Addis Ababa University

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A study on bionomics and pathogen infection status of *Aedes* mosquitoes in Metema District, Northwest, Ethiopia

By

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I, the undersigned, hereby declare that this thesis, entitled *"*A Study on the Bionomics and Pathogen Infection Status of Aedes Mosquitoes in Metema District, Northwest Ethiopia," is my original work and has not been submitted or presented elsewhere. All sources of information and materials used in the preparation of this thesis have been properly acknowledged

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Abstract

Background: Female *Aedes* species transmit viral diseases such as yellow fever, dengue fever, chikungunya and Zika in humans. Dengue fever and chikungunya have been reported in Metema district, northwest, Ethiopia However, entomological evidence scares to guide vectors control strategies. Therefore, this study examined the physicochemical characteristics of larval breeding habitats, biting time, resting habitats, and Wolbachia and viral infectious state of *Aedes* mosquitoes.

Methdology: Entomological studies were conducted in Metema-Yohannes, Kokit and Gend-Wuha towns, Metema distnict, northwestern Ethiopia from January 2022-December, 2023. A longitudinal entomological study was carried out on the physico-chemical characterstics of larvae/pupae habitats, adult biting hours, resting habit and cross section study was carried out viral and Wolbachia strains infection status of *Aedes aegypti*. Physical characterstics of larvae/pupae habitat were observed in situ and chemical composition of habitat's water sample was analyzed under laboratory condition. *Aedes* mosquitoes were identified to species under microscope using a taxonomic key and confirmed using molecular technique based on mitochondrial cytochrome oxidase subunit -1 (COI-1) and internal transcribed spacer -1 & 2 (ITS-1 & 2) genes. Biting hour of adult *Ae. aegypti* and *Ae. vittatus* was deterred by CDC-LT collection from indoor and outdoor each hour for 24hrs and their host seeking behavior was determined by collecting them from human (indoor & outdoor), cattle, goat, sheep and donkey shelters overnight. Adult *Aedes* species were collected from their resting places by Prokopack aspirators, pyrethrum spray catches and Mouth Aspirator. dengue virus, chikungunaya viruse and Zika viruse were detected by SYBR Green RT-PCR and also wMel strain Wolbachia was detected by conventional RT-PCR. Statistical method used to data analysis was considered significant when to $p < 0.05$.

Results: *Aedes* larvae/pupae were abundant in Metema-Yohannes, Kokit and Genda-Wuha towns during wet seasons. Breteau, house, and container indices exceeded the World Health Organization (WHO) risk levels for arboviral diseases. *Aedes* larvae/pupae density increased in habitat with high water temperature, total hardness, sulphate, and with the decrease distance between households and habitat. Adult *Ae. aegypti* larvae/pupae were the most abundant (56.77%; 5106/8993) followed by *Aedes vittatus* (37.25%; 3350/8993), *Aedes communis* (2.39%; 215/8993), *Aedes opok* (0.66%; 60/8993) and *Aedes albopictus* (0.26; 24/8993). The mean ranks of *Ae. aegypti* emerged from the larvae/pupae of artificial, algae positive and tadpole free habitats were analyzed by Mann-Whitney U test. Results were considered significant when $p < 0.05$ higher than the corresponding results from the natural, algae free, and tadpole infested, respectively. Adults of *Ae. vittatus* followed a similar trend. The mean number of *Ae. aegypti* emerged from larvae/pupae of habitats closer to human habitations, not exposed to sunlight, free of emergent vegetation and tyre substrates were analyzed by Kruskal Wallis H test. Results were considered significantly ($p<0.001$) higher than from habitats with no these attributes. *Aedes aegypti* and *Ae. vittatus* exhibited two peak biting rates were observed indoor and outdoor at sunrise and sunset. Both *Ae. aegypti* and *Ae. vittatus* was abundantly collected from nearby human sleeping arrangements than from the animal shelters like cattle, sheep, goats, and donkeys. The highest proportions of *Ae. aegypti* (91.21%) and *Ae. vittatus* (89.87%) was unfed. A total of 4,054 *Aedes* mosquitoes were collected that belonged to *Ae. aegypti* (3026; 58.49%), *Ae. vittatus* (n = 723; 29.08%), *Ae. communis* (n= 186; 7.24%), *Ae albopictus* (n = 77; 3.30%), and *Ae. opok* (n = 42; 1.88%). A greater number of *Aedes* species were collected during the wet season (June to September) than during the dry (October to May), 2022-2023. *Ae. aegypti* and *Ae. vittatus* occurred in Metema-Yohannes, Kokit, and Genda-Wuha, *Aedes communis* in Genda-Wuha and Kokit, but *Ae. albopictus* and *Ae. opok* only in Metema-Yohannes. Large

numbers of *Ae. aegypti, Ae. vittatus* and *Ae. communis* was collected resting in cattle shade, while *Ae. albopictus* within larvae hatching containers but *Ae. opok* was collected from inside bridge. The *wMel* strain of *Wolbachia* was detected in both the first and second generations of *Aedes aegypti* under laboratory conditions, indicating successful transovarian transmission. Additionally, all *Ae. aegypti* pools were tested negative for DENV, CHIKV and ZIKV.

Conclusion: The predominant *Aedes* species identified in the study area was *Ae. aegypti*, followed by *Ae. vittatus*. Other *Aedes* species was observed in the towns included *Ae. communis*, *Ae. albopictus*, and *Ae. opok*. Larvae and pupae of *Ae. aegypti*, *Ae. vittatus*, and *Ae. albopictus* were primarily found in discarded tyres, while *Ae. communis* was associated with metal containers, and *Ae. opok* with tree holes. Peak biting activity for *Ae. aegypti* and *Ae. vittatus* occurred during the morning and early night hours. The resting habitats of *Aedes* species varied with seasonal changes. The pathogenic infection status of *Ae. aegypti* may be influenced by natural suppressive agents. Findings from this study can serve as a baseline for implementing targeted *Aedes* mosquitoes control programs, particularly in managing larval and pupal breeding containers within the study towns. Additionally, control efforts should include community engagement on the elimination of common larval and pupal breeding habitats.

Key words/phrase: *Aedes* species, physico-chemical, biting time, resting habitat, arbovirus, Metema district, wMel-Wolbachia.

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List of Abbrevation

YFV:	Yellow Fever Virus;
DENV:	Dengue Virus;
CHIKV:	Chikungunya Virus;
ZIKV:	Zika Virus;
HI:	House Index;
CI:	Container Index;
BI:	Breteau Index;
ANOVA:	Analysis of Variance;
PCR:	Polymerase Chain Reaction;
COI:	Cytochrome Oxidase Subunit 1;
ITS:	Internal Transcribed Spacer.
ITNs:	Insecticide-Treated Nets,
LLINs:	long-lasting ITNs
IRS:	Indoor Residual Spraying
IIT:	Incompatible Insect Technique
EIP:	Extrinsic Incubation Period

List of Appindices

Glossary of Terms/Words

Mortality: The state or condition of being subject to death

Morbidity: The presence of illness or disease within a population

Vector: Any organisms enhance to transmit diseases (pathogens) to humans or animals

Arbovirus: Any virus that is transmitted to humans and other animals by arthropods

Autogenous: Some mosquitoes species that can produce and lay a batch of eggs without taking a blood meal.

Chapter One: Introduction

1.1 Background

Aedes mosquitoes transmit viral diseases such as dengue, zika, chikungunya, and yellow fever that affect human health and economic activities severely in tropical and subtropical regions of the world (Nigatu *et al.,* 2020). However, low attention is paid to the diseases in Africa due to factors including lack of diagnostic facilities (Mohamed *et al.*, 2024), lack of vaccines or antiviral treatments (Halstead and Wilder-Smith, 2019), the global climate change, increasing unplanned urbanization (Ajlan *et al*., 2019; Robert *et al.,* 2020), low political commitment, and economic challenges (Marcos-Marcos *et al*., 2018; Wilder-Smith *et al.,* 2017). Control of *Aedes* mosquitoes is being considered a key strategy in the prevention and control of the diseases.

In recent years, arboviral diseases outbreaks occurred in Ethiopia most notably YF, DEN and CHIK (Degife *et al.*, 2019; Ferede *et al.*, 2018a; Geleta, 2019). Yellow fever was first reported in the southwestern part of the country between 1960 and 1962, with 100,000 cases and 30,000 fatalities. The second outbreak was in south Omo between 2012 and 2013 (Burkett-Cadena *et al*., 2008; Lilay *et al*., 2017). In October 2018, it claimed the lives of ten individuals in Wolaita, southwest Ethiopia (Tufa *et al*., 2020). Repeated dengue and chikungunya outbreaks have been documented in Eastern Ethiopia (Mesfin *et al*., 2022; Shimelis *et al.*, 2023). In Dire Dawa, the first dengue outbreak infected more than 11,000 individuals (Degife *et al*., 2019) and more recently chikungunya outbreak infected over 40,000 individuals (Geleta *et al*., 2020). Zika virus was reported in the Gambela region of southwest Ethiopia using a seroprevalence technique. (Asebe *et al.*, 2021)

There have also been chikungunya (Ferede *et al*., 2021) and dengue (Ferede et al., 2018b) infections in Metema District, northwestern Ethiopia. Some people move seasonally from the

highlands to the Metema district and back. Similarly, Sudanese people come from endemic areas of arboviral diseases in the east Sudan to visit the district briefly for trade (Siam *et al*., 2022). In the district, most people, including visitors, sleep outside of homes during dry seasons due to high temperature, pressure, and lack of air conditioning and ventilation systems inside homes. Such outdoor sleeping arrangements, without long-lasting insecticide treated mosquito nets, favor uninterrupted biting activity for *Aedes* species (Delatte *et al.*, 2010) and hence lead to the transmission of viral diseases from infected to susceptible individuals in the area. Furthermore, it enhances establishment of the transmission in the highlands.

These viruses are transmitted by *Aedes* species which are widely distributed in the globe (Calle-Prieto *et al*., 2024) *Aedes* species exhibit diverse behavior including day and night bitting activities (Rund *et al.*, 2020), diverse types of larval habitats (Ferede *et al.,* 2018a), outstanding desication resistance capacity of deposited eggs (Prasad *et al*., 2023), susceptability to viruses (Gómez *et al*., 2022) and feed on diverse vertebrate hosts (Rashidi *et al.*, 2022). Moreover, *Aedes* species exhibit diverse resting places in wet and dry seasons that contribute to their transition from favorable to unfavorable condtion and adaption to diverse ecological niches (Sauer *et al*., 2021). *Aedes aegypti* and *Ae. albopictus* exhibited two peak biting hours; one in the morning and the other late afternoon when sunlight intensity is too weak. *Aedes* species are reported from various parts of Ethiopia (Yared *et al*., 2024) of which *Ae. simpsoni* complex in Southwest Ethiopia (Waldetensai *et al.*, 2023), *Ae. simpsoni* and *Ae. africanus* in the Omo and Didessa river valleys (Neri *et al.*, 1968), *Ae. aegypti* in Dire Dawa and the Southern Afar regions in Eastern Ethiopia (Seid et al., 2024a; Waldetensai et al., 2021b) and *Ae*. *hirsutus* in Somalia region of the country (Yared *et al*., 2024). *Ae. aegypti* and *Ae. vittatus* were distributed in northwest, Ethio[i]pia (Ferede *et al.*, 2018a)

Aedes larvae control is largely dependent on the knowledge of the spatio-temporal distribution and characteristics of their habitats. *Aedes* larvae are found in man-made water containers and natural habitats (García-Betancourt *et al*., 2015). Their typical habitats include rain pools, river edges, hoof prints, tree holes, flower pots, swimming pools, storm water, bamboo sticks, plant axils, groove stones, discarded tires, plastics, metals, cements, clay pots and any standing water (Dida et al., 2018). These along with geographical location, season and physico-chemical features of their local habitats determine the abundance and species composition of *Aedes* mosquitoes (Jeffrey Gutiérrez *et al.*, 2020; Medeiros-Sousa *et al*., 2020).

The physical characteristics of *Aedes* larvae breeding habitats can positively or negatively influence the distribution and abundance of *Aedes* mosquitoes. Factors that promote their proliferation include the presence of artificial containers, shaded breeding sites, algae, and organic matter. In contrast, conditions that inhibit *Aedes* larvae development include high temperatures, direct sunlight exposure, the presence of tadpoles and aquatic plants, water movement, and other natural predators (Dissanayake *et al.*, 2021). Chemical characteristics of breeding habitats were influenced on *Aedes* larvae density in both positive and negative ways. Factors such as dissolved oxygen, total hardness, and total alkalinity are generally associated with increased larval density. In contrast, high salinity levels can inhibit larval growth and turbidity often show negative correlations with larval density, although these relationships can vary depending on specific environmental conditions (Ouédraogo *et al.*, 2022). These characteristics affect abundance, survival, body size, behavior and susceptibility to pathogen infection of adult *Aedes* mosquitoes (Imam and Deeni, 2015). Furthermore, the larval habitat characteristics may impact the detoxifying enzymes and density of larvae which potentially lead to the emergence of insecticide-resistant genes in the population (Joy *et al*., 2010). Seasonal variables such as temperature,

precipitation, and humidity also affect survival and vectorial role of the mosquitoes (Jeffrey Gutiérrez *et al.*, 2020; Selvan *et al.*, 2015).

In addition to pathogenic viruses, *Aedes* mosquitoes are associated with microbial community during their life time. There has been increased interest towards the application of genetic control methods such as incompatible insect technique (IIT) to control *Aedes* species. Wolbachia is a maternally-transmitted obligate intracellular Alpha proteobacterium which is widespread in different mosquito species (Damiani *et al.*, 2008; Zhang *et al*., 2015). Almost 65% of *Aedes* species are believed to be naturally infected with it (Joubert *et al*., 2016). Once entered to a wild mosquito population, it is transmitted from generation to generation through the reproductive process (Flores and O'Neill, 2018). Under laboratory condition, wAlbB Wolbachia strain infected *Culex tarsalis* induced increasing infectious rate of West Nile virus compared to uninfection mosquitoes (Dodson *et al*., 2014). On the other hand, *Aedes aegypti* infection with Wolbachia strains such as wMel, wAlbA, wAlbB and wMelPop inhibited viral replication effect against DENV, YFV and CHIKV (Pereira *et al.*, 2018). Moreover, Wolbachia strains reduce adult lifespan, alter locomotors and blood-feeding behavior, metabolism, reproduction, and long-term egg viability and have detrimental effects on fecundity and fertility (Rashidi *et al.*, 2022).

1.2 Statement of problem

Arboviruses infect millions of people worldwide and represent a growing public health concern, both as emerging and re-emerging threats (Tajudeen *et al.,* 2022). In African countries, including Ethiopia, the arboviral diseases such as yellow fever, dengue fever, chikungunya , Rift Valley fever , West Nile fever, and Zika cause high rates of morbidity, mortality, disability and considerable economic burden (Marchi *et al.,* 2018; Massengo *et al.,* 2023; Venter, 2018). The impact of such

diseases is expected to rise in relation to the climate change, rapid population growth, and increased human mobility and trade. These factors contribute to the geographic spread and transmission of these viruses (Caminade *et al.*, 2012; Gedefie *et al*., 2025).

CHIKV (Ferede *et al*., 2021) and DENV (Ferede *et al.*, 2018b) were documented in Metema district of northwestern Ethiopia. Similarly, chikungunya virus infections were reported at Kassala Teaching Hospital of neighboring eastern, Sudan regions (Bower *et al.*, 2021). However, adequate entomological and epidemiological data remains scarce partly due to factors including limited diagnostic facility (Garishah *et al.*, 2023), as well as lack of confirmatory tests of samples (Brault *et al.*, 2004). In view of these problems, a detailed study was carried out on the abundance and spatio-temporal distribution of *Aedes* larvae and their habitats, the biting and resting times of adult *Aedes* species, and the infectious rates of Wolbachia bacteria and viruses in *Ae. aegypti* in Metema district, which has a high risk of arboviral disease transmission. The purpose of the study was to provide baseline data for policy makers and public health professionals.

1.3 Significance of the study

This study examined the physico-chemical characteristics of larvae/pupae habitats and their influence on the distribution, abundance, and species composition of adult *Aedes* species. The research assessed the biting and host-seeking behavior of *Aedes* species to determine their peak biting times and preferred blood meal host. In addition, the study explored the resting habits of *Aedes* mosquitoes during dry and wet seasons. Furthermore, infection rates of *Ae. aegypti* with arboviruses and *Wolbachia* bacterial were assessed in the study sites. These findings provide valuable evidences for policy makers and public health professions in the attempt to develop effective control strategies against arbovirus transmitting *Aedes* species in Metema area and

elsewhere. Ultimately, the results will contribute to efforts to combat arboviral disease transmission at the local, national, and international levels.

1.4 Research questions

What types of habitats support immature *Aedes* species in Metema district, West Gondar zone, Northwestern Ethiopia?

What are the physical and chemical characteristics of habitats that influence distribution and abundance of *Aedes* larvae/pupae?

What is the most preferred biting time of *Aedes* species?

Where do the *Aedes* species rest during the wet and dry seasons?

What are the viral species infection rates of *Aedes aegypti*?

What are the Wolbachia bacteria strains infection rates of *Aedes aegypti*?

1.5 Objectives

1.5.1 General objective

To investigate larval habitat characteristics, resting habitats, biting hours and Wolbachia and viral infection rates of *Aedes* species in Metema district, Northwest Ethiopia

1.5.2 Specific objectives

- To assess physical and chemical characteristics of habitats that determine abundance and composition of *Aedes* species in Metema District, Northwest Ethiopia

- To investigate the preferred resting habitats of adult *Aedes* species during wet and dry seasons
- To identify the peak biting hours of *Aedes* species in the study sites
- To determine Wolbachia bacteria and arbovirus species/strains infection rates of *Aedes* species.

Chapter Two: Litrature reiview

2.1 Global burden of arboviral diseases

Arboviral diseases are rapidly emerging and re-emerging posing a major impact on human health. Mosquitoes in the genus *Aedes* transmit the viral diseases such as YF, DEN, CHIK, and ZIK to humans through their bites (LaBeaud *et al.*, 2011). Dengue causes approximately five million infections and more than 5000 deaths in over 80 countries annually (Pourzangiabadi *et al.*, 2025). YFV, which is mainly a problem in Africa (Garske *et al*., 2014) and South America causes over 200,000 global cases and 30,000 deaths (Mutebi and Barrett, 2002). CHIKV is responsible for millions of cases while the majority recover within weeks. CHIKV was first isolated during the 1952–53 outbreak in southern Tanzania (Khongwichit *et al.*, 2021). Today, CHIKV is widespread in more than 110 countries of the world and is a global public health concern (Weaver, 2014). The virus is causing outbreaks in various regions around the world. The expansion of arboviral diseases is attributed to the movement of infected hosts and mosquito invasions to new foci facilitated by global changes, trade of goods, and trave (Mariconti *et al.*, 2019). Furthermore, changes in the human environment due to urbanization, globalization, and greater human mobility contributed to increased arboviral infections.

Aedes aegypti and *Ae. albopictus* transmit several arboviruses including DENV (Simmons *et al*., 2012), YFV and CHIKV(Leparc-Goffart *et al*., 2014). *Aedes vittatus* plays a crucial role in transmitting YFV, CHIKV and DENV in Senegal (Diallo *et al*., 1999). Additionally*, Ae. vittatus* has the vectorial capacity to transmit other species of arboviruses like Japanese encephalitis, WNV in rural areas of tropical Africa *(Sudeep et al*., 2020). *Ae. opok* are arboviral vector and transmitting several arboviruses such as YFV circulates in African forests from monkey to monkey, transmitted by forest *Aedes* species including *Ae. opok* with incursions into villages (Fontenille and Powell,

2020; WHO, 1986). *Cs. longiareolata* a vector of avian malaria (Schoener *et al.*, 2017), tularemia (Maslov *et al.,* 1989), and arboviruses such as West Nile fever (Bisanzio *et al.,* 2011).

