UNIVERSITY OF CAPE COAST

ASSESSMENT OF THE POTENTIAL OUTBREAK OF *AEDES*-BORNE DISEASES DURING THE COVID-19 RESTRICTION PERIOD IN CAPE COAST

JOANA AYETTEY

2022

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BY

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Thesis submitted to the Department of Conservation Biology and Entomology, School of Biological Sciences, University of Cape Coast, in partial fulfillment of the requirements for the award of Master of Philosophy degree in Entomology.

OCTOBER 2022

DECLARATION

Candidate's Declaration

I hereby declare that this thesis is the result of my own original research and that no part of it has been presented for another degree in this university or elsewhere.

Candidate's Signature:.. Date:..........................

Name: Joana Ayettey

Supervisors' Declaration

We hereby declare that the preparation and presentation of the thesis were supervised in accordance with the guidelines on supervision of thesis laid down by the University of Cape Coast.

Principal Supervisor's Signature:................................... Date:.........................

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Name: Prof. B. A. Mensah

ABSTRACT

Vector-borne diseases transmitted by *Aedes* mosquitoes pose a significant public health threat worldwide. This study focused on assessing the risk of *Aedes*-borne disease outbreaks during the Covid-19 restriction period based on entomological indices and investigating the contributing factors, with a particular emphasis on the role of artificial containers as breeding sites. A household larval survey was conducted in a small community called Duakor in Cape Coast, evaluating three larval indices (Container, House, and Breteau Index). Mosquito larvae collected from containers were reared and tested for insecticide resistance against four commonly used insecticides which were Deltamethrin (0.05%), DDT (4%), Fenitrothion (0.1%) and Bendiocarb (0.1%). The findings indicated that the risk of *Aedes*-related disease outbreaks during the Covid-19 restriction period was significantly lower compared to a previous assessment in 2017 (Breteau Index = 2.02, House Index =34%, and Container Index =22.5%). The sampled *Aedes aegypti* population showed resistance to all four tested insecticides, and the study reported the detection of a novel V410L kdr mutation in Ghana, in addition to the previously known F1534C and V1016I mutations in *Ae. aegypti* population in the country.

The lower risk observed during the restriction period was attributed to frequent community clean-up exercises, aligning with the notion that sanitation improvement plays a crucial role in controlling *Aedes aegypti*. Given the emergence of multiple insecticide resistance, the study emphasizes the need to encourage non-insecticide-based control tools such as sanitation improvement and proper water storage practices.

KEY WORDS

Mosquitoes

Ecological succession,

Water storage containers

Covid-19

Larval Indices

Predator-prey

Insecticide resistance

ACKNOWLEDGMENTS

My heartfelt gratitude goes to Almighty God for being my anchor throughout my life and education, and for enabling the successful completion of this work. To God be the glory now and forever.

I would like to express my sincere appreciation to my supervisors, Dr. Andreas Kudom and Prof. B. A. Mensah from the Department of Conservation Biology and Entomology, for the opportunity given to me, their mentorship, valuable comments, contributions, and for being exceptional guides.

I am also deeply grateful to the CIH-LMU One Health Scholarship for their support in funding my research work. I would like to acknowledge the dedication of Mr Francis Abortri for his invaluable assistance in field sampling of mosquitoes.

Lastly, I extend my heartfelt thanks to my family for their prayers and unwavering encouragement. May the Lord shower them with boundless blessings.

DEDICATION

Dedicated to my parents, siblings, and the entire Ayettey family.

TABLE OF CONTENTS

Content **Page**

LIST OF TABLES

Table **Page**

LIST OF FIGURES

Figure **Page**

LIST OF ABBREVIATIONS

WHO	World Health Organization
Ae.	*Aedes*
Cx.	*Culex*
An.	*Anopheles*
GPS	Global Positioning System
SIT	Sterile Insect Technique
RIDL	Dominant lethal gene
BI	Breteau Index
CI	Container Index
HI	House Index

CHAPTER ONE

INTRODUCTION

1.1 Background to the Study

In recent years, the global impact of *Aedes*-borne diseases, such as Zika, Dengue, yellow fever, and Chikungunya, has raised significant concerns for public health (Ogunlade et al., 2021; Puntasecca et al., 2021). These diseases are mainly transmitted by the *Aedes aegypti* mosquito, and their outbreaks have been reported in various regions worldwide. The emergence of dengue cases, particularly, has been a cause for alarm, with a growing number of incidents reported in recent years (Eltom et al., 2021; Yang et al., 2021). Additionally, the World Health Organization (WHO) declared a public health emergency in 2016 due to neurological complications associated with Zika virus infection, highlighting the urgent need for effective control strategies (Hasan et al., 2019; Mullen et al., 2020). The Covid-19 pandemic further compounds the challenges posed by *Aedes*-borne diseases. Notably, during the Covid-19 restriction period, there has been a surge in the incidence of *Aedes*-borne arboviral diseases in several countries, with millions of cases reported (PAHO, 2020). The coexistence of the Covid-19 pandemic and *Aedes*-borne disease outbreaks has the potential to cause devastating consequences, particularly in low-resource countries. Therefore, it is imperative to enhance preparedness and response measures to address the potential outbreak risk of *Aedes*-borne diseases during the Covid-19 restriction period.

In urban areas, *Aedes aegypti* mosquitoes commonly breed in water storage containers and other man-made receptacles that can hold water (Getachew et al., 2015; Kudom, 2020). The increased demand for water storage containers during the pandemic, due to measures such as frequent handwashing, may inadvertently lead to an upsurge in *Aedes aegypti* breeding habitats within households. This raises concerns about the potential risk of disease transmission in communities with limited access to piped water (Trewin et al., 2013). Understanding the impact of water storage practices and poor sanitation conditions on *Aedes* mosquito population density is crucial for developing effective control strategies.

Furthermore, insecticide-based strategies, particularly the use of pyrethroid insecticides, have been the primary approach for emergency control of *Aedes* mosquitoes. However, the emergence and development of insecticide resistance among mosquito vectors, including *Aedes aegypti*, pose a significant challenge to the effectiveness of these control measures (Kudom et al., 2020; Kawada et al., 2016). Continuous monitoring of insecticide resistance status, identification of resistance mechanisms, and development of resistance management strategies are essential to ensure the success of control efforts.

1.2 Statement of the Problem

Aedes-borne diseases, including Zika, Dengue, yellow fever, and Chikungunya, pose significant health burdens to populations in endemic regions. The increasing number of dengue cases and the potential for outbreaks of these arboviral diseases have raised concerns globally (Leta et al., 2018).

Aedes mosquitoes have adapted to breed in various environments, including urban settings, which has amplified the magnitude and impact of their disease outbreaks. In 2016, the World Health Organization declared a public health emergency due to neurological complications from Zika virus infection, while yellow fever outbreaks spread across urban centers in Africa and Brazil, emphasizing the ongoing risk to global public health. To address this alarming trend, robust vector surveillance is crucial to understand the dynamics of *Aedes*-borne diseases and enable effective control measures. In addition to surveillance, understanding the factors influencing container productivity for *Aedes aegypti* and other container-dwelling mosquitoes is essential for effective control strategies. Water storage practices and sanitation conditions significantly impact the breeding habitats of *Aedes* mosquitoes. The increased demand for water in households during the Covid-19 pandemic, combined with limited access to piped water in some communities, can result in increased use of water storage containers. If these containers are not properly managed, they can become potential breeding sites for *Aedes* mosquitoes. Furthermore, the effectiveness of insecticide-based control strategies during epidemics relies on the susceptibility of mosquito populations. In Ghana, reports of high levels of pyrethroid resistance in mosquitoes highlight the potential limitations of insecticide-based approaches (Kudom et al., 2018; Mugenzi et al., 2022). Continuous monitoring of insecticide resistance status and understanding of the major resistance mechanisms among *Ae*. *aegypti* populations are crucial for developing effective resistance management strategies.

1.3 Justification

The COVID-19 pandemic has had a profound impact on public health worldwide, with significant disruptions to various aspects of society. In the context of vector-borne diseases, such as those transmitted by *Aedes aegypti* mosquitoes, the pandemic has introduced new challenges and complexities. Understanding the interplay between the COVID-19 pandemic and *Aedes* mosquito ecology is essential for developing effective strategies to mitigate disease transmission.

Ghana, like many other countries, has been dealing with the consequences of the COVID-19 pandemic. The emergence of COVID-19 in Ghana led to the implementation of various containment measures, including lockdowns, travel restrictions, and changes in human behavior. These measures may have inadvertently influenced the dynamics of *Aedes* mosquito populations and their breeding habitats, leading to potential changes in disease transmission patterns.

Of particular concern is the potential infestation of *Aedes* larvae in artificial containers used for water storage. With many households experiencing increased water storage needs due to COVID-19 preventive measures, containers can become breeding grounds for *Aedes* mosquitoes if not properly maintained. The disruption of regular vector control efforts during the pandemic may have further exacerbated this issue.

Considering these challenges, there is an urgent need to investigate the impact of the COVID-19 pandemic on *Aedes* mosquito ecology in Ghana. A comprehensive entomological survey that considers the influence of pandemic-related factors on vector breeding sites, water storage practices, and human-vector interactions is crucial. By understanding how the pandemic has influenced *Aedes* mosquito populations and their distribution, targeted and effective vector control measures can be implemented to reduce the risk of disease transmission.

1.4 Research objectives

This research, therefore, aimed to investigate *Aedes aegypti* vector factors that could potentially increase in the risk of outbreak and transmission of *Aedes*-borne diseases during the Covid-19 restriction period in 2020, a small community in Cape Coast.

The specific objectives were to:

1. Determine the infestation level of *Aedes* and other container dwellers in Duakor using entomological indices.
2. Determine the spatial resistance status of *Aedes aegypti* mosquitoes from Cape Coast.
3. Investigate the succession pattern and predatory behaviour of *Aedes aegypti* and other container-dwelling mosquitoes.

1.5 Significance of the study

The study holds significant implications for public health and disease prevention. By assessing the risk of *Aedes*-borne disease outbreaks during the Covid-19 restriction period, the research aims to contribute to improved public health outcomes. Understanding the factors influencing larval breeding, insecticide resistance, and larval ecology can inform the development of effective vector surveillance and control strategies. This knowledge can help reduce transmission rates, mitigate the impact of *Aedes*-borne diseases, and ultimately protect the health of communities.

Furthermore, the study's findings have the potential to influence policy development and decision-making processes. By providing evidence-based insights into larval ecology, container productivity, and the effectiveness of current control strategies, policymakers and public health authorities can make informed decisions to optimize resource allocation and prioritize interventions. This research can contribute to the design and implementation of targeted measures to prevent *Aedes*-borne disease outbreaks.

1.6 Delimitations of the study

The study did not check the chemical cues that attracted mosquitoes regarding the ecological succession. Although ecological succession was studied using yeast, the content of the yeast that attracted the mosquito was not investigated.

1.7 Limitations

Some of the mosquito species sampled could only be identified to the genus level, rather than to the species level. This limitation arises from the inherent challenges in distinguishing between closely related mosquito species based solely on external morphological characteristics.

1.8 Definition of terms

Ecological succession: How a biological community's structure changes over time

Interaction: Organisms living together in a community and having an influence on one another

Predation: Process whereby a predator kills its prey in an interaction

Outbreak: sudden rise in the sum of cases of an ailment.

Biological Control: The use of natural enemies to control pest populations.

Breeding: Reproduction with the aim to produce an offspring

Instar: the name given to the developmental stage of an arthropod between molts

Larvicidal: Insecticides used by mosquito control programs to kill mosquito larvae.

Adulticidal: Insecticides used by mosquito control programs to kill adult mosquitoes.

Dwelling: A place where an insect or organism lives

1.9 Organization of the study

This thesis is separated into six chapters. Chapter 1 describes the study's broad definition, providing background information and stating the study's issue, intent, objectives, and significance. The second chapter delves into the literature applicable to the thesis in depth. A comprehensive analysis of the literature on identifying container dwelling mosquitoes and their ecological importance around the world is given. The methods are discussed in Chapter 3.

The study areas are well-described, as are the statistical methods and applications used to analyze the collected data.

The findings and discussion are presented in Chapters 4 and 5, respectively. In Chapter 4, the research findings are described in graphs, charts, and tables with brief explanations. In Chapter 5, a detailed description of the findings and inferences is presented in the form of a discussion. Chapter 6 concludes with conclusions and recommendations. A list of sources and appendices are among the other parts included in this thesis.

1.10 Chapter summary

In this chapter, the study's underlying rationale is discussed, along with justification and the significant information contribution to global science communities and the country from larval prey-predator interactions among container-dwelling mosquitoes towards biological control of *Aedes aegypti* mosquitoes. To assist readers, the goals of the study have been outlined, and terms have been defined.

CHAPTER TWO

LITERATURE REVIEW

During the COVID-19 pandemic, the dynamics of artificial containers and their impact on mosquito populations, particularly *Aedes aegypti* mosquitoes, have gained significant importance. This review focuses on the interactions within artificial containers, including predator-prey relationships, ecological succession, and species colonization. By understanding these interactions, we can gain insights into the factors influencing mosquito populations amidst the pandemic. Furthermore, the implications of these interactions on the transmission of vector-borne diseases are explored, considering the disruptions and changes caused by COVID-19. This review sheds light on the ecological context of mosquito-borne diseases and provides valuable insights for effective disease surveillance and control measures during this unprecedented time.

2.1 The COVID-19 Pandemic

The COVID-19 pandemic, caused by the novel coronavirus SARS-CoV-2, emerged in December 2019 in the city of Wuhan, Hubei province, China (Lu et al., 2020; Yang et al., 2020). The rapid global spread of the virus has led to a significant impact on public health systems worldwide (Baloch et al., 2020). The highly contagious nature of the virus and its ability to cause severe respiratory illness has necessitated stringent public health measures, including lockdowns, travel restrictions, and social distancing protocols (Baker et al., 2020; Khanna et al., 2020).

The COVID-19 pandemic has posed unprecedented challenges to healthcare systems globally, with healthcare facilities becoming overwhelmed by the influx of patients requiring medical care. The shortage of hospital beds, medical equipment, and healthcare workers has strained the capacity of healthcare systems in many countries, leading to difficulties in providing adequate care for COVID-19 patients as well as other medical conditions (Sen-Crowe et al., 2021). The impact on public health extends beyond the direct consequences of the virus, as delays in accessing healthcare services and disruptions to routine immunization programs have also been observed (Alene et al., 2020).

In addition to the immediate health effects, the COVID-19 pandemic has had far-reaching socioeconomic implications. The implementation of lockdowns and travel restrictions has resulted in the closure of businesses, loss of jobs, and economic downturns in many countries (Kalogiannidis, 2020). Disruptions in global supply chains have led to shortages of essential goods and services, further exacerbating the socio-economic impact of the pandemic. Vulnerable populations, including low-income individuals, migrant workers, and marginalized communities, have been disproportionately affected, with increased risks of poverty, food insecurity, and social inequities (Clark et al., 2020; Wolfson & Leung, 2020).

The global response to the COVID-19 pandemic has highlighted the importance of international cooperation and the need for robust public health measures. Efforts to control the spread of the virus have included widespread testing, contact tracing, quarantine measures, and the development and deployment of vaccines (WHO, 2020). However, challenges such as vaccine distribution inequities, vaccine hesitancy, and the emergence of new variants continue to pose obstacles in effectively managing the pandemic. The ongoing monitoring, research, and implementation of evidence-based strategies are crucial in mitigating the impact of the COVID-19 pandemic on public health systems and preventing further spread of the virus.

