae (s.l.)* larvae decreased with increasing temperature

The survival of mosquito larvae decreased with increasing temperature. A possible explanation for the decreased larval survival at higher temperatures might be due to denaturation of proteins, interactions with oxygen supply, increased metabolic rate, disruption of membrane structure, and dehydration (Neven, 2000; Klose & Robertson, 2004; Chown & Terblanche, 2006). Mosquito larvae likely increase their metabolic rates to overcome any thermal stress experienced in the breeding habitat, resulting in higher energy expenditure (Ukubuiwe et al., 2018; Shah et al., 2020). The increased metabolic rates could exceed oxygen supply from the

environment leading to reduced performance, lowered tolerance to thermal stress (Pörtner et al., 2017), and the death of the larvae. Other studies have linked high larval mortalities and reduced larval survival time with increasing temperatures in other mosquito species such as *An. gambiae (s.s.)* (Bayoh & Lindsay, 2003; Christiansen-Jucht et al., 2014), *An. arabiensis* and *An. quadriannulatus* (Davies et al., 2016), *Culex quinquefasciatus* (Ukubuiwe et al., 2018), and *Aedes aegypti* (Carrington et al., 2013). In a future warmer temperature, it is possible that larval populations could decrease because of high larval mortalities. Estimating the effects of higher temperatures on larval survival could provide critical information in controlling larval populations in future warmer temperatures.

5.2.3 Pupation success of mosquitoes decreased with increasing temperature

Pupation success, which is the proportion of larvae that pupated from the total number of larvae, decreased with increasing temperature. According to Clements (1992), it is a prerequisite for mosquitoes and other holometabolous insects to reach a certain critical body mass in the course of larval development before they can pupate, and this mass decreases with increasing temperature (Chambers & Klowden, 1990). In this current study, the highest pupation success was recorded at 28 ºC, a finding similar to that of Mamai et al. (2018), who reported higher pupation success of *An. arabiensis* mosquitoes at 27 ± 1ºC. The failed metamorphosis of larvae to pupae and adults could be attributed to the fact that higher temperatures increase development rate, therefore, resulting in rapid uptake of nutrients and faster metabolism (Davies et al., 2016). This requirement may be physiologically demanding, leading to insufficient body mass needed for eclosion (emergence of the adult from the pupal stage) (Chambers & Klowden, 1990).

5.2.4 Larval and pupal size decreased with increasing temperature

Larval and pupal size decreased with increasing temperatures. This could possibly be due to the short duration in development of immature stages; there is reduced food intake by the larvae (Calkins & Parker, 2005) resulting in decreased larval size and consequently pupal size. These are similar to observation of Christiansen-Jucht et al. (2015) and Sasmita et al. (2019), in which rearing temperature significantly influenced larval and pupal size, with lower temperatures resulting in larger larvae and pupae and vice versa. According to Keena and Moore (2010), temperature has an influence on larval weight therefore the ability of the larvae to pupate. In addition, the effect of temperature goes beyond the immature stages into adult stage. For example, previous studies have also reported a link between high temperatures and small larval size which consequently resulted in small adults (Joy et al., 2010; Phasomkusolsil et al., 2011; Yeap et al., 2013; Bond et al., 2017). This could suggest that the temperature of the larval environment may have a profound impact on the adult stage. It is possible that in a future warmer temperature, the size of mosquitoes could reduce, and this can affect almost all aspects of its physiology, performance, morphology, and fitness (Kingsolver & Huey, 2008; Barreaux et al., 2018).

5.2.5 Number of adult mosquitoes produced decreased with increasing temperature

The number of adults produced (production capacity) decreased with increasing temperature, with fewer female mosquitoes emerging at high temperatures, compared to males. These results are consistent with those of Bayoh and Lindsay (2003) who reported that the number of *An. gambiae (s.s.)* mosquitoes produced decreased with increasing temperature. In addition, the findings on sex ratio are in agreement with the findings of Monteiro et al. (2007), where there was an equilibrated male-female ratio at 25 °C. However, at 30 °C, the production of males was about two (2) times that of females. It is possible that a high proportion of female larvae and pupae were killed before becoming adults, leading to the production of few female

mosquitoes at high temperatures. Further investigations are needed to provide greater insight into the mechanism underlying the production of few female mosquitoes at higher temperatures. It is likely that in a future warmer temperature, mosquito populations could reduce because there would be few female mosquitoes available for mating and egg-laying.

5.3 Effect of temperature on the growth and development of adult *An. gambiae (s.l.)* mosquitoes

5.3.1 Longevity of mosquitoes decreased with increasing temperature

Longevity of mosquitoes decreased with increasing temperature. This observation could be attributed to teneral reserves acquired from the immature stages in progressing to adulthood. Teneral reserves determine the amount of energy accessible for most adult life traits such as longevity, body size, vitellogenesis, and flight (Ukubuiwe et al., 2019). Larger mosquitoes (those that emerge at low temperatures) usually have more reserves and are likely to survive longer than smaller mosquitoes (those that emerge at high temperatures) (Barreaux et al., 2018). Another possible explanation for the reduced longevity at high temperatures is the fast depletion of energy reserves because of the prolonged periods of high metabolic rates (Storey & Storey, 2004). Higher temperatures may accelerate the reaction rate of various metabolic processes that affect the growth and development of the mosquito. The increased reaction rate could amplify the damage caused by the by-products of metabolism, such as reactive oxygen species (ROS) (Keil et al., 2015), leading to the death of the mosquito. Adult longevity influences the number of gonotrophic cycles and may limit disease transmission (Menge et al., 2005). These results support those of previous studies that have reported a decrease in longevity of *An. coluzzii* (Faiman et al., 2017) and *A. aegypti* (Marinho et al., 2016) with increasing temperatures.

The study also assessed the effect of blood meal on longevity of mosquitoes and found that mosquitoes fed on blood meal in addition to sugar solution had shorter longevity than those not

fed with blood (these were fed on sugar only) in all the temperature regimes. The decreased longevity among blood-fed mosquitoes may be attributed to the intensity of oviposition, which requires much energy and is likely to decrease longevity (Marinho et al., 2016). A blood meal provides the female mosquitoes with the protein needed to synthesize yolk and develop their eggs (Nikbakhtzadeh et al., 2016). In a future warmer temperature, it is possible that adult mosquitoes may not be able to survive longer to reproduce and transmit diseases. This could help in the eradication of the vector and the disease it transmits.

5.3.2 Gonotrophic cycle length and biting rate of mosquitoes were unaffected by increasing temperature

The associations between gonotrophic cycle length and biting rate of *An. gambiae (s.l.)* mosquitoes did not statistically change with increasing temperature. Previous studies have reported a significant decrease in the gonotrophic cycle length of *Anopheles* mosquitoes with increasing temperature (Paaijmans et al., 2013a; Christiansen-Jucht et al., 2015; Shapiro et al., 2017). Also, Shapiro et al. (2017) found that biting rate significantly increased with increasing temperature. It is entirely possible that the difference in the findings between this study and previous studies may be attributable to the type of blood meal used to feed mosquitoes. In the studies above, mosquitoes were allowed to feed on human blood, compared to the above where mosquitoes were fed on guinea pigs. It has been reported that host blood source as food for mosquitoes could affect the fecundity and gonotrophic cycle length of mosquitoes (Shehata, 2018). In this regards, high temperatures was found to speed up digestion of blood meals and reduce the gonotrophic cycle length (Afrane et al., 2006). Increased biting rate and reduced gonotrophic cycle length of *An. gambiae (s.l.)* mosquitoes could suggest that mosquitoes may feed more frequently, increasing egg-laying frequency and increasing their ability to transmit diseases (Afrane et al., 2012; Mala et al., 2014).