2.1.1 Arboviral disease distribution in Africa

In Africa, arboviral diseases outbreaks are increasing due to poor management of used tyres, plastics and metals, poor environmental management, limited viral and entomological surveillance, absence of sustainable vector management strategy and weak capacity of health care systems (Elduma and Osman, 2014). Additionally, the success of arbovirus transmission is influenced by several driving factors, including the vector's competence, the infectivity of the virus strain, vector population density, biting behavior, survival rate, and host availability (Manouana *et al., 2024)* .

The major arboviral diseases being transmitted are YF, ZIK, DEN and CHIK (Struchiner *et al*., 2015). DEN was first reported in Africa in the late 19th and early 20th centuries from Burkina Faso, Zanzibar, Senegal, Egypt and South Africa (Amarasinghe *et al*., 2011). More than 90% of the cases occur in sub-Saharan Africa, where about 51,000 to 380,000 severe cases are reported each year with 19,000 to 180,000 deaths (Garske *et al.*, 2014). CHIK cases were documented in Ethiopia, Sudan and Kenya (Were, 2012).

2.1.2 Arboviral disease transmission in Ethiopia

There have been increasing incidences of arboviral diseases in Ethiopia in recent years. The diseases include YF, DEN, CHIK and ZIK since they were detected first in the 1960-62 (Mwanyika *et al.*, 2024). In the past year, unexpected epidemics of dengue fever infections occurred in the eastern, southeastern, southern and northwest parts of the country (Degife *et al.*, 2019). CHIK has been reported from southeastern, southern, and northwestern Ethiopia (Alayu *et al*., 2020; Geleta

et al., 2020). In the country, the first confirmed YF outbreak occurred in 1959 in Wollega; and also observed between 1960 and 1962 in almost all the rural districts of Gamu-Goffa, Shoa, Kaffa. In 1966, a yellow fever epidemic near Lake Abaya and around Akobo town in Illubabor, infected a total of 2200 individuals and killed 450 of them (Mekonnen and Kloos, 2019). Recently, there was YF outbreak in the Gurage zone in southwestern Ethiopia (Waldetensai *et al.*, 2020). Although YF is endemic in the country, vaccination is not part of the childhood immunization program. Therefore, children and others living outside of vaccinated areas remain at risk for future YF outbreak. Several factors such as unplanned urbanization and absence of standardized public health services, poor water supply, poor sewage and poor waste disposal contribute to proliferation of *Aedes* species that transmit the diseases (Andral *et al.*, 1968).

Serological tests revealed chikungunya virus (Ferede *et al.*, 2021) and dengue virus infections (Ferede *et al.,* 2018b) among febrile cases who attended outpatient departments at health centers in the Metema district, Northwest Ethiopia. Despite the significant public health implications and recent outbreaks of arboviral diseases in the district, there is limited information on the bionomics and vectoral roles of the *Aedes* species thriving in the area.

2.2 Global distribution of *Aedes* species

2.2.1 *Aedes* species diversity and distribution

Aedes is a genus of mosquitoes in the family Culicidae**,** comprising over 950 recognized species. These mosquitoes are medically significant as vectors of many arboviruses, including dengue**,** Zika**,** chikungunya**,** and yellow fever (Sinclair, 2023)**.** The genus is known for its global expansion, driven by human activity, climate change, and increasing urbanization and cross borderline movement of man (Li *et al*., 2022). The genus *Aedes* is divided into several subgenera to aid in the

classification of species based on morphological and genetic characteristics. The subgenus *Stegomyia* includes species such as *Aedes aegypti* and *Aedes albopictus*, which are distributed across tropical and subtropical regions worldwide. The subgenus *Finlaya* comprises species like *Aedes japonicus* and *Aedes koreicus*. *Ochlerotatus* includes species such as *Aedes canadensis* and *Aedes vexans*. The subgenus *Diceromyia* contains species like *Ae. furcifer*, while *Aedimorphus* includes *Aedes argenteopunctatus*, *Aedes minutus*, and *Aedes punctothoracis* (Diouf *et al*., 2020; Harbach, 2007).

Ecological diversity of *Aedes* species inhabit a range of environments: urban, suburban, rural, forest, and coastal areas (Thongsripong *et al.*, 2013). *Aedes* species have diverse habitats that found in both artificial containers such as discareded tyres, plastices and metals and natural habitats like to tree holes, leaf axils, ground pools and others. *Aedes* species are highly diverse and adaptability to various climates and urban settings, combined with increasing global connectivity to makes them significant public health threats. Therefore, entomological surveillance, ecological studies, and vector control strategies are essential to manage their spread and mitigate disease transmission risks (Wilson *et al*., 2020).

Aedes aegypti (Linnaeus, 1762) and *Ae. albopictus* (Skuse, 1895) are two of the common vector species in the world. They are found on every continent except the Antarctic and played major roles in various vector-borne disease outbreaks over the last century (Kraemer *et al.,* 2015). Currently, their suitable habitats are found in Antarctica, Canada, Russia, Scandinavia, Sahara Desert, southern Ar gentina, the Australian deserts, and the New Guinea. Europe showed no suitability, except for scattered coastal areas in Portugal, Spain, and Italy (Laporta *et al.*, 2023) (Fig 1). *Ae. aegypti* and *Ae. albopictus* are less associated with southern regions but considerably

as sociated with the northern hemisphere, including regions of the United States, Europe, China and Japan. However, in African and Asia will be continued arboviral vector distribution.

Most of the Amazon basin of Brazil will become unsuitable for *Ae. aegypti* in the future. Europea will show suitable areas in Portugal, Spain, France, and Italy, while parts of sub-Saharan Africa would have fewer suitable areas. Some areas of China, Japan, and southern Australia showed suitability for *Ae. aegypti* in the coming time. *Ae. albopictus* was shown to be more associated with the northern hemisphere and have higher tolerance to cooler areas than *Ae. Aegypti*. Future climate change scenarios showed that it will likely expand in the United States. However, South America would possess fewer suitable areas, particularly in the Amazon basin. *Ae. albopictus* will likely expand towards northern areas in Europe. On the contrary, sub-Saharan Africa will possess less suitable habitats in the future. *Ae. albopictus* will be expanded in the future to the southern hemisphere of Indonesia but will remain unchanged in Asia (Laporta *et al.*, 2023).

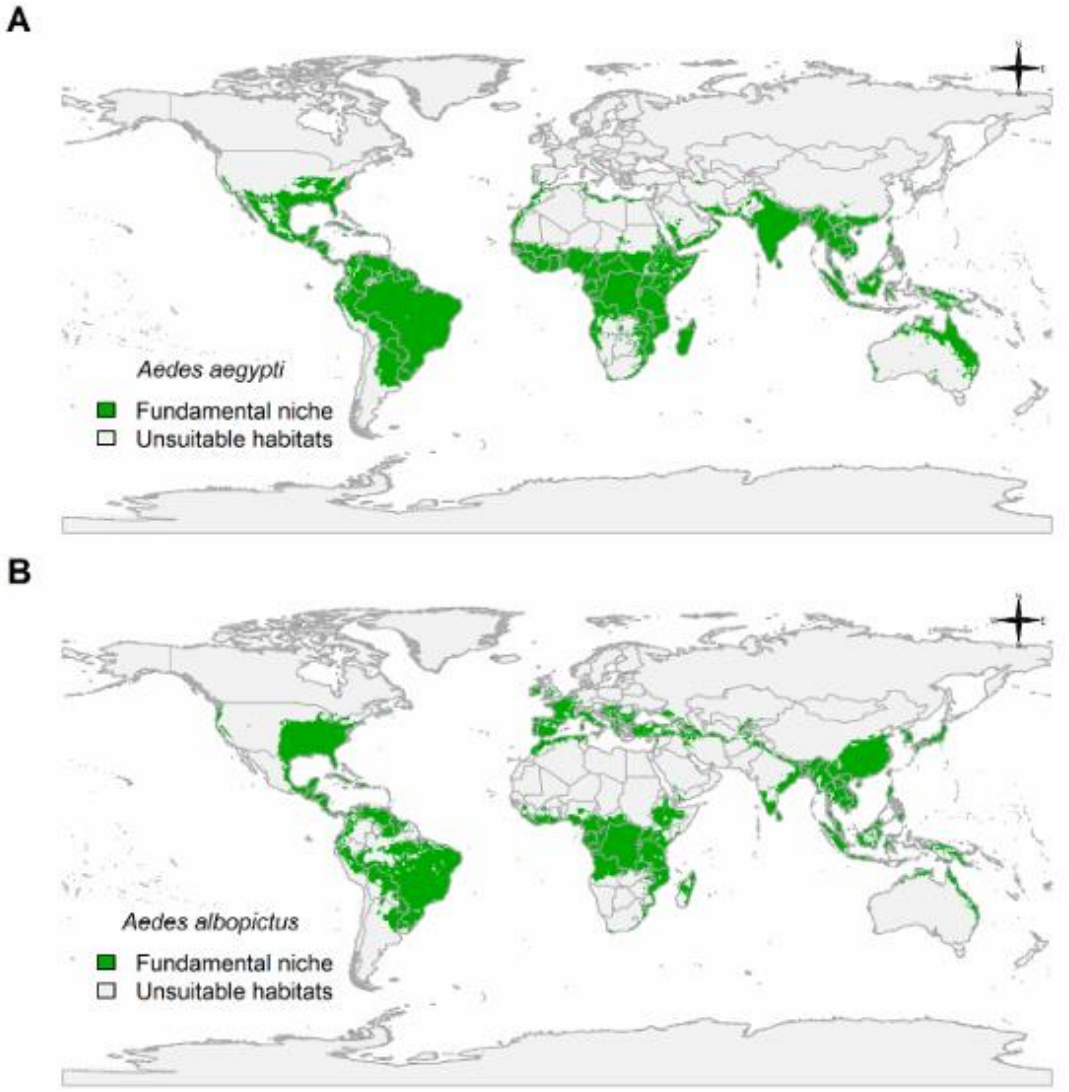


Figure 1: Distribution of *Aedes aegypti* and *Aedes albopictus* in worldwide (Padonou *et al.*, 2023)

2.2.2 *Aedes* species diversity and distribution in Africa

Africa, as the ancestral home of several *Aedes* species, plays a critical role in the global epidemiology of arboviral diseases (Braack *et al*., 2018). West Africa has diverse species including *Ae. aegypti*, *Ae. albopictus, Ae. vittatus*, and *Ae. luteocephalus (*Vulu *et al.,* 2024*)*. Central Africa has dense forest regions harbor sylvatic species like *Ae. africanus*, East Africa: *Ae. simpsoni* complex, *Ae. aegypti* and *Ae. vittatus.* Southern Africa: lower diversity and limited range for

species like *Ae. aegypti* and *Ae. vittatus*. Northern Africa: less number of *Aedes* species presence due to arid climate, but *Ae. aegypti* exists in some urban areas (Weetman *et al*., 2018). Factors influencing distribution of *Aedes* mosquitoes are climate, temperature, rainfall. urbanization and across borderline movement. Africa has high information about the *Aedes* species and to understanding the distribution and ecology of these species is essential for disease prevention and control (Zhang, 2024).

2.2.3 *Aedes* species diversity and distribution in Ethiopia

In East Africa including Ethiopia, *Ae. aegypti*, *Ae. vittatus Ae. simpsoni, Ae. africanus, and Ae. hirsutus* are associated with forest, rural, and urban transmission cycles of the viruses (Ferede et al., 2018a; Waldetensai *et al.*, 2021a; Yared *et al*., 2024). These species are widely distributed across various regions, influenced by several factors such as unplanned urbanization, discarded materials that serve as larval and pupal habitats, limited access to standardized public health services and inadequate waste disposal systems. Additionally, insecticide-resistant gene develop in the vector population and limited entomological surveillance (Seid *et al.*, 2024b).

Aedes aegypti is documented in various resting habitats in Dera-Diwa, Eastern Ethiopia (Yared *et al.,* 2024), while *Ae. simpsoni* complex on the leaves of Abyssinian banana (*Ensete ventricosum*) in southwestern Ethiopia (Waldetensai *et al.*, 2023). *Ae. simpsoni* and *Ae. africanus* were documented in Omo and Didessa River valleys (Neri *et al*., 1968). In 2024, entomological surveillance identified *Ae. aegypti*, and *Ae. hirsutus* in the Somali region of Ethiopia (Yared *et al.*, 2024). Similarly, *Ae. aegypti* and *Ae. vittatus* were reported in the Metema district, Northwest Ethiopia (Ferede *et al.*, 2018a).

2.3 Types of larval habitat

Larvae of *Aedes* species occur in different types of aquatic habitats (Ngugi *et al*., 2017). Immature stages of mosquitoes are entirely aquatic and require water for their development. Various water-holding containers ranging from small to large and natural to manmade serve as immature mosquito habitats (Troyo *et al.*, 2008). Female *Aedes* mosquito select their appropriate habitats for egg deposition that is a key element for their productivity, population survival and dynamics (Rashidi *et al.*, 2022). Habitat type and characteristics determine development of larvae and pupae and successful development into adult mosquitoes (Mamai *et al.*, 2021). Therefore, knowledge of larval habitats helps to develop appropriate larval control strategies.

Aedes species are often associated with temporary or permanent habitats in urban and rural areas and distribute from lowlands to high lands due to increasing global temperature that make suitable habitats both for their immature and adult stages. Artificial habitats include flower vases, water storage drums or tanks, and discarded plastic, tyres or metal containers and clay pots. The preference of habitat by female *Aedes* mosquitoes largely depends on the habitat's physical and chemical properties and also is linked with its stability, temperature, humidity and rainfall (Costa *et al.*, 2010).

2.3.1 Physical characteristics of larval habitats

Physical characteristics of habitats influences the magnitude and competence of the larval and adult mosquito population (Haidy Massa *et al.,* 2025). The characteristics include habitat perimeter, water depth, exposure to sunlight, speed of water flow, distance to the nearest house, vegetation cover, habitat permanence, presence/absent of algae and tadpole, surface debris coverage, emergent plant coverage, habitat type and substrate type. Physical characterstics of breeding habitats were determined to the density of immature mosquitoes and hence the adult

mosquito population size (Mwangangi *et al.*, 2010). *Aedes* larvae/pupae habitat is influenced by climatic factors such as temperature, precipitation, and humidity (Jeffrey Gutiérrez *et al.,* 2020; Selvan *et al.,* 2015). Typically, greater numbers of mosquitoes were produced in water bodies within so much less water flows, higher temperatures, and higher organic content than in those having good water flows, lower temperatures, and lower organic content (David *et al.*, 2021).

2.3.2 Chemical composition of larval habitats

The chemical characteristics of habitats also influence the distribution and abundance of immature and adult stages of the mosquitoes. These in turn affect control strategies of disease transmitting mosquitoes. Some of the characteristics are pH, salinity, dissolved oxygen, total dissolved solids, ammonium, ammonia, sulphate, phosphate and nitrite which have been shown to influence the density of immature stage of mosquitoes (Minakawa *et al*., 1999; Nikookar *et al.*, 2017). The chemical characteristics of the breeding habitats in which *Aedes* larvae live have both positive effects, supporting their survival and development such as dissolved oxygen, organic matter and total hardness. Negative effects, which can be exploited for control measures including high level of ammonia and phosphate, and low value of PH. The overall effect depends on the specific chemical compounds present and their concentration in the larvae breeding habitat (Mbanzulu *et al.,* 2022).

Moreover, habitats are affected by industrial hazard chemicals, agriculture fertilizers, pesticides and insecticides used in vector control. Pesticides and insecticides in larval habitats induce insecticide resistance in disease transmitting mosquitoes. Thus, distribution and abundance of immature stage of *Aedes* species are influenced by physical and chemical characteristics of habitats (Jeffrey Gutiérrez *et al.*, 2020; Medeiros-Sousa *et al.*, 2020). Furthermore, chemicals in habitats

may impact detoxifying enzymes of the larvae which potentially lead to the emergence of insecticide-resistant genes in the population (Joy *et al.*, 2010).

2.4 Behavior of Adult *Aedes* mosquitoes

2.4.1 Biting behavior

Aedes mosquitoes are diurnal and most active during the early morning and late afternoon hours. This contrasts with malaria transmitting anophelines mosquitoes that bite mostly at night (Lima-Camara, 2010). *Aedes* females often take multiple small blood meals from different hosts in one feeding period. This behavior increases the chance of transmitting viruses between hosts, as it allows them to pick up and spread pathogens more effectively. They show a strong preference for humans (anthropophilic), particularly *Aedes aegypti*. *Aedes* mosquitoes are attracted by: Carbon dioxide (CO_2) exhaled by hosts, body heat, Lactic acid, sweat, and certain skin odors and dark clothin. After feeding, the female rests for a few days while her eggs develop and then lays its eggs near water sources (Perrins, 1996),

Various vector control measures are applied in order to suppress mosquito populations. Such measures depend on the knowledge of mosquitoes' biting behavior mostly expressed as biting rate. Mosquito biting rate refers to the density of a mosquito species involved in biting a host in a given time period. Information on biting activities of vectors facilitates the selection of protection measures that would prevent humans from being bitten by mosquitoes (Korgaonkar *et al.*, 2012).

Aedes species have diverse blood meal sources including domestic animals (dogs, cattle, goat, cats, horses, pigs), wild animals (monkey, rat, mongooses, birds and fowl) and humans. Blood feeding activity of *Aedes* mosquitoes is influenced by their age, body size, time of the day, blood

engorgement state, carbohydrate availability and environmental condition (Xue *et al.,* 2001). Sugar and blood are essential for survival and reproduction in female mosquitoes. The number of host contact events by a single female mosquito can affect vectorial capacity (Xue *et al*., 2001). Adults of male and female *Aedes* mosquitoes feed on nectar of plants. Females also feed on blood including on humans in order to produce eggs. *Aedes* mosquitoes readily bite during the day and indoors as well as outdoors.

A female mosquito produces 100 to 200 eggs per batch depending on the size of the blood meal. It can produce up to five batches of eggs during its lifetime. Eggs are laid singly on damp surfaces in areas likely to be temporarily flooded, such as tree holes and man-made containers (Clements, 2000). Most eggs are deposited at varying distances above the water line (Foster and Walker, 2019). Eggs can survive desiccation for months and hatch to first instar larvae at different times once submerged in water (Foster and Walker, 2019).

Humans are at the highest risk of exposure to pathogen infected *Aedes* mosquito bites, which is crucial for transmission of arboviral diseases. *Aedes* mosquitoes are most active for about two hours after sunrise and several hours before sunset, but they can also bite during the night in well-lit areas. These mosquitoes tend to bite unnoticed, as they approach from behind and typically target the ankles and elbows. *Ae. aegypti* primarily prefers biting humans, but it can also bite dogs and other domestic mammals. *Ae. albopictus* exhibited three peak biting periods: 08:00–09:00, 16:00–17:00, and 19:00–20:00 (Chadee and Martinez, 2000). At the University of Malaya student hostel, the peak biting hours observed were 10:00–11:00, 16:00–17:00, and 18:00–19:00 (Chen *et al.,* 2014). In contrast, only two peaks were observed at the Taman Melati location: 06:00–07:00 and 18:00–19:00 (Waldetensai *et al*., 2023). *Ae. albopictus* showed bimodal activity patterns, the higher attack rates being in the morning (08:00) and evening (14:00–20:00)(Zahid *et al.*, 2023).