2.1.1 Impact of COVID-19 on Vector-Borne Diseases

The COVID-19 pandemic has had indirect consequences on the transmission dynamics of vector-borne diseases. With the implementation of lockdowns and social distancing measures, there has been a significant reduction in human mobility and interaction, which can influence the transmission of vector-borne diseases (Lim et al., 2021; Nouvellet et al., 2021). For instance, decreased travel and limited outdoor activities may lead to a decrease in human exposure to vectors and subsequent transmission of diseases such as dengue, Zika, and malaria (Jansen et al., 2021). Conversely, the disruption of routine vector control programs during the pandemic, including the reduction in insecticide spraying and larval control activities, may result in an increase in vector populations and disease transmission (Reegan et al., 2020). The COVID-19 pandemic has also posed challenges to the surveillance and control efforts of vector-borne diseases. The diversion of healthcare resources and personnel to COVID-19 response has impacted the capacity for vector surveillance, diagnosis, and treatment (McKay et al., 2021). Many countries have experienced disruptions in routine vector surveillance activities, such as entomological surveys and disease reporting systems, leading to potential delays in detecting outbreaks and implementing timely control measures (Brady & Wilder-Smith, 2021). Additionally, the prioritization of COVID-19 testing and treatment may result in reduced access to diagnostic tests and treatment for vector-borne diseases, further hindering effective disease management (Nyaruhirira et al., 2022).

Furthermore, the COVID-19 pandemic has highlighted the interconnectedness of human, animal, and environmental health in the context of vector-borne diseases. The spillover of zoonotic diseases, such as COVID-19 itself, emphasizes the need for a One Health approach in disease surveillance and control (Sparrer et al., 2023). The disruptions in wildlife trade and habitat encroachment during the pandemic may lead to shifts in vector populations and their interactions with animal reservoirs, potentially influencing disease transmission patterns (Lawler et al., 2021). Understanding the complex interactions between human activities, environmental changes, and vector-borne diseases is essential for effective preparedness and response strategies in both the COVID-19 pandemic and future disease outbreaks.

2.1.2 COVID-19 Mitigation Measures on Vector-Borne Disease Transmission

The implementation of COVID-19 mitigation measures, including lockdowns, travel restrictions, and changes in human behavior, has had potential effects on the transmission of vector-borne diseases. Lockdowns and restrictions on movement have resulted in reduced human outdoor activities and limited opportunities for vector-human interactions, potentially leading to a decrease in disease transmission (Fernandez et al., 2021). For instance, studies have shown a decline in reported cases of dengue and chikungunya during periods of strict lockdowns, attributed to reduced vector breeding sites and decreased human exposure to vectors (Prakash et al., 2021; Simpson et al., 2023). However, it is important to consider the potential resurgence of vector populations and disease transmission once the measures are lifted.

The changes in human behavior and practices during the COVID-19 pandemic may have also influenced vector populations and breeding habitats. Increased hygiene practices, such as frequent handwashing and proper waste management, can contribute to the reduction of potential breeding sites for mosquitoes (Sharma et al., 2020). The reduced use of public spaces and closure of establishments like hotels and resorts have led to a decrease in artificial breeding sites for mosquitoes, such as swimming pools and water storage containers (Sharma et al., 2020). However, the increased time spent at home during lockdowns may have resulted in individuals creating new breeding sites in and around their residences, leading to localized increases in vector populations (Lim et al., 2021). Understanding the interplay between human behavior and vector ecology is crucial for effective vector-borne disease control strategies.

Moreover, the disruption of vector control programs during the pandemic has also had implications for vector populations and disease transmission. The redirection of resources and personnel towards COVID-19 response has impacted the capacity for routine vector surveillance and control activities (WHO, 2020). The suspension of insecticide spraying campaigns, distribution of bed nets, and other vector control interventions may have allowed vector populations to rebound and increase in certain areas (Mbunge et al., 2021). The delay or reduction in vector control efforts can lead to an accumulation of susceptible individuals within the population, increasing the potential for disease outbreaks once control measures are reinstated (WHO, 2020). It is essential to develop strategies that balance the prioritization of COVID-19 control with the ongoing need for effective vector-borne disease management.

2.1.3 Covid-19 situation in Ghana

In Ghana, the COVID-19 pandemic had a significant impact on the country's healthcare system and public health measures (WHO, 2020). The first case of COVID-19 was reported in March 2020, leading to a sharp rise in infections across various regions. To mitigate the spread of the virus, the government of Ghana implemented strict measures, including the closure of schools, banning public gatherings, and imposing partial lockdowns in certain areas. These measures aimed to reduce human interactions and limit the transmission of the disease.

During the COVID-19 restriction period, which lasted from July to September 2020, this study was conducted in the community of Duakor in Cape Coast, Ghana. While schools remained closed, final-year students were allowed to return to write their final examinations. It is important to note that piped water was provided free of charge by the government as a relief measure during this period, considering the importance of adequate water supply for hygiene practices and disease prevention.

The COVID-19 pandemic and the associated restrictions may have affected the dynamics of vector-borne diseases in Ghana. The interplay between the reduced human activities, changes in water availability, and potential disruptions to vector control efforts during the COVID-19 restriction period could have influenced the prevalence and transmission of vector-borne diseases. Assessing these impacts and understanding the environmental and behavioral factors that contribute to disease transmission during the pandemic is essential for developing targeted and effective control measures to minimize the burden of vector-borne diseases in Ghana.

2.2 Mosquitoes

Mosquitoes belong to the class Insecta which is the most dominating group of the Phylum Arthropoda (Rueda, 2007; Usman et al., 2017). Mosquitoes are classified as insects because their bodies are divided into three segments (Head, thorax and abdomen). They also possess wings that aid in their flight and proboscis for feeding (Dennis et al., 2019; Foster & Walker, 2019). Generally, mosquitoes feed on glucose, using their proboscis as the sucking mouthpart. However, the female may also go for a blood meal to develop its eggs. This may result in the biting of both humans and animals for the blood meal. Mosquitoes are found in almost every part of the world. They are, however, absent in some islands and Antarctica (Sallum et al., 2000; Vinogradova, 2000). They can survive in a wide range of environments in biotic communities such as tropical forests and arctic tundra. Figure 1 shows images of some notable mosquito species. Mosquitoes are important because their bites have been associated with disease transmission such as malaria, dengue and filariasis. (Jelinek, 2009; Marzal et al., 2022). They are often regarded as the major arthropod affecting the health of humans. Apart from their bites, they are known to cause great discomfort to humans through itching, swelling, and soreness to the bitten area. The disease that occur as a result of bites from these tiny insects may lead to deaths indirectly reducing the productivity of humans (Hong, 2011). Globally, millions of monies are spent yearly on control interventions.

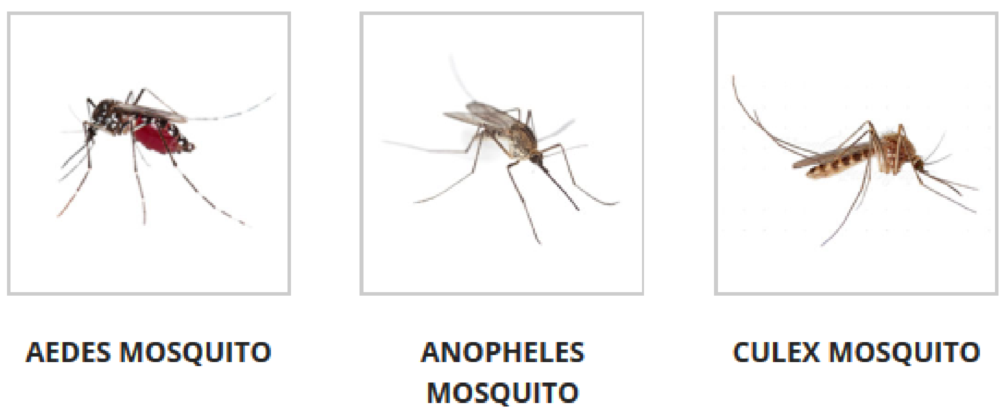

Figure 1: Images of different species of mosquitoes. Image source:https://cdn1.npcdn.net/userfiles/21083/image/Mosquitoes.png

2.3 Taxonomy

Mosquitoes, belonging to the family Culicidae, are characterized by their blood-feeding behavior. They are classified under the infraorder Culicomorphs due to their possession of a long proboscis used for biting and sucking. With approximately 3500 identified species, mosquitoes represent a diverse group, although many species are yet to be discovered (REF). Within the family Culicidae, two subfamilies are recognized: Culicinae and Anophelinae (Figure 2). The subfamily Culicinae comprises most mosquito genera. These subfamilies can be distinguished based on their morphological features. Anopheline larvae lack air tubes and rely on spiracles for respiration, while their adults possess palps that are equal in length to their proboscis. In contrast, Culicine larvae possess air tubes, and the length of their palps exhibits variation.

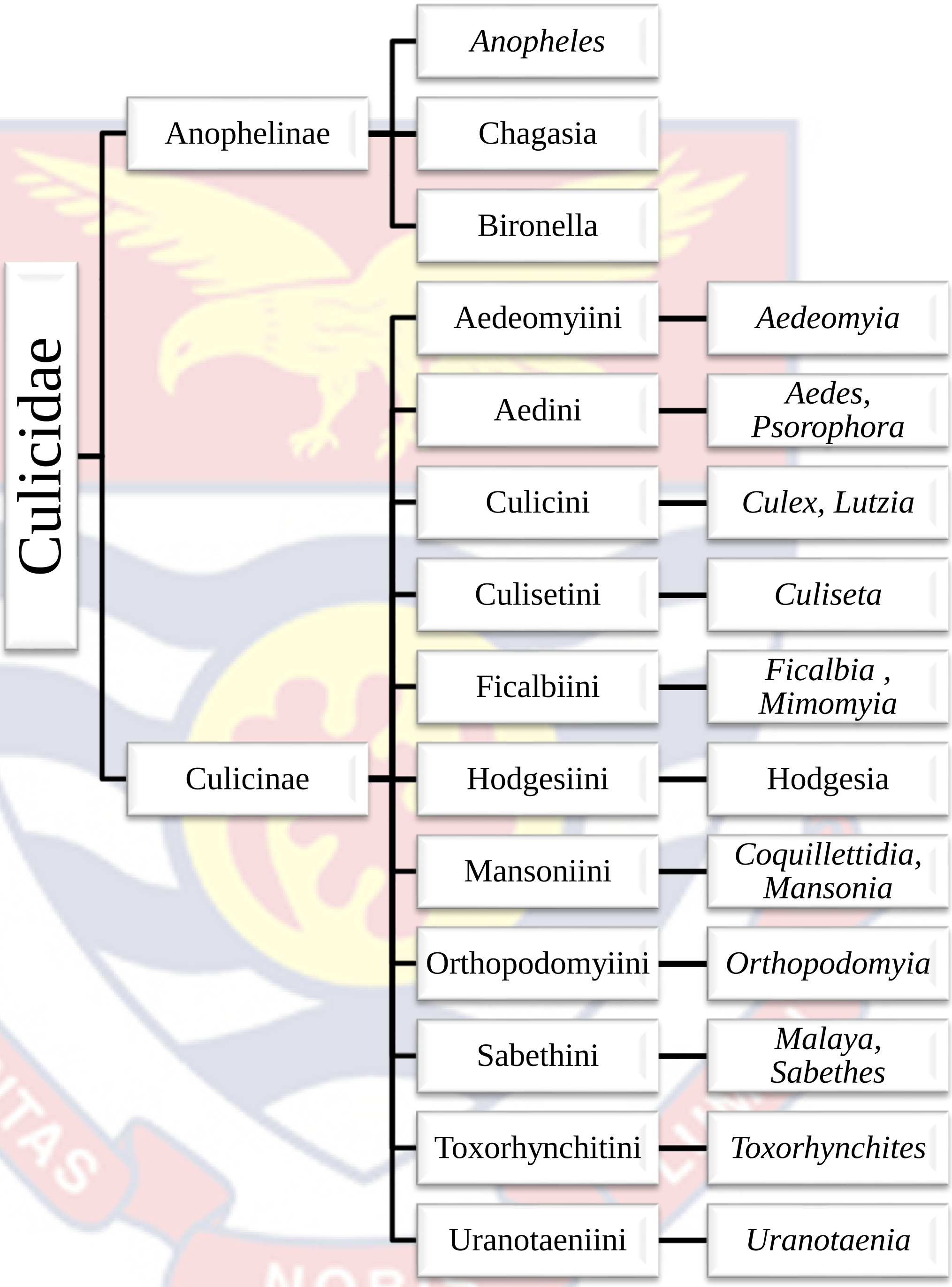

Figure 2: Classification of mosquitoes into subfamilies and genera (Harbach, 2007).

Globally, *Aedes* subgenus stegomyia, *Anopheles*, and *Culex* are recognized as the most significant groups of mosquitoes (Becker et al., 2020). Within the *Anopheles* complex groups, certain species play a crucial role as vectors of diseases such as malaria and lymphatic filariasis. The *Culex* species are also important disease vectors, transmitting diseases such as West Nile Virus, St. Louis encephalitis, and bird malaria (Becker et al., 2020). Of medical importance within the subgenus Stegomyia are *Aedes aegypti* and *Aedes albopictus*. *Aedes aegypti*, known for its role in the transmission of yellow fever, dengue, and Zika virus, has a global distribution in tropical and subtropical regions (Akiner et al., 2016). Within *Ae. aegypti*, there are two forms: *Aedes aegypti formosus* and *Aedes aegypti aegypti*. *Aedes aegypti formosus* is a wild or undomesticated species primarily found in the interior parts of Africa, where they inhabit tree holes and feed on various animals (Futami et al., 2020). Although they are not typically associated with domestic environments, studies have shown that some populations have adapted to domestic habitats in parts of Africa, particularly breeding in rain-filled containers (Futami et al., 2020). On the other hand, *Ae. aegypti aegypti* is known as a domestic mosquito, predominantly found in coastal areas of Africa and sparsely distributed in southern parts of Asia and warmer regions of the southern United States (Ding et al., 2018) . This species is well-known for its preference for breeding in hand-filled containers throughout the year and, in areas where it coexists with formosus, it also utilizes rain-filled containers (Ngingo, 2020)

2.4 Morphology

Mosquitoes exhibit distinct morphological characteristics in their different life stages, namely the larval and adult stages. Each stage showcases unique features, although they all possess notable appendages such as the head, thorax, and abdomen. The life cycle begins with the hatching of eggs, which are typically laid on the surface of water. From these eggs, larvae emerge and undergo various molting stages as they develop in aquatic environments.

2.4.1 Egg

Mosquitoes' eggs are spindled-shaped, ovoid, elongated, rhomboid or spherical. The chorion which is the outer layer of the eggshell often has a diagnostic pattern of a particular species. Figure 3 shows images of mosquito eggs of various species.