5.3.3 Fecundity of mosquitoes decreased with increasing temperature

Fecundity, which is the number of eggs laid per female mosquito, decreased with increasing temperature. However, mosquitoes kept at 32 and 34 ^{o}C failed to lay eggs. The reason for the decreased fecundity is not clear but it may have something to do with the size of mosquitoes as higher temperatures result in smaller adult mosquitoes. Temperature affects mosquito body size, which consequently influences mosquito's first meal choice. Smaller mosquitoes are more likely to take sugar meals than blood meals (Barreaux et al., 2018). Another possible reason for the inability of mosquitoes to lay eggs at 32 and 34 ^{o}C could be that small mosquitoes (those that emerged at higher temperatures) were pre-gravid; that is, mosquitoes need at least two blood meals to complete the first gonotrophic cycle (Lyimo & Takken, 1993) and such mosquitoes are not likely to live long enough. The findings are in line with those of previous studies (Aytekin et al., 2009; Phasomkusolsil et al., 2011; Christiansen-Jucht et al., 2015; Ezeakacha & Yee, 2019) that have reported a decrease in mosquito fecundity with increasing temperature.

According to Christiansen-Jucht et al. (2015), when higher temperature affects mosquitoes fecundity, it also decreases the probability of mosquitoes to lay eggs. At high temperatures (32 and 34 ^{o}C), the few mosquitoes that took blood meal even died within two days' post-feeding. It is possible that eggs may be present in the ovaries, just that mosquitoes did not live long enough to lay eggs (Ezeakacha & Yee, 2019). However, this study did not dissect ovaries of dead mosquitoes to check insemination and egg development. These findings suggest that in a future warmer temperature, it is possible that the number of potential vectors may decrease (Charlwood & Bragança, 2012) because of reduced fecundity of mosquitoes.

5.3.4 Body size and proboscis length of mosquitoes decreased with increasing temperature

The effects of temperature on adult size and proboscis length could be attributed to the conditions experienced during larval development. Larval rearing temperature plays a significant role in shaping the overall size of adult mosquitoes (Ezeakacha & Yee, 2019). The reduced body size of mosquitoes may have negative effects on fitness components such as reproduction, competition and stress tolerance (Kingsolver & Huey, 2008; Chidawanyika & Terblanche, 2011). The results are in agreement with previous studies (Aytekin et al., 2009; Phasomkusolsil et al., 2011; Charlwood & Bragança, 2012; Christiansen-Jucht et al., 2015; Barreaux et al., 2016b; Barreaux et al., 2018) that reported reduced body size of *Anopheles* mosquitoes with increasing temperature. This current study did not investigate how temperature affected the ability of mosquitoes to insert proboscis into the host. It will be interesting for further studies to consider whether mosquitoes with the short proboscis (those kept at high temperatures) can reach the blood vessels of its host and acquire blood meal for egg development.

5.4 Effects of temperature on insecticide susceptibility and metabolic enzyme expression

5.4.1 Susceptibility of mosquitoes to pyrethroids decreased with increasing temperature

Susceptibility of mosquitoes to pyrethroids decreased with increasing temperature. This may be attributed to higher expression of enzymes at high temperatures (Matzrafi, 2019). At high temperatures, the metabolism of insects is significantly faster (Canola Council of Canada, 2015), leading to rapid breakdown of insecticides, thereby making them relatively ineffective. Finding is in agreement with that of Oliver and Brooke (2017), in which the susceptibility of *An. arabiensis* (SENN DDT strains) to pyrethroids decreased with increasing temperatures. Another important finding was that deltamethrin induced higher mortality of mosquitoes than permethrin, indicating that mosquitoes were resistant to permethrin based on WHO criteria (WHO, 2016a). These findings are likely to be linked to the chemistry of the insecticides;

deltamethrin belongs to the group of type II pyrethroids, which are more toxic than type I pyrethroids such as permethrin (Dalefield, 2017). This could explain the high mortalities of mosquitoes to deltamethrin insecticides across all the temperature regimes. This finding is in agreement with the findings of Dadzie et al. (2017) which showed that *An. gambiae (s.l.)* mosquitoes were more susceptible to deltamethrin than permethrin. With projections of warmer temperatures in coming years, especially in sub-Saharan Africa, high resistance to pyrethroids with increasing temperatures could affect the effectiveness of malaria control programs using pyrethroids (Ochomo et al., 2013). In Ghana, most mosquito control programs rely mostly on pyrethroid insecticides (Agyekum, 2017), and any future resistance will not bode well.

5.4.2 Expression of metabolic enzymes in mosquitoes increased with increasing temperature

Metabolic enzyme (mixed-function oxidases (MFO) and Glutathione S-transferases (GSTs)) levels in mosquitoes that were not exposed to insecticides (pyrethroids) increased with increasing temperature from 25 to 32 ^{o}C, On the other hand, enzyme expression levels decreased at 34 ^{o}C, suggesting that enzyme expression may be impaired above a certain optimum temperature range (Tripathi et al., 2011). In addition, very high temperatures could disrupt the shape of the active site of an enzyme, hence reducing enzyme level by preventing its formation (BBC, 2021). When insects are faced with harsh conditions such as heat stress, there is an increase in the expression of enzymes such as GST and catalase in order to overcome the stress (González-Tokman et al., 2020). This could be a possible explanation for the increased enzyme levels at high temperatures observed in this study. These findings are corroborated by that of Kristensen and colleagues who reported increased expression of proteasomal proteins (proteins involved in repair and degradation of oxidatively damaged proteins) in *Drosophila melanogaster* adapted to high temperatures than those acclimatized to low temperatures (Kristensen et al., 2016). The elevated levels of enzyme associated with

higher temperatures could provide a useful understanding of the role that future warmer temperatures could play in the evolution of insecticide resistance (Polson et al., 2012).

Comparing the enzyme levels of mosquitoes that were not exposed and those exposed to pyrethroids, MFO, GST, α-esterase and β-esterase were higher in those exposed to pyrethroids than in those that were not exposed, especially at 25 and 30 ^{o}C. The high resistance of mosquitoes to insecticides might have involved metabolic detoxification due to the elevated enzyme expression in mosquitoes exposed to pyrethroids (Ochomo et al., 2013). Findings are consistent with the results of Ochomo et al. (2013), who observed that the level of MFO, GST, and β-esterase in *An. gambiae (s.s.)* mosquitoes exposed to permethrin insecticides were higher than in mosquitoes that were not exposed to insecticides.

The study further revealed that MFO levels were the least expressed enzyme in mosquitoes. The findings are in agreement with Alemayehu et al. (2017), who observed higher levels of GSTs in *An. arabiensis* mosquitoes than the levels of MFO. However, the findings of this current study are contrary to those reported by Leong et al. (2019), who found more elevated MFO levels in *A. aegypti* mosquitoes than α-esterase, β-esterase and AChE levels. These findings raise the possibility that the levels of metabolic enzymes may differ among mosquito species (Farouk et al., 2021). Therefore, understanding the relationship between temperature and expression of metabolic enzymes in a future warmer temperature would be of great contribution to ascertain the different metabolic enzymes involved in detoxification of insecticides.

CHAPTER SIX

CONCLUSION AND RECOMMENDATIONS

6.1 Conclusion

Understanding how temperature variability affects the biology of mosquitoes is key to understanding many aspects of the diseases it transmits and the control of those diseases, especially in a future warmer temperature. This study investigated the influence of elevated temperatures on the growth, development and susceptibility of *An. gambiae (s.l.)* mosquitoes to pyrethroids.