Mosquito biting behavior and rates are highly variable with time, temperature, relative humidity, rainfall, location (indoors and outdoors) (Thavara *et al*., 2001), and across rural and urban habitats (Yasuno and Tonn, 1970).. The peak biting activity of *Ae. simpsoni* complex sharply declined after 10h in the morning and 18h in the afternoon (Waldetensai *et al.*, 2023). Lower temperature slows down blood-meal digestion and leads to a longer gonotrophic cycle (Pant and Yasuno, 1973), which reduces mosquito's daily biting rates. Based on temperature variations, the gonotrophic cycle duration can vary by up to four days (Delatte *et al*., 2009). Rainfall also impacts mosquitoes' biting activities (Thavara *et al.*, 2001). Therefore, human biting time is influenced by environmental condition and other factors. So, such information is critical to develop appropriate vector control strategy.

2.4.2 Resting behavior

Aedes mosquitoes select resting habitats based on a combination of factors that maximize their chances of survival and reproduction. These factors include humidity, temperature, shelter, proximity to water, and protection from predators and harsh environmental conditions. *Aedes* mosquitoes prefer areas with high humidity because they are susceptible to desiccation. They are typically found in humid environments that provide shelter from direct sunlight and wind, which helps them conserve moisture. *Aedes* mosquitoes are more likely to rest in areas with warm temperatures, as they are adapted to tropical and subtropical climates. These mosquitoes thrive in environments where the temperature remains within the optimal range for their activity (typically between 25°C and 30°C) (Schmidt *et al.*, 2018). *Aedes* mosquitoes seek shaded locations to rest, as these areas provide cooler and more humid conditions. Shaded places like underneath furniture, tree canopies, bushes, or roof eaves offer protection from both heat and wind.

Aedes mosquitoes tend to avoid areas with strong wind or open spaces. Wind increases evaporation rates and also hinders their ability to rest comfortably. They prefer sheltered spots where the airflow is minimal and where they are less exposed to predators (Burkett-Cadena *et al.*, 2013). Areas with dense vegetation or shelter are also beneficial as they reduce the risk of predation from natural enemies, such as spiders, frogs, and birds. Conditions like proximity to buildings, vegetation, and water bodies create localized microclimates ideal for mosquito survival (Liu *et al*., 2022).

Aedes species rest in diverse places that contribute to their adaptation to a wide range of ecological conditions. Adult mosquito collection from different resting sites helps to assess their behavioral diversity, blood meal sources, ecological niches, genetic diversity and adaptation mechanisms (Service, 1971). A key factor for pathogen transmission through mosquitoes is the environmental temperature, affecting mosquito's life history traits and pathogen development rate in a mosquito (Mordecai *et al.,* 2017). Hence, understanding mosquito-specific resting site and microclimate preferences can refine epidemiological data (Haider *et al.*, 2017).

2.5 Viral transmission by *Aedes* species

Arboviral diseases are rapidly emerging and re-emerging in tropical and subtropical regions of the world affecting its economic, political and health conditions (LaBeaud *et al.*, 2011). Vectors of arboviral diseases are associated with more than 3 billion people living in endemic regions. Global warming, deforestation, urbanization, international travel, shipping and industrialization worsen the problem as infected mosquitoes and eggs colonize new ecological niches (Coffey *et al.,* 2013; Li *et al.,* 2014). The global burden of arboviruses is significant, with up to 700,000 deaths (Ketkar *et al.,* 2019). Among the arboviruses, dengue virus (DENV) causes over 390 million cases annually, mainly affecting Asia and Latin America (Wilder-Smith *et al.,* 2017). YFV, which is

mainly a problem in Africa (Kraemer *et al.*, 2017) and South America (Vasconcelos *et al.*, 2004) infects over 200,000 people globally and claims 30,000 lives annually (Chippaux and Chippaux, 2018). In Uganda, serological studies by Henderson *et al.* reported on the arbovirus of WNV, YFV, and ZIKV have 16.8% prevalence (Byaruhanga *et al.,* 2023)

In Africa, arboviral diseases are increasing due to increasing global temperature, use of plastic materials and poor environmental management that favor conditions for the breeding of vectors. Moreover, limited viral and entomological surveillance, absence of sustainable vector management strategy and weak capacity of health care systems contribute to increased occurrence of the diseases (Elduma and Osman, 2014). The major arboviral diseases undergoing most striking resurgence in this century are dengue, yellow fever and chikungunya. They frequently outbreak in urban or peri urban areas where *Aedes* mosquitoes are abundant (Struchiner *et al.,* 2015). In Africa, laboratory diagnostics is not done for most of the arboviral diseases. Thus, there is limited data on arbovirus transmission and the incidence and prevalence of the diseases. The first report of dengue fever in Africa was in the late 19th and early 20th centuries from Burkina Faso, Zanzibar, Senegal, Egypt and South Africa (Amarasinghe *et al.,* 2011). In Africa, a few available data suggest that *Aedes* mosquitoes are present in all countries (Were, 2012). More than 90% of the cases occur in sub-Saharan Africa, where an estimated 51,000 to 380,000 severe cases of yellow fever occur each year with 19,000 to 180,000 deaths (Garske *et al.*, 2014).

Aedes species control is the main strategy to prevent transmission of viral diseases. According to WHO vector control strategies larvicide application along larvae breeding sites, indoor residual insecticide sprays (IRS) and long-lasting insecticide-treated nets (LLINs) are useful to reduce their population (Ocampo *et al.,* 2011). However, these strategies are less effective to control *Aedes*

species than the malaria vectors. This is due to their day and night biting behaviors, change their behavior, eggs resist outstanding desiccation and develop insecticide resistance (Faull *et al.*, 2016).

2.6 Factors affecting arbovirus transmission by *Aedes* species

2.6.1 Temperature, humidity, rainfall

Temperature is among the most important drivers of the transmission of mosquito-borne pathogens. The body temperature of mosquitoes, as ectothermic insects, is equivalent to the ambient temperature (Sauer *et al.,* 2022). Temperature strongly affects both the biological life history traits of mosquitoes (e.g. adult mortality and biting rates) and the pathogen development rate. The inverse of the pathogen development rate is referred to as the extrinsic incubation period (EIP), i.e. the time it takes for a pathogen to develop or disseminate in a vector until it can be transmitted (Samuel *et al.*, 2016). Each pathogen has a specific temperature-dependent development rate and temperature threshold preventing further development, e.g. 14–15 °C for WNV (Shocket *et al.*, 2020). In addition, other environmental factors such as optimum relative humidity and rainfall are influencing on vector survival, production, growth, abundance and dispersal (Chepkorir *et al.*, 2014)

2.6.2 Transovarian transmission

Arbovirus transmission to susceptible *Aedes* species could be vertical and/or horizontal (Diniz *et al.*, 2017). The horizontal mode of transmission is divided into two: 1) Arboviruses transfer from water bodies to larvae through the feeding process, 2) During blood-feeding females get viruses together with the blood meal. During blood feeding, adult female mosquitoes get viruses from infected person or animal and transmit them during subsequent feeding on susceptible hosts or transovarial through their generations (Diniz *et al.*, 2017). The virus develops within the vector

and infects a new vertebrate host during a subsequent blood meal. Vertical transmission of arboviruses in mosquitoes is maintained through two main mechanisms: when the virus infects the germinal tissues of the female mosquitoes' reproductive organs and trans-egg transmission when the virus infects the egg during oviposition time inside the female reproductive organ. Horizontal transmission of arboviruses to humans is mainly through the bite of infective female *Aedes* species. The *Aedes* mosquito acquires the virus when it ingests blood from a viremic host (Lequime *et al.*, 2016). Vertical transmission can maintain the viruses for a long time even in the absence of horizontal transmission which could serve as a source of human infection.

Arbovirus transmission often occurs through highly complex transmission networks that include various hosts and vectors (Diaz *et al.,* 2013). During the dry season in tropical areas or the cold season in temperate regions, the absence or low density of adult mosquitoes is less likely to support continuous host-to-vector (horizontal) transmission and the viruses survive through vertical transmission in the vector. Vertical transovarial transmission occur when the virus infects the germinal tissues of the female mosquito, whereas trans-egg takes place during oviposition in the fully formed egg (Ebert, 2013). The rate of vertical transmission is influenced by factors such as climate, vector immunity, anthropogenic activities and genetics (Behura and Severson, 2012).

2.6.3 Wolbachia bacteria infection in *Aedes* species

Wolbachia bacteria is described in *Culex*, *Anopheles* and *Aedes* mosquitoes. However, it is interesting that Wolbachia is frequently detected in the arbovirus vectors such as *Ae. albopictus* (Zhang *et al.*, 2015). Wolbachia is a genus of obligate intracellular alphaproteobacteria within the family Rickettsiaceae (Werren *et al.*, 2008). It causes cytoplasmic incompatibility (CI), a phenomenon that results in sperm and eggs being unable to form viable offspring. Wolbachia is an

endosymbiotic intracellular bacterium naturally present in about 65% of all insect species including *Ae. albopictus, Ae. aegypti, Ae. fluviatilis, Cx. quinquefasciatus, Cx. Pipiens, An. gambiae s.s. and An. Coluzzii* (Andrews *et al.,* 2014). Wolbachia is commonly transmitted vertically from parent to offspring but horizontal transmission between host populations is rare. (Werren *et al.*, 2008). Some strains of Wolbachia bacterial (wAlbA, wAlbB & wMelPop) are used to manage a variety of mosquito species without any environmental contamination (Shirozu *et al.,* 2024). *Aedes aegypti* and *Ae. albopictus* infected with wMelPop that induced to decreased adult lifespan, altered locomotors, blood-feeding behavior, metabolism, reproduction, long-term egg viability and having particularly detrimental effects on fecundity and fertility (Brelsfoard and Dobson, 2011). Wolbachia strains have varying levels of pathogenicity to *Ae. aegypti* and other *Aedes* species. The effect of wMel strain of Wolbachia infectious of *Ae. aegypti* (Ant *et al.*, 2018) has limiting effect to adult female and male longevity compared to uninfected lines (Fraser *et al.,* 2017). Moreover, a slight delay in larval developmental time has been observed when wMel-infected and uninfected lines were reared within the same tray in laboratory session (Ross *et al.*, 2014). wAlbB causes minor negative effect on egg and adult longevity when introduced to *Ae. aegypti* but has no impact on its fecundity, larval development or mating success compared to wild type mosquitoes. In contrast, wMelPop-CLA is highly virulent on the *Ae. aegypti* to decreasing adult female lifespan as well as reduce blood-feeding success, fecundity, fertility and egg viability (McMeniman and O'Neill, 2010). The application of the cytoplasmic incompatible technique is dependent on environmental factors, mosquito species and Wolbachia bacteria strains. A specific Wolbachia strain has targeted to mosquito species under the appropriate habitat that proper effective manage to adult mortality, reduced fecundity, prolonged egg and larval development time, and reduced survival of eggs (Ross *et al.*, 2014).

Aedes albopictus, *Ae. aegypti*, and *Ae. polynesiensis* are susceptible to Wolbachia strains (wMel, wMelPop and wAlbB) that reduce susceptibility to DENV, YFV, CKIV and WNV or inhibit viral replication in the vector (Bian *et al.,* 2010). Trans infection of Wolbachia into mosquito species has been successful for *Ae. aegypti, Ae. albopictus, Ae. polynesiensis, Cx. pipiens,* and *An. Stephens* (Blagrove *et al.,* 2012). Previous studies indicated that Wolbachia strains infectious of arboviral mosquitoes that induced to block viral replication and reduce ZIKV, DENV, CHIKV, and YFV transmission to susceptible mosquitoes (Mousson *et al.,* 2012) and interruption various physiological activities of mosquito (Hughes and Rasgon, 2014).

Chapter Three: Materials and Methods

3.1 Study area

The research was conducted in the towns of Metema-Yohannes (12.950649N, 36.147112E), Kokit (12.822325N, 36.326846E), and Genda-Wuha (12.822333N, 36.326861E) in Metema Woreda (District), northwestern Ethiopia (Fig 2). Metema district is located at 925 kms northwest of Addis Ababa and 180 kms west of Gondar. The district shares more than 60 kms long boundary with Sudan (Kendie *et al.,* 2018) and it is endemic to malaria. The average altitude of the woreda is 734 meters above sea level. The daily temperature ranges from 25^0C and 30^0C in June to September and 280C and 42^0C in October to May. The yearly average rain fall is about 625 mm *(Gashaw et al*., 2023). The local people's income is primarily generated from livestock farming and cash crop production. Additionally, people in both countries frequently cross border for various purposes. Three towns, Metema-Yohannes, Kokit, and Genda-Wuha were selected purposively in consultation with local health bureaus, based on recent reports of dengue fever and chikungunya cases. Within each town, residential houses were selected to ensure adequate geographic coverage.

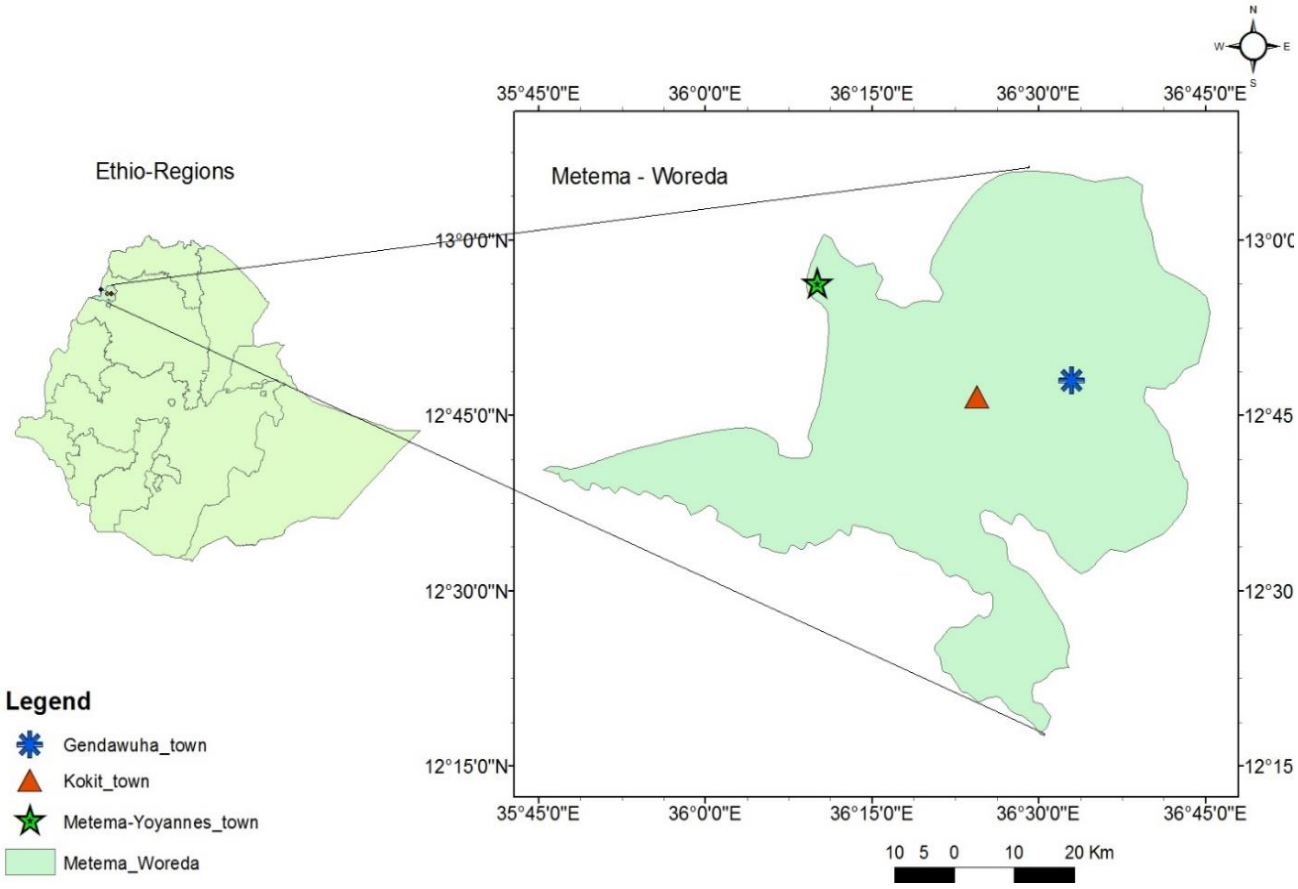


Figure 2: Geographical location of Genda-Wuha, Kokit and Metema-Yohannes towns, Metema Woreda (District), Northwestern Ethiopia

3.2 Immature mosquito collection and rearing

The study was conducted in each town over three consecutive days per month for a period of 24 months, from January 2022 to December 2023, using a longitudinal design. Surveys of *Aedes* larvae and pupae were conducted between 9:00 AM and 12:00 PM. The dry season, in the study area, spans from October to May, while the wet season from June to September. Metema-Yohannes had 9,810 households, Kokit 8,940, and Genda-Wuha 13,560. From these, a sample of 327 houses in Metema-Yohannes, 298 in Kokit, and 452 in Genda-Wuha were inspected. The first house in each town was selected randomly, and thereafter, every 30^{th} house was surveyed for potential *Aedes* larvae habitats. Larval sampling was conducted within a 200-meter radius of each reference house. Verbal consent was obtained from the head of the reference household prior to conducting the survey. The same houses were used for data collection during the study period.

Larval sampling was carried out using a standard 350 ml dipper (Bio Quip Products, Inc., California, USA) from both artificial and natural larval habitats (Mattah *et al.*, 2017; Mereta *et al.*, 2013). In cases where the dipper could not be used, such as in small or confined habitats, pipettes were employed to collect larvae/pupae (Minakawa *et al.*, 1999). Collected larvae and pupae were separated from debris and predators, placed in labeled plastic jars, and transported to the insectary at the Department of Biology, University of Gondar, Ethiopia. In the insectary, larvae were reared to adulthood under controlled conditions: a temperature of 27 ± 2°C, 75% relative humidity, and a 12:12-hour light-dark cycle, with fish food provided as nourishment. Adult *Aedes* mosquitoes were identified to species level using a taxonomic key based on morphological characteristics (Coetzee, 2020; Rueda, 2004a; Supryono et al., 2023; Yamany et al., 2024)

3.3 Characterizing physical and chemical properties of larval/pupal habitats

Physical characteristics of larval and pupal habitats: The physical attributes assessed included water volume, distance to the nearest household, sunlight exposure, habitat type, temperature, substrate type, habitat permanence, and presence of algae, tadpoles, and emergent vegetation. Habitat volume was categorized as follows: (1) small (≤ 20 liters), (2) medium (21–100 liters), and (3) large (≥ 101 liters). The distance of habitat to the nearest household was measured using a tape measure and grouped into three categories: (1) ≤ 50 m, (2) 51–200 m, and (3) ≥ 201 m. Habitats were considered temporary if they retained water for two weeks or less after rainfall, and permanent if they held water for more than two weeks (Mwangangi *et al.,* 2010). Habitat types were classified into artificial and natural categories. Artificial habitats included discarded tyres, plastic, metal scraps, drums, cans, and clay pots (Fig. 3). Natural habitats comprised surface pools, river bank, hoof prints, and tree holes.