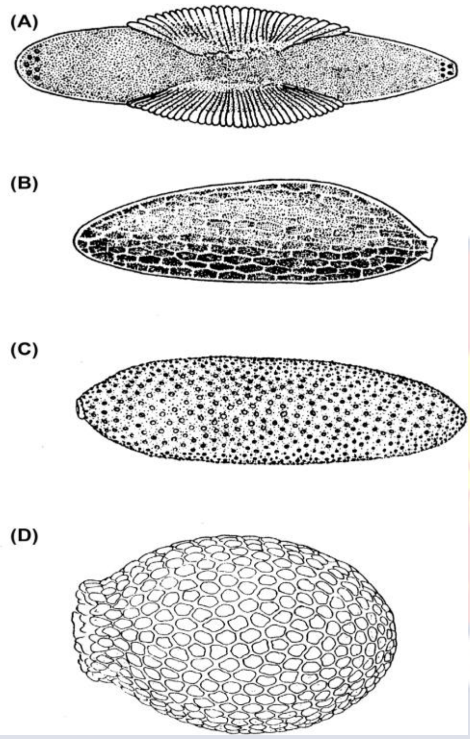

Figure 3: chorionic sculpturing of mosquito’s eggs, displaying shape variations (A) *Anopheles*. (B) *Culex*. (C) *Aedes aegypti*. (D) *Toxorhynchites brevipalpis* (Foster & Walker, 2019)

2.4.2 Larvae

Mosquito larvae undergo four developmental (instar) stages. At each instar, the larvae moult and increase in size. The larval body has three distinct divisions (the head containing the mouth parts, the broader thorax and the abdomen (Becker et al., 2020). The head has a capsule which bears a set of ocelli (simple eyes) and a pair of antennae of different lengths and shapes (Bar & Andrew, 2013). Their mouthparts are modified for chewing, while they feed with sweepers, brushes or combs located around the mouth. The abdomen of the larvae has eight segments with a set of spiracles opening on the dorsal side. Anophelines lack siphons and therefore obtain oxygen with the help of the spiracles (figure 4A). Culicines have their spiracular structures forming a siphon in the anal region, to obtain air from the surface of the water (figure 4B).

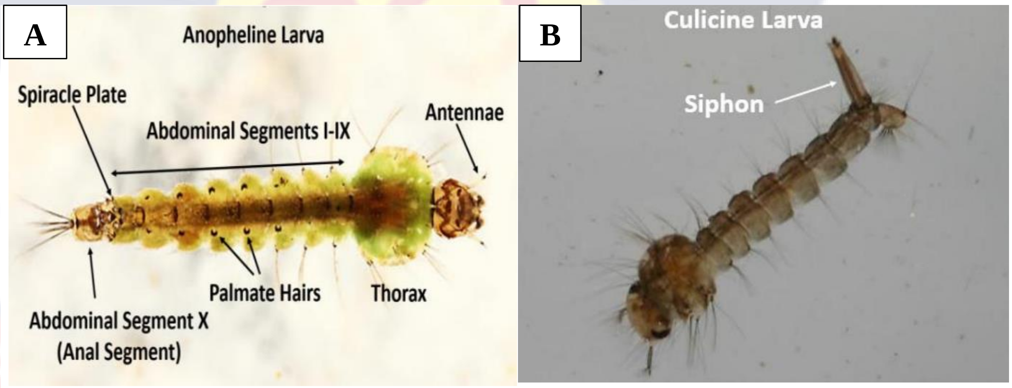

Figure 4: Mosquito Larval morphology with siphon and spiracles. (A) Anopheline (B) Culicine (Wucherer, 2021).

2.4.3 Pupae

Mosquito pupae are comma-shaped and possess a cephalothorax (head and thorax fused) and abdomen (Figure 5). They also have air trumpets through which they obtain oxygen at the surface of the water. The cephalothorax has an air pocket that helps the pupa to maintain buoyancy on the surface of the water. Unlike other insects, mosquito pupae show a bit of motion (Becker et al., 2020). Upon disturbance of a water surface, the pupae can flap downward with the help of their flexible abdominal segment. The pupae float back to the surface after the dive, unlike larvae which swim. Pupae do not feed and can even tolerate harsh conditions of the breeding site, unlike larvae.

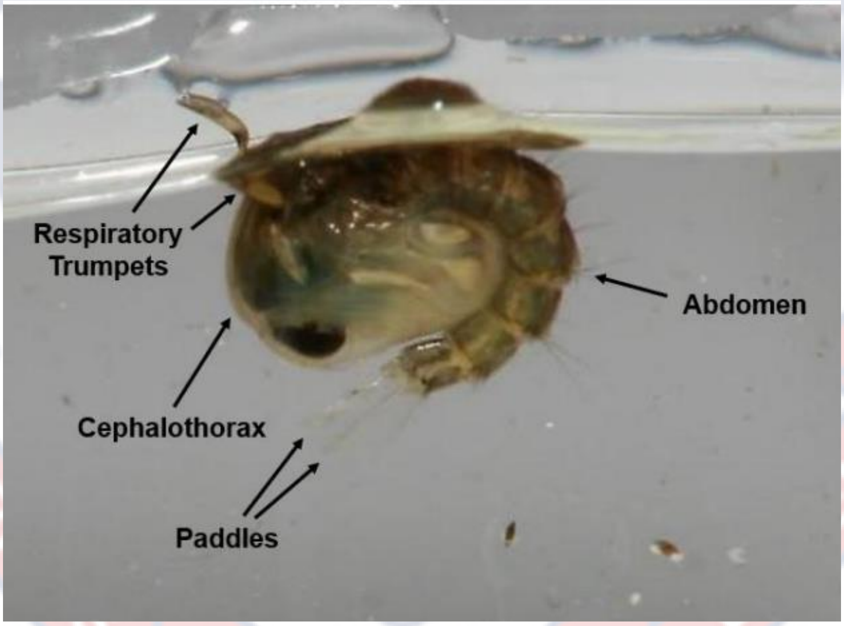

Figure 5: Lateral view of Mosquito pupae (Wucherer, 2021).

2.4.4 Adult

Adult mosquitoes exhibit distinct anatomical features that are essential for their survival and reproductive functions (Becker et al., 2020). They possess a pair of compound eyes that enable them to perceive their surroundings, antennae that serve as sensory organs for detecting odors and sounds, and three pairs of walking legs for locomotion (Becker et al., 2020). The body of an adult mosquito is covered with scales, fine piles, and setae, giving each species unique markings and patterns of colors (Becker et al., 2020).

There are notable differences between male and female mosquitoes. Male mosquitoes are not sexually mature upon emergence and undergo a maturation process that involves spinning their hypopygium before they can mate (Becker et al., 2020). This maturation process takes approximately a day, and as a result, males typically emerge 1-2 days earlier than females to ensure they reach sexual maturity at the same time (Becker et al., 2020). Male mosquitoes have plumose antennae, which they use to grasp the female during mating (Becker et al., 2020). In contrast, female mosquitoes possess a distinctive proboscis that projects outwardly to about two-thirds the length of their abdomen (Becker et al., 2020). The proboscis is a piercing and sucking mouthpart used by females to penetrate the skin of their hosts (Becker et al., 2020).

The wings of adult mosquitoes are crucial for their movement. Mosquito wings have a characteristic pattern of veins and are covered with scales, with the hind margin forming a fringe (Becker et al., 2020). The wings also possess halters, small club-like structures that aid in flight control (Becker et al., 2020). Following emergence, adult mosquitoes are ready to resume their life cycle, which includes seeking hosts for blood meals (in the case of females) and engaging in mating activities (Becker et al., 2020).

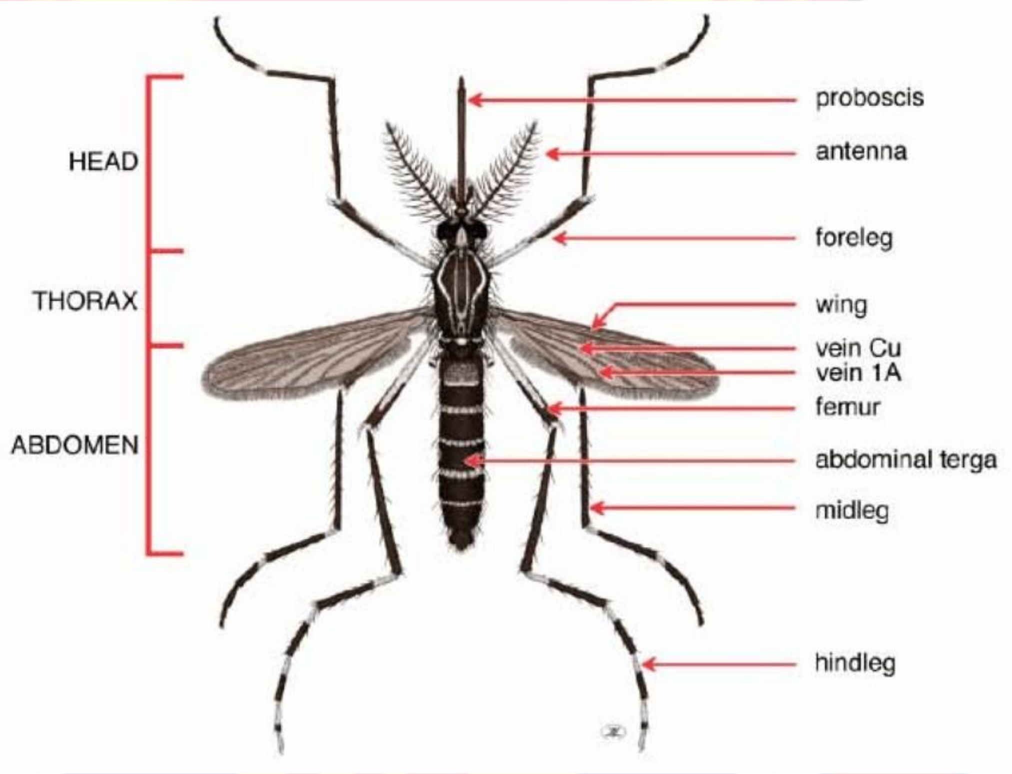

Figure 6: Dorsal view of adult female mosquito-*Aedes (Stegomyia) aegypti* (Wucherer, 2021)

2.5 The life cycle of mosquitoes

Mosquitoes go through a complete metamorphosis; thus, they go through four stages of development (egg, larvae, pupa, and adult). They have aquatic and terrestrial life forms as well. Here, the life stages for eggs, larvae and pupa are widely distributed in aquatic environments whereas the adults are terrestrial. The only absolute requirement for larval and pupa development is to maintain at least a film of water in their growth period (Ramasamy & Surendran, 2012). The type of water in which these mosquitos' larvae are found gives an idea of the kind of species that are present in the water (Mala & Irungu, 2011). Different mosquitoes can be found inhabiting different environments with their breeding sites ranging from stagnant sunlit waters, gutters, tree holes, and small pools of water collected in holes and discarded containers (Mattah et al., 2017). A lot of mosquitoes have been reported to co-exist with other species and breed in very polluted habitats (Bhattacharya et al., 2016; Juliano, 2009). *Anopheles* species, which are mainly clear water breeders have recently been found breeding in polluted habitats (Gunathilaka et al., 2013). These polluted habitats have formerly been known as the preferred breeding sites of *Culex* species. Again, *Anopheles* has been seen co-habiting with *Culex* and *Aedes* in containers (Yee et al., 2015). *Aedes* are the main container dwellers, which have long-established themselves by breeding in artificial containers filled with water. For the study, this review will focus on the biology, ecology, and behaviour of *Aedes aegypti* mosquitoes.

2.6 Biology of *Aedes aegypti* Species

The adult female *Aedes aegypti* mosquitoes undergo mating, feeding and oviposition to begin another life cycle.

2.6.1 Mating

Mating in *Aedes aegypti* typically occurs two to three days after the emergence of adult mosquitoes. During mating, males employ swarm markers, which are prominent objects or visual cues in the environment that help attract females. Male mosquitoes in a swarm can emit volatile compounds that serve as long-range attractants for females (Becker et al., 2020). In the case of *Aedes aegypti*, the female uses the host as the primary swarm marker. When a female enters a male swarm, the male detects the frequency of her wing beat using its plumose antennae and Johnston's organ, and then flies towards her (Becker et al., 2020). Mating can occur either in the air or on a solid surface, and it is during this process, known as copulation, that the reproductive structures of the male and female merge (Becker et al., 2020). Copulation typically lasts for a brief period, usually not exceeding 30 seconds, during which the male deposits spermatozoa into the bursa copulatrix of the female (Becker et al., 2020).

After mating, female *Aedes aegypti* mosquitoes seek a blood host for a blood meal, which is essential for their egg development. Once the female has fed on blood, she searches for a suitable water source to lay her eggs. *Aedes aegypti* mosquitoes commonly choose containers as preferred sites for oviposition. Upon finding a suitable location, the female deposits her eggs singly on the surface of the water (Day, 2016). A gravid female mosquito is capable of laying approximately 100 eggs (Day, 2016). Remarkably, the eggs of *Aedes aegypti* are resilient and can survive under extreme conditions such as drought. The eggs, upon water immersion can hatch into larvae, initiating the next stage of the mosquito life cycle (Venkataraman, 2022). This adaptive behavior of *Aedes aegypti*, allowing their eggs to withstand adverse conditions and hatch upon water immersion, presents a significant challenge in mosquito control efforts (Venkataraman, 2022).

2.6.2 Dispersal and Host-seeking behaviour

Mosquitoes use a wide range of volatile chemicals to locate their hosts. These host attractants include octanol, carbon dioxide, acetone, caproic acid, butanone, phenolic compounds and lactic acid (Becker et al., 2020). Fatty acids and other compounds also play a crucial role in the attraction of most mosquito species (Smallegange & Takken, 2010). Females are equipped with many receptors on their antennas to respond to these attractants. The *Aedes aegypti* mosquito requires a blood meal to complete oogenesis and vitellogenesis, and therefore has developed complex host-seeking behaviour for locating hosts. These host-seeking behaviours have been grouped into 3 phases (Becker et al., 2020).

1. Non-oriented dispersal behaviour
2. Oriented host location behaviour
3. Attraction to an appropriate host candidate

Female mosquitoes exhibit a complex behavioral pattern that involves both non-oriented dispersal and host-oriented behavior. In non-oriented dispersal, females are driven by derived stimuli to increase the likelihood of encountering a potential host. These stimuli act as guiding factors for the mosquito's flight, leading it towards areas where hosts are likely to be present. However, it is the contact with the host that intensifies the stimuli and strengthens the attraction between the mosquito and the host. When the host and the mosquito are in proximity, the derived stimuli become more potent, prompting the female to transition from non-oriented flight to a focused, host-oriented pattern. This shift in behavior signifies the mosquito's ability to identify and track the host within its surrounding environment.

When mosquitoes can spot sites with the resources they need, they fly in that direction. Temperature, female physiological stage, humidity, light, and wind speed affect flight behaviour. *Aedes aegypti* migrates during twilight in a period when humidity is high, and temperatures are low. They are very active on moonlight nights either exhibiting passive migration (drifting) or active dispersal (flying) (Becker et al., 2010). The average flight speed of *Aedes aegypti* is about 5.4km/hr. They avoid flight during windy periods, they only move during this time when they are aided by a tailwind (Becker et al., 2010), either crosswind or downwind, depending on the speed of the wind. They fly upwind when there is less wind speed compared to their flight speed. Flying against the wind may increase the chances of meeting a stimulus deriving from the host. The compound eyes of the mosquito help in visual location by detecting and noting the difference between movement, colour, light, and contrasts. If they are not able to detect this visual information, they rely on perceptible odors and fly with the available cues perceived and move to the source. This source can be drinking water or a nectar source. Mosquitoes are very sensitive to minute environmental change, especially to carbon dioxide concentration. The female mosquito is stimulated by the host odor only if they occur in a unique mixture unique to the host which is why it can distinguish between diverse host organisms (Paaijmans et al., 2013). Through dispersal, these blood-sucking insects become close to appropriate signals from a host animal. It is, therefore, likely, that species in areas where hosts are few tend to have stronger migration urges than those in host-prone environments. *Aedes aegypti* which breeds in the vicinity of its host usually migrate less than 500m for this same reason (Paaijmans et al., 2013).