Lower temperatures (< 30 ºC) prolonged the hatching of *Anopheles gambiae (s.l.)* eggs than higher temperatures (30 – 38 ºC), but no egg hatched at 40 ºC. However, mosquitoes could not breed beyond temperatures at 36 ºC. So, if the ambient environmental temperatures rise (or are elevated) to 36 ºC as a consequence of climate change, it is likely malaria transmission will be inhibited, which may therefore result in its suppression and potential eradication in future warmer temperatures.

High temperatures reduced the susceptibility of *An. gambiae (s.l.)* mosquitoes to deltamethrin and permethrin and increased resistance of mosquitoes to these insecticides. In addition, metabolic enzyme levels were elevated at a higher temperature than lower temperatures. The high levels of metabolic enzymes at high temperatures could pose a significant threat to malaria control interventions. However, in a future warmer temperature, it is possible that a considerable proportion of emerging mosquitoes may not survive longer, and numbers of potential vectors may decrease. These results supplement the limited studies examining the

effects of environmental temperature on the development and growth of the adult *An. gambiae (s.l.)* mosquitoes.

6.2 Recommendations

The following are recommended based on the findings of the study;

- Ghana Health Service through the national vector control program under the Public Health Division should aim at reducing the larval population at breeding sites to significantly reduce vector population before becoming adults. This can reduce the population of mosquitoes and make vector control effective.

- Ghana Health Service and the Media should intensify public education on environmental sanitation to reduce the breeding sites of mosquitoes, and the proper use of insecticides to reduce mosquitoes' resistance to insecticides.

- Beyond temperature, future studies should consider varying other climate parameters simultaneously to determine the overall effects on the development of *An. gambiae (s.l.)* mosquitoes.

- Future studies should explore the effects of temperature on the mating success of *An. gambiae (s.l.)* mosquitoes.

6.3 Strength and limitations

6.3.1 Strength

- This is one of the first studies that have assessed the effects of different rearing temperatures on the susceptibility of *An. gambiae (s.l.)* mosquitoes to insecticides and the expression of metabolic enzyme activities.
- One of the strengths of this study is that it represents a comprehensive assessment of the growth and development of both the immature and adult *An. gambiae (s.l.)* mosquitoes.

6.3.2 Limitations

- The data reported in this thesis refer to just two sibling species (*An. gambiae (s.s)* and *An. coluzzii*) in the *An. gambiae* complex, and the possibility that the impact of temperature on development may vary between populations within the *An. gambiae* complex cannot be excluded.
- This study did not take into account temperature fluctuations or differences in humidity that would affect mosquito development and survival in the field.
- The study only considered the effects of temperature on phenotypic and biochemical resistance of mosquitoes and did not consider target site resistance.

6.4 Future research direction

- The effects of temperature on *An. gambiae (s.l.)* mosquitoes reported in this study could result from plasticity. It is essential to establish whether there could be a genetic modification in *An. gambiae (s.l.)* mosquitoes due to temperature variability by assessing the effects of elevated temperatures on mosquitoes over generations and conducting genotypic analysis to ascertain the differences.

- This study assessed the effects of different rearing temperatures on the susceptibility of *An. gambiae (s.l.)* mosquitoes to only deltamethrin and permethrin (both pyrethroid insecticides). This can be expanded to include other insecticide classes and products used for malaria control, including mosquito coils. Though mosquito coils are not officially part of malaria control interventions, they are highly patronized in many African countries.

- Future studies should assess the influence of elevated temperatures on the dynamics of the malaria parasite. An increase or decrease in the time before a mosquito becomes infectious is key to malaria transmission.

6.5 Contribution to knowledge

This study has provided information that could guide the design of malaria control interventions in a future warmer temperature. The study also contributes to the knowledge on the relationship between temperature and *An. gambiae (s.l.)* mosquitoes and provides valuable information to guide vector control in a future warmer climate. To the best of my knowledge, this study could be the first study to evaluate the effects of different rearing temperatures on metabolic enzymes levels in *An. gambiae (s.l.)* mosquitoes that were exposed and those that were not exposed to pyrethroid insecticides.

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APPENDICES

Appendix I: Search terms and search results from databases

Database	Search Term	Search Results
Scopus	TITLE-ABS-KEY ("*Anopheles* mosquito" OR "malaria") AND ("temperature" OR season) AND ("development time" OR "survival" OR "longevity" OR "fecundity" OR "gonotrophic cycle" OR "biting rate" OR "enzyme" OR "insecticide")	**5,926**
PubMed	("*Anopheles* mosquito" OR "malaria") AND ("temperature" OR season) AND ("development time" OR "survival" OR "longevity" OR "fecundity" OR "gonotrophic cycle" OR "biting rate" OR "enzyme" OR "insecticide")	**1,156**
Science Direct	("*Anopheles* mosquito") AND ("temperature" OR "season") AND ("longevity" OR "fecundity" OR "gonotrophic cycle" OR "development time" OR "enzyme" OR "insecticide")	**1,403**
Google Scholar	("*Anopheles* mosquito") AND ("temperature" OR "season") AND ("longevity" OR "fecundity" OR "gonotrophic cycle" OR "development time" OR "enzyme" OR "insecticide")	**8,130**
ProQuest	("*Anopheles* mosquito") AND ("temperature" OR "season") AND ("longevity" OR "fecundity" OR "gonotrophic cycle" OR "development time" OR "enzyme" OR "insecticide")	**850**
Web of Science	("*Anopheles* mosquito") AND ("temperature" OR "season") AND ("longevity" OR "fecundity" OR "gonotrophic cycle" OR "development time" OR "enzyme" OR "insecticide")	**17**
Hand search		4

Appendix II: List of studies excluded with reasons

Sn	Author name	Reason for exclusion
1	Abiodun et al. (2016)	1
2	Abiodun et al. (2017)	1
3	Abiodun et al. (2018)	1
4	Agusto et al. (2015)	1
5	Beck-Johnson (2013)	1
6	Beck-Johnson et al. (2013)	1
7	Beck-Johnson et al. (2017)	1
8	Blanford et al. (2013)	2
9	Charlwood and Bragança (2012b)	3
10	Christiansen-Jucht et al. (2015b)	1
11	Culler et al. (2015)	1
12	Ewing et al. (2016)	1
13	Glunt et al. (2013)	2
14	Gonçalvez De Carvalho et al. (2002)	3
15	Lindsay et al. (1991)	4
16	Lunde et al. (2013)	1
17	Lyons et al. (2014)	1
18	Mordecai et al. (2013)	2
19	Mukhtar et al. (2019)	1
20	Murdock et al. (2016)	2
21	Murdock et al. (2012b)	2
22	Okechukwu et al. (2011)	4
23	Okuneye et al. (2019)	1
24	Owiti and Christopher (2017)	3
25	Owusu et al. (2017)	2
26	Paaijmans et al. (2012)	3
27	Paaijmans et al. (2010)	2
28	Paaijmans et al. (2009)	1
29	Parham and Michael (2010b)	1
30	Parham and Michael (2010a)	1
31	Parham et al. (2012)	1
32	Shimaponda-Mataa et al. (2017)	1
33	Wang et al. (2016)	1
34	Wei-Ming et al. (2011)	4
35	Yamana and Eltahir (2013)	1
36	Zakharova et al. (1990)	4

1 = mathematical models; 2 = Opinions/Letters/Reports; 3 = Study did not report on any of the outcomes of interest; 4 = Full-text not available