Water sample was collected from each *Aedes* positive habitat using labeled sterilized plastic bottle, transported to the Environmental Science Laboratory, Department of Environmental Science Gondar University and analyzed for its chemical properties. Water pH, conductivity (μS/cm) and total dissolved solids (mg/l) were recorded using Orion 5-star portable multiparameter meter (Thermo Scientific). Turbidity (NTU) was measured by placing a water sample in glass test tube and holding it against a white background. Dissolved oxygen (%) was measured by Dissolved Oxygen Portable Meter and water chemical composition (Amonia (NH_3), Amomium (NH_4), Phosphate (PO_4), Sulphate (SO_4), Nitrate (NO_3), Nitric (NO_2) and total hardness using) were measured using Muiti-Parameter Photometer-Palintest 7100 (Minakawa *et al.,* 1999).

Figure 3: *Aedes* larvae/pupae habitats: Artificial containers(a-d) such as (a) discarded plastics, (b) discarded tyres, (c) discarded metal, (d) clay pots. Natural habitats: (e) rain pools, hoof prints, aquatic algae and plant emergent

3.4 Determination biting hour and host seeking behavior of *Aedes* mosquitoes

Biting hour and host-seeking behavior of adult female *Aedes* species were assessed in the three towns. Ten households were selected from each town based on certain criteria, such as the presence of larvae-holding containers and the availability of at least four domestic animals within the

compound. Data were collected from June to September 2023. Female *Aedes* species that attempted to bite humans (indoors and outdoors) were trapped using CDC light trap (CDC-LT) (Model 512 (John W. Hock Company, Gainesville, FL, USA) that operated close to a man sleeping under an insecticide-free mosquito net every hour for 24 h (daylight hour: 06:00–18:00, night hour: 18:00–06:00). The collection was made for three consecutive days per month. In the same time, female *Aedes* species that attempted to host seeking on cattle, sheep, goats, and donkeys in their shelters within a 50-m radius of the houses were trapped overnight (18:00–06:00) CDC-LT (Figure 4). The trapped mosquitoes were put into labeled holding cups.

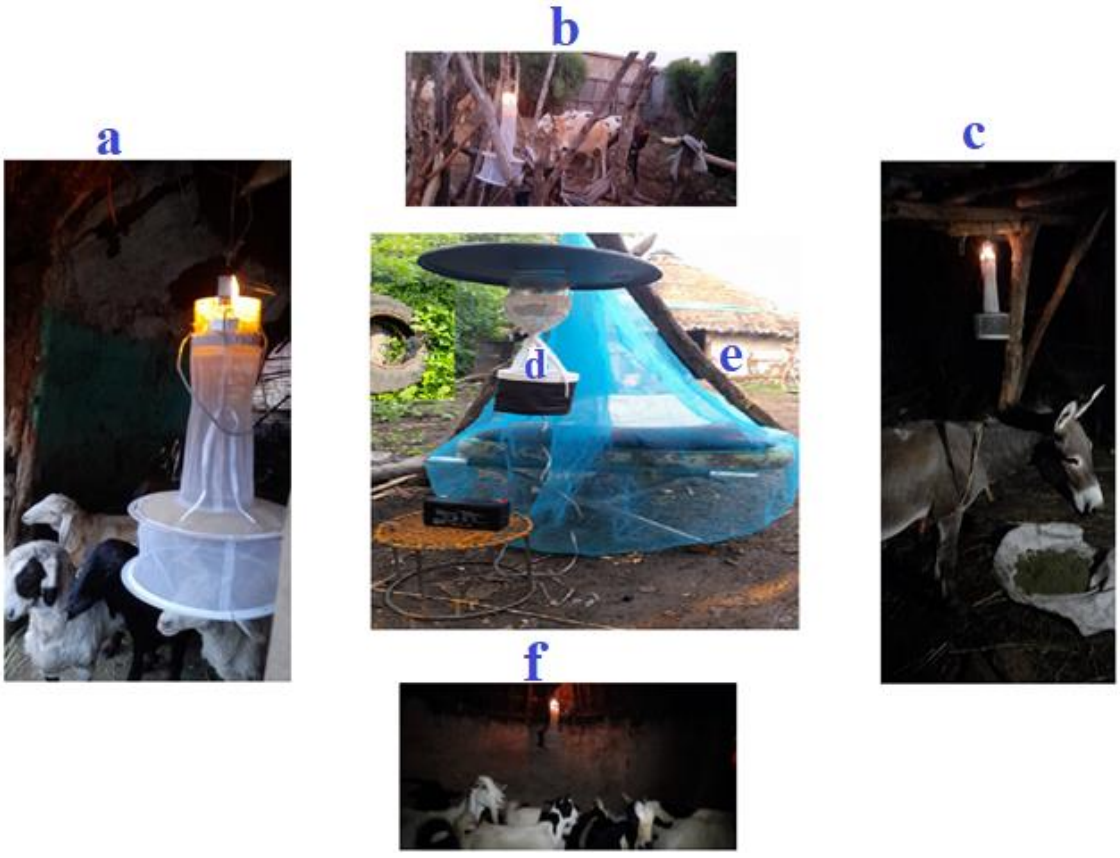


Figure 4: Collection of *Aedes* species from animal shelters using CDC light trap within a 50 m radius from the target house. (a) sheep, (b) cattle, (c) donkey, (d) outdoor (human), and (e) indoor (human), (f) goat.

3.5 Collection of adult *Aedes* mosquitoes from their resting habitats

Adult mosquitoes were collected from human homes (indoor and outdoor (grain storage)), cattle shade, inside larval containers, deep grooves, and inside bridges. Data were Collected from 1st January to 30th December, 2023 using Pyrethrum Spray Sheet, Prokopack Aspirator and Mouth Held Aspirator collection (Fig, 5).

3.5.1 Collection by pyrethrum Spray Sheet

Indoor resting mosquito collection was conducted three days per month in ten randomly selected houses in each of the three towns, accordingly, WHO indoor residual spraying operational guideline (WHO, 2011). The floors, beds and furniture were covered with white sheets, and food items were removed and properly closed before spraying an aerosol insecticide (Baygon, SC Johnson and Son Inc., Racine, WI, USA) . Additionally, windows, doors and other openings were closed to prevent mosquitoes from escaping. The collections were performed early in the morning (06:00-07:00am) and about 15min post-spraying, mosquitoes were collected, lebeled, and indivdually preserved in eppendorf tube for further study.

3.5.2 Collection by prokopack aspirator

Adult mosquitoes were collected from different resting habitats including indoor and outdoor, animal shelter, containers, deep grooves and under bridge using prokopack aspirators according to the WHO guidelines (Manzambi *et al.,* 2023). Each *Aedes* mosquito was transferred to a labeled Eppendorf tube and placed in large zip-lock plastic bag with slica gel. The samples were transported to insectary, University of Gondar, Ethiopia where identified to the species level by using morphological taxonomic key (Rueda, 2004b) (Fig. 5).

3.5.3 Collection by mouth aspirator

Mouth Held Aspirator, model 412 (John W. Hock Company, Gainesville, FL, USA), using for the collection of adult mosquitoes from different resting places such as grain storage, indoor, cattle shelter, inside container, deep groove and inside bridge. A collector spent 10 minute at each site searching for resting mosquitoes. Additionally, abdominal status of female mosquitoes was classified as unfed, blood fed, semi-gravid and graved (Yared *et al.,* 2024). Individual mosquito sample was maintained in a vial and transferred to a big zip-lock plastic bag with silica gel. Adult mosquitoes resting habitats were characterized. .

Figure 5: Adult *Aedes* mosquito collection from the resting places (A, outdoor & B, indoor) at Metema-Yohannes, Kokit and Genda-Wuha towns, in Metema district, northwestern Ethiopia.

3.6 Identification of *Aedes* species

3.6.1 Morphological identification

Initially, *Aedes* larvae were identified and separated from their other genus level by observing key features of the head (antennae, location of brushes, and compound eyes), thorax (rounded and comprising the prothorax, mesothorax, and metathorax), and abdomen (number of segments, presence of tufts, arrangement of comb spines, respiratory structures, pecten row, and structure of the siphon) (Kumar *et al.*, 2022; Nelson, 1986).

Aedes and *Culiseta* genus have several morphological similarities at immature stage including head: antennae, median brush, lateral brush, thorax, abdomen, siphon, and anal papillae. The larvae have a pair of antennae that are straight, wide at the base, and narrow towards the tip. The compound eyes are prominent laterally. A pair of mouth brushes at the anterolateral margin emerging from the clypeal/labral area. The larvae have a narrow, cylindrical, webbed neck connecting the head and thorax. The thorax is round and consists of the pro, meso and meta thorax. In the meso and metathoracic regions, a pair of large spine-like processes is seen dorsally. The larval abdomen consists of eight segments, long, transparent and cylindrical. Lateral tufts of hair emerge from the abdominal segments in varying numbers on each segment. Abdomen segment VIII of the larva has a row of comb spines and a respiratory tract or siphon. At the end of the siphon are the respiratory openings, the spiracles, which are surrounded by peri-spiracular lobes. The siphon has a row of pecten teeth on either side laterally and has basal denticles. The end or terminal part of the abdomen contains the anus. The anal segment has a ventral brush with five pairs of setae of variable size and four equal-sized anal papillae. The anal papillae are transparent and boat-shaped (Fig. 6).

Figure 6: Morphological structure of *Aedes* and *Culiseta* larval in the study sites

Adult *Aedes* species identified using stereomicroscope following the morphological identification key (Azari-Hamidian and Harbach, 2009; Huang, 2004; Supriyono *et al.,* 2022). There were several features focused on white scales on the occiput and vertex of the head, white spots and stripes on the scutum, white bands on the abdomen, the presence or absence of a white band and the number of segments on the palps, and claws on the legs characterized by pale, spirally striped tarsi and tarsal claws. The wings were examined for the presence of white pale bands and for colorless or black scales along the wing venation (Fig.7). Each *Aedes* specimen was preserved individually in a perforated 1.5-mL microcentrifuge tube and stored in a zip-lock plastic bag containing silica gel for subsequent molecular confirmation.

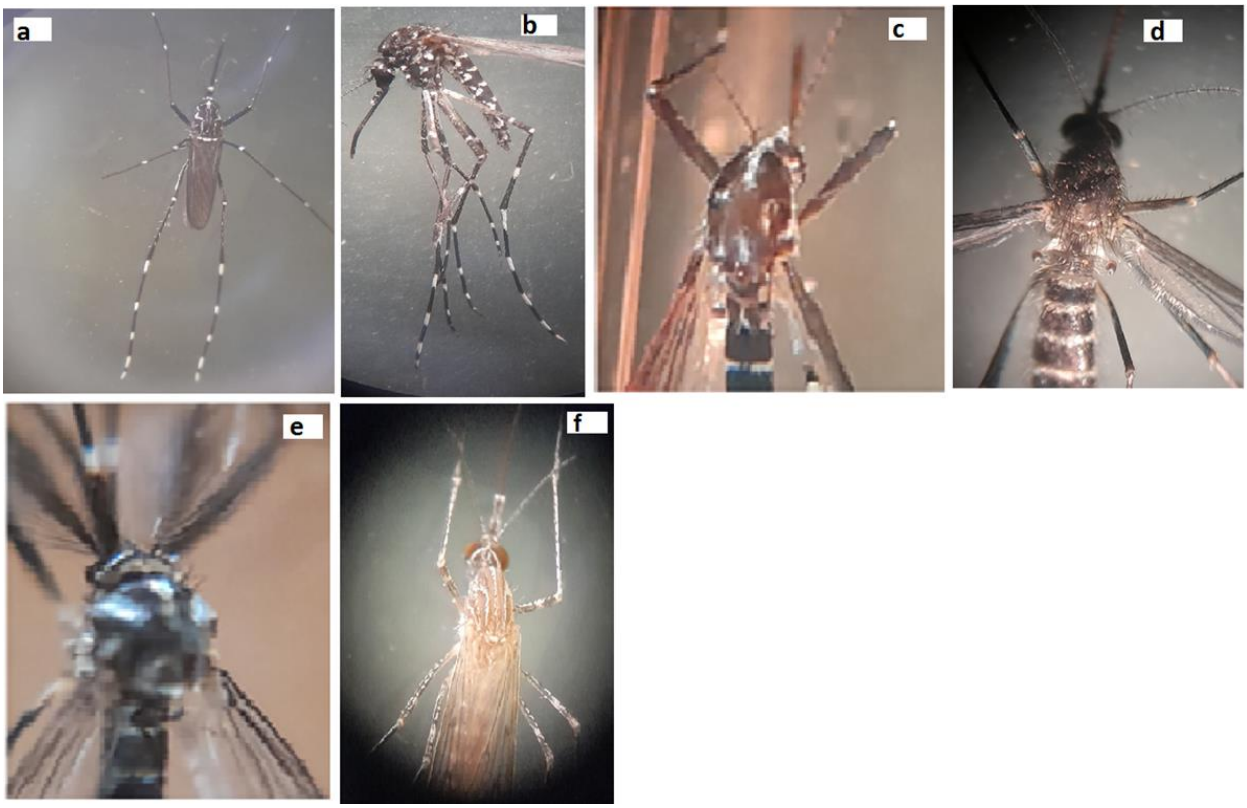


Figure 7: Four *Aedes* species (a) *Aedes aegypti,* (b) *Aedes vittatus*, (c) *Aedes albopictus*, (d) *Aedes communis*, (e) *Aedes opok* and (f) *Culiseta longiareolat*) were identified in the study sites.

3.6.2 Molecular identification of *Aedes* species

3.6.2.1 DNA extraction

The legs of each pereserved *Aedes* mosquito was washed briefly in 70% alcohol in a Petri dish, transferred to a filter paper and dried at room temperature for approximately 1 min. DNA was extracted from the fore-, mid-, and hind legs using Phenol-Chloroform-Isoamyl method (Gupta and Preet, 2012; Vitale *et al.,* 2023). Prepared *Aedes* mosquito legs were collected to microtube; added 12.5µl lysis buffer solution and then crushed by glass rod; spined with vortex mixer for 1 minute. 25µl of proteinase K and 437.5µl buffer lysis were added and mixed by vortex mixer and placed to incubator in the thermomixer at 65ºC for 30 minutes. It was taken and added 500µl of PCI (25µl phenol: 24 µl chloroform: 1µl Isoamyl alcohol), spined at 550 rpm for 10 minutes.

Centrifuged at 15,000 rpm for 5 minute and then the supernatant was transferred to new microtube and the precipitated pellet was discarded. A 14.5µl of 5MNaCl solution was added and centrifuged at 15,000 rpm for 1 minute and the supernatant was transferred to new microtube. 700µl of 96% ethanol was added and mixed by inversion with hand holding and then placed into incubator at -20ºC for overnight. It was centrifuged at 15,000 rpm for 1 minute. 500µl ethanol was added to microtube and incubated at -20ºC for 1 minute and then centrifuged for 5 minutes. Supernatant was discarded and the microtube was upside dawn on the soft paper for dehydration. Extracted DNA was resuspended by add 50µl Tri-EDTA. Suspended DNA was preserved at -20ºC for further study.

3.6.2.2 Amplification of species specific genes by PCR

Polymerase chain reaction (PCR) amplification was made to species specific barcode genes such as the mitochondrial cytochrome oxidase subunit 1 (COI-1) for *Aedes aegypti*; internal transcribed spacer 1 (ITS1) for *Ae. vittatus* and internal transcribed spacer 2 (ITS2) for *Cs. longiareolata*. A 735 bp region flanking the mitochondrial COI gene was amplified by PCR using the following primers: forward 5-GGATTTGGAAATTGATTAGTTCCTT-3 and reverse 5-AAAAATTTT AATTCCAGTTGGAACAGC -3. A polymerase chain reaction consist of Master mix PCR tube was 12.5 µl; primary forward 1.5 µl; reverse primer 1.5 µl; template DNA 2 µl, and as many as 7.5 µl ddH2O with a total volume of 25 µl. Time required for PCR method for 1 hour 48 minutes. The process of PCR performed 3 stages: initiation, annealing, and a final extension. Initiation process for 5 minutes at a temperature of 95 °C, followed by denaturation i.e. 35 cycling stages 94 ºC for 50 seconds, annealing 56 ºC for 1 minute, the extension of 72 ºC for 1 minutes followed by a final extension at 72ºC for 10 minutes(Gupta *et al.,* 2016)

The second gene was a 700 bp region bordering internal transcribed spacer1 (ITS1) using the following primers: forward 5-GAAGTAAAAGTCGTAACAAGG-3 and reverse 5-CGACCCTCAGACAGGCGTGGC-3. PCR amplification was performed with a thermocycler (Veriti, Applied Biosystems, Taipei, Taiwan). Each PCR reaction mixture consisted of primary forward 0.5 µl and reverse primers 0.5 µl, Master mix PCR tube was 11.5 µl; template DNA 2 µl, and as many as 5.5 µl ddH2O with a total volume of 20 µl. The PCR conditions included denaturation at 94 °C for 5 min, followed by 35 cycles of denaturation at 94 °C for 1 min, annealing at 53 °C for 1 min, extension at 72 °C for 1 min, and final extension at 72 °C for 10 min. The third step was setting the temperature to 4 °C for an infinite period of time.

The third gene was targeted to the internal transcribed spacer 2 (ITS2) gene and amplified by PCR using the following primers: forward 5-CAGGTTATGCGAACATCGAC-3 and reverse 5-GTCTTACCTGATCTGAGGTC-3. The PCR amplification was performed with a thermocycler (Veriti, Applied Bioosystems, Taipei, Taiwan). Each PCR reaction mixture consist of primary forward 0.5 µl and reverse primers 0.5 µl, Master mix PCR tube was 11.5 µl; template DNA 2 µl, and as many as 5.5 µl ddH2O with a total volume of 20 µl. The PCR conditions included denaturation at 94 °C for 5 min, followed by 35 cycles of denaturation at 94 °C for 1 min, annealing at 53 °C for 1 min, extension at 72 °C for 1 min, and final extension at 72 °C for 10 min. The third step was setting the temperature to 4 °C for an infinite period of time (Mousson *et al.,* 2005).

3.6.2.3 Amplified gene of *Aedes* species detect by gel electrophoresis

A total of 100 mL of Tris-Acetate-EDTA (TAE) buffer was added to a glass vial containing 1.5 g agarose powder. The mixture was heated in a microwave for five minutes until fully dissolved. After cooling slightly, 3 µl of ethidium bromide was added and mixed thoroughly. The solution was then poured into a gel casting tray with a comb and allowed to solidify for 30 minutes. Next,

2 µl of a molecular weight marker (DNA ladder) was added to serve as a reference for base pair (bp) comparison. For each sample, 2 µl of loading dye was mixed with 4 µl of PCR product on parafilm. The prepared mixtures were carefully loaded into the wells of the solidified gel using a micropipette. The gel was placed in an electrophoresis chamber and run at 120 volts and 100 milliamperes for 45 minutes. DNA bands were visualized using an ultraviolet (UV) imaging system to confirm the presence of amplified DNA (Kuldeep Gupta *et al.*, 2016). Positive PCR products were sent to Macrogen Biotechnology Company Ltd. in South Korea for purification and sequencing. The obtained sequences were edited using Bio Edit software and submitted to the GenBank database. A phylogenetic tree was constructed using multiple sequence alignment with Clustal W in MEGA 6, and compared with available sequences in GenBank about covering percentage and similar identity (Tamura *et al.*, 2013). Finally requested annotation number from the BLAST company.