2.6.3 Feeding and Foraging of *Aedes aegypti.*

Upon emergence, adult mosquitoes require a source of energy to respond to stimuli and sustain their activities. Both male and female mosquitoes obtain carbohydrates from various sources such as plant juice, tree sap, honeydew, floral nectar, and plant stems and leaves (Kessler et al., 2015). Among these sources, honeydew and nectar are particularly important as they contain amino acids and fructose, which contribute to the longevity of mosquito species (Kessler et al., 2015). In addition to carbohydrates, female mosquitoes also require a blood meal for egg maturation. Their specialized mouthparts, including the proboscis composed of the stylet surrounded by the labium, enable them to pierce the skin of a host. The stylet consists of the labroepipharynx (food duct), hypopharynx (saliva duct), maxillae, and mandibles, with the mandibles playing a role in breaking the skin for the stylet to penetrate. Once successfully inserted, the mosquito utilizes the cibarial and pharyngeal pump to ingest the blood/plant juice mixture (Becker et al., 2020). To ensure the blood remains in a liquid form, female mosquitoes inject saliva containing anticoagulant into the wound. It is this saliva that triggers an immune response in the host, leading to inflammation and the sensation of itching (Becker et al., 2020).

2.6.4 Oviposition

With the help of visual and chemical cues, females can locate suitable oviposition sites. These cues are derived from reflective surfaces, humidity, temperature, salts and organic chemicals (Guha et al., 2012). Chemical characteristics of a habitat, which the mosquito can perceive include ammonia, phosphate, nitrate and dissolved solids (Onchuru et al., 2016). Adult female mosquitoes of *Aedes aegypti* are mostly found breeding in and around households (Overgaard et al., 2017). They are also capable of breeding in the wild (Bargielowski et al., 2011). *Aedes* mosquitoes require a standing water medium to lay their eggs. Therefore, they inhabit containers that have water standing for some time (Dom et al., 2013). Breeding containers include concrete tanks, tyres, discarded bottles and earthen wares (Hasnan et al., 2016; Shultis, 2009). The probability that some eggs may fail to hatch or offspring may not survive to the adult stage is solved by distributing eggs over several breeding sites (Overgaard et al., 2017). The breeding of mosquitoes also depends on the conditions of water that are found in the container types. Mosquitoes breed in the water that has been stored for some time and is devoid of direct sunlight. Certain chemical characteristics of habitats such as temperature and dissolved oxygen are capable of attracting mosquitoes to breed in a particular place (Olayemi et al., 2010).

2.7 Artificial containers as a breeding ground for *Aedes aegypti*

According to Eilers et al. (2013), insects exhibit a preference for oviposition sites that provide optimal conditions for the growth and development of their offspring. This is particularly important because once introduced into unsuitable habitats, juvenile mosquitoes have limited mobility. While natural containers can serve as breeding sites, artificial containers are widely recognized as significant breeding grounds for mosquito species, especially in urban areas (Zahouli et al., 2017). These artificial containers range from discarded items to household objects such as buckets, bowls, tires, barrels, jerricans, and plastic drums, which accumulate water over time due to natural (rainfall) or intentional (water storage) processes (Getachew et al., 2015).

Various mosquito species exhibit the ability to breed in artificial containers, including *Aedes aegypti*, *Aedes albopictus*, and *Culex*, which are important disease vectors worldwide. Among these species, *Aedes aegypti* is commonly encountered, depending on factors such as the type of larval habitat and level of urbanization (SNR et al., 2011). Additionally, other species belonging to the genera *Ochlerotatus*, *Culex*, *Toxorhynchites*, Culiseta, Armigeres, *Lutzia*, *Uranotaenia*, and *Tripteroides* have been found in artificial containers (Vezzani, 2007). Interspecific interactions have also been observed among container-dwelling species, such as the association between *Ae. japonicus* with *Aedes albopictus* and *Ae. Triseriatus* (Armistead et al., 2014). These associations are likely facilitated by adaptive mechanisms that promote coexistence in artificial containers, as highlighted by (Armistead et al., 2014) who found higher abundances of late instars and pupae in *Aedes japonicus* compared to *Aedes albopictus*, contributing to their coexistence.

The presence of physical factors such as precipitation, temperature, and evaporation, as well as biological factors including predation, parasitism, competition, and feeding, collectively influence the number of emerging adult mosquitoes in varied aquatic container habitats (Booth, 2018). Mosquito species possess distinctive abilities to locate these containers based on cues such as rainfall and temperature, which indicate the presence of standing water (Booth, 2018). Although predation and parasitism can occur, their regulatory impact on artificial containers in urban areas may be limited due to isolation from natural areas, lack of vegetation cover, and container dryness, which can affect the development of natural enemies. Food availability also plays a role in regulating mosquito densities, as the presence of stored water used by humans necessitates regular cleaning and maintenance of containers, thereby limiting the habitat suitability for immature mosquitoes (Zahouli et al., 2017).

2.8 Prey-Predator interaction in artificial containers

Predators can have a substantial impact on the diversity of communities and the growth of prey populations (Alto et al., 2012; Benard, 2004). The fundamental environmental basis, including the existence of other species, chemical pollutants, and habitat complexity, may greatly affect how predators affect prey (Booth, 2018). The capture and devouring of prey, a direct deadly action, is responsible for the predators' most evident effects (Alto et al., 2012). The fear caused by predators, however, may have non-lethal impacts that change the phenotypic features of prey and even affect sample size of non-prey animals (Fill et al., 2012). Predators' non-lethal effects may include influencing changes in behaviour, development, growth, morphology and physiology (Werner & Peacor, 2003). Predator-induced alterations in phenotypic features of prey are generally protective tactics (Barry, 1994). It is frequently believed that phenotypic plasticity is responsible for changes in prey features brought about by predators, whether through lethal or non-lethal pathways (Alto et al., 2012). Changes in prey features, however, may result from other phenotypic-changing mechanisms, such as selection among individuals with various phenotypes (Alto et al., 2012). Theoretically and practically, it has been shown that a predator may change the distribution of prey features in a population by selectively removing some prey morphologies (Reznick et al., 1996). Plastic responses within an individual's lifetime may be reversible (behavioral changes) whereas if the traits are heritable, selection effects occur across generations (Alto et al., 2012).

Numerous mosquito species live in relatively uncomplicated communities found in water-filled containers. Predation and competition are examples of biotic interactions that frequently influence mosquito populations and may have an impact on adult features associated with pathogen transmission (Juliano, 2009). Intra- and interspecific competition, for example, have an impact on the performance of the sample size as a whole as well as the Arthropod-borne virus vector competence like the Dengue viruses (Alto et al., 2008). The presence of predators, including *Culex* and *Aedes*, as well as predator cues in the absence of capture and consumption, influence mosquito behavior and life history features (Beketov & Liess, 2007). Therefore, it is anticipated that females selecting oviposition sites will be drawn to locations with abundant resources, few conspecifics and competitors, and few predators. For some mosquitoes, avoiding areas where there are predators is a common and powerful habit (Albeny-Simoes et al., 2014). Particularly the genus *Aedes* appears to exhibit little avoidance of oviposition in the presence of predators (Vonesh & Blaustein, 2010). Nevertheless, numerous mosquitoes live as larvae in relatively tiny, isolated environments (containers), where predators that eat the larvae have the potential to indirectly increase the microorganisms in the water—the mosquito larvae's main food—via a trophic cascade (Albeny-Simoes et al., 2014). Also, upsurges in bacteria could result from the addition of uneaten prey and predator feaces to the water, which acts as substrates for bacterial growth. This effect is most likely to happen in relatively small bodies of water because a small number of predators can have a significant impact on these substrates (Albeny-Simoes et al., 2014; Stav et al., 2000). Many inter and intra predation have been reported from containers. For example, the mosquito *Toxorhynchites rutilus* is noted to consume a wide variety of mosquito larvae including *Aedes albopictus* and *Ochlerotatus triseriatus* (Lounibos & Machado ‐ Allison, 1983). The corethrellid midge (Corethrella appendiculate) is also a small ambush predator that significantly reduces the abundance by consuming mosquito larvae (Alto et al., 2005; Lounibos & Machado ‐ Allison, 1983). Other known known predators of mosquitoes include tadpoles, larvivorous fish, dragonflies, and damselflies (Bowatte et al., 2013; Cavalcanti et al., 2007; Sebastian et al., 1980). Predation rates of the sexes may vary depending on behavioural, morphological, and physiological modifications that exist between male and female insect prey. Predation may have different effects on the sexes for prey that is sexually dimorphic and protandrous, such as many mosquitoes.

2.9 Medical importance of *Aedes* mosquito

Pathogen transmission and acquisition are greatly enhanced by the blood-feeding habits of mosquitoes. The sucking of blood introduces medically important parasites and pathogens which include viruses, protozoans, bacteria, and nematodes (Becker et al., 2020). These disease-causing microorganisms are responsible for some grave human diseases like malaria, Zika, yellow fever, dengue, chikungunya, West Nile and filariasis (Becker et al., 2020; Caraballo & King, 2014). Over 3 billion people comprising people from tropical and subtropical regions are threatened by the existence of mosquitoes. These vectors have influenced the economic, social and political scope of mankind (WHO, 2017). One of the blood-sucking mosquitoes that transmit dengue, yellow fever, and zika, which have reemerged in developing nations and constitute a concern for epidemic outbreaks in developed nations, is *Aedes aegypti*.

2.9.1 Yellow Fever

Yellow fever (YF) virus is spread in central and southern America as well as broad portions of Africa despite the availability of an effective vaccine (Becker et al., 2020). It is historically the most significant and dangerous infection caused by a mosquito which gained its ‘yellow’ name from the jaundice signs that affect patients. The yellow fever virus occurs in both the enzootic (kept in monkey populations by forest mosquitoes) and an epidemic form (spread through human populations by domestic forms of *Ae. aegypti*) (Monath, 1994). The epidemic form is the most efficient because it entails an urban cycle where the mosquitoes can readily enter a house, feed on humans, and find oviposition sites in artificial containers. Yellow fever is a hemorrhagic disease that causes about 5-75% per cent mortality in infected humans (Thomas et al., 2012). The virus first appeared among monkeys in Central Africa and mosquitoes that dwelled in the forest canopy (Barrett & Higgs, 2007). The virus was first isolated in Ghana in the year 1927, when a rhesus monkey was injected with blood with yellow fever. The spread of the virus from Africa to other parts of the world occurred over 400 years ago. The trading of slaves in ships in virus-infested cargoes of slaves and the water barrels found in these cargoes helped in the rapid establishment of the vector and the virus. In recent decades, the rapid development of cities provides a wide range of habitats for these mosquitoes. Recent outbreaks have been reported in some African countries (the Democratic Republic of the Congo, Nigeria, Angola, Uganda, and Brazil) (WHO, 2016b). In Asia, the virus is absent probably because of human exposure to other related viruses or the vectors may be less efficient (Sacchetto et al., 2020; Vasconcelos & Quaresma, 2022)

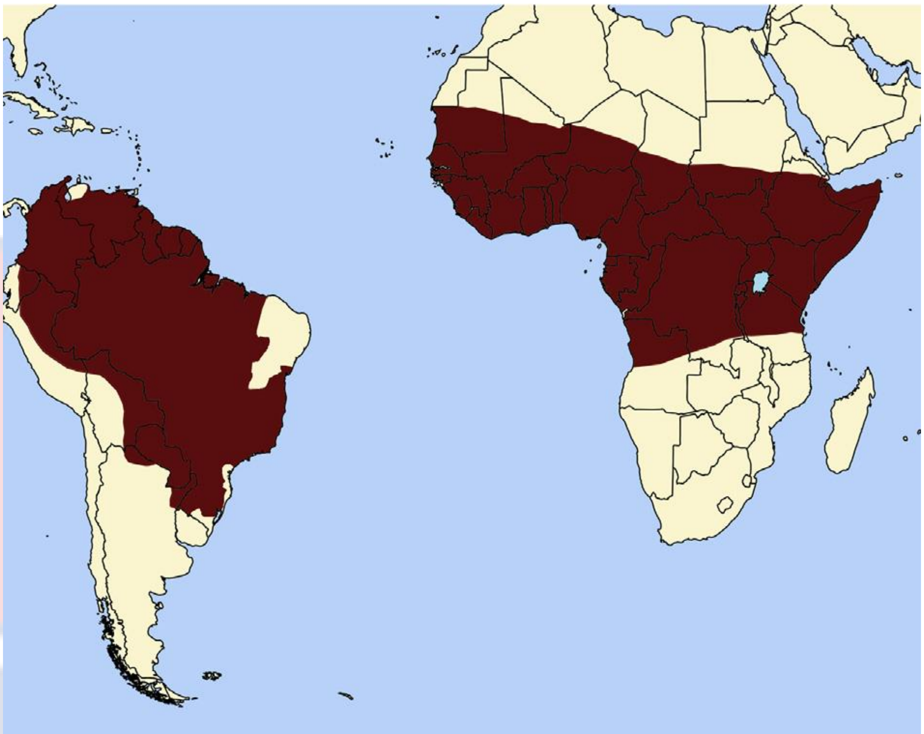

Figure 7: Human yellow fever cases distributed geographically. Based on information from the Centers for Disease Control and Prevention in the United States and the World Health Organization (WHO, 2016b)

2.9.2 Dengue virus

The dengue virus is represented by four serotypes (DEN 1, DEN 2, DEN 3, DEN 4) which are closely related (Gubler, 1998; Innis et al., 1988). Dengue virus infestations are known to be found in Southeast Asia, Africa, Central America and the Caribbean basin (Ramos-Castañeda et al., 2017). It is characterized by severe headache, rash, fever, and excruciating pain in muscles and joints starting 5-7 days after an infective bite (Ramos-Castañeda et al., 2017). Whereas rashes may appear on the lower limb and chest for others, some might have widely spread rashes over the body. If one can recover from one dengue serotype, a long-life immunity against that virus may occur. However, immunity against the other three serotypes might only be partial and following infections with different serotypes maximizes the risk of dengue hemorrhagic fever (DHF). DHF is a potentially deadly complication involving high fever and haemorrhagic conditions (liver enlargement and circulation failure). The sudden rise in temperature marks the start of the illness which sometimes is followed by facial rush and convulsion. This can last for about 2-7 days. When all these symptoms and signs take place without any medical intervention, the patient can quickly enter a critical state of shock leading to death within 12-24 hours (Becker et al., 2020; Martina et al., 2009). However, the patient can recover when the appropriate medication is taken. Dengue and DHF are predominantly in urban and many semi-urban areas. Every year, there are over 390 million dengue cases, of whom 90 million have clinical symptoms, 500,000 have severe symptoms, and 2.5% of cases result in death, with a disproportionately high percentage of children (Bhatt et al., 2013). The virus is mostly amplified by infected people who act as a source of the infection for uninfected mosquitoes. During the 2–7 days of a fever, the virus circulates in the blood of the infected person. When feeding on the person during this time, *Aedes* females contract the virus.

2.9.3 Zika virus

Zika virus was initially found in Uganda in 1947, from a rhesus monkey in the Zika Forest (Wikan & Smith, 2016). The first human cases were documented in Africa in 1947 and the first major outbreak of humans occurred in Micronesia in 2007 (Wikan & Smith, 2016). Now, it has spread throughout major parts of Africa, southern Asia and the South Pacific Island. Zika infection in humans presents itself as a mild illness, including headache, rash, fever and sometimes joint pains (Yuan et al., 2017). More than a million probable cases were recorded in Brazil in 2015, while Colombia was the source of indigenous transmission the same year. By 2016, at least 33 other nations and territories in the Americas had also been infected with the virus (Becker et al., 2020). An unusual characteristic feature of the Zika virus is that it is capable of being transmitted sexually between humans (Sikka et al., 2016). Since a vaccine is not yet available, treating symptoms, preventing mosquito bites, and reducing sexual transmission are the main focuses of Zika treatment, prevention, and control.