Appendix III: Risk of bias in included studies using the SYRCLE tool

Author/year	Sequence generation (selection bias)	Baseline characteristics (selection bias)	Allocation concealment (selection bias)	Incomplete outcome data (attrition bias)	Selective reporting (reporting bias)	Other bias (Rearing of mosquito)	Other bias (Funding source)
Aytekin et al. (2009)	High risk	Low risk	Unclear risk	Low risk	Low risk	Low risk	High risk
Barreaux et al. (2016b)	High risk	Low risk	Unclear risk	Low risk	Low risk	Low risk	High risk
Barreaux et al. (2018)	High risk	Low risk	Unclear risk	Low risk	Low risk	Low risk	Low risk
Bayoh and Lindsay (2003)	High risk	Low risk	Unclear risk	Low risk	Low risk	Low risk	Low risk
Bayoh and Lindsay (2004)	High risk	Low risk	Unclear risk	Low risk	Low risk	Low risk	Low risk
Charlwood and Bragança (2012)	High risk	Unclear risk	Unclear risk	Low risk	Low risk	High risk	High risk
Christiansen-Jucht et al. (2014)	High risk	Low risk	Unclear risk	Low risk	Low risk	Low risk	Low risk
Christiansen-Jucht et al. (2015)	High risk	Low risk	Unclear risk	Low risk	Low risk	Low risk	Low risk
Davies et al. (2016)	High risk	Low risk	Unclear risk	Low risk	Low risk	Low risk	Low risk
Faiman et al. (2017)	High risk	Low risk	Unclear risk	Low risk	Low risk	Unclear risk	Low risk
Glunt et al. (2018)	High risk	Low risk	High risk	Low risk	Low risk	High risk	Low risk
Glunt et al. (2014)	High risk	Low risk	High risk	Low risk	Low risk	High risk	Low risk
Impoinvil et al. (2007)	High risk	Low risk	Unclear risk	Low risk	Low risk	Low risk	Low risk
Kirby and Lindsay (2009)	High risk	Low risk	Unclear risk	Low risk	Low risk	Low risk	High risk
Lyons et al. (2013)	High risk	Low risk	High risk	Low risk	Low risk	Low risk	Low risk
Lyons et al. (2012)	High risk	Low risk	High risk	Low risk	Low risk	High risk	Low risk
Mala et al. (2014)	Low risk	Low risk	High risk	Low risk	Low risk	High risk	Low risk

NB: Performance (Random housing and Blinding) and Detection (Random outcome assessment and Blinding) biases were not applicable

Appendix III: Continued

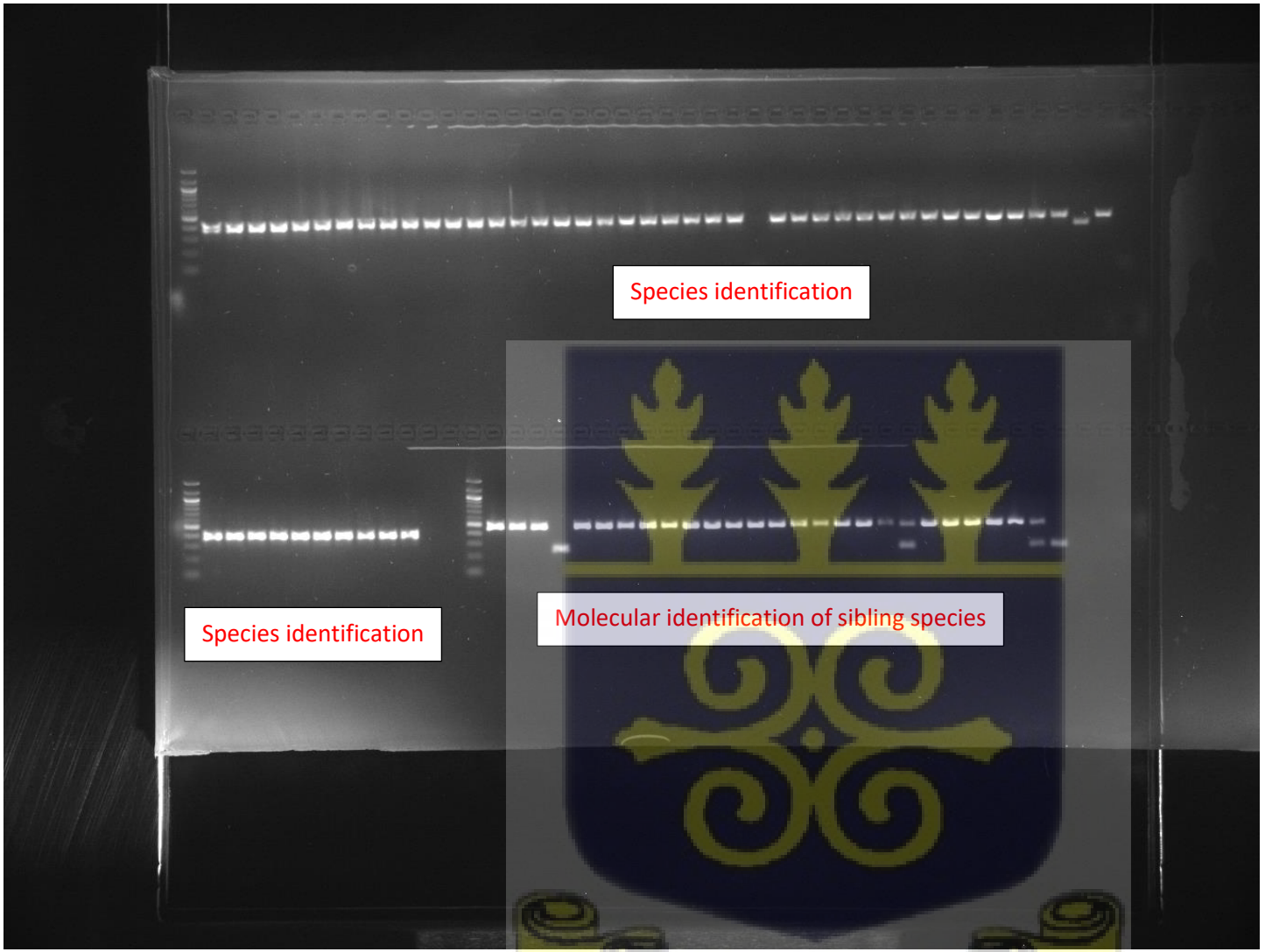
Author/year	Sequence generation (selection bias)	Baseline characteristics (selection bias)	Allocation concealment (selection bias)	Incomplete outcome data (attrition bias)	Selective reporting (reporting bias)	Other bias (Rearing of mosquito)	Other bias (Funding source)
Mamai et al. (2018)	High risk	Low risk	High risk	Low risk	Low risk	Low risk	Low risk
Murdock et al. (2014)	High risk	Low risk	High risk	Low risk	Low risk	High risk	Low risk
Murdock et al. (2013)	High risk	Low risk	High risk	Low risk	Low risk	High risk	Low risk
Murdock et al. (2012a)	High risk	Low risk	High risk	Low risk	Low risk	High risk	Low risk
Olayemi et al. (2011)	High risk	Unclear risk	High risk	Low risk	Low risk	High risk	High risk
Oliver and Brooke (2017)	High risk	Low risk	High risk	Low risk	Low risk	Low risk	Low risk
Paaijmans et al. (2013a)	High risk	Low risk	High risk	Low risk	Low risk	Low risk	High risk
Paaijmans et al. (2013b)	High risk	Low risk	High risk	Low risk	Low risk	Low risk	Low risk
Phasomkusolsil et al. (2011)	High risk	Low risk	High risk	Low risk	Low risk	Low risk	High risk
Rúa et al. (2005)	High risk	Low risk	High risk	Low risk	Low risk	Low risk	High risk
Shapiro et al. (2017)	High risk	Low risk	High risk	Low risk	Low risk	Low risk	Unclear risk
Wallace and Merritt (1999)	High risk	Low risk	High risk	Low risk	Low risk	Low risk	Low risk