3.7 Detection of arboviruses in *Aedes aegypti*

3.7.1 Rearing and processing of *Aedes aegypti*

The study was conducted by cross section design to detect arboviral infectious status of *Ae. aegypti.* Larvae and pupae collected from the study sites and transported to insectary room, University of Gondar. The larvae were kept in plastic trays (25 cm x 25 cm x7 cm) filled to a depth of 5 cm with distilled water. After eclosion, the larvae were provided with finely powdered Tetramin® fish food as a source of food. After the larvae reached the pupal stage, the pupae were separated every day and introduced into a beaker half-filled with distilled water and kept inside the adult emergence cages. Filter paper was provided in distilled water-filled cups for oviposition. The adult mosquitoes were provided with 10% sucrose solution (w/v) as food. At 3 to 5 days old,

adults were first starved at eight hours and then provided with a rabbit blood feeding for 30 min. The culture was maintained at 27 ± 1°C, 65-70% RH and on a 12:12 h photo-period. Three days post-blood feeding, filter paper was placed in glasses containing distilled water to facilitate oviposition. This procedure was repeated across the first, second, and third generations of *Ae. aegypti* (McLean-Cooper *et al.*, 2008). Specimens from each generation were preserved separately at -80°C for subsequent detection of transovarial transmission of arboviral infection of *Ae. aegypti*

3.7.2 RNA extraction

TRIzol is a monophasic solution of phenol and guanidinium isothiocyanate, which simultaneously solubilizes biological material and denatures proteins. Application of chloroform, the solution undergoes phase separation: proteins partition into the organic phase, DNA localizes at the interphase, and RNA remains in the aqueous phase. This allows for the simultaneous isolation of RNA, DNA, and protein from a single sample. In addition to total RNA, TRIzol is also effective in isolating small RNA species, including microRNAs, piwi-interacting RNAs (piRNAs), and endogenous small interfering RNAs (siRNAs) (Ries *et al.,* 2024)

Total Nucleic Acid Extraction: Female *Ae. aegypti* samples were prepared 21 pooled for three generation and each pool of microtube (Eppendorf tube) consist of 20 female *Ae. aegypt*. Eppendorf tubes were labeled according to the first, second, and third generations of female *Aedes aegypti* mosquitoes. 0.2 g of zirconia/silica beads and 20 females *Aedes aegypti* were put to Eppendorf tube and 200 µL of molecular-grade water was added to each tube. Mosquitoes were homogenized using a Mini-Bead Beater machine at 3,800 rpm for 20 seconds. The homogenate was then centrifuged at 2,000 rpm for 1 minute, and the supernatant was transferred to a new labeled Eppendorf tube. Next, 1,000 µL of TRIzol™ Reagent was added to each tube. The samples

were incubated at room temperature for 5 minutes to allow complete dissociation of the nucleoprotein complexes. Followed to incubation samples were centrifuged at 12,000 × g for 3 minutes at 4 °C, and the resulting supernatant was transferred to a new Eppendorf tube. Then, 200 µL of chloroform was added to each tube. The tubes were shaken vigorously by hand for 15 seconds to mix, incubated at room temperature for 2–3 minutes, and centrifuged again at 12,000 × g for 15 minutes at 4 °C. After centrifugation, the samples separated into three distinct phases: Top layer (clear aqueous phase): contains RNA; Middle layer (white interphase): contains DNA and Bottom layer (red organic phase): contains proteins (Hebbale, 2015).

Pure RNA Extraction: The top aqueous layer from each sample, containing RNA, was carefully transferred to a new Eppendorf tube. 500 µl of 100% isopropanol was added by angling the tube at 45° and pipetting the solution along the side. The tubes were inverted five times to mix and incubated at room temperature for 10 minutes. Samples were centrifuged at 12,000 × g for 10 minutes at 4 °C. The supernatant was carefully removed using a pipette, ensuring the RNA pellet was not disturbed (Lope Choque, 2020)

RNA Washing and Resuspension: The RNA pellet was washed with 1ml of 75% ethanol, mixed gently by occasional inversion. The samples were briefly homogenized, then centrifuged at 7,500 × g for 5 minutes at 4 °C. After centrifugation, the supernatant was discarded, and the RNA pellet was air-dried for approximately 15 minutes. The dried RNA pellet was resuspended in 50 µL of molecular-grade water by pipetting up and down. The tubes were incubated in a heat block at 55 °C for 10–15 minutes to fully dissolve the RNA. Finally, the quantity and quality of the extracted RNA were assessed using a Nano Drop spectrophotometer (Die and Román, 2012)

3.7.3 Detection of dengue, chikungunya and Zika virus infection of *Ae. aegypti*

Arbovirus infectious of *Aedes aegypti* was detected using SYBR Green TR-PCR technique. A total of 1,260 female *Ae. aegypti* from the first, second, and third generations were screened to arboviral infectious. Each generation comprised 21 pools, each pool contained to 20 female *Ae. aegypti* mosquitoes. Three negative extraction controls were included per generation. The TaqMan™ Arbovirus Triplex Kit was used to detect viral RNA from the samples. This kit is designed to simultaneous detection of ZIKV, DENV and CHIKV and included specific primers, positive control and TaqMan™ probes (Ries *et al*., 2024). The reaction setup followed the manufacturer's protocol (Panmei *et al.,* 2025). The total RNA sample volume was adjusted to 25 µL per reaction using nuclease-free water. The steps were as follows: The cap of the 8-tube strip was removed and discarded; 25 µL of RNA was added to each tube; a new optical cap strip was applied firmly; the contents were mixed briefly by vortexing and centrifuged to collect the mixture; dye detectors were assigned to each tube based on the layout and the tube strips were loaded into the real-time PCR instrument. Thermal cycling conditions for the assay were reverse transcription: 50 °C for 20 minutes (1 cycle); Enzyme activation: 95 °C for 2 minutes (1 cycle) and amplification: 40 cycles of 95 °C for 15 seconds and 60 °C for 1 minute (Wilca-Cepeda *et al.*, 2025).

3.8 Detection of Wolbachia infection *Aedes aegypti*

3.8.1 DNA extraction

DNA extraction was performed using the Cetyl Trimethyl Ammonium Bromide (CTAB) method and the following the protocol was described by Tel-Zur *et al*. (1999). The CTAB extraction buffer was prepared by combining the following components: (a) 100 mL of 1 M Tris-HCl (pH 8.0) to achieve a final concentration of 100 mM; (b) 20 mL of 0.5 M EDTA to reach 10 mM; (c) 81.8 g

of NaCl for a final concentration of 1.4 M; (d) 20 g of CTAB for a 2% solution; (e) sterile water to bring the final volume to 1 liter. Eppendorf tubes were labeled for first-, second-, and third-generation *Aedes aegypti*. For each generation, pools of ten female mosquitoes were placed into individual tubes, and 200 µL of molecular-grade water was added to each. The mosquito samples were then homogenized using a mini-Leadbeater. The samples were centrifuged at 2,000 rpm for 1 minute. The supernatant was then transferred to a new, labeled Eppendorf tube. Next, 200 µL of CTAB buffer was added to each tube containing the mosquito homogenate. The samples were incubated in a dry heat block at 65°C for 10 minutes. After incubation, 200 µL of chloroform was added to each tube. The tubes were gently inverted 10 times to mix, followed by centrifugation at 12,000 rpm for 5 minutes. Using a new pipette tip for each sample, 75 µL of the upper aqueous layer was carefully transferred to a fresh tube, while the chloroform layer was discarded. To each supernatant, 200 µL of isopropanol was added and mixed by inverting 10 times. The mixture was then centrifuged at 12,000 rpm for 5 minutes. The isopropanol supernatant was discarded, and the resulting DNA pellet at the bottom of the tube was retained. Each pellet was washed with 200 µL of 70% ethanol and centrifuged again at 12,000 rpm for 5 minutes. The ethanol was discarded, and the tubes were placed in a dry heat block at 45°C until the pellets were completely dry and no ethanol remained.

Finally, 40 µL of molecular-grade water was added to each pellet to resuspend the DNA. The tubes were incubated at 55°C for 5–10 minutes in a heat block and then centrifuged at 2,000 rpm for 1 minute. The extracted DNA was stored at -4°C for short-term use and at −20°C for long-term storage.

3.8.2 Detection of Wolbachia strains

Four designed primers Wolbachia genes were used to detect infectious statues of *Ae. aegypti* such as wsp, wMel, wAlbB, and wMwA (Table 1) and using conventional RT-PCR techniques. A total of 630 female *Ae. aegypti* mosquitoes were molecularly screened across the first, second, and third generations. Each generation consisted of 21 pools and individual pool containing 10 female *Aedes* mosquitoes' samples. Four designed primer genes were amplification DNA extracted from preserved *Aedes aegypti*. A polymerase chain reaction consist of Master mix PCR tube was 6.4 µl; primary forward 0.5 µl; reverse primer 0.5 µl; template DNA 2µl, and as many as 10.6 µl ddH2O with a total volume of 20µl. Time required for PCR method for 1 hour 48 minutes. Thermocycle profile: Reverse transcription 50°C for 10 min, initial denaturation: 95°C for 3 min, 40 cycles, denaturation: 95°C for 15 sec, annealing: 60°C for 60 sec. Use a negative control and non-template control to validate in the reaction. The Ct value is automatically calculated and known by positive strain of background. Accordingly, the previous reported a positive sample detection of Wolbachia strain has a CT value < 35.

Table 1: Wolbachia bacteria genes and primers amplified using PCR

No.	Designed Wolbachia bacterial gene	Forward & reverse primer
1	Wolbachia surface protein (wsp) gene, 139 bp	FP = (5′- GCATTTGGGTTAYAAAATGGACGA-3′) RP = (5`- GGAGTGATAGGCATATCTTCAAT-3`)
2	wMel gene, 68 bp	FP = (5`- CAAATTGCTCTTGTCCTGTGG-3`) RP = (5`-GGGTGTTAAGCAGAGTTACGG-3`)
3	wAlbB gene, 109 bp	RP = (5`-CCTTACCTCCTGCACAACAA-3`)

		RP = (5`-GGATTGTCCAGTGGCCTTA-3`)
4	wMwA gene, 155 bp	RP = (5`- GAAGTTGAAGCACAGTGTACCTT-3`) RP = (5`-GCTTGATATTCCTGTAGATTCATC-3`)

3.9 Data analysis

Data were analyzed using IBM SPSS statistical software for Windows (IBM Corp., Armonk, NY) version 20.0. Habitat infestation with *Aedes* larvae and pupae was described using Stegomyia indices as House Index (HI; number of infested houses divided by the total number of houses inspected), Container Index (CI; number of positive containers divided by number of containers inspected) and Breteau Index (BI; number of positive containers per 100 houses inspected) (Ngugi *et al*., 2017). Species diversity was determined as $H = -\sum [Pi * \ln(Pi)]$ when, H = Shanon diversity index, Pi=proportion of individual species, ln = natural logarithm, Pielou's evenness Index (J) = H/Ln(S) when S = number of species) (Krebs, 1972; Lande, 1996) in three study towns

Mann-Whitney U test was used to compare larval density between artificial and natural habitats, with and without algae, presence or absence of tadpole, and temporary and permanent. Kruskal–Wallis H test was used to compare larval density among more than two groups. Spearman correlation coefficient was used to compare association between larval density and physical/chemical properties of larval habitats. Multiple regression analysis was used to investigate the impact of larval habitat variables on larval density.

Data were checked by the Shapiro-Wilk test for being normally distributed, and those not normally distributed were log-transformed [log (n + 1)] to fit the normal distribution curve and analyzed by one way ANOVA using Tukey Kramer multiple comparison with the post hoc t-test. The biting rates were compared among the study sites, biting hours, host groups, and abdominal status of

female *Aedes* mosquitoes. The independent sample t-test was used to compare *Aedes* species biting rates between two biting hours in both outdoor and indoor setting. Mosquito biting rate (MBR) is determined as the total number of female *Ae. aegypti* or *Ae. vittatus* per person per hour. Phylogenetic analysis was performed for selected *Ae. aegypti* and *Ae. vittatus* samples, in which case their gene sequences were aligned with CLUSTAL W software version 1.6, examined using the program MEGA (Molecular Evolutionary Genetics Analysis) version 7.1.16 Neighbor joining (NJ) and their phylogenetic trees were constructed. Statistical values were considered significant when corresponding $p < 0.05$ (Chow *et al.,* 1998).

Chapter Four: Results

4.1 Physical and chemical characterstics of *Aedes* larval and pupal habitats

4.1.1. Indices of *Aedes* larvae and pupae

A total of 2028 habitats were surveyed of which 512 (25.24%) were infested with immature stage of *Aedes* mosquitoes. The highest number of *Aedes* larvae infested houses (highest house index; HI) was observed in Metema Yohannes town (31.53%), followed by Koki (27.81%) and Genda-Wuha (20.53%) (Fig. 8). On the other hand, the highest container index (CI) was observed in Genda-Wuha town (28.43%), followed by Kokit (26.59%) and Metema Yohannes (21.27%). Similarly, the highest number of positive containers per 100 houses inspected (BI) was observed in Metema-Yohannes (49.84%) followed by Kokit (47.65%) and Genda-Wuha (45.96%). There was a significant difference (Post hoc test, $F_{(2, 345)} = 59.055$; $P < 0.000$) among the three towns to their house infestation.

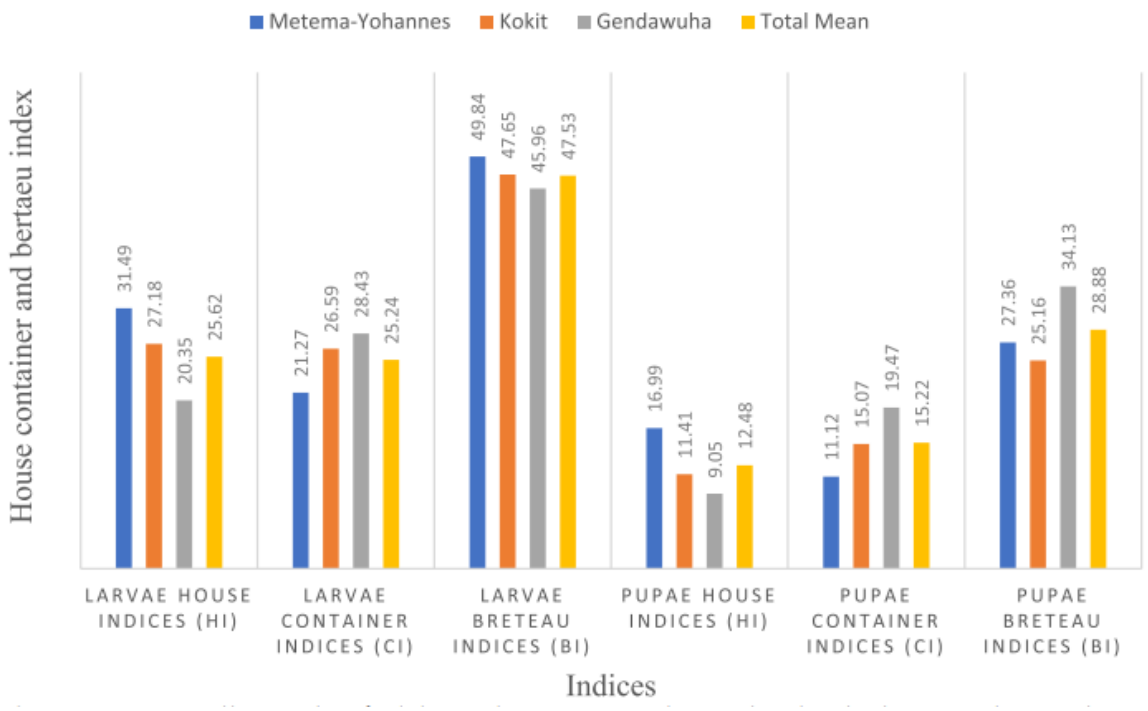


Figure 8: Infestation status of house, container and breteau indices of Aedes larvae and pupae in Metema-Yohannes, Kokit and Genda-Wuha towns, northwestern Ethiopia, January, 2022 - December, 2023

4.1.2 Distribution and abundance of *Aedes* larvae and pupae

A total of 9325 Aedes larvae and 683 pupae were collected from different breeding containers in the study towns (Table 2). About 43.77% (4082) of the larvae were from Metema-Yohannes, 25.52% (2380) from Kokit, and 30.70% (2863) from Genda-Wuha. There was no significant difference in larval/pupae abundance between the towns ($X^2 = 0.142$; $P = 0.868$). The density of *Aedes* larvae in artificial habitats was significantly higher than in natural habitats ($U = 2.337$; $p = 0.021$). The number of larvae collected was significantly higher in discarded tyres (78.18%; 6799) than in the other container types ($X^2 = 25.858$; $P = 0.001$) such as in plastics (8.72%; 813), metals (7.51%; 723) and clay pots (5.58%; 505). The highest density of *Aedes* larvae occurred in tree-holes (52.65%; 290), followed by in rain pools (32.98%; 127), footprints (8.28%; 39), and river banks (6.15%; 29) and the difference was significant ($X^2 = 7.100$; $P = 0.012$). The abundance of

Aedes pupae also followed a similar trend although their numbers were comparatively lower compared to the larvae.

Table 2: Habitat types and density of *Aedes* larvae/pupae in Metema-Yohannes, Kokit, and Genda-Wuha towns, northwest Ethiopia, Jan.2022-Dec. 2023

Study site	Habitat types	Total No. dips	Total No. larvae (%)	Total No. pupae (%)	Density larvae	Density Pupae
Metema-Yohannes	Discard Tyres	65	3001(73.5)	187(58.2)	46.17	2.87
	Discard Plastic	24	369 (9.1)	73(22.7)	15.37	3.04
	Discard Metal	39	297(7.3)	28 (8.8)	7..62	0.72
	Clay Pots	19	231(5.6)	17 (5.2)	12.15	0.89
	Rain Pool	60	44(1.1)	4 (1.2)	0.73	0.06
	Hoof Print	18	14(0.3)	0 (0.0)	0.77	0
	River Bank	17	20(0.5)	0 (0.0)	1.18	0
	Tree Hole	9	106(2.5)	12 (3.7)	11.77	1.3
Total		251	4082	321	88.14	8.88
Kokit	Discard Tyres	53	1628(68.4)	112(65.8)	30.72	2.11
	Discard Plastic	29	294(12.3)	22(12.9)	10.14	0.76
	Discard Metal	41	148(6.2)	16(9.4)	3.61	0.39
	Clay Pots	19	130(5.4)	11(6.4)	6.84	0.58
	Rain Pool	49	67(2.8)	7(4.1)	4.46	0.46
	Hoof Print	36	18(0.7)	0(0.0)	0.5	0
	River Bank	19	0(0.0)	0(0.0)	0	0
	Tree Hole	15	95(3.9)	2(1.1)	1.94	0.04
Total		261	2380	170	58.21	4.34
Genda-Wuha	Discard Tyres	51	2170(75.8)	109(56.7)	42.54	2.13
	Discard Plastic	27	150(5.2)	24(12.5)	5.55	0.88
	Discard Metal	51	278(9.7)	31(16.1)	5.45	0.61
	Clay Pots	21	144(5.1)	23(11.9)	6.85	1.09
	Rain Pool	44	16(0.5)	0(0.0)	0.36	0
	Hoof Print	30	7(0.2)	0(0.0)	0.23	0
	River Bank	15	9(0.3)	0(0.0)	0.6	0
	Tree Hole	13	89(3.1)	5(2.6)	6.84	0.38
Total		252	2863	192	68.42	5.09

4.1.3 Composition of *Aedes* species among the larvae/pupae

A total of 8993 adult *Aedes* mosquitoes were reared from larvae and pupae of which 56.77% (5106) were *Ae. aegypti,* 37.25% (3350) *Ae. vittatus*, 0.26% (24) *Ae. albopictus*, 2.39% (215) *Ae. communis,* 0.66% (60) *Ae. opok* and 2.64% (238) *Cs. longiereolata* (Table 3). Both *Ae. aegypti* and *Ae. vittatus* was present in all the surveyed towns; however, *Ae. albopictus* and *Ae. opok* occurred in Metema-Yohannes, while *Ae. communis* occurred in Genda-Wuha and Kokit. *Ae. aegypti* exhibited the highest abundance, followed by *Ae. vittatus* in all the study sites. *Ae. opok* and *Ae. albopictus* were the least abundant in Metema-Yohannes town. Additionally, more evenness distribution (J value = 0.71) was observed in Kokit followed by Genda-Wuha (0.65) and Metema-Yohannes (0.59).