2.10 Vector control and Insecticide resistance

The conscious effort to manage vector populations in order to reduce or bare the transmission of vector borne diseases has in recent years failed to prevent epidemics and halt geographical spread of important arboviruses (Achee et al., 2019). These methods of controlling vectors include the elimination of larval habitats, larviciding with insecticides, the use of biological agents and application of adulticides (WHO, 2009). The use of insecticide among these strategies has been the go-to option because of how quickly it can inactivate the vector. These insecticides are administered in the form of mass spraying, indoor residual spraying, insecticide treated nets and long-lasting insecticide treated bed nets. Nevertheless, there have been various challenges that accompanies the use of insecticides and renders them ineffective (Zhu et al., 2016). Insecticide resistance has been a major hindrance to vector control strategies of arboviral diseases considering the high dependence on insecticide usage (Kawada et al., 2016). According to Zhu et al. (2016), insecticide resistance not only compromises the effectiveness of vector control but also impacts the volume and frequency of insecticide application. Mosquitoes have developed this resistance as an evolutionary mechanism, taking advantage of their short life cycles and high reproductive capacity, which allows for rapid selection of resistant individuals (Naqqash et al., 2016; Karunaratne et al., 2018). The development of insecticide resistance is influenced by a combination of genetic, biological, and environmental factors (Karunaratne et al., 2018). Resistant mosquitoes can survive exposure to insecticide doses that would be lethal to other mosquitoes of the same species. This poses a significant challenge to the effectiveness of insecticide-based control strategies and underscores the need for alternative approaches in vector management.

2.10.1 Insecticide resistance in *Aedes* mosquitoes

About six groups of insecticides including carbamates, organophosphates, organochlorines, pyrroles, pyrethroids and phenyl pyrazoles are employed worldwide in mosquito vector control programmes (WHO, 2016a). According to Karunaratne et al. (2018), resistance has been reported towards organophosphates, organochlorines, and carbamates. From these three groups of insecticide came the well-known DDT (organochlorine) which usage was later discouraged and reverted by WHO in 1976. Mosquitoes continues to develop resistance to pyrroles, pyrethroids and phenyl pyrazoles. Many *Aedes* mosquitoes have garnered mechanisms to evade insecticide toxicity through resistance thereby affecting the success of control measures. Worldwide report on insecticide resistance in *Ae. aegypti* and *Ae. albopictus* to almost all six groups of insecticide have been reported. For instance, *Ae. albopictus* from Sri Lanka (Dharshini et al., 2011) and Cameroon (Kamgang et al., 2011) showed resistance to 4% DDT after exposure for 1 h. Even though many insecticides exist, pyrethroids are the common ones used for the control of *Aedes* mosquitoes. Resistance to pyrethroids by both *Ae. aegypti* and *Ae. albopictus* are sporadic with *Ae. aegypti* showing active resistance particularly against neurotoxic active ingredients (Vontas et al., 2012). In Africa, reports on pyrethroid resistance by *Ae. aegypti* has been reported. A study conducted by Kudom (2020), found varying level of resistance to four pyrethroid insecticide including Deltamethrin 0.05%, Permethrin 0.75%, Cyfluthrin 0.15%, Etofenprox 0.5%. Also, recent studies conducted in Ghana found *Aedes aegypti* populations highly resistance to DDT (Owusu-Asenso et al., 2022). According to Kawada et al. (2016), high frequencies of point mutations at the voltage-gated sodium channel (F1534C) and one heterozygote of the other mutation (V1016I) which is the first detection on the African continent were detected in some resistance colonies. Insecticide resistance of *Aedes aegypti* in Ghana may be attributed to F1534C kdr-mutation with an allele frequency of 35% and metabolic detoxifying enzyme activities (Kudom, 2020).

2.11 Control of Mosquitoes

Mosquito control is important because it helps to minimize the population of mosquitoes in a place. It also helps to prevent mosquitoes from causing nuisance and biting people by minimizing mosquito-vertebrate contact. In recent times, the main objective in mosquito control is the reduction of mosquito abundance and prevalence of diseases using control programs such as integrated pest management. Nonetheless, other means of mosquito control exist, and they include personal protection, chemical control methods, biological control methods and genetic control methods.

2.11.1 Personal protection

A crucial element in the control of mosquito nuisance and disease carried by mosquitoes is personal protection (Becker et al., 2010). Personal protections are usually the most simple and basic approach used against mosquito species. Window screens have been used in houses and shelters for animals to prevent mosquitoes from entering. Furthermore, measures such as wearing clothes to cover bare skin and staying indoors are advised to be used. Again, people have been encouraged to use bed nets to prevent mosquito bites. These nets have been treated with synthetic pyrethroids that kill or repel mosquitoes that come around it. There are also mosquito repellent creams that are applied to the skin to prevent mosquitoes from landing on the skin.

2.11.2 Chemical control method

Chemical control methods are commonly used to combat mosquito populations by employing insecticides that target either the larvae or the adult mosquitoes (Becker et al., 2010). Larvicides are specifically designed to be applied to water bodies or stagnant water where mosquito larvae are developing, while adulticides are used to treat the air or resting places of adult mosquitoes. Various equipment and techniques are utilized for the application of these chemicals, such as hand-operated compression sprayers, mist blowers, aerosol generators, thermal foggers, and aerial application equipment (Becker et al., 2010). The residual insecticide can persist on surfaces for a period, providing extended effectiveness. However, the development of resistance to insecticides by both larval and adult mosquitoes has been reported (Cui et al., 2006; Liu et al., 2006). Moreover, adult mosquitoes can modify their behavior, such as changing their feeding or resting habitats, to avoid contact with the chemicals.

Despite their efficacy, the use of chemical control methods has raised concerns regarding their potential environmental harm, including contamination of land, water, and air, as well as their adverse effects on non-target populations, particularly humans (Esmaili et al., 2021). The emergence of insecticide resistance has further compounded these challenges. Additionally, the application of synthetic repellents to exposed skin is a commonly employed strategy to prevent mosquito bites and reduce the transmission of mosquito-borne diseases (Norris & Coats, 2017). However, there are ongoing concerns about the safety and potential toxicity associated with the use of these repellents.

2.11.3 Biological control method

Biological control strategies, involving the use of pathogens, competitors, predators, and toxins, have been implemented to reduce mosquito populations while minimizing adverse effects on the ecosystem (Becker, 1997). The focus of many studies on mosquito biological control has been primarily on the aquatic stages of mosquitoes, aiming to protect human populations from mosquito-borne diseases while preserving biodiversity and avoiding ecotoxicological impacts (Chapman, 1985; Hemingway, 2005). Various predators, including birds, hydra, flatworms, insects, and dragonflies, have been observed to feed on mosquito larvae (Becker et al., 2020; Lamborn, 1890). Additionally, introduced aquatic predators such as mosquito fish and killifish have demonstrated effective predation on mosquitoes in water bodies (Benelli et al., 2016; Walton, 2007). Predatory copepods have also shown successful implementation as mosquito predators (Lacey & Orr, 1994; Rey et al., 2004). Notably, *Toxorhynchites* larvae, known as predatory mosquito larvae, have exhibited high predatory efficiency for biological control purposes (Lacey & Orr, 1994). *Lutzia trigripes* larvae, a predatory mosquito species of the *Culex* genus, have demonstrated effective predation on *Aedes aegypti* larvae compared to *Anopheles gambiae* and *Culex quinquefasciatus* (Appawu et al., 2000). A key advantage of biological control methods is the preservation of existing predators, which continue to prey on newly hatched mosquito larvae even after control operations have ceased, thereby enhancing the effectiveness of the control measures.

2.11.4 Genetic control of mosquitoes

Due to the high rate of sexual reproduction and genomic plasticity in mosquitoes, traditional approaches to mosquito control have proven largely unsuccessful (Wilke & Marrelli, 2012). Concerns about environmental impact and the development of tolerance to toxins have limited the effectiveness of insecticide-based methods. As an alternative, genetic control strategies are being explored to regulate mosquito populations by introducing heritable factors that reduce pest harm (Alphey, 2014). These genetic control tactics rely on the dissemination of desired traits through mating or inheritance within the target population.

Genetic control measures differ from other biological control strategies, such as the use of predators, parasitoids, or infectious agents, as they specifically depend on mating and vertical transmission. Instead of employing external agents, the mosquitoes themselves are genetically modified to possess desired traits, effectively turning them into biocontrol agents. The Sterile Insect Technique (SIT) is one such genetic control strategy that involves mass rearing, radiation-mediated sterilization, and the release of large numbers of sterile male insects (Wilke & Marrelli, 2012). While conventional SIT has encountered limitations in mosquito control, the release of insects harboring a dominant lethal gene (RIDL) offers a promising solution by combining species-specific benefits with ecological advantages, thereby overcoming many of the challenges associated with traditional SIT methods.

2. 12 Chapter summary

This chapter explores the impact of the COVID-19 pandemic, with a focus on the biology and ecological aspects of *Aedes aegypti* mosquitoes. It highlights the implications of the pandemic on disease surveillance and control efforts, particularly for mosquito-borne diseases. The medical significance of *Aedes aegypti* as disease vectors is emphasized, along with the importance of effective prevention and control measures.

CHAPTER THREE

MATERIALS AND METHOD

This chapter discusses the materials and methods used in this study. The sections below contain detailed descriptions of the materials and methods used in the experiment.

3.1 Study Area

A field survey was conducted in Duakor, a small community located between latitude 5°06′N and 5.1°N and longitude 1°15′W and 1.25°W in the Cape Coast Metropolitan Area in the Central Region of Ghana. The study area is located along the coast of Cape Coast and shares a direct border with the Gulf of Guinea (Figure 8). The area is located on the southern outskirts of the semi-deciduous rainforest, which has two wet seasons per year. With an annual mean rainfall of about 980 mm, the rainfall pattern follows the double maxima (bimodal) rainfall distribution experienced in most parts of southern Ghana. The major rain season begins in March and ends in July, while the minor rain season begins in September and lasts until mid-November. The community was selected based on a previous study (Kudom, 2020) which assessed the same entomological indices before the covid-19 pandemic. This study was conducted from July to September (ending of the major rainy season). The rainfall during the present study and that of the previous study were 12.8 mm and 125.6 mm respectively (Figure 9).

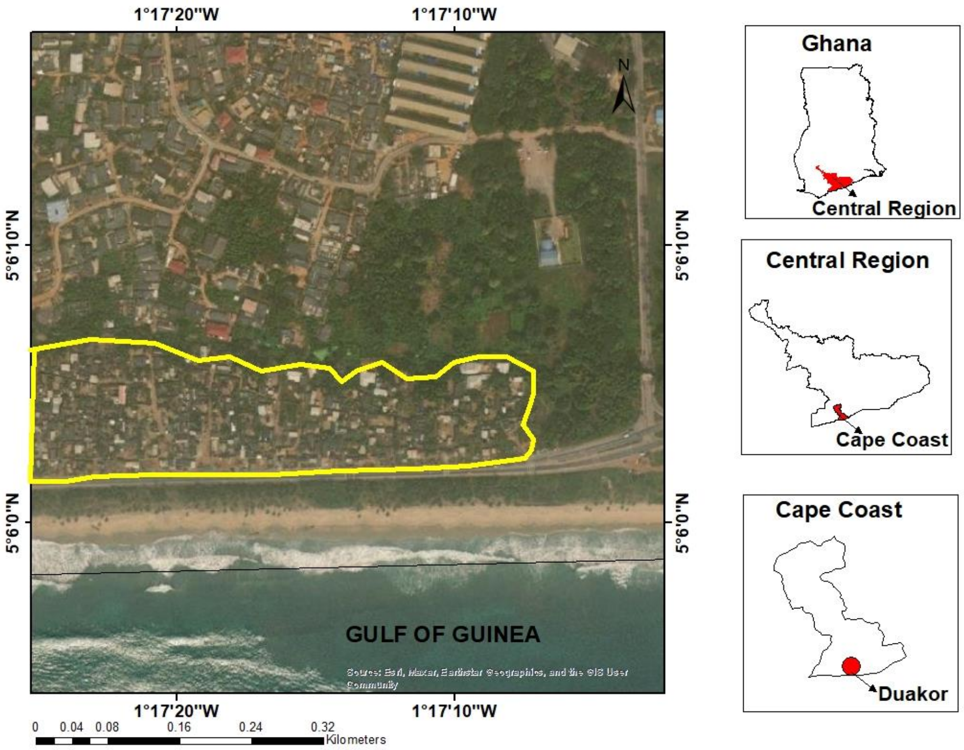

Figure 8: Map showing the Duakor community.

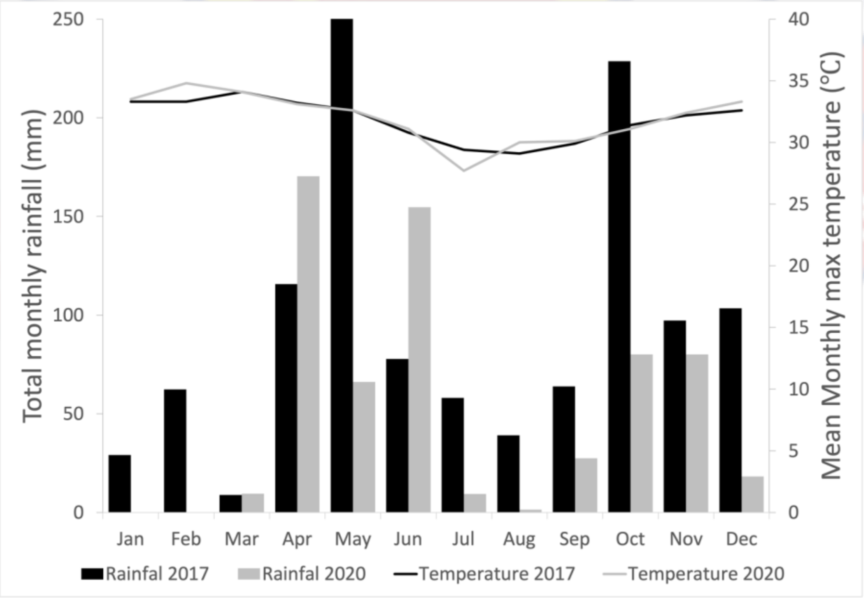

Figure 9: Total monthly rainfall and mean monthly maximum daily temperature recorded for Cape Coast in 2017 and 2020.

3.2 Study design

Surveys of larval and pupal productivity were carried out in households and public spacing following guidelines stated by WHO 2011. Before the survey, a preliminary survey was carried out to count the number of houses in the community for baseline information. The houses were then chosen using a simple random sampling technique.

3.3 Entomological survey

The entomological survey was conducted following the guidelines outlined by the World Health Organization (WHO, 2011) to assess larval and pupal productivity in households and public spaces. A total of 100 houses were randomly selected using a simple random sampling technique. In each selected house, all containers were thoroughly examined for the presence of water, and any containers with water were further inspected for immature forms of mosquitoes. The containers were classified into two categories based on their usage: category A for actively used containers and category B for unused containers. Information on the number of occupants in each house and the types of containers present was recorded. Additionally, the GPS coordinates of each house were obtained, and samples containing mosquito larvae and pupae were collected and transported to the laboratory for further analysis. This entomological survey aimed to comprehensively evaluate mosquito breeding sites, considering container usage, population density, and geographical distribution, in order to gather reliable data on the productivity of mosquito larvae and pupae in the study area, following established guidelines and employing random sampling techniques as recommended by WHO (2011).