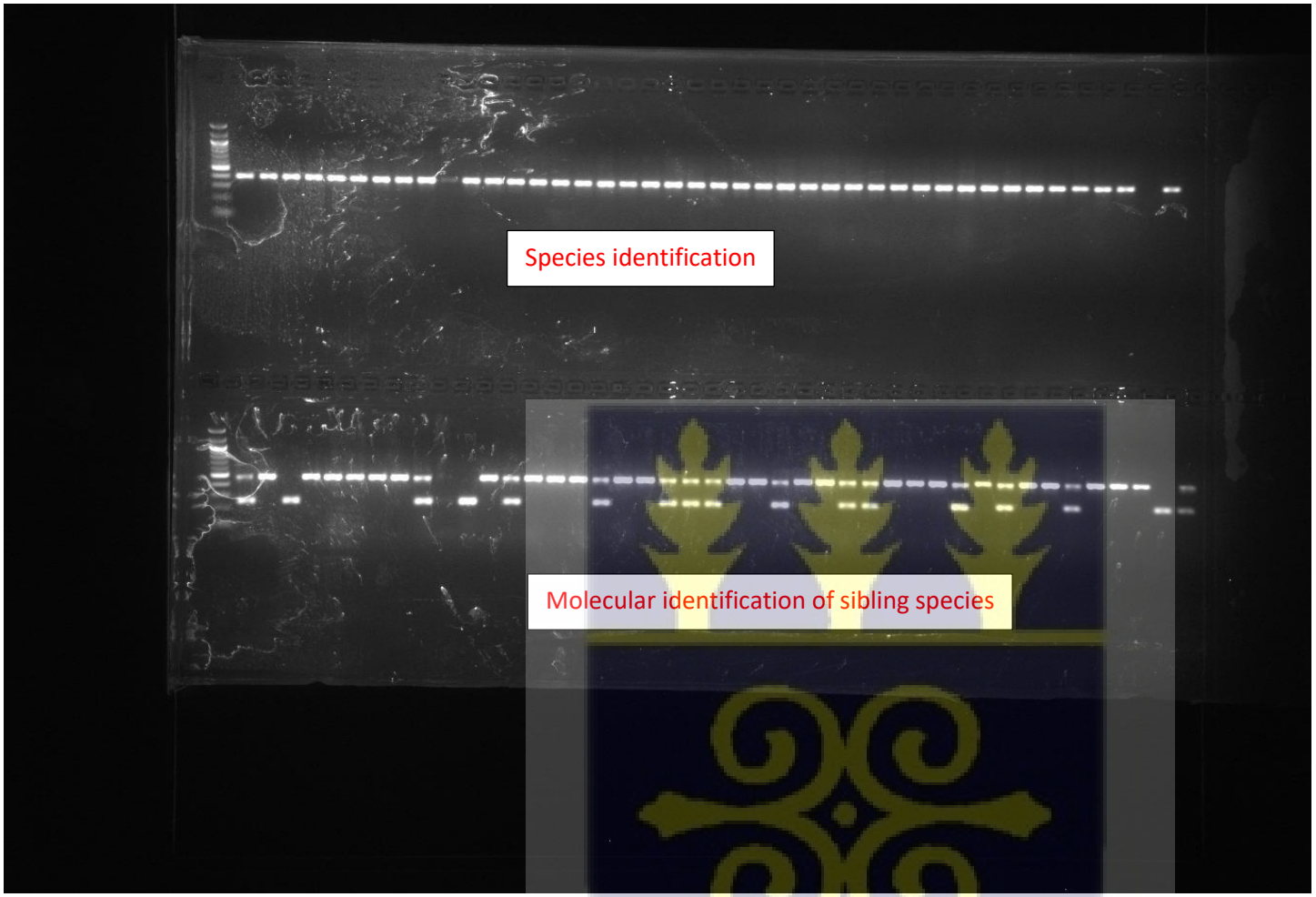
NB: Performance (Random housing and Blinding) and Detection (Random outcome assessment and Blinding) biases were not applicable

Appendix IV: Ambient and rearing water conditions for each temperature regime

Temperature regime (°C)	Ambient conditions in the incubators		Rearing water temperature (°C)
	Temperature (°C)	Relative Humidity (%)	
25	26.17 ± 0.30	84.67 ± 4.90	24.54 ± 0.15
28	27.98 ± 1.31	81.00 ± 4.66	27.41 ± 0.82
30	31.03 ± 0.25	84.05 ± 4.27	29.46 ± 0.14
32	33.05 ± 0.28	86.33 ± 4.54	30.86 ± 0.13
34	35.19 ± 0.50	84.59 ± 4.43	32.79 ± 0.12
36	36.97 ± 0.24	85.29 ± 3.78	34.79 ± 0.09
38	38.88 ± 0.30	80.40 ± 5.20	36.29 ± 0.10
40	40.95 ± 0.40	87.03 ± 6.62	38.41 ± 0.12

Appendix V: Gel photographs of the PCR performed





Appendix VI: Ethical approval for the study

UNIVERSITY OF GHANA

University of Ghana Institutional Animal Care and Use Committee (UG-IACUC)

Phone:
Email: UG-IACUC@ug.edu.gh

P.O. Box LG 581
Legon, Accra
Ghana

Office Location: Department of Animal Experimentation Building, Noguchi Memorial Institute for Medical Research (NMIMR), University of Ghana

28/09/2020

ETHICAL CLEARANCE
(UG-IACUC 001/20-21)

Your protocol for an ethical clearance has been reviewed by the University of Ghana Institutional Animal Care and Use Committee and has been approved as follows:

TITLE OF PROTOCOL: Effects of temperature on the development and survival of adult Anopheles mosquitoes

PRINCIPAL INVESTIGATOR: Thomas Peprah Agyekum

Please note that the final review report must be submitted to the Committee at the completion of the study. Your research records may be audited at any time during or after the implementation.

Any modification of this research project must be submitted to UG-IACUC for review and approval prior to implementation.

Please report all serious adverse events related to this study to UG-IACUC within seven (7) days verbally and in writing within fourteen (14) days.

This certificate is valid till 1st September, 2021. You are to submit annual reports for continuing review.

Signature of Chairperson
Prof. Major (Rtd.) George A. Asare

Appendix VII: Pairwise comparisons of the development of the immature stages of *An. gambiae (s.l.)* mosquitoes

Temperature regime (°C)	Development time (in days)		Time to pupation (in days)		Pupation Success (%)		Pupal mortality (%)		Adults produced (%)		Sex ratio (M/F)	
	t	p-value	t	p-value	H	p-value	H	p-value	H	p-value	H	p-value
28 vs 25	-1.77	0.010*	-1.81	0.481	-0.90	0.184	3.09	0.001**	-0.90	0.183	-0.90	0.184
30 vs 25	-4.10	< 0.001*	-3.25	0.035*	0.90	0.184	2.48	0.007	0.90	0.183	-1.84	0.033
32 vs 25	-5.63	< 0.001*	-6.50	< 0.001*	1.87	0.031	1.98	0.024	1.83	0.033	-2.70	0.004
34 vs 25	-7.17	< 0.001*	-7.22	< 0.001*	2.89	0.002**	1.44	0.075	2.66	0.004	-3.56	< 0.001**
36 vs 25	-10.57	< 0.001*	-9.75	< 0.001*	3.32	< 0.001**	-0.90	0.185	3.60	< 0.001**	-4.50	< 0.001**
30 vs 28	-2.32	0.001*	-1.44	0.701	1.80	0.036	-0.61	0.271	1.80	0.036	-0.94	0.175
32 vs 28	-3.86	< 0.001*	-4.70	0.001*	2.77	0.003**	-1.11	0.133	2.73	0.003**	-1.80	0.036
34 vs 28	-5.39	< 0.001*	-5.42	< 0.001*	3.79	< 0.001**	-1.65	0.049	3.56	< 0.001**	-2.66	0.004
36 vs 28	-8.80	< 0.001*	-7.95	< 0.001*	4.22	< 0.001**	-3.99	< 0.001**	4.49	< 0.001**	-3.60	< 0.001**
32 vs 30	-1.53	0.032*	-3.25	0.035*	0.97	0.166	-0.50	0.308	0.93	0.175	-0.86	0.194
34 vs 30	-3.07	< 0.001*	-3.97	0.007*	1.99	0.023	-1.04	0.149	1.76	0.039	-1.73	0.042
36 vs 30	-6.48	< 0.001*	-6.50	< 0.001*	2.43	0.008	-3.38	< 0.001**	2.70	0.004	-2.66	0.004
34 vs 32	-1.54	0.031*	-0.72	0.977	1.02	0.153	-0.54	0.295	0.83	0.204	-0.86	0.194
36 vs 32	-4.94	< 0.001*	-3.25	0.035*	1.46	0.073	-2.87	0.002**	1.76	0.039	-1.80	0.036
36 vs 34	-3.41	< 0.001*	-2.53	0.155	0.43	0.333	-2.34	0.010	0.93	0.175	-0.94	0.175