Table 3: Composition of adult *Aedes* species reared from larvae in Metema District, Northwest Ethiopia, Jan. 2022 – Dec. 2023

Town	Breeding habitat	*Ae. aegypti*	*Ae. vittatus*	*Ae. albopictus*	*Ae. communis*	*Ae. opok*	*Cs. longiareolata*	Total
Metema-Yohannes	Discard Tyres	1649	1305	10	0	0	0	2964
	Discard Plastic	225	92	5	0	0	0	322
	Discard Metal	108	131	3	0	0	0	242
	Clay Pots	112	62	1	0	15	0	190
	Rain Pool	11	4	5	0	24	0	44
	Hoof Print	14	0	0	0	0	0	14
	River Bank	15	2	0	0	0	0	17
	Tree Hole	53	18	0	0	21	0	92
	Total	2187	1614	24	0	60	0	3885
Kokit	Discared Tyres	893	703	0	12	0	27	1635
	Discared Plastic	128	123	0	9	0	31	291

	Discared Metal	43	40	0	25	0	35	143
	Clay Pots	51	52	0	17	0	11	131
	Rain Pool	46	36	0	3	0	0	85
	Hoof Print	13	5	0	0	0	0	18
	River Bank	0	0	0	0	0	0	0
	Tree Hole	27	30	0	0	0	5	62
	Total	1201	989	0	66	0	109	2365
Genda-Wuha	Discared tyres	1455	548	0	71	0	36	2110
	Discared plastic	50	32	0	38	0	30	150
	Discared metal	104	78	0	35	0	41	258
	Clay Pots	53	58	0	0	0	13	124
	Rain Pool	9	7	0	0	0	0	16
	Hoof Print	2	5	0	0	0	0	7
	River Bank	3	1	0	5	0	0	9
	Tree Hole	42	18	0	0	0	9	69
	Total	1718	747	0	149	0	129	2743

4.1.4 Physical characteristics of breeding habitat on the density of *Aedes* species

The number of adult *Ae. aegypti* emerged from larvae and pupae from artificial habitats was significantly higher than those from the natural habitats ($p < 0.001$). The number of *Ae. aegypti* emerged from algal habitats was greater than the number from the algae free habitats ($p < 0.001$) and the number from tadpole free habitats was greater than the number from tadpole infested habitats ($p < 0.001$). *Ae. vittatus* was shown to be more frequent in artificial habitats ($p < 0.001$), algae-containing habitats ($p < 0.001$), and tadpole-free environments ($p < 0.001$) (Table 4).

Table 3: Effect of habitats' physical characteristics on the density of *Aedes aegypti* and *Aede Vitatus,* Metema-Yohannes, Kokit and Genda-Wuha Towns Northwest Ethiopia, January, 2022 - December, 2023

		Ae. aegypti		*Ae. vittatus*	
Habitat characterstices	Variables	Mean rank	Mann-Whitney & P-value	Mean rank	Mann-Whitney & P-value
Habitat Types					
	Artificial	83.69	U = 185.500	84.86	U = 108.000
	Natural	30.5	P = 0.000	29.04	P = 0.000
Permanence					
	Permanent	61.01	U = 1044.000	60.62	U = 1081.500
	Temporary	56	P = 0.524	57.56	P = 0.697
Algae present or absent					
	Absent	36.48	U = 534.500	37.93	U = 608.500
	Present	61.35	P = 0.000	59.67	P = 0.000
Tadpole present or absent					
	Absent	44.36	U = 287.500	44.68	U = 271.500
	Present	24.48	P = 0.000	23.81	P = 0.000

The mean number of adult *Ae. aegypti* reared from larvae/pupae collected from habitats closer to residential houses (< 50 m) was higher than that from habitats located > 50 m away ($p < 0.001$). Habitats not exposed to light have the highest mean number of adult *Ae. aegypti* compared to partial and fully exposed habitats ($P < 0.001$). The highest number of *Ae. aegypti* was obtained from environments without emergent vegetation compared to the vegetated (grass and weed) habitats ($P < 0.001$). Discarded tyres supported more *Aedes* larvae ($P < 0.001$) than the other habitat types. The abundance of *Ae. vittatus* was similar to that of *Ae. aegypti* (Table 5).

Table 4: Physical characteristics of habitats and mean number of *Ae. aegypti* and *Ae. vitatus* larvae in Metema-Yohannes, Kokit and Genda-Wuha Towns, northwestern Ethiopia, January, 2022 - December, 2023

		Ae. aegypti		Ae. vitatus	
Habitat characterstices	Variables	Mean rank	Chi-square & p-value	Mean rank	Chi-square & p-value
Water volume (litter)					
	< 20lit	59.41	X2 =1.283	59.08	X2 = 59.08
	20 - 100 lit	67.86	P = 0.527	67.17	p = 0.617
	≥100 lit	56		57.56	
Distance nearest house					
	< 50 m	82.42	X2 = 29.293	80.8	X2 = 23.231
	50 - 200 m	58.9	p = 0.000	58.07	p = 0.000
	≥ 200 m	33.08		37.26	
Sunlight condition					
	No exposed	100.36	X2 = 57.500	98.88	X2 = 53.787
	Partial exposed	63.65	p = 0.000	63.76	p = 0.000
	Exposed	43.12		43.68	
Emergent plant coverage					
	Absent	72	X2 = 35.004	72.4	X2 = 37.382
	Grass	27.18	p = 0.000	32.36	p = 0.000
	Weeds	35.36		31.54	
	Grass+ weeds	29.8		26.1	
Substrate type					
	Tyre	105.08	X2 = 94.481	104.67	X2 = 98.468
	Plastic	67.32	p = 0.000	65.44	p = 0.000
	Metal	72.05		79.27	
	Clay pot	70.9		73.65	
	Wood	54.88		49.13	
	Stone	22.8		18.1	
	Mud	23.44		23.86	

4.1.5 Association between habitat's chemical characteristics and larval density

Chemical characterstics of the artificial habitats such as to temprature (r = 0.762, p = 0.008), dissolved oxygen (r = 0.581, p = 0.050), conductivity (r = 0.871, p = 0.001), Amonia (r = 0.766, p = 0.008), Amonium (r = 0.615, p = 0.039), Phosphate (r = 0.875, p = 0.001), Sulphate (r = 0.856, p =0.002) and Total hardness (r = 0.760, p = 0.009). Interestingly, most of natural habitats including groove stone, rain pools, hoof prints, river edges, and tree holes were fully exposed to sunlight. There was significant positive correlation between temperature (r = 0.797; P = 0.005), dissolved oxygen (r = 0.781; P = 0.006), Conductivity (r = 0.767; P = 0.008) and Total hardness (r = 0.699; P = 0.024) and the density of *Aedes* larvae (Table 6).

Table 5: Correlation between habitat characteristics and density of *Aedes larvae,* in Metema district, northwestern Ethiopia, January, 2022 -December, 2023

Habitat	Artificial			Natural habitat		
	Mean ± SE	Correlation coefficient	P- value	Mean ± SE	Correlative coefficient	P- value
Temperature (°C)	29.30 ± 0.52	0.762	0.008*	29.11 ± 0.52	0.797	0.005*
water PH	7.48 ± 0.21	0.416	0.133	8.41 ± 0.14	0.441	0.118
Dissolved oxygen	1.07 ± 0.16	-0.581	0.050*	4.11 ± 0.27	0.781	0.006*
Turbidity (NTU)	120.72 ± 42.26	-0.157	0.344	49.62 ± 14.27	-0.192	0.311
Conductivity(µs/cm)	215.37 ± 32.86	0.871	0.001*	372.45 ± 48.25	0.767	0.008*
TDS(ppm)	156.11 ± 16.08	0.38	0.156	354.84 ± 31.45	0.321	0.199
Ammonia (NH_3)	2.94 ± 0.58	0.766	0.008*	3.38 ± 0.49	-0.081	0.492
Ammonium (NH_4)	3.04 ± 0.61	0.615	0.039*	3.56 ±0.52	-0.134	0.366
Phosphate (PO_4)	2.52 ± 0.84	0.875	0.001*	1.08 ± 0.18	0.214	0.391
Sulphate (SO_4)	11.55 ± 3.31	0.856	0.002*	14.32 ± 1.18	-0.276	0.236
Nitrate (NO_3	1.73 ± 0.17	-0.567	0.056	5.91 ± 1.97	-0.368	0.166
Nitric (NO_2)	0.27 ± 0.14	0.260	0.249	0.47 ± 0.04	-0.094	0.405
Total hardness (mg/l)	73.16 ± 12.17	0.760	0.009*	155.93 ± 8.59	0.699	0.024*

*Statisticaly different at P < 0.05. df dgree of freedom

4.1.6 Association between *Aedes* larval density and physico-chemical characteristics of habitats

The best predictors of *Aedes* larval density in the breeding habitats to that determine by several independent variables of physico-chemical characterstics to analysis by model (Table 7). Multiple linear regression revealed that Aedes larvae/ pupae densities were positively related to temperature (β = 4.640, p = 0.003), turbidity (B = 0.048, P = 0.031), dissolved oxygen (B = − 0.048, P = 0.009) and sulphate (B = 0.385, P = 0.035) of water breeding containers. Similarly, total hardness (B = − 0.175, P = 0.042) of water-holding containers were negatively related

Table 6: Association between habitat chemical parameters and *Aedes* larvae density, northwestern Ethiopia, Jan.2022-Dec. 2023.

Parameter	B	95% CI for B		T	*P-value*
		Lower bound	Upper bound		
Temperature	4.640	1.772	7.509	3.355	0.003*
PH	-1.605	-6.274	3.065	-0.713	0.484
Dissolved oxygen	4.972	-8.593	-1.351	-2.848	0.009*
Turbidity	0.048	0.005	0.091	2.304	0.031*
Conductivity	-0.017	-0.063	0.028	-0.784	0.442
Total Dissolved Substrate	0.050	-0.002	0.102	1.997	0.058
Ammonia (NH3)	0.825	-2.361	4.011	0.537	0.597
Ammonium (NH4)	0.273	-2.969	3.513	0.175	0.863
Phosphate (PO4)	1.262	-0.630	3.154	1.383	0.185
Sulphate (SO4)	0.385	0.030	0.740	2.247	0.035*
Nitrate (NO3)	0.174	-0.488	0.835	0.544	0.592
Nitric (NO2)	2.793	-5.172	10.758	0.727	0.475
Total hardness	-0.175	-0.343	-0.007	-2.157	0.042*
Constant	-97.891	-190.107	-5.687	-2.202	0.038*

B the unstandardized coefficient value

4.1.7 Effects of season, temperature and humidity on the density of *Aedes* larvae

In general, density of *Aedes* larvae remained closer to zero from the first week of November to the end of April and increased starting from the beginning of May to the end of October (Figure 7).

The density remained between 40 and 50 from June to October. *Aedes* larvae were abundant in Metema-Yohannes town from the 15th June to 20th September, in Genda-Wuha town from the 25th May to the 10th of October. During the period from 15th October - 20th May, there was no water-holding habitat and also *Aedes* larvae in all the towns. Density of *Ae. vitatus* was 36.24 in June and gradualy decreased until the end of October. Monthly, the density of larvae showed a significant positive correlation with humidity *(*r = 0.896*, P* = 0.000) and negative correlation with averaege monthly temprature (r = -0.770, *P* = 0.003).

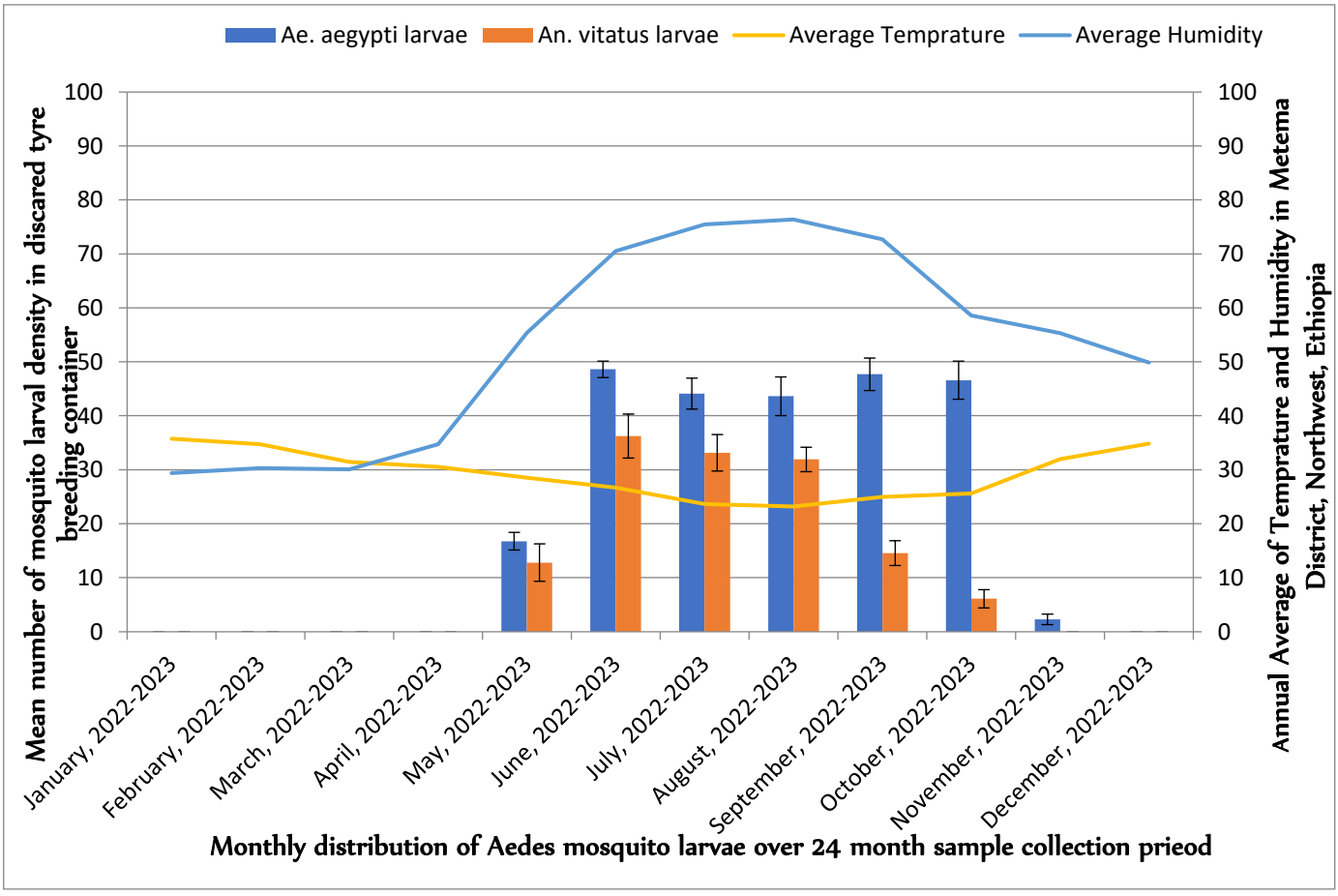


Figure 9: Monthly density of *Aedes aegypti* and *Aedes vittatus* in Metema district, Northwest Ethiopia, January, 2022-December, 2023

4.2 Biting time and host seeking behavior

4.2.1 Composition of Mosquito Species

A total of 16,324 female mosquitos were collected from three study towns, of which 42.3% (n = 6915) were from Metema-Yohannes, 24.1% (n = 3946) from Kokit, and 33.4% (n = 5463) from Genda-Wuha town (Table 8). There was no significant difference in the total number of mosquitoes catches among the towns (F = 0.386; df = 2.00; p = 0.695). The mosquitoes comprised the genus *Culex* (55.4%; n = 9056), *Aedes* (39.2%; n = 6411), *Anopheles* (4.9%; n = 800), and *Culiseta* (0.3%; n = 57). Of which 6411 *Aedes* mosquitoes, 67.5% (n = 4333) were *Ae. aegypti*, 29.5% (n = 1895) *Ae. vittatus*, 1.6% (n = 107) *Ae. communis*, 0.7% (n = 48) *Ae. albopictus*, and 0.4% (n = 28) *Ae. opok*. Similarly, the 800 *Anopheles* were composed of 60.2% (n = 482) of *An. gambiae s.l.,* 25.8% (n = 207) of *An. stephensi,* 8.7% (n = 70) of *An. pretoriensis*, 3.0% (n = 24) of *An. christyi* and 2.1% (n = 17) of *An. cinereus*. Among all the mosquitoes (n = 16,324) caught by the CDC-LT, 79.5% (12,492) were caught during night hours and 23.4% (3832) during the day light hour. All the *Anopheles*, *Culex*, and *Culiseta* were caught during night hours, whereas *Aedes* species were caught during both night and daylight hours

Table 7: Mosquitoes collected during day light hour and day night hour in Metema District, Northwest Ethiopia from June to September, 2023.

Study Town	Species	Day Light time (6:00 - 18:00)		Day Night time (18:00 - 6:00)		
		Indoor	Outdoor	Indoor	Outdoor	Total
Metema-Yohannes	*Ae. aegypti*	309	839	295	561	2004
	Ae. vittatus	147	380	133	184	844
	Ae. albopictus	17	19	0	12	48
	Ae. opok	13	9	0	6	28

	An. gambiae s.l.	0	0	76	123	199
	An. stephensi	0	0	37	65	102
	An. pretoriensis	0	0	11	23	34
	An. christyi	0	0	5	7	12
	An. cinereus	0	0	3	6	9
	Culex spp.	0	0	1220	2415	3635
Total		486	1247	1780	3402	6915
Kokit	*Ae. aegypti*	163	494	107	396	1160
	Ae. vittatus	87	242	67	172	568
	Ae. communis	17	29	0	19	65
	An. gambiae s.l.	0	0	42	107	149
	An. Stephensi	0	0	15	26	41
	An. Pretoriensis	0	0	3	7	10
	An. christyi	0	0	0	5	5
	An. Cinereus	0	0	0	3	3
	Cs. Longiareolata	0	0	0	25	25
	Culex spp.	0	0	768	1152	1920
Total		267	765	1002	1912	3946
	Ae. aegypti	193	519	87	370	1169
	Ae. vittatus	87	256	28	112	483
	Ae. communis	0	12	13	17	42
	An. gambiae s.l.	0	0	53	81	134
	An. stephensi	0	0	17	47	64
	An. Pretoriensis	0	0	10	16	26
	An. chrisityi	0	0	0	7	7
	An. Cinereus	0	0	0	5	5
	Cs. Longiareolata	0	0	0	32	32
	Culex spp.	0	0	1341	2160	3501
	Total	280	787	1549	2847	5463

4.2.2 Hourly human seeking behavior of *Aedes* species

In general, the outdoor biting rate of *Ae. aegypti* was higher than its indoor activity during both the day light hour and day night hour (Fig. 10). During the day light hour, *Ae. aegypti* exhibited peak biting rate between 07:00 and 08:00 with the biting rate of 4.5/person/hour followed by from 17:00 to 18:00 with the rate of 3.75/person/hour. The biting rates differed significantly across the collection hours (F= 480.630; P = 0.001). The peak indoor biting rate of *Ae. aegypti* was observed from 19:00 to 20:00 with biting rate of 2.00 bites/person/hour followed by from 08:00 to 09:00 with the biting rate of 1.50 bites/person/hour. The indoor biting rates differed significantly (F = 240.046; P = 0.001) across the hours. Moreover, its peak indoor biting activity in the morning hours was late by one hour compared to the corresponding peak outdoor biting hours and the values did not differ significantly.