3.4 Collection of Larvae and Pupae

A dipper and pipette were used to collect the larvae. For containers that had more than 20 litres of water, pupae were collected by filtering the water with a mesh sieve. Pupae from containers containing fewer than 20 litres of water were picked using a pipette and those from very small containers were emptied into trays. All larvae collected were kept in plastic containers and pupae were kept in emerging chambers, labelled accordingly, and transported to the laboratory. The labels included the location, house ID, container type, date collected, and the GPS taken. All immatures collected were counted and the number recorded.

Figure 10: Various artificial containers in homes from which samples were collected.

3.5 Ecological succession of mosquitoes in plastic domestic containers

A total of 9 plastic containers of size 15L were established. The containers were distributed in a transect and spaced 1m apart (Fig 11). Each container was filled with pipe-borne water to 70% of the total capacity, following a protocol by Andrade (2015) with slight modifications. In the first 3 containers, 2g of yeast was added to the water. The second 3 containers had a lower amount of yeast (0.02g), and the last set of containers had no yeast (0g) in them. The containers were monitored daily for 14 days for the presence of larvae and pupae. The container contents were sieved with mesh and the water was returned to the container. Pupae collected were kept in small cups for emergence. Adults that emerged were taxonomically identified to genus and species level. This experimental setup was repeated once.

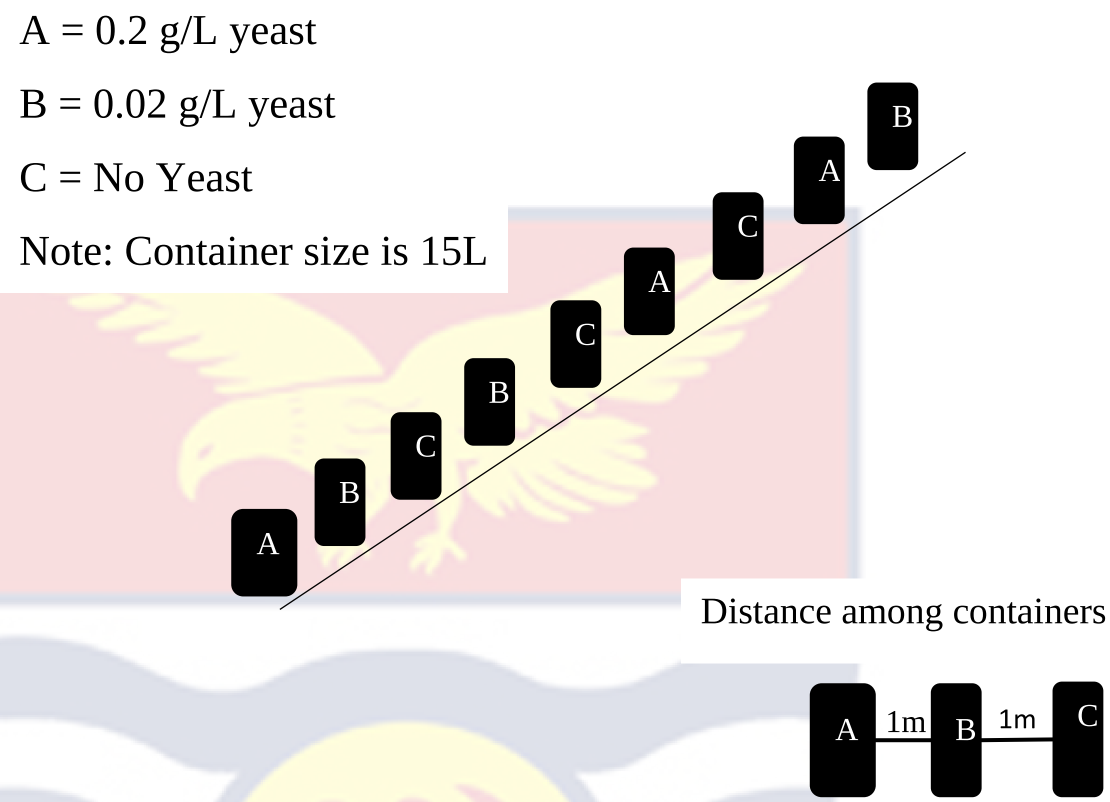

Figure 11: Semi-field experimental design illustration

3.6 Insecticide resistance bioassay

Insecticide resistance levels of *Ae. aegypti* to Deltamethrin (0.05%), DDT (4%), Fenitrothion (0.1%) and Bendiocarb (0.1%) were determined following WHO standard protocols using the WHO impregnated papers and test kits (WHO, 2016a). The first emergence (F0) of *Ae. aegypti* collected as larvae from household water storage containers (HWSC) found inside houses as well as abandoned car tires (ACT) and abandoned plastic containers found outside the residential houses were used for the susceptibility bioassay. About twenty-five, unfed 3-to-5-day-old females were exposed to each insecticide with four replications for each of the three vector populations. The laboratory conditions for the bioassay were 29 ± 1 °C and relative humidity of 75 ± 10%. Mosquitoes were transferred to holding tubes after an hour of exposure, fed with 10% sugar solution and knockdown rates read after one hour. Total death or mortality was read after 24 hours. For control, mosquitoes were exposed to paper without any insecticides.

3.7 Screening for kdr mutations

DNA of one hundred adult female mosquitoes from the three populations (HWSC, ACT, APC) was extracted using the QuantaBio® Extracta kit according to the manufacturer's direction. qPCR melting curve analysis was used to screen for knock-down resistance (kdr) mutations. The Saavedra-Rodriguez et al. (2018) (V410L) and Estep et al. (2017) (V1016 and F1534C) protocols were optimized and modified for this work. For V410L detection, a total of 20µL reaction contained 0.05µM of V410fw and L410fw primers and 0.1µM of primer 410rev, 9.5µL of 2x Sybr Hi-Rox Mix (Bioline), 1µL of genomic DNA and DNase-free water. V1016I detection was done in a 20µL reaction consisting of 8.2µL of 2x Sybr Hi-Rox Mix (Bioline), 0.15µM of Val1016f primer, 0.2µM each of lle1016f and lle1016r primers, 2µL DNA template and DNase-free water. The cycling condition for this procedure is 95ºC for 3minutes, 40x (95 ºC :10sec, 60 ºC:10sec,72 ºC :30sec) 95 ºC with the melting condition of 65º-95º inc 0.2 ºC per 10sec. Each 20µL reaction for V1016I consisted of 8µL of 2x Sybr Hi-Rox Mix (Bioline), 0.15µM of Val1016f primer, 0.2µM each of lle1016f and lle1016r primers, 2µL DNA template and DNase-free water, with the same cycling conditions as the V410L. F1534C was screened in a reaction volume of 20µL containing 8µL of 2x Sybr Hi-Rox Mix (Bioline), 0.3µM of Cys1534+ primer, 0.3µM each of Phe1534+ and 1534- primers, 2µL DNA and DNase-free water. The cycling conditions for F1534C included 3min at 95°C, 37 cycles of (10 sec at 95°C, 10 sec at 57°C, 30 sec at 72°C) and 95 ºC for 10 seconds. A melting curve analysis was performed at the end of the PCR cycle at 65°C - 95 ºC. The melting curve peaks for the mutant gene (resistant) and wild type (susceptible) were 83°C and 86°C for V410L, 80°C and 86°C for V1016I, and 86°C and 82°C for F1534C, respectively.

3.8 Predatory experiments

In the predator-prey experiment, the interactions among larvae of *Aedes aegypti*, *Anopheles coluzzi*i, and *Culex sp* were investigated. The experimental setup involved utilizing both live and dead larvae as prey for the predators. These prey conditions were tested in separate experiments to assess their impact on predator behaviour and feeding patterns. Each experiment consisted of a 200 ml plastic cup filled with 50 ml of tap water, ten first instar larvae as prey and one-third of instar larvae as predators. The prey and the predator tested together were of different species. In separate experiments, live and dead prey from each species (*Aedes aegypti*, *Anopheles coluzzi*i, or *Culex* species) were tested against each predator among the three mosquito species. In the second larval bioassay, ten larvae of first to third instars of *Anopheles coluzzi*i and *Culex* larvae were also used as prey and third instar larva of *Aedes aegypti* as a predator was tested in a separate experiment. Each experiment was replicated five times and each test cup was provided with fishmeal as a source of food. The number of missing prey was recorded for 72 hours. The experiments were conducted under laboratory conditions with a temperature of 29 ± 1 °C, relative humidity of 75 ± 10%, and a 12:12 photoperiod.

Figure 12: Laboratory setup for predator-prey interaction

3.9 Data Analysis

The larval indices were calculated using the following formulas: The House Index (HI) represents the percentage of households that had *Ae. aegypti* larvae; the Container Index (CI) represents the percentage of containers infested with larvae and pupae; and the Breteau index represents the number of positive containers per 100 inspected dwellings (BI). The Mann Whitney and Kruskal Wallis tests were used to determine whether there was a difference in means between interactions. The statistical significance level was set at $p < 0.05$.

CHAPTER FOUR

RESULTS

4.1 Household container survey

In the 100 houses surveyed, 850 water-holding containers were counted. 22% of the containers in 32 houses were positive for mosquito larvae (Figure 8). A total of 2829 larvae and 481 pupae were collected from the positive containers (Table 1). Water storage containers in the houses (Category A containers) harboured 84% and 96% of total larvae and pupae that were collected respectively. *Aedes* larvae constituted 66.8% of the total larvae with 27.4% being *Culex* while 4.3% and 1.45% were *Lutzia* and *Anopheles* larvae respectively. The pupae collected were mainly *Aedes* and *Culex* mosquitoes. The estimated larval indices in the study community were: House index – 34%, Container index – 22.35%, and Breteau index – 2.02.

Table 1: Number of containers found in households with infestations of mosquito larvae and the total number recorded.

Category	**Type of container**	**Number of containers**	**Number of items infested**	**Number of larvae**				**Total larvae**	**Total pupae**
				Aedes	***Culex***	***Lutzia***	***Anopheles***		
A	bucket	306	9	65	0	0	0	65	265
	gallon	18	11	213	340	16	0	569	0
	cement tank	39	20	628	366	53	41	1088	138
	barrel/drum	54	10	629	0	27	0	656	59
	bowls/jars	24	0	0	0	0	0	0	0
Total		441	50	1535	706	96	41	2378	462
B	tires	13	5	214	0	27	0	241	15
	coconut shell	313	0	0	0	0	0	0	0
	bowls/jars	15	5	30	70	0	0	100	0
	discarded bottles	170	130	110	0	0	0	110	4
Total		511	140	354	70	27	0	451	19

4.2 Ecological succession of mosquitoes in plastic domestic containers

Eggs were laid in all the 18 containers that were used for the experiment albeit at different times. Twelve of the containers were first colonized by *Aedes* species while the other of the six containers were first colonized by *Culex* species. A total of 873 pupae were collected in 15 out of the 18 containers at the end of the two 14-day periods. *Culex* mosquitoes constituted 93% of the total pupae collected while *Aedes* mosquitoes made up 5.4% and 1.6% *Lutzia* species. The set of plastic containers that had fresh pipe water without yeast infusion had the least number of mosquitoes. The containers with water having 0.02g/L yeast infusion had the highest number of mosquitoes. Out of these numbers, *Aedes* pupal productivity was highest in the 0.02g/L infusion (3.44) and least in the 0g/L infusion (0.69).

In terms of succession, *Aedes* mosquitoes were the first and only mosquitoes to colonize the containers that had fresh water. Again, *Aedes* mosquitoes were first to colonize the containers with water containing 0.02g/L of yeast infusion followed by *Culex* mosquitoes. The containers with water containing 0.2g/L yeast infusion were first colonized by *Culex* mosquitoes in 5 out of the 6 containers followed by *Aedes* and later *Lutzia* mosquitoes (Table 2).

Table 2: Succession of mosquitoes within a 14-day period in water infused with different concentrations of yeast.

Infusion	**Mean ± SD number of days for first colonizing of mosquito**[*]				**Number of adult mosquitoes**			**Pupal productivity for *Aedes***
	Egg	**Larvae**	**Pupae**	**Species**[#]	**Total**	**Male**	**Female**	
(0.2g/L yeast)	2.8 ± 1.7^{ab}	5.2 ± 1.5^{ab}	10 ± 2.3^{a}	*Aedes*^{2}	15	14	1	1.72
				Culex^{1}	800	397	403	
				Lutzia^{3}	17	15	2	3.44
(0.02g/L of yeast)	1.3 ± 0.8^{b}	3.0 ± 1.1^{b}	6.7 ± 3.6^{a}	*Aedes*^{1}	30	18	12	
				Culex^{2}	4	1	3	0.69
(No yeast)	5.2 ± 3.5^{a}	7.2 ± 3.5^{a}	7.0 ± 5.7^{a}	*Aedes*^{1}	6	0	6	

[*] Values in columns not sharing the same letters are significantly different at the 5% level.

[#] Species with the subscript 1,2 or 3 denotes 1st, 2nd or 3rd to colonize the containers in the experimental groups respectively.

4.3 Insecticide resistance in *Aedes aegypti* population collected from different habitat.

In total, *Ae. aegypti* from the study community were resistant to the four insecticides (Fig 13). However, resistance differed in the *Ae. aegypti* collected from the different breeding habitats (Fig 14). Besides deltamethrin, *Ae. aegypti* collected from the household water containers (HWSC) were more resistant to DDT (P = .002), Fenitrothion 1% (P = .005), and Bendiocarb 0.1% (P = 0.002) than those collected from the abandoned automobile tires (ACT) or plastic containers (APT) outside residential houses. It was more pronounced in Bendiocarb and DDT insecticides (Fig 14). A mean %mortality of 32 ± 20 and 24 ± 14.9 of Bendiocarb and DDT were recorded for the vector population collected from household water storage containers, which were significantly lower than 94 ± 2.8 and 74.7 ± 9.2 for the population collected from automobile tires and 88 ± 45.7 and 60 ± 12.7 for those collected from abandoned plastic containers against the same insecticides respectively. Similarly, the vector population collected from outside the residential houses were almost susceptible (ACT: 97.3 ± 4.6; APC: 98 ± 2.3) to Fenitrothion whereas the insecticide caused a mean %mortality of 74 ± 12.4 to the vector population collected from HWSC. Among the 100 mosquitoes, 98, 97 and 96 were successfully genotyped for the F1534C, V410L and V1016I kdr mutations, respectively (Table 2). The homozygote mutant at codons 1016 and 410 was absent whereas the wild type of F1534C was rarely (2%) observed. F1534C mutation was widely distributed in the study population with the heterozygote F1534C genotype mutant dominating in the population. Higher allelic frequency of F1534C mutation was observed in the *Ae aegypti* population collected from HWSC compared to those collected outside residential houses (ACT and APC) (Table 2) (X2 = 33.93, df = 2, p < .0001).