H = Kruskal-Wallis test statistics; t = One-Way ANOVA test statistics; single asterisk (*) and double asterisk (**) represent significant difference at $P < 0.05$ (according to Tukey post hoc test) and $p < 0.003$ (according to Dunn's multiple range test), respectively.

Appendix VIII: Two-group comparisons and the overall trend of the effect of temperature on *An. gambiae (s.l.)* larval survival

Temperature regime comparison	Cox proportional hazard			The overall effect of temperature on larval survival	
	Hazard ratio	95% CI	p-value		
25 vs. 28	1.35	1.13, 1.61	0.001*	Log-rank test	5353.12
25 vs. 30	2.63	2.26, 3.06	<0.001*	p-value	<0.001*
25 vs. 32	5.62	4.79, 6.59	<0.001*		
25 vs. 34	11.37	9.61, 13.45	<0.001*		
25 vs. 36	51.07	42.33, 61.61	<0.001*		
25 vs. 38	215.85	175.45, 265.54	<0.001*		

A single asterisk (*) represents a significant difference at $P < 0.05$; 95% CI = 95% Confidence Interval

Appendix IX: Two-group comparisons and an overall trend of the effect of temperature on adult *An. gambiae (s.l.)* longevity

Temperature regime comparison	Cox proportional hazard			The overall effect of temperature on larval survival	
	Hazard ratio	95% CI	p-value		
Non-blood-fed					
25 vs. 28	2.89	2.43, 3.44	<0.001*	Log-rank test	1163.60
25 vs. 30	6.86	5.62, 8.37	<0.001*	p-value	< 0.001*
25 vs. 32	16.46	13.28, 20.40	<0.001*		
25 vs. 34	22.65	18.15, 28.26	<0.001*		
Blood-fed					
25 vs. 28	1.26	1.07, 1.49	0.005*	Log-rank test	904.15
25 vs. 30	2.71	2.28, 3.23	<0.001*	p-value	< 0.001*
25 vs. 32	5.27	4.37, 6.36	<0.001*		
25 vs. 34	11.16	9.11, 13.67	<0.001*		
Effect of blood meal on longevity					
Log-rank test; 217.63, $P < 0.001$*					

A single asterisk (*) represents a significant difference at $P < 0.05$; 95% CI = 95% Confidence Interval

Appendix X: Comparison by sex across temperature and an overall trend of the effect of sex on adult *An. gambiae (s.l.)* longevity

Comparison by sex across temperature	Blood-fed		Non-blood-fed	
	Log-rank test	p-value	Log-rank test	p-value
Male (25 °C) vs. Female (25 °C)	3.36	0.067	18.13	< 0.001*
Male (28 °C) vs. Female (28 °C)	11.63	0.001*	8.86	0.003*
Male (30 °C) vs. Female (30 °C)	7.29	0.007*	31.76	< 0.001*
Male (32 °C) vs. Female (32 °C)	1.20	0.274	9.79	0.002*
Male (34 °C) vs. Female (34 °C)	5.08	0.024*	5.22	0.022*
Overall	*925.98*	*< 0.001**	*1198.52*	*< 0.001**

A single asterisk (*) represents significant difference at $P < 0.05$

Appendix XI: Pairwise comparisons of gonotrophic cycle length, biting rate and fecundity

Temperature regime (ºC)	Gonotrophic cycle length		Biting rate		Fecundity	
	t	p-value	t	p-value	t	p-value
28 vs 25	-0.00	1.000	0.24	0.968	-1.31	0.393
30 vs 25	-1.22	0.462	1.22	0.466	-2.63	0.029*
30 vs 28	-1.22	0.462	0.97	0.607	-1.32	0.392

t = One-Way ANOVA test statistics; a single asterisk (*) represents significant difference at $P < 0.05$

Appendix XII: Mortality of *An. gambiae (s.l.)* mosquitoes exposed to pyrethroids

Mosquito strain	Temperature regime (ºC)	Insecticide	Number of death	Total	Mortality (%)
Tiassalé	25	Deltamethrin	93	94	98.94
		Permethrin	28	88	31.82
		Control	0	42	0.0
	28	Deltamethrin	85	85	100.00
		Permethrin	71	87	81.61
		Control	0	41	0.0
	30	Deltamethrin	83	89	93.26
		Permethrin	67	89	75.28
		Control	0	41	0.0
	32	Deltamethrin	75	95	78.95
		Permethrin	46	92	50.00
		Control	5	41	12.20*
	34	Deltamethrin	60	60	100.00
		Permethrin	60	60	100.00
		Control	18	41	43.90**
Kisumu	25	Deltamethrin	85	85	100.00
		Permethrin	92	92	100.00
		Control	0	48	0.0
	28	Deltamethrin	84	84	100.00
		Permethrin	89	89	100.00
		Control	0	40	0.0
	30	Deltamethrin	81	81	100.00
		Permethrin	81	81	100.00
		Control	4	42	9.52
	32	Deltamethrin	83	83	100.00
		Permethrin	82	82	100.00
		Control	3	46	6.52
	34	Deltamethrin	-	-	-
		Permethrin	-	-	-
		Control	-	-	-

Mortality in control replicates of Tiassalé strain kept at 34 ºC exceeded 20 %, hence, results were excluded in the final analysis; Kisumu mosquitoes kept at 34 ºC failed to develop, hence no test conducted

Appendix XIII: Median levels of metabolic enzyme levels in *An. gambiae (s.l.)* mosquitoes reared at different temperature regimes

Mosquito status	Temperature regime (°C)	Level of enzyme levels (mole/min/mg protein)			
		MFO (IQR)	GST (IQR)	α-EST (IQR)	β-EST (IQR)
Unexposed to pyrethroids	25	7.21×10^{-10} (1.43×10^{-10})	1.18×10^{-3} (6.25×10^{-4})	2.52×10^{-7} (7.30×10^{-8})	1.36×10^{-7} (6.10×10^{-8})
	28	8.85×10^{-10} (1.50×10^{-10})	1.31×10^{-3} (1.05×10^{-3})	2.83×10^{-7} (4.32×10^{-7})	1.38×10^{-7} (1.82×10^{-7})
	30	1.49×10^{-9} (2.26×10^{-9})	1.37×10^{-3} (1.43×10^{-3})	2.12×10^{-7} (4.10×10^{-7})	1.30×10^{-7} (2.93×10^{-7})
	32	4.55×10^{-9} (4.13×10^{-9})	8.16×10^{-3} (1.30×10^{-2})	1.32×10^{-6} (9.41×10^{-7})	2.87×10^{-7} (3.21×10^{-7})
	34	1.94×10^{-9} (3.80×10^{-10})	1.52×10^{-3} (7.18×10^{-3})	4.04×10^{-7} (1.56×10^{-7})	3.56×10^{-7} (1.54×10^{-7})
Exposed to pyrethroids	25	2.07×10^{-9} (7.50×10^{-10})	2.72×10^{-3} (1.56×10^{-3})	2.94×10^{-7} (1.20×10^{-7})	2.47×10^{-7} (1.32×10^{-7})
	28	1.34×10^{-9} (2.50×10^{-10})	1.35×10^{-3} (6.59×10^{-4})	1.44×10^{-7} (2.40×10^{-7})	1.11×10^{-7} (6.85×10^{-8})
	30	3.15×10^{-9} (6.40×10^{-10})	2.62×10^{-3} (1.69×10^{-3})	3.12×10^{-7} (1.17×10^{-7})	2.15×10^{-7} (1.59×10^{-7})
	32	2.40×10^{-9} (1.07×10^{-9})	3.55×10^{-3} (2.63×10^{-3})	3.13×10^{-7} (1.81×10^{-7})	2.64×10^{-7} (1.63×10^{-7})