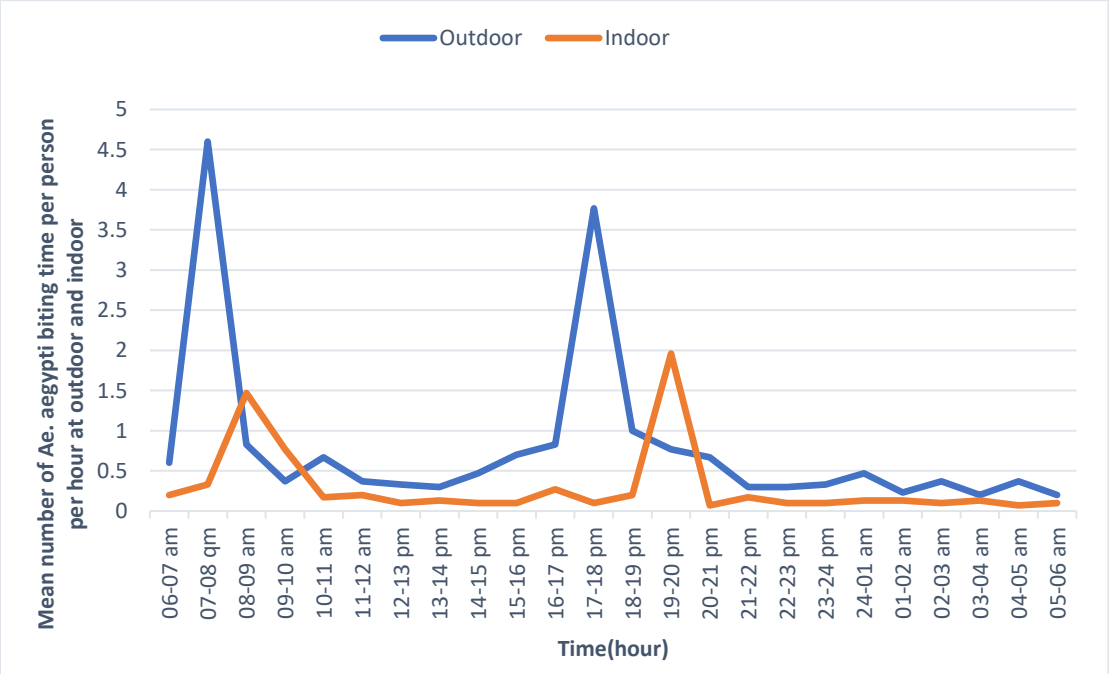


Figure 10: Human biting behavior of *Aedes aegypti*, Metema district, northwest, Ethiopia, Jun - Sept, 2023

The outdoor peak biting rate of *Ae. vittatus* was observed from 08:00 to 09:00 in the morning with 4.00 bites/person/hour followed by from 18:00 to 19:00 in the afternoon with 3.25 bites/person/hour (Fig. 11). Similarly, its peak indoor biting rate was observed between 09:00 and 10:00 in the morning with a bite rate of 1.75/hour/person followed by from 19:00 to 20:00 in the afternoon with a rate of 1.50 bites/person/hour. The biting rates differed significantly (F =217.752; P =0.001) across the hours. However, no significant difference was observed between two consecutive peaks outdoors and also indoors.

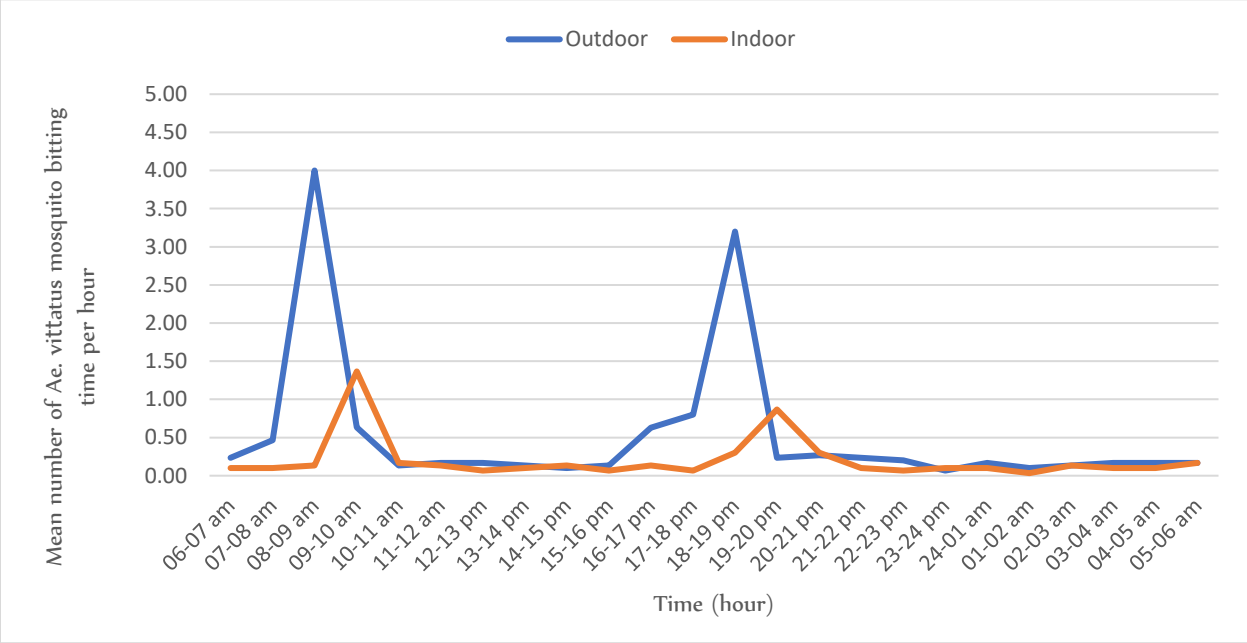


Figure 11: Human biting behavior of *Aedes vittatus* Metema district, northweast, Ethiopia, Jun – Sept, 2023

4.2.3 Host seeking behavior and physiological status of *Ae. agypti* and *Ae. vitatus*

A total of 6,411 female *Aedes* mosquitoes were collected using CDC-LT in the towns, of which 76.68% (4928/6411) were from the households (indoor and outdoor) with human stimulants and

20.27% (1300/6411) from domestic animal (cattle, sheep, goat, and donkey) shelters located in the compound. The highest percentage (82.1%) of *Ae. aegypti* preferred human, followed by cattle (6.7%), sheep (4.6%), goat (3.87%), and donkey (2.7%) (Table 9) and it showed significant difference ($P < 0.001$) compared to other hosts in the study sites. Similarly, the highest number of *Ae. vittatus* was collected from nearby humans (74.5%), followed by cattle (10.9%), sheep (6.0%), goat (4.7%), and donkey (4.0%) with significant difference ($P < 0.001$).

The highest proportion of *Ae. aegypti* was unfed (91.21%) followed by the blood fed (6.20%) and gravid (2.58%). There was significant difference ($p < 0.001$) among the abdominal status of *Ae. aegypt*. Similarly, the highest proportion of *Ae. vittatus* was unfed (89.87%), followed by the blood fed (7.86%) and gravid (2.27%) physiological condition. There was also significant difference ($p< 0.001$) among the abdominal status of female *Ae. vittatus*.

Table 8: Host seeking behavior and physiological status of *Aedes aegypti* and *Aedes vittatus* in Metema district, Northwest Ethiopia, Jun-Sept, 2023.

Ae. aegypti					*Ae.vittatus*			
	Unfed n(%)	Blood fed n(%)	Gravid n(%)	Total N	Unfed n(%)	Blood fed n(%)	Gravid n(%)	Total N
Human	3318(81.8)	232(86.2)	90(80.4)	3640(82.1)	1329(74.5)	118(79.2)	24(55.8)	1471(74.5)
Cattel	276(6.9)	18(6.7)	6(5.3)	300(6.7)	197(11.6)	14(9.4)	4(9.3)	215(10.9)
Sheep	194(4.9)	7(2.6)	4(3.6)	205(4.6)	104(6.1)	7(4.7)	7(16.3)	118(6.0)
Goat	153(3.9)	8(2.9)	7(6.2)	168(3.8)	82(4.8)	6(4.0)	4(9.3)	92(4.7)
Donkey	114(2.9)	4(1.5)	5(4.5)	123(2.7)	71(4.2)	4(2.7)	4(3.3)	79(4.0)
Total	4055(91.4)	269 (6.1)	112 (2.5)	4436	1783 (90.3)	149 (7.5)	43(2.2)	1975

4.3 Resting habitat

4.3.1 Mosquito species composition and their relative abundance in different resting places

A total of 9,936 resting adult mosquitoes were collected utilizing prokopack aspirator, mouth held aspirator, and Pyrethrum spray sheet collections (PSC) (Table 10). These mosquitoes belonged to four genera and 13 species. Mosquitoes collected from Metema-Yohannes, Kokit, and Genda-Wuha towns were 3678(37.02%), 3035(30.54%) and 3223 (32.43%), respectively. There was no significant difference ($P > 0.05$) among the distribution of the mosquitoes in towns. Resting adult mosquitoes belonged to the genera *Culex* (n = 5024; 50.56%), *Aedes* (n = 4054; 40.80%), *Anopheles* (n = 702; 7.06%) and *Culiseta* (n = 156; 1.57%). The five *Aedes* species collected from six resting places were *Ae. aegypti* (n = 3026/4054; 74.64%), *Ae. vittatus* (n = 723/4054; 27.26%), *Ae. communis* (n = 186/4054; 7.02%), *Ae. albopictus* (n = 77/4054; 2.90%), and *Ae. opok* (n = 42/4054; 1.58%) (Fig 12A). Furthermore, six *Anopheles* species were caught from their various resting locations namely *An. gambiae* s.l (n = 184/702; 26.21%), *An. stephensi* (n = 135/702; 19.23%), *An. pharonsis* (n = 53/702; 7.54%), *An. pretoriensis* (n = 144/702; 20.51%), *An. christyi* (n = 84/702; 11.96%) and *An. cinereus* (n = 102/702; 14.52%) which differed significantly in their abundance ($P < 0.05$).

Table 9: Distribution of adult mosquito species in different resting places of Geda-Wuha, Kokit and Metema-Yohannes towns, January to December, 2023

Study Town	*Mosquito* Species	Outdoor collections					Total outdoor	Total indoor	Grand Total
		Grain storage	Deep groove	Cattle shelter	Larval container	under bridge			
Metema-Yohannes	*Ae. aegypti*	190	74	324	301	94	983	98	1081
	Ae. vittatus	63	13	95	87	43	301	6	307
	Ae. albopictus	8	20	17	22	10	77	0	77
	Ae. opok	0	11	9	7	15	42	0	42
	An. gambiae s.l.	32	0	17	0	0	49	19	68
	An. stephensi	17	8	14	0	0	39	13	52
	An. pharensis	9	0	3	0	0	12	6	18

	An. pretoriensis	12	10	16	0	4	42	7	49
	An. christyi	10	4	11	0	3	28	2	30
	An. cinereus	9	1	15	0	4	29	3	32
	Culex spp.	163	517	400	521	204	1805	117	1922
	Total	**516**	**647**	**923**	**939**	**375**	3400	**278**	**3678**
Kokit	*Ae. aegypti*	150	53	300	280	75	858	77	935
	Ae.vittatus	43	12	73	85	4	217	26	243
	Ae. communis	13	17	36	24	4	94	7	101
	An. gambiae s.l.	23	9	14	0	0	46	13	59
	An. stephensi	21	0	5	0	0	26	12	38
	An. pharensis	7	1	5	0	0	13	4	17
	An. pretoriensis	15	11	10	0	4	40	6	46
	An. christyi	3	6	12	0	3	24	0	24
	An. cinereus	9	1	7	0	4	21	3	24
	Cs. longiareolata	7	5	11	13	2	38	3	41
	Culex spp.	203	307	321	414	157	1402	105	1507
	Total	**494**	**422**	**794**	**816**	**253**	2779	**256**	**3035**
Genda-Wuha	*Ae. aegypti*	170	63	312	292	86	923	87	1010
	Ae.vittatus	52	10	45	37	3	147	26	173
	Ae. communis	22	8	10	19	9	68	17	85
	An. gambiae s.l.	25	5	14	0	0	44	13	57
	An. stephensi	17	8	11	0	0	36	9	45
	An. pharensis	9	0	3	0	0	12	6	18
	An. pretoriensis	12	10	16	0	4	42	7	49
	An. christyi	10	4	11	0	3	28	2	30
	An. cinereus	17	0	19	0	4	40	6	46
	Cs. longiareolata	37	28	14	21	0	100	15	115
	Culex spp.	206	257	393	462	164	1482	113	1595
	Total	**577**	**393**	**848**	**831**	**273**	2922	**301**	**3223**

4.3.2 Distribution and abundance of *Aedes* species in their resting places

Among the 4054 *Aedes* species, the highest proportion (3026; 74.6%) belonged to *Ae. aegypti.* The highest proportion of *Ae. aegypti* found in the cattle shelters that compared to inside larvae breeding containers (873; 28.8%), local grain storage (510; 16.8%), indoor (262; 8.65%), under bridge (255; 8.42%), and deep groove (190; 6.27%). Similarly, *Aedes vittatus* were preferred cattle shade (213; 29.4%) to compared tyre breeding containers (209; 28.9%), local grain storage (158; 21.8%), indoor (58; 8.0%), bridge (50; 6.9%), and deep groove (35; 4.8%). Whereas *Ae. communis* were preferred to cattle shelter (46; 24.7%) and follow by larvae containers (43; 26.8%), local

grain storage (35; 17.74%), indoor (24; 12.90%), deep groove (25; 10.75%), and inside bridge (13, 6.98%). In addition, a higher number of *Ae. albopictus* were preferred tyre breeding containers (22; 33.7%) and follow by to cattle shelter (20; 22.0%), deep groove (17; 19.48%), local grain storage (10; 15.58%), bridge (8; 9.09%), and indoor 0(0.00%). Surprisingly, a higher number of *Ae. opok* was preferred to under bridge place (15; 35.7%) compared to the deep groove (11; 26.2%), cattle shade (9; 21.4%), breeding container (7: 16.6%), local grain storage (0; 0.0%) and indoor (0; 0.0%) (Table 10). All five *Aedes* species were significant different ($P < 0.001$) observed distribution to across six resting places.

4.3.3 Monthly distribution of *Aedes* species in their resting places

Density of *Ae. aegypti* and *Ae. vittatus* peaked in June and September (in the wet season), but remained very low from October - May (in the dry season) (Fig 12 & 13). However, *Ae. opok* was observed in the last two weeks of June and *Ae. communis* was higly abundant in September and the first week of October. In the wet season, the temprature reached 26 -28 ^{o}C and relative humidity 48-55 %. In the dry season, water holding containers became dry. The environmental temprature ranged from 31.53°C to 39.37°C and the corresponding relative humidity from 35 % to 47 %. Thus, a few numbers of *Ae. aegypti*, *Ae. vittatus* and *Ae. albopictus*, *Ae. communis and Ae. opok* were found in moisture producing places especially under deep groove and underside bridge surface. Monthly average temperature and relative humidity were significantly different ($p < 0.001$). Mann–Whitney U test revealed high statistical difference ($p < 0.001$) observed in average temperature and relative humidity between wet and dry seasons across the research sites.

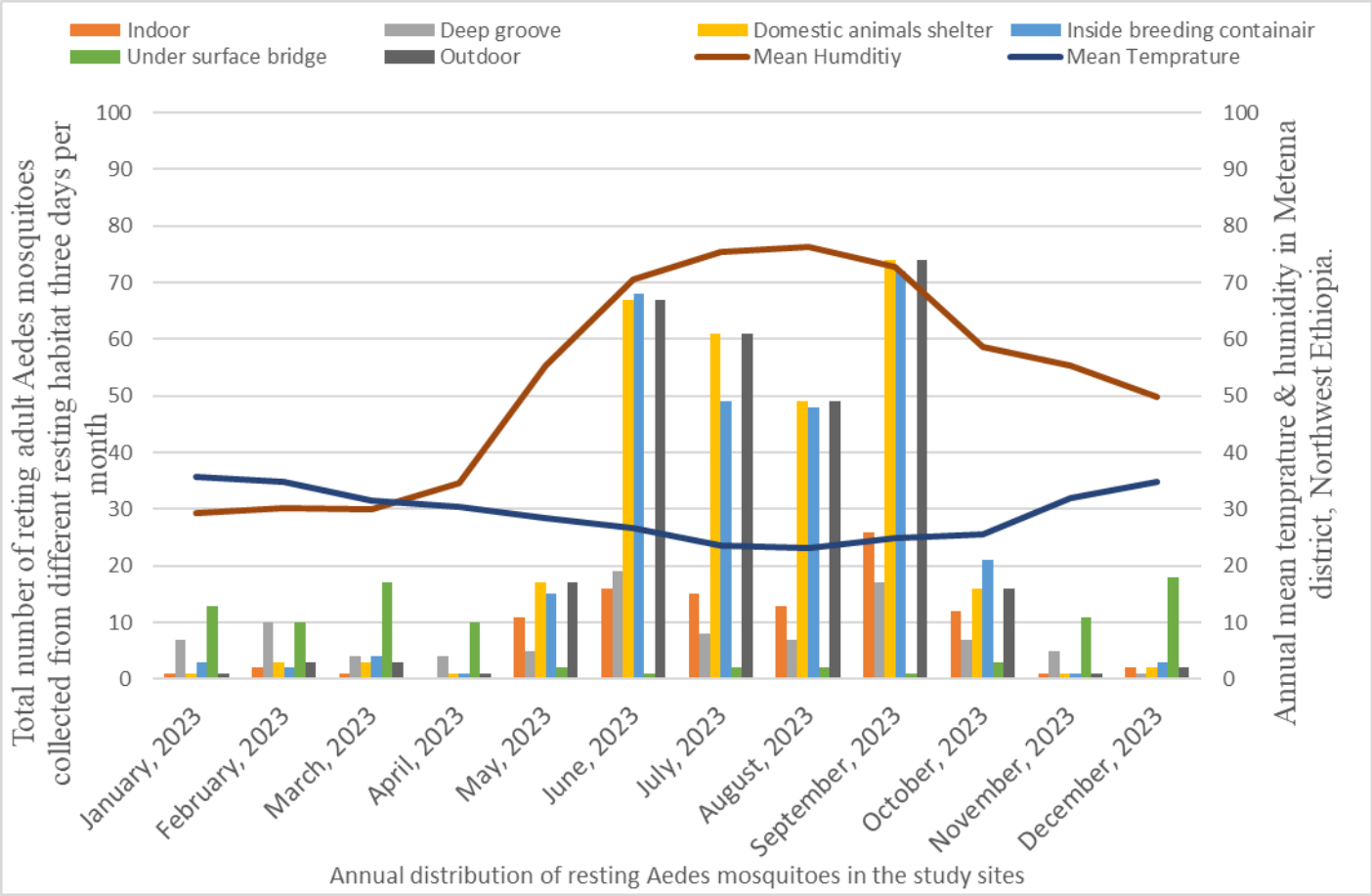


Figure 12: Monthly distribution of *Aedes* species in their resting places in Metema district, Northwest, Ethiopia, January, 2023-December, 2023