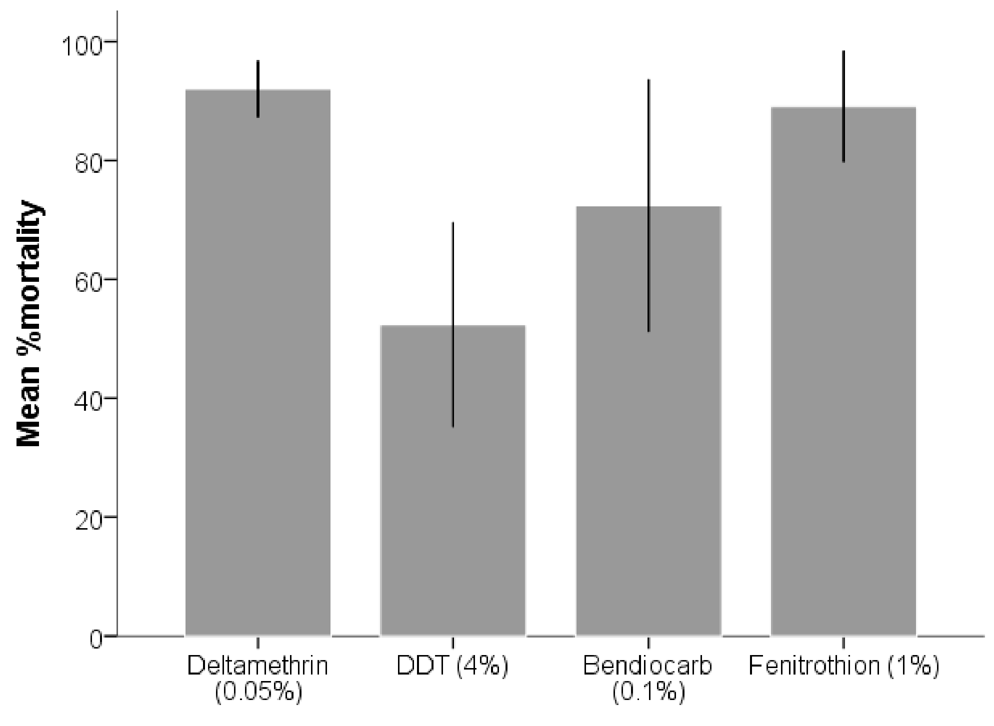

Figure 13: Susceptibility of female adult *Aedes aegypti* from Cape Coast to Deltamethrin (0.05%), DDT (4%), Fenitrothion (0.1%) and Bendiocarb (0.1%) using WHO test kits.

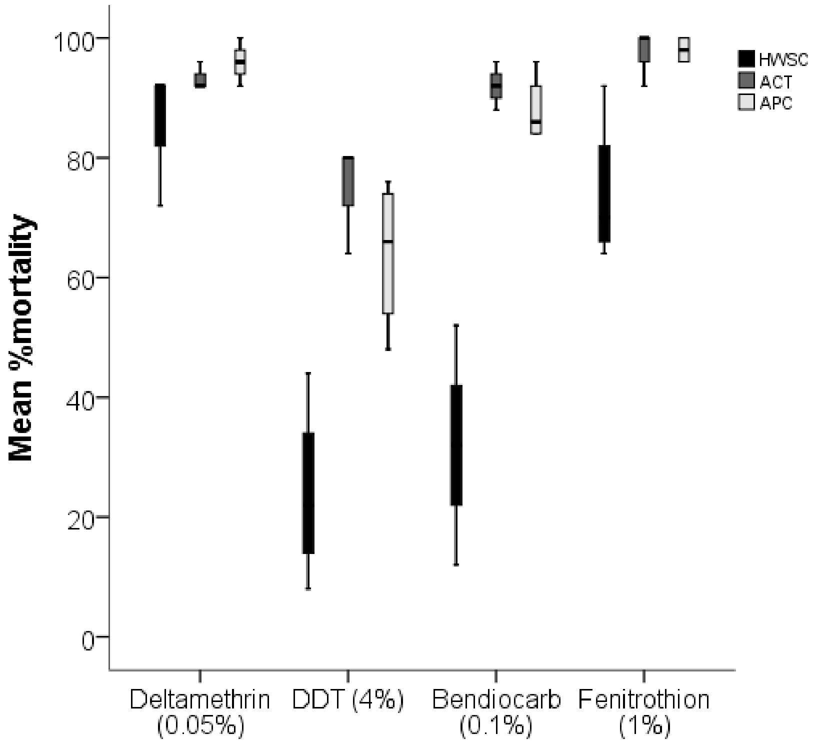

Figure 14: Susceptibility of female adult *Aedes aegypti* collected from household water storage containers (HWSC) (inside houses) and those collected from abandoned car tires (ACT) and abandoned plastic containers (APC) (outside residential houses) to Deltamethrin (0.05%), DDT (4%), Fenitrothion (0.1%) and Bendiocarb (0.1%) using WHO test kits.

However, V1016I kdr mutation was absent in the population from the HWSC whereas low allele frequency was observed in the populations collected outside the residential houses (APC and ACT). Nine genotypes across the three kdr mutations were identified from the 93 *Ae. aegypti* population from Cape Coast (Fig 15). The genotype FC/VL/VV was the most frequently observed (37%, n = 93). Individuals that were homozygous for the three kdr mutations were absent. Overall, the genotype frequency distribution of V1016I was consistent with Hardy-

Weinberg equilibrium (X2 = 0.233, df = 1, p = .63). However, the genotype frequency distribution of F1534C and V410L were not in Hardy-Weinberg equilibrium (X2 = 7.6, df = 1, p = .006; X2 = 28.3, df = 1, p < .0001).

Table 3: V1016I, V410L and F1534C genotype numbers and the allelic frequency of the I, L and C mutations of *Ae. aegypti* collected from different breeding habitats.

Source of mosquito	**V1016I**			**V410L**			**F1534C**			**Kdr allele frequencies**		
	V/V	**V/I**	**I/I**	**V/V**	**V/L**	**L/L**	**F/F**	**F/C**	**C/C**	**I**	**L**	**C**
Household water storage containers	23	0	0	10	12	0	0	2	22	0	0.27	0.96
Abandoned car tires outside houses	43	7	0	9	41	0	2	32	16	0.07	0.41	0.64
Plastic containers outside houses	21	2	0	10	15	0	0	16	8	0.04	0.30	0.67
Total	87	9	0	29	68	0	2	50	46	0.05	0.35	0.72

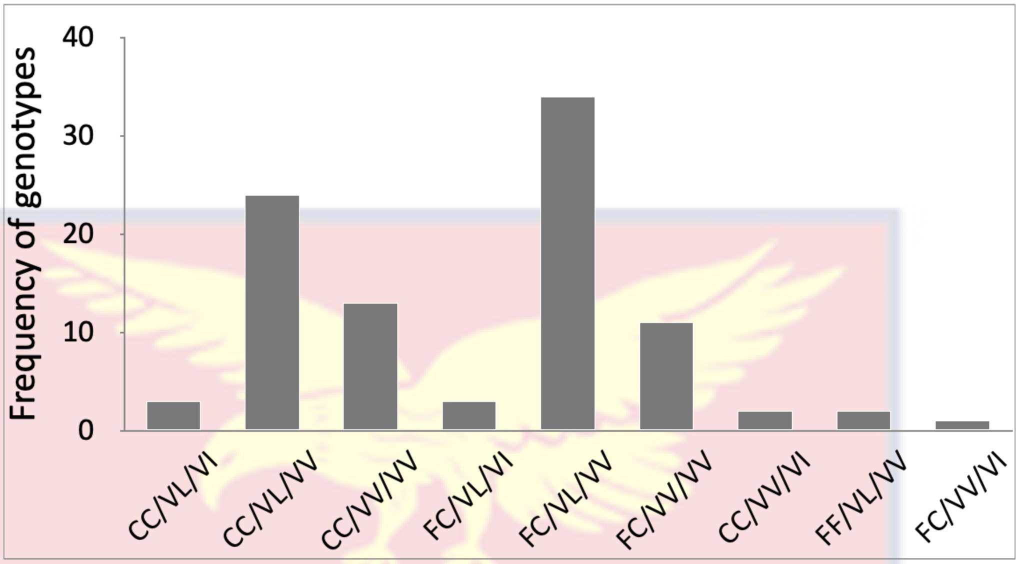

Figure 15: Frequency of the combined V1016I, V410L and F1534C genotypes of *Aedes aegypti* from Cape Coast.

4.4 Predator-Prey interactions among *Anopheles*, *Culex*, and *Aedes* larvae in a laboratory setting

The first part of the experiment on the predator-prey interactions among *An*. *coluzzi*i, *Ae*. *aegypti* and *Culex* species showed that the 3rd instar larvae of each species consumed the 1st instar larvae of the other species. The 3rd instar of *Ae*. *aegypti* consumed more than 8 first instar larvae of either *An*. *coluzzi*i or *Culex* species within 72 hours. *An*. *coluzzi*i, on the other hand, consumed 1-4 of either *Ae*. *aegypti* or *Culex* prey whilst *Culex* consumed 4-8 of the prey within 72 hours. However, *Culex* as a predator consumed 2 times more of the *An*. *coluzzi*i larvae than *Ae*. *aegypti* larvae (T-test, p = .029). Interestingly, when dead prey was offered, *An*. *coluzzi*i, *Culex* sp. and *Ae*. *aegypti* as predators consumed the same number of prey (8-10 larvae) within 72 hours (ANOVA, p = .77). *An*. *coluzzi*i as a predator consumed about 2.5 times more of the dead prey than the live prey (Figure 16).

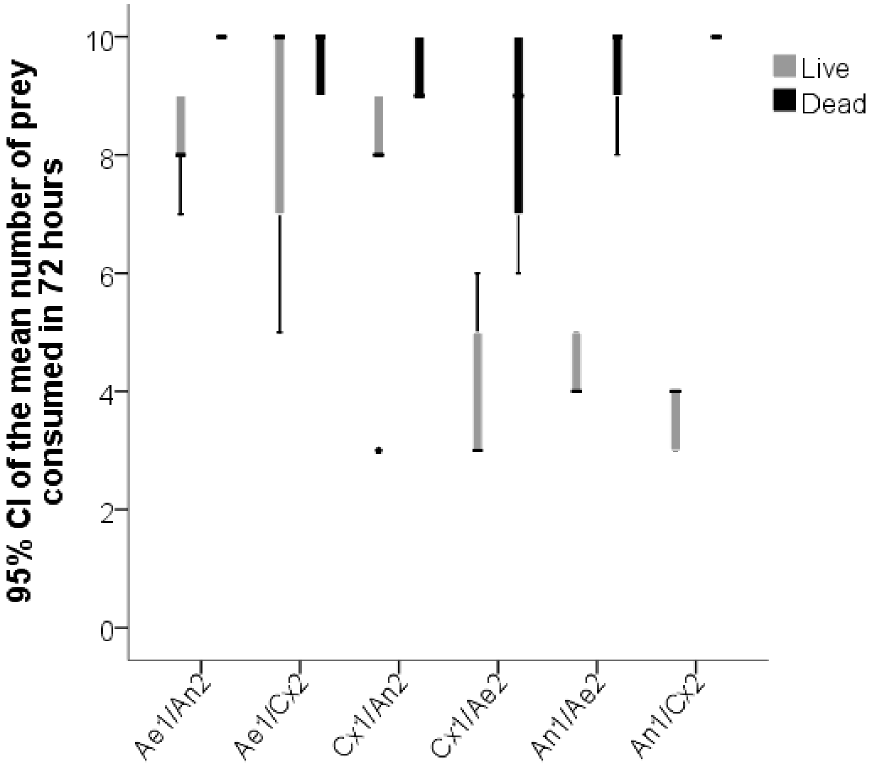

Figure 16: Boxplot of the number of dead or live first instar larvae ingested within 72 hours by a single-third instar larva of *Aedes aegypti*, *Anopheles coluzzi*i and *Culex*. {(*Ae*: *Aedes aegypti*; *An*: *Anopheles coluzzi*i; *Cx*: *Culex* species) (species with number 1 in front indicates the predator while the species with number 2 indicates the prey)

CHAPTER FIVE

DISCUSSION

5.1 The risk of an outbreak of *Aedes*-borne diseases in Covid 19 restriction period.

The study assessed the risk of transmission of *Aedes*-borne arboviruses in the Duakor community in Cape Coast during the Covid-19 lockdown period in 2020 based on three larval indices: container index, house index and Breteau index. The results were compared with a previous study conducted in 2017 in the community before the Covid-19 pandemic. The assumption was that Covid-19 mitigation strategies could increase the demand for water, which in turn, might influence significant changes in householder behavior towards water storage practices. A change in behavior that would lead to increased use of water storage containers may promote the breeding of *Ae*. *aegypti* and other container-breeding mosquitoes. This may lead to an outbreak of mosquito-borne diseases. Among the three indices determined in the study, only the container index marginally exceeded the WHO threshold. Both Breteau and house indices were below the WHO threshold. Contrary to the assumption made, the result on the larval indices suggested a lower risk of transmission of *Aedes*-borne diseases in the Covid-19 restriction period compared to the 2017 survey period. In the previous study in Cape Coast in 2017, discarded and abandoned containers/receptacles (category B) were important breeding grounds in all the four communities and accounted for 60% of the infested containers. Water storage containers were less important in the three communities that had better access to pipe water. However, water storage containers together with discarded items (category B items) were equally important in Duakor community (the study area of the present study), which had the least access to piped-borne water. Duakor accounted for 77% of infested household water storage containers among the four study communities (Kudom, 2020) In this study, fewer category B items were observed to have been infested with mosquito larvae, and this may have contributed to the lower larval indices compared to the study before the pandemic (Kudom, 2020). The category B containers contributed only 4% to the total pupae collected in the study. During the Covid-19 restriction period, there were frequent clean-up exercises in both public and residential areas (Asante & Mills, 2020) leading to the removal of discarded

items from the compounds. This may have improved the sanitation condition in the study community resulting in fewer mosquito-infested category B items. The total precipitation during the present survey period (July-September) was extremely lower than the survey period (April-July) in the previous study (Fig 9). This could also have contributed to the differences in the infested category B items recorded between the two studies. However, we think precipitation might not have contributed much on the infestation of category B items in the present study. For precipitation to influence the level of infestation of category B items, discarded containers must first be available. Unlike the previous study, discarded items were extremely low in this study due to the clean-up exercise and other factors mentioned earlier. It is worth mentioning that the bottles and coconut shells, which formed 95% of category B items in the present study were technically not discarded or abandoned items. The bottles and the coconut shells were packed because of restricted movement during that period. They were waiting to be transported to their destinations. Furthermore, the Government of Ghana's free water initiative greatly improved water accessibility in Ghana during the Covid-19 restriction period and this initiative may have minimized water stress in the community. The limitation of this study was that the larval survey was conducted only in one community. A larval survey from different communities with different levels of accessibility to pipe-borne water may have provided a better picture of the situation than one community. However, Covid-19 restrictions made it very difficult to include other communities during the study period. Another limitation of the study was that the infectious status of the mosquitoes collected were not determined. Although, entomological surveillance tools such as the use of larval or pupal indices to assess the risk of outbreak of *Aedes* borne diseases remain important, especially in low endemic areas like Ghana, information on the infectious status of the mosquitoes could have given a better picture of the situation. A future study that would determine the infectious status of the vector together with the entomological indices may give a better risk assessment of the situation in the study area. Notwithstanding, health education on mosquito prevention in the community is still needed. All the houses that were surveyed in the study community had water storage containers that were either partially covered or not covered at all. The practice of storing water has been reported to be a reason for the increase in mosquito productivity (Forsyth et al., 2020; Overgaard et al., 2017). It was therefore not surprising that the majority (86%) of the mosquitoes collected in the study came from water storage containers. Health education such as the promotion of the use of lids to cover long-term water storage containers can drastically reduce the presence of *Aedes* larvae in the community (Pinchoff et al., 2021). The promotion of this simple technique could have been incorporated into the Covid-19 public health campaigns. A recent study in Cape Coast found that intensive public health education during the pandemic has greatly improved health knowledge among the population (Saah et al., 2021). However, the improved knowledge was related to chronic diseases, nutrition, hygiene, and risky health behaviors. Improving the knowledge of anti-mosquito strategies in the community may also help further lower the population density of *Aedes* mosquitoes and ultimately the risk of an outbreak of *Aedes*-borne diseases.