MFO = Mixed-Function Oxidase, GST = Glutathione-S-transferase, α-EST = α-Esterase, β-EST = β-Esterase, and IQR = Inter Quartile Range

Appendix XIV: Pairwise comparisons of enzyme levels in *An. gambiae (s.l.)* mosquitoes reared at different temperature regimes

Mosquito status	Temperature regime (°C)	MFO		GST		α-esterase		β-esterase	
		H	p-value	H	p-value	H	p-value	H	p-value
Unexposed to pyrethroids	28 vs 25	-3.10	0.001*	-2.23	0.013	-1.36	0.087	-0.70	0.241
	30 vs 25	-5.33	< 0.001*	-2.42	0.008	-0.18	0.430	-0.86	0.194
	32 vs 25	-1.12×10^{1}	< 0.001*	-9.03	< 0.001*	-8.56	< 0.001*	-3.18	0.001*
	34 vs 25	-7.37	< 0.001*	-2.856	0.002*	-3.47	< 0.001*	-6.14	< 0.001*
	30 vs 28	-2.24	0.013	-0.17	0.431	1.18	0.120	-0.17	0.433
	32 vs 28	-8.11	< 0.001*	-6.82	< 0.001*	-7.23	< 0.001*	-2.44	0.007
	34 vs 28	-4.45	< 0.001*	-0.73	0.234	-2.18	0.015	-5.30	< 0.001*
	32 vs 30	-5.90	< 0.001*	-6.68	< 0.001*	-8.35	< 0.001*	-2.24	0.013
	34 vs 30	-2.35	0.010	-0.56	0.286	-3.28	< 0.001*	-5.04	< 0.001*
	34 vs 32	3.26	0.001*	5.81	< 0.001*	4.69	< 0.001*	-2.73	0.003*
Exposed to pyrethroids	28 vs 25	3.23	0.001**	2.89	0.002**	2.36	0.009	4.82	< 0.001**
	30 vs 25	-5.83	< 0.001**	0.86	0.195	-0.69	0.247	0.95	0.171
	32 vs 25	-2.87	0.002**	-1.96	0.025	-1.20	0.115	-1.71	0.044
	30 vs 28	-7.63	< 0.001**	-2.14	0.016	-2.76	0.003**	-3.80	< 0.001**
	32 vs 28	-5.40	< 0.001**	-4.14	< 0.001**	-3.27	0.001**	-6.08	< 0.001**
	32 vs 30	3.23	0.001**	-2.59	0.005**	-0.40	0.343	-2.48	0.007**

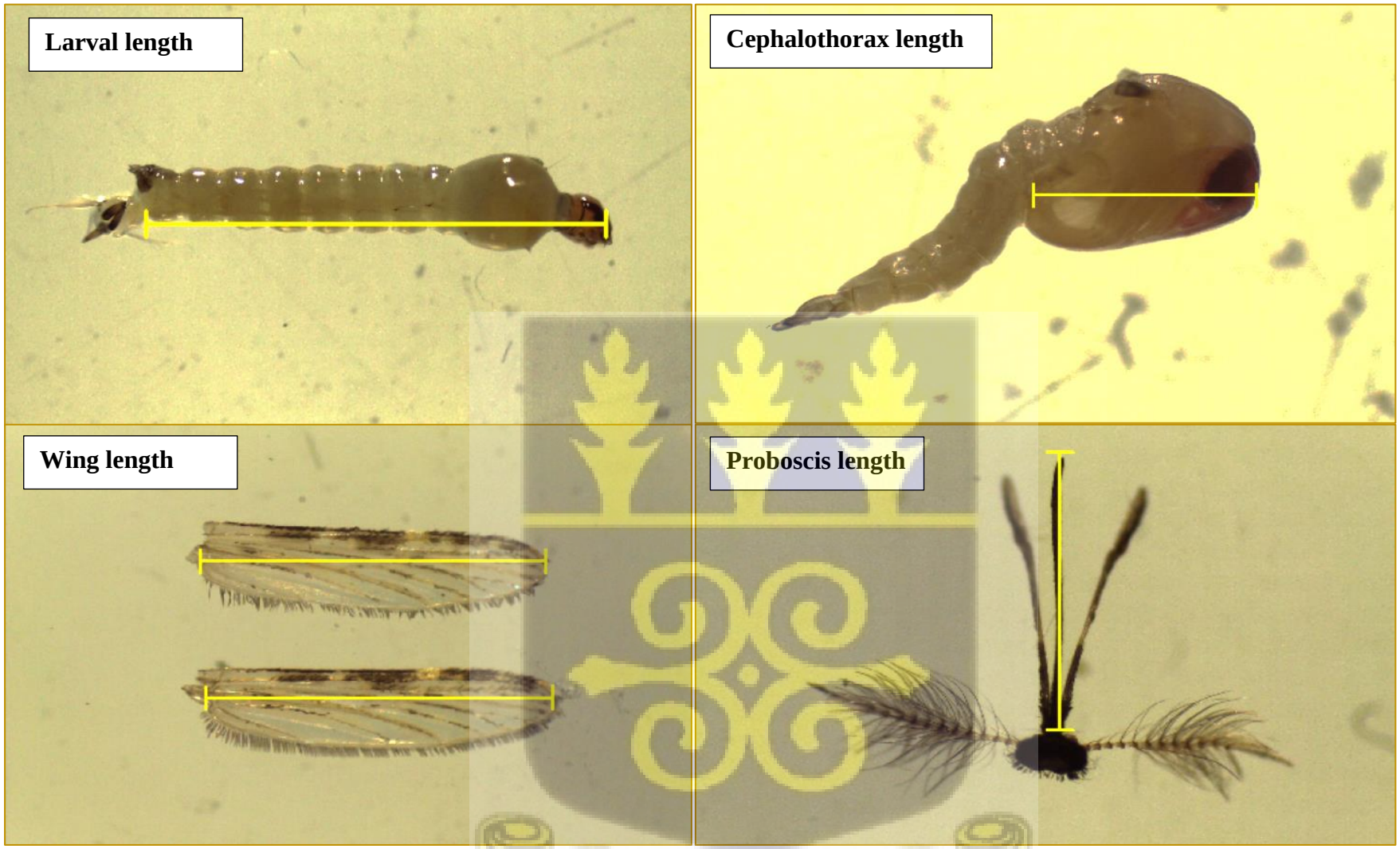
H = Kruskal-Wallis test statistics; single asterisk (*) and double asterisk (**) represent significant differences at $p < 0.005$ and $p < 0.008$ respectively (according to Dunn's multiple range test).

Appendix XV: Mann-Whitney U Test between mosquitoes that were not exposed and those exposed to pyrethroids

Metabolic enzyme	Temperature regime (°C)	z-value	p-value
MFO	25	-7.72	< 0.001*
	28	-5.33	< 0.001*
	30	-4.68	< 0.001*
	32	5.12	< 0.001*
GST	25	-8.12	< 0.001*
	28	-0.52	0.605
	30	-2.48	0.013*
	32	3.79	< 0.001*
α-esterase	25	-2.42	0.016*
	28	2.26	0.024*
	30	-1.53	0.127
	32	6.94	< 0.001*
β-esterase	25	-6.03	< 0.001*
	28	1.41	0.159
	30	-1.46	0.144
	32	-0.90	0.368

MFO = Mixed-Function Oxidase; GST = Glutathione-S-transferase; single asterisk (*) represents significant difference at $P < 0.05$

Appendix XVI: Measurements of *An. gambiae (s.l.)* mosquito body parts using Leica application Software



Appendix XVII: Abstracts of publication related to this study

International Journal of
Environmental Research and Public Health

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Review

A Systematic Review of the Effects of Temperature on *Anopheles* Mosquito Development and Survival: Implications for Malaria Control in a Future Warmer Climate

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Abstract: The rearing temperature of the immature stages can have a significant impact on the life-history traits and the ability of adult mosquitoes to transmit diseases. This review assessed published evidence of the effects of temperature on the immature stages, life-history traits, insecticide susceptibility, and expression of enzymes in the adult *Anopheles* mosquito. Original articles published through 31 March 2021 were systematically retrieved from Scopus, Google Scholar, Science Direct, PubMed, ProQuest, and Web of Science databases. After applying eligibility criteria, 29 studies were included. The review revealed that immature stages of *An. arabiensis* were more tolerant (in terms of survival) to a higher temperature than *An. funestus* and *An. quadriannulatus*. Higher temperatures resulted in smaller larval sizes and decreased hatching and pupation time. The development rate and survival of *An. stephensi* was significantly reduced at a higher temperature than a lower temperature. Increasing temperatures decreased the longevity, body size, length of the gonotrophic cycle, and fecundity of *Anopheles* mosquitoes. Higher rearing temperatures increased pyrethroid resistance in adults of the *An. arabiensis* SENN DDT strain, and increased pyrethroid tolerance in the *An. arabiensis* SENN strain. Increasing temperature also significantly increased Nitric Oxide Synthase (NOS) expression and decreased insecticide toxicity. Both extreme low and high temperatures affect *Anopheles* mosquito development and survival. Climate change could have diverse effects on *Anopheles* mosquitoes. The sensitivities of *Anopeheles* mosquitoes to temperature differ from species to species, even among the same complex. Notwithstanding, there seem to be limited studies on the effects of temperature on adult life-history traits of *Anopheles* mosquitoes, and more studies are needed to clarify this relationship.

Keywords: *Anopheles* mosquito; body size; fecundity; gonotrophic cycle; immature stage; insecticide; longevity; temperature

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RESEARCH ARTICLE

Effects of elevated temperatures on the development of immature stages of *Anopheles gambiae (s.l.)* mosquitoes

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Abstract

Objective: This study investigated the effects of temperature on the development of the immature stages of *Anopheles gambiae (s.l.)* mosquitoes.

Methods: Mosquito eggs were obtained from laboratory established colonies and reared under eight temperature regimes (25, 28, 30, 32, 34, 36, 38 and 40°C), and 80 ± 10% relative humidity. Larvae were checked daily for development to the next stage and for mortality. Pupation success, number of adults produced and sex ratio of the newly emerged adults were recorded. Larval survival was monitored every 24 h, and data were analysed using Kaplan–Meier survival analysis. Analysis of variance was used where data followed normal distribution, and a Kruskal–Wallis test where data were not normally distributed. Larval and pupal measurements were log-transformed and analysed using ordinary least square regression with robust standard errors.

Results: Increasing the temperature from 25 to 36°C decreased the development time by 10.57 days. Larval survival ($X^2(6) = 5353.12$, $p < 0.001$) and the number of adults produced ($X^2(5) = 28.16$, $p < 0.001$) decreased with increasing temperature. Increasing temperatures also resulted in significantly smaller larvae and pupae ($p < 0.001$). At higher temperatures, disproportionately more male than female mosquitoes were produced.

Conclusions: Increased temperature affected different developmental stages in the life cycle of *An. gambiae (s.l.)* mosquitoes, from larval to adult emergence. This study contributes to the knowledge on the relationship between temperature and *Anopheles* mosquitoes and provides useful information for modelling vector population dynamics in the light of climate change.

KEYWORDS
Anopheles gambiae, development time, immature stage, larval and pupal size, survival, temperature

INTRODUCTION

Anopheles mosquitoes are responsible for transmitting several diseases, such as malaria and lymphatic filariasis. They are among the well-known vector species due to their crucial role in transmitting *Plasmodium falciparum* – a malaria parasite [1]. The ecology of mosquitoes is essential as conditions present can affect the development of mosquitoes [2, 3]. Biotic and abiotic factors and their interaction could influence the ecology of mosquitoes and subsequently affect the growth and development of mosquitoes [4, 5]. Biotic factors such as larval nutrition, competition for resources, predation by other

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Research

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Vector-Borne Diseases, Surveillance, Prevention

Effects of Elevated Temperatures on the Growth and Development of Adult *Anopheles gambiae (s.l.)* (Diptera: Culicidae) Mosquitoes

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Abstract

Higher temperatures expected in a future warmer climate could adversely affect the growth and development of mosquitoes. This study investigated the effects of elevated temperatures on longevity, gonotrophic cycle length, biting rate, fecundity, and body size of *Anopheles gambiae (s.l.)* (Diptera: Culicidae) mosqui-

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Parasites & Vectors

RESEARCH Open Access

Relationship between temperature and *Anopheles gambiae* sensu lato mosquitoes' susceptibility to pyrethroids and expression of metabolic enzymes

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Abstract

Background: Malaria remains one of the most devastating diseases globally, and the control of mosquitoes as the vector is mainly dependent on chemical insecticides. Elevated temperatures associated with future warmer climates could affect mosquitoes' metabolic enzyme expression and increase insecticide resistance, making vector control difficult. Understanding how mosquito rearing temperatures influence their susceptibility to insecticide and expression of metabolic enzymes could aid in the development of novel tools and strategies to control mosquitoes in a future warmer climate. This study evaluated the effects of temperature on the susceptibility of *Anopheles gambiae* sensu lato (s.l.) mosquitoes to pyrethroids and their expression of metabolic enzymes.

Methods: *Anopheles gambiae* s.l. eggs obtained from laboratory-established colonies were reared under eight temperature regimes (25, 28, 30, 32, 34, 36, 38, and 40 °C). Upon adult emergence, 3- to 5-day-old female non-blood-fed mosquitoes were used for susceptibility tests following the World Health Organization (WHO) bioassay protocol. Batches of 20–25 mosquitoes from each temperature regime (25–34 °C) were exposed to two pyrethroid insecticides (0.75% permethrin and 0.05% deltamethrin). In addition, the levels of four metabolic enzymes (α-esterase, β-esterase, glutathione S-transferase [GST], and mixed-function oxidase [MFO]) were examined in mosquitoes that were not exposed and those that were exposed to pyrethroids.

Results: Mortality in *An. gambiae* s.l. mosquitoes exposed to deltamethrin and permethrin decreased at temperatures above 28 °C. In addition, mosquitoes reared at higher temperatures were more resistant and had more elevated enzyme levels than those raised at low temperatures. Overall, mosquitoes that survived after being exposed to pyrethroids had higher levels of metabolic enzymes than those that were not exposed to pyrethroids.

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