The most productive containers are identified by determining the relative contribution that a particular container type makes to the overall production of *Aedes* pupae (WHO, 2011). The most productive containers from this study were concrete tanks and medium to large plastic containers. However, buckets were the most productive contributing to more than 50% of the pupae collected in this study. This is consistent with the findings from other countries where concrete washbasins, drums and buckets are the most productive household containers for *Aedes* mosquitoes (Islam et al., 2019; Quintero et al., 2014). Mosquito larvae feed primarily on aquatic microorganisms that colonize detritus in breeding habitats and the chemicals produced by the microorganisms also influence the oviposition behaviors of adult female mosquitoes (Walker et al., 1991). Under normal conditions, it may take some time for microorganisms to colonize and build up their population in water-filled containers. It is therefore not surprising that this study and many other findings have shown containers (e.g., cement tanks, barrels) that are used to store water for a long period to be more productive than containers (e.g., bowls, jars) that are mostly used to store water for short period (Islam et al., 2019; Overgaard et al., 2017; Romero-Vivas et al., 2006). Targeting these containers for vector control may greatly reduce the population of *Aedes* in the community. Although *Ae. aegypti* constituted the major mosquito larvae collected, *Culex*, *Anopheles* and *Lutzia* mosquitoes were also found in the household containers. These species are known to co-exist in breeding habitats in Ghana. *Lutzia* is a well-known mosquito predator whilst *Aedes*, *Culex, Anopheles* are also known to exhibit interspecies predatory activities (Appawu et al., 2000; Muturi et al., 2014). Indeed, the fourth instar *Lutzia* larva can consume up to 24 fourth instar *Aedes* larvae per day (Pramanik et al., 2016). In a mixture of different mosquito larvae, Appawu et al. (2000) reported that *Lutzia* larvae exhibited a significant preference for Ae. *aegypti* larvae compared to *An. gambiae s. I.* and *Cx quinquefasciatus.* Interaction of these mosquito larvae in the containers may have a significant influence on the resulting adult populations of *Ae. aegypti*. Further study is needed to elucidate the impact of such complex interaction among mosquito larvae in household containers on *Aedes*’ productivity.

5.2 Insecticide resistance and Kdr mutations

Chemical control remains an important part of most control measures against mosquito vectors. In this study, *Ae. aegypti* population from Cape Coast was highly resistant to deltamethrin (pyrethroid), DDT (organochlorine) and bendiocarb (carbamate). But exhibited moderate resistance to fenitrothion (organophosphate). Resistance of the mosquitoes to the different classes of insecticides is worrying and could affect the efficacy of insecticide-based control tools (Marcombe et al., 2011). The level of resistance to deltamethrin and DDT is consistent with the results from other parts of the country (Kawada et al., 2016; Kudom, 2020; Owusu-Asenso et al., 2022). However, the high level of resistance to carbamate (bendiocarb) was unexpected. Unlike pyrethroid insecticides, carbamate insecticides are not normally employed in household vector control tools. Furthermore, urban agriculture with its associated insecticide use are not important activity in Cape Coast Metropolis. Thus, the source of resistance particularly to carbamates is not clearly known. In fact, the source of resistance in *Ae. aegypti* population from Ghana and many African countries remains less obvious (Weetman, Kamgang, et al., 2018). Nonetheless, domestic use of insecticides could be a very important source, particularly for pyrethroid insecticides (Boakye et al., 2009; Kudom et al., 2013; Toé et al., 2022). The result from the insecticide bioassay showed that the mosquitoes collected from containers located inside houses were more resistant to the insecticides than the mosquitoes collected from containers located outside houses (Fig 12). This differential resistance could be explained by the heavy use of insecticides in houses (Boakye et al., 2009; Kudom et al., 2013; Toé et al., 2022). Most houses depend on daily use of insecticide-based tools such as insecticide treated nets and mosquito coils for protection against mosquito bites. This could put *Ae. aegypti* that lives and oviposit in containers located in houses under higher insecticide selection pressure than the mosquitoes that breed in containers outside houses. Understanding the spatial distribution of insecticide resistance among the mosquito population may help improve resistance management strategies.

In this study, F1534C, V1016I, and V410L kdr mutations were detected from the *Ae*. *Aegypti* population from Cape Coast. Single or multiple of these kdr mutations have been associated with resistance to different pyrethroid insecticides and DDT (Toé et al., 2022; Vera-Maloof et al., 2015; Yanola et al., 2011). F1534C mutation was previously detected in Cape Coast and other parts of Ghana and remains the most widespread mutation in *Ae*. *aegypti* population in Africa (Weetman, Djogbenou, et al., 2018). We report for the first time the detection of V410L and V1016I mutations in *Ae*. *aegypti* population outside Accra, Ghana. In the first report of the detection of V1016I mutation in Africa about six years ago (Kawada et al., 2016), a single *Ae*. *aegypti* mosquito from Accra (Ghana) was found with the mutation. Thus, it is alarming to observe the spread of the mutation together with the V410L in Cape Coast within this short time. The combination of F1534C and V1016I has been shown to generate high levels of resistance to pyrethroid [22,41]. In fact, Vera-Maloof et al. (2015) suggested that high pyrethroid resistance in *Ae*. *aegypti* requires the sequential evolution of F1534C and V1016I mutations. It is for this reason that the high frequency of the F1534C mutation recorded in this study in addition to the V1016I mutation is worrying. However, the contribution of V410L to the pyrethroid resistance remains unclear. This mutation was recently detected in a population from Accra (Kwame Amlalo et al., 2022) as well as Ghana’s neighboring countries of Cote d’Ivoire (Konan et al., 2021) and Burkina Faso

(Toé et al., 2022). However, all the three studies did not find the V410L contribution to pyrethroid resistance in their respective countries. Nevertheless, V410L alone or in combination with the F1534C mutation has been shown to reduce the sensitivity of mosquito sodium channels expressed in Xenopus oocytes to pyrethroids (Haddi et al., 2017). A recent study found high frequencies of the V410L kdr mutation to be associated with pyrethroid resistance and its combination with F1534C and V1016I was also found to influence the survival of *Ae. aegypti* after exposure to pyrethroid insecticide in a field cage tests (Hernandez et al., 2023) .

Like the results from the bioassay, higher frequency of the resistant allele of F1534C mutation was found in the mosquito population collected inside houses than those collected outside the houses (Table 3). This supports the earlier suggestion that household use of insecticide may be contributing to the selection of resistance in the vector population. However, the three kdr mutations detected in this study cannot fully explain the multiple insecticide resistance found in the mosquito populations from Cape Coast. The resistance to bendiocarb and fenitrothion in the vector population indicates the existence of other important mechanisms. Previous study in Cape Coast detected metabolic resistance through elevated activity of mixed function oxidase, esterase and glutathione-S-transferase from biochemical assays in *Ae. Aegypti* population (Kudom, 2020). Similar result has also been reported in *Ae. aegypti* population from Accra (Kwame Amlalo et al., 2022). This mechanism could explain some of the resistant phenotypes found in this study. Further study is needed to elucidate all the important resistance mechanisms and the potential source of resistance for *Ae. aegypti* in the study area.

5.3 Ecological succession in oviposition sites and predatory behaviour of *Aedes aegypti* in artificial containers

The results from the semi-field experiment showed that the container with high yeast infusion had the highest abundance and diversity of mosquitoes. Among these mosquitoes, *Culex* was the first colonizer in containers with high yeast concentrations. Increased oviposition by *Culex* quinquefasciatus has been found in polluted water samples more than in clean water samples (Emidi et al., 2017). Therefore, it was expected to find more *Culex* species in it. *Aedes* larvae were mainly the first colonizers in containers with low and no concentrations of yeast.

The containers that received nutrient input were more productive and colonised two times faster than the containers that did not receive nutrient input. The level of nutrient input also affected the succession pattern and species assemblages in the household containers. The presence of nutrients in aquatic habitats is one of the key factors that can influence oviposition behaviour and larval performance. This may explain why the experimental containers with nutrient input were more productive than the containers that did not receive such input. Similar results have been reported for other types of container habitats such as tree hole and car tire ecosystems (Kling et al., 2007; Walker et al., 1991). Walker et al. (1991) linked the increased mosquito productivity in a temperate tree-hole ecosystem to nutrient input. Furthermore, Kling et al. (2007) found high input of nutrients to be responsible for high species composition and abundance of mosquitoes in car tires.

Aedes aegypti was the only mosquito species that colonised the containers that did not have any nutrient input whereas *Culex* mosquitoes were the dominant species and the first to colonise containers that received the high nutrient input. This finding is consistent with the habitat preference of both mosquito species. *Ae. aegypti* is known to inhabit containers that have water with poor nutrient content (OECD, 2018) whereas *Culex* mosquitoes are known to inhabit breeding sites with high nutrient content or organically polluted waters (Service, 2008). In a preference-performance oviposition bioassay in response to a substance that mimics decaying organic matter, *Culex* mosquito preferred the high concentration to the low concentration while *Aedes* mosquitoes did not differentiate between the two concentrations (Allgood & Yee, 2017), which is consistent with the result from this study.

Productive containers have been associated with several factors, including location, water purpose, cover, frequency of use, shade, water volume, and frequency of refilling and emptying (Philbert & Ijumba, 2013). Most of these factors could have some degree of influence on food production in the containers. Mosquito larvae feed primarily on aquatic microorganisms that colonise detritus in breeding habitats and the chemicals produced by the microorganisms also influence the oviposition behaviours of adult female mosquitoes (Walker et al., 1991). The location of the container could influence the chance of detritus falling into the water, which could catalyze food production. Furthermore, under normal conditions, it may take some time for microorganisms to colonise and build up their population in household water-filled containers. It is therefore not surprising that containers that are used to store water for long periods are more productive than containers that are mostly used to store water for short periods (Mwakutwaa et al., 2023). The results from this study support control interventions such as the use of lids to cover long-term water storage containers (Pinchoff et al., 2021) and regular washing of storage containers with soap (Overgaard et al., 2017). These interventions could prevent or reduce food production in the containers, which in turn could reduce the productivity of *Ae. aegypti*.

The mosquitoes that colonised the water-filled household plastic containers in this study are known to co-exist in containers in Ghana (Appawu et al., 2000; Kudom, 2020). From the prey-predator bioassay, the late instar of either *Ae. aegypti*, *An. coluzzi*i or *Culex* were found to prey on the early instar of each other. This result agrees with an earlier report on the occurrence of prey-predator activities between *An. gambiae s.s.* and *Cx. Quinquefasciatus* (Muturi et al., 2014). However, it was observed from this study that *Ae. aegypti* was substantially more predatory and less susceptible to attack whereas *An. coluzzi*i was very susceptible to attack and less predatory. Interestingly, when offered dead prey, *Ae. aegypti*, *An. coluzzi*i and *Culex* as predators consumed the same number of preys. This may suggest that the differences in consumption of the live prey among the predators could not be a dislike of the prey as food. Rather, the strength and behaviour of the different mosquito species may have played a significant role in predatory activities. The high predatory activity of *Ae. aegypti* could be attributed to its relatively large size, which could make it less likely to be eaten or stronger in its escape response. This could also explain why the third instar of *Ae. aegypti* successfully preyed on the third instar of both *An. coluzzi*i and *Culex* species.

Interaction of these mosquito larvae in the containers may have consequences for the productivity of individual mosquito species. For instance, the productivity of *Anopheles* species could be affected severely in containers that may also harbour *Aedes* and *Culex* mosquitoes. This could explain the general absence of *Anopheles* larvae in household containers. However, the detection of *Anopheles* in containers needs further studies, especially the species involved (Chinery, 1984; Kudom, 2020) (Kudom 2020, Chinery 1984). This is particularly important due to the recent detection and spread of *Anopheles stephensi* in Africa, which is known to breed in household containers (WHO/HTM/GMP/2019). It is also highly probable that *Aedes*' productivity could be affected in containers that have been first colonised by *Culex* mosquitoes. From the study, the productivity of *Ae. aegypti* was higher in the containers that it first colonised than the containers that were first colonised by *Culex* species. This notwithstanding, the containers that were first colonised by *Culex* also harboured *Lutzia*, which is also a well-known mosquito predator, and it has been shown to consume many *Ae. aegypti* larvae (Appawu et al., 2000; Pramanik et al., 2016). Due to its predatory behaviour, it is understandable that *Lutzia* mosquitoes mainly colonized containers that already had a high abundance of other larvae. Appawu et al. (2000) reported that *Lutzia tigripes* exhibited a significant preference for *Ae. aegypti* larvae compared to *An. gambiae sI.* and *Cx quinquefasciatus*. Thus, predatory activities of both *Culex* and *Lutzia* could be responsible for the lower productivity of *Ae. aegypti* in the containers that received high food input than containers that received moderate nutrient input.

CHAPTER SIX

SUMMARY, CONCLUSION AND RECOMMENDATION

6.1 Summary

The study's major goal was to determine the infestation level of *Aedes aegypti* and other container dwellers, the insecticide resistance status of *Aedes*, larval prey-predator interactions among container-dwelling mosquitoes towards biological control of *Aedes aegypti* mosquitoes. From the sampled sites, the study showed that the risk of a possible outbreak of *Aedes*-related diseases in the Covid 19 restriction period was much lower than in 2017 when a similar assessment was conducted. The *Aedes aegypti* sampled in the study community were resistant to the four insecticides tested. The study reports for the first time the detection of V410L kdr mutation in Ghana in addition to F1534C and V1016I mutations that are already known in *Ae*. *aegypti* population in the country.

Aedes aegypti infestation status, the ecological succession of container dwelling mosquitoes, and their predator-prey interaction have been presented. the result of this study showed that food resources and predatory activities could affect mosquito productivity in household containers. Factors that could enhance food resources can in turn affect the rate of colonization, succession pattern, species composition and larval abundance in water-filled household container ecosystems. This result may help identify productive household containers for vector surveillance and control. Owing to the insecticide resistance in Ae.

aegypti population in the country (Kudom 2020, Ablode et al 2023), proper management of productive household containers could support control efforts.

6.2 Conclusion

The study found the risk of an outbreak of *Aedes*-borne diseases lower in the covid-19 lockdown period than before the pandemic period. Although the study sample is very limited, valuable lessons could be drawn from it concerning the control of *Ae. aegypti*. In the previous study in the community, a high number of discarded items were infested with mosquito larvae, and this contributed to the high larval indices. However, improved sanitation conditions through the clean-up exercise during the restriction period in the community may have caused lower larval indices than what was observed in 2017 before the pandemic. Multiple insecticide resistance coupled with three kdr mutations among the *Ae. aegypti* population in Cape Coast could affect the effectiveness of control measures, especially in emergency situations. The study supports sanitation improvement as a tool to control *Ae. Aegypti* (Lindsay et al., 2017; Overgaard et al., 2017) and could complement insecticide-based tools in controlling this vector.

6.3 Recommendation

The following suggestions are given based on the findings of this study:

1. Intensive education on good water storage practices to reduce the number of container-breeding mosquitoes is needed.
2. Comprehensive mosquito surveillance with an emphasis on *Aedes* species be conducted.

3. Further study is needed to elucidate all the important resistance mechanisms and the potential source of resistance for *Ae. aegypti* in the study area.
4. Health education on mosquito prevention in the community is still needed.

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