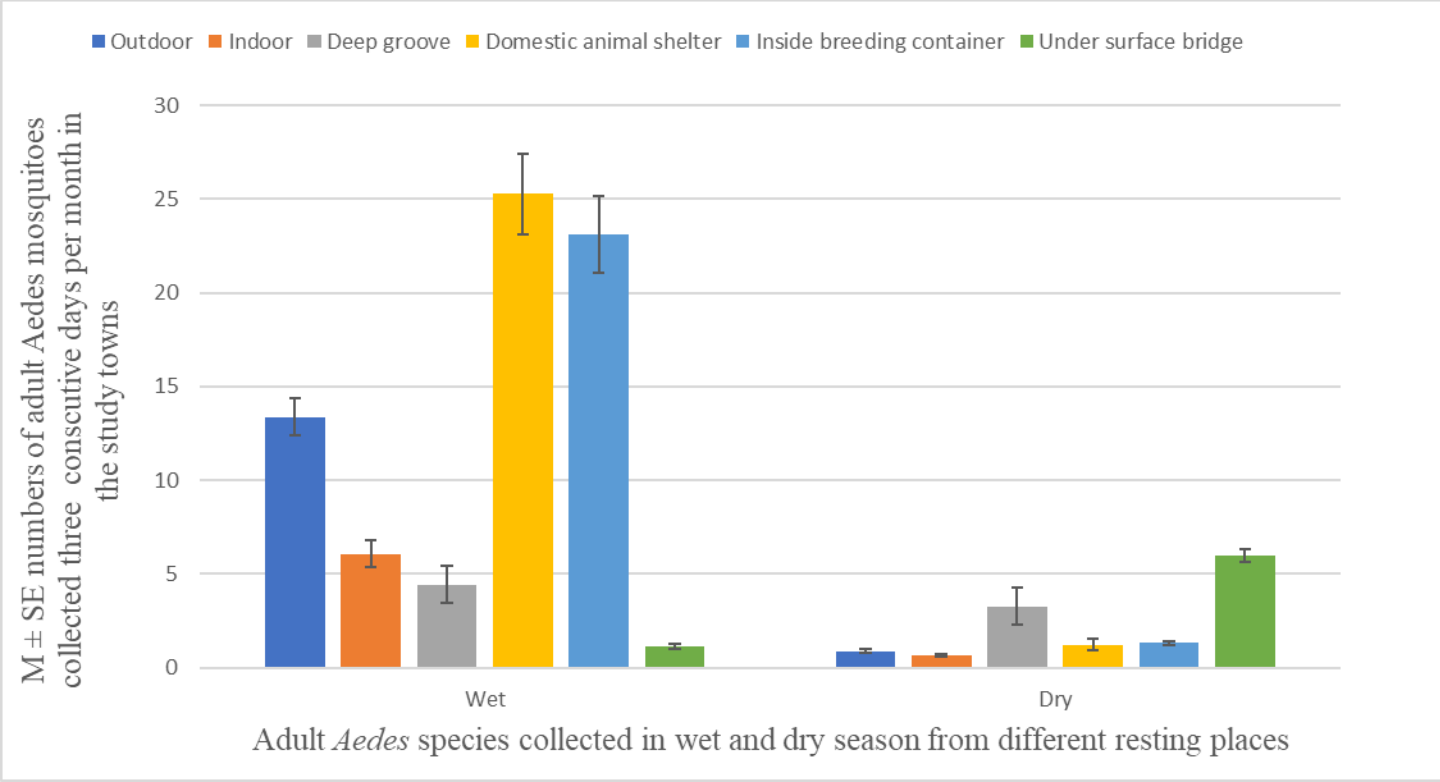


Figure 13: Distribution of *Aedes* species in different resting place in wet and dry season, Metema District, northwestern Ethiopia January, 2023 to December, 2023

4.3.4 Blood feeding status of *Aedes* species in the resting places

A total of 2076 female *Aedes* species were collected from the towns. Their abdominal conditions comprised unfed (n=1880, 90.5%), blood fed (n = 120, 6.3%), semi-gravid (n = 42, 2.2%) and gravid (n = 34, 1.8%). The hihgest number of blood fed *Ae. aegypti* were collected from cattle shade (n= 35, 41.6%), followed by outdoor (n = 23, 27.3%), indoor (n = 19, 22.6%), deep groove (n= 4, 4.7%) and underside bridge (n = 3.5%). Similarly, high number of blood feds *Ae. vittatus* were collected from cattle shade (n = 11, 45.8%) and followed by indoor (n = 6, 25.0%), outdoor (n = 4, 16.6%) and insige the breeding container (n = 3, 12.5%). High number of blood fed *Ae. communis* were collected from cattle shade (n = 4, 50.0%) followed by outdoor (n = 2, 25.0%) and indoor (n = 2, 25.0%). Better number of blood fed *Ae. albopictus* was collected from cattle shade

(n = 2, 50.0%) than from underside of artificial containers (n = 2, 50.0%). High number of blood fed *Ae. opok* (n = 2, 100%) were collected from inside bridge and the remianing resting place had not avialable (Fig 13).

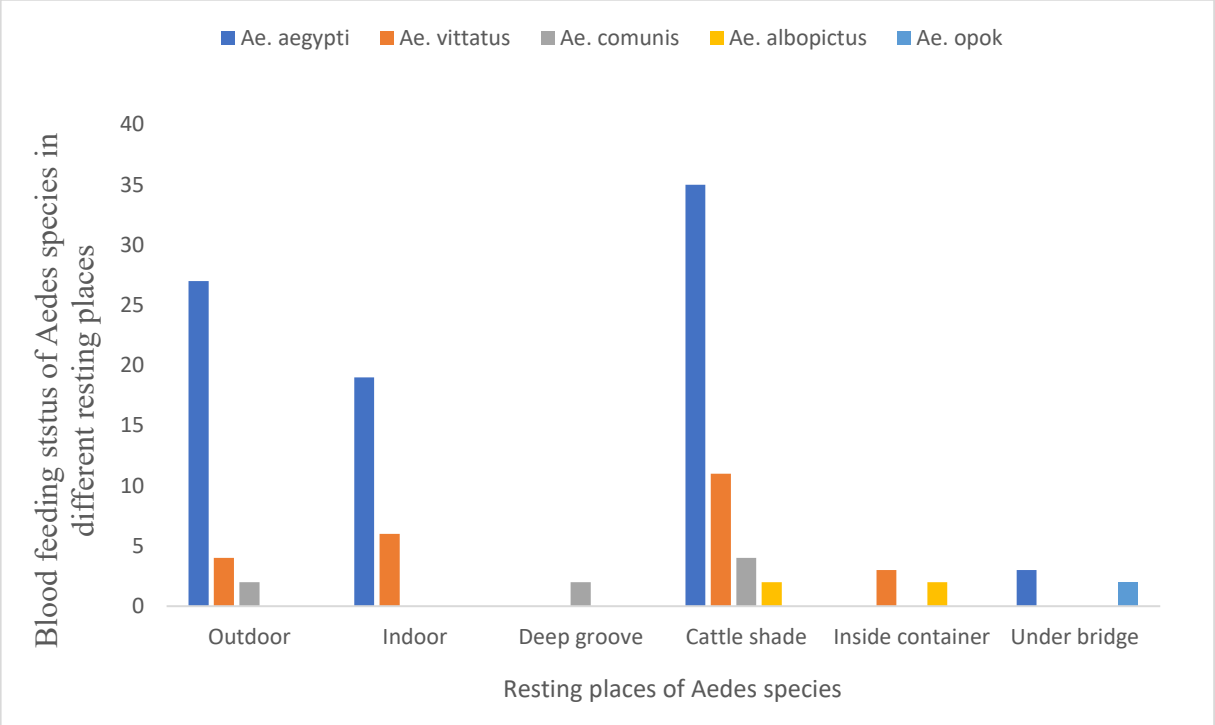


Figure 14: Blood feeding status of *Aedes* mosquitoes in different resting places

4.4 Viral infection status of *Aedes aegypti*

In the study sites, cases of arboviral diseases were reported at the health center through serological testing, which confirmed the presence of DEN and CHIK viruses in the study district. Additionally, DEN, ZIK, CHIK, and YF viruses were recorded at the health center in eastern Sudan. *Ae. aegypti* was reared in the laboratory until to three consecutive generations that assess their infection status of Ae. aegypti. Unfortunately, out of the 63 pools of *Ae. aegypti* was not tested positive samples for any of the viruses. (Fig. 15 & 16).

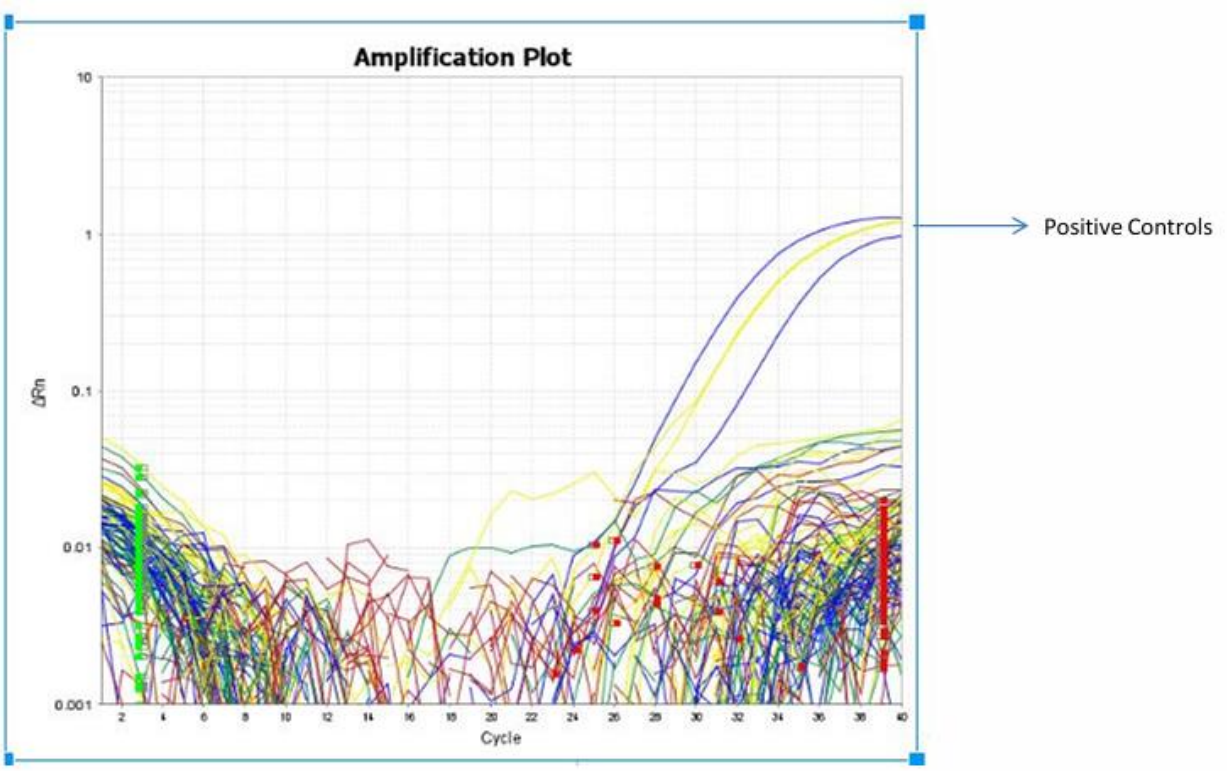


Figure 15: View of viral gene amplification plots

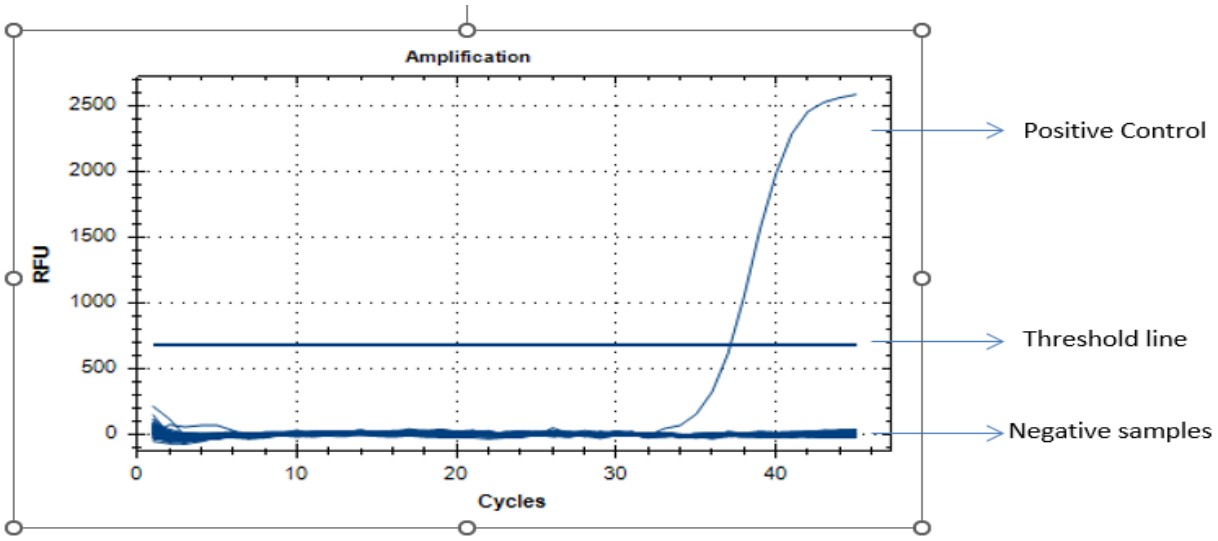


Figure 16: All *Aedes aegypti* samples were not infected to DENV, CHIKV and ZIKV.

4.5 Wolbachia bacteria infection status of *Aedes aegypti*

This study used conventional RT-PCR to detect four antiviral Wolbachia bacterial strain genes in *Ae. aegypti*, including wMel, wsp, wAlbB, and wMwA. The three successive generations of female *Ae. aegypti* samples were prepared to 63 pools, each pool which contains 10 *Ae. aegypti.* The results showed that the *wMel* strain of the *Wolbachia* gene was detected in the first generation, with five positive individuals identified across 21 pools. In the second generation, three positive individuals were similarly detected from another set of 21 pools. However, no positive samples were found in the third generation (Fig. 17). Overall, 26.26% of *Aedes aegypti* individuals were infected with *Wolbachia*, Additionally, transovarian transmission of *Wolbachia* was observed in the field at the study sites

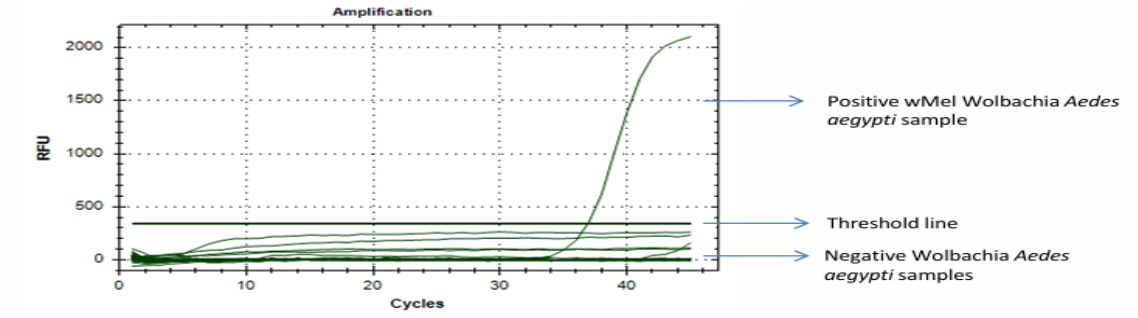


Figure 17: wMel strain of Wolbachia showed positive signal.

4.6 Identification of mosquitoes to species

4.6.1 Molecullar identification

Approximately 25 *Ae. aegypti* samples were identified based on to their morphologically characterstics and also confirm molecularly using PCR targeting the mitochondrial COI gene. All samples were confirmed *Ae. aegypti* of which three of the *Ae. aegypti* that showed better bands

sent to macro gene company for purification and sequenced. After that it received, it was edited, and aligned using the basic local alignment search tool (BLAST) analysis (Fig. 18). Finally, accession code of *Ae. aegypti* isolate AEAEM1 (PQ350417.1) got from BLAST company. The result was revealed to *Ae. aegypti* isolated in the study site had 0.0 E-value, query coverage 100% and per identity 100% when compared with *Ae. aegypti* isolated in India and West African countries.

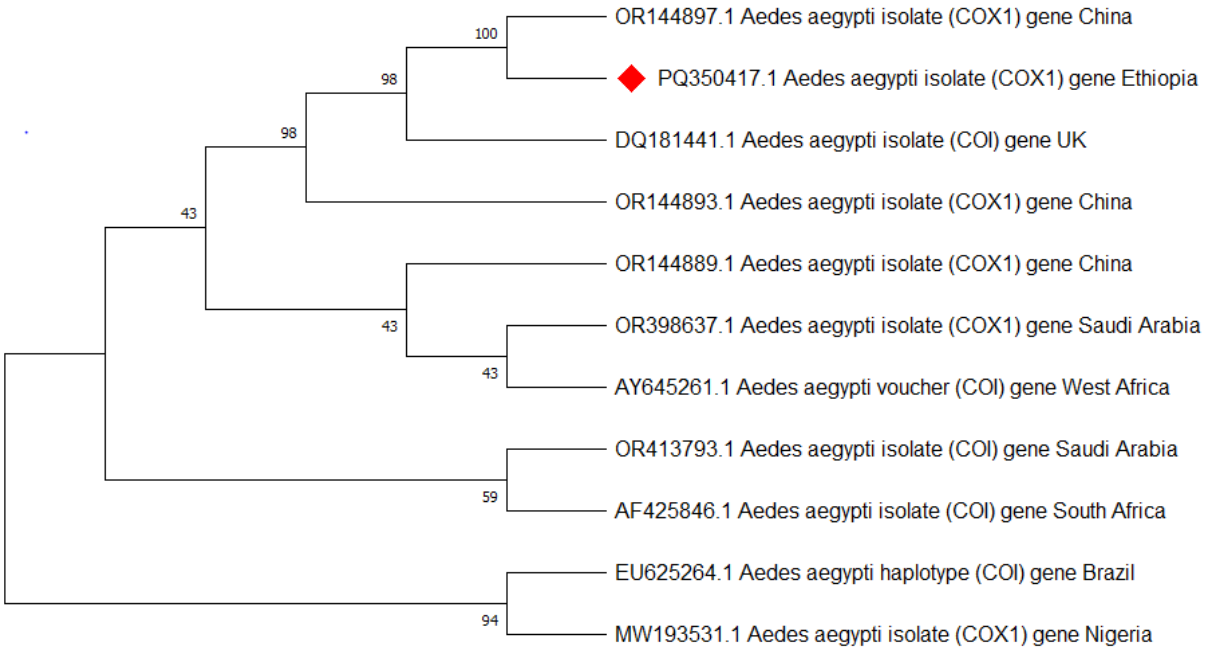


Figure 18: Phylogenetic tree representing of *Aedes aegypti* COI gene of Ethiopia sequence is indicated by red color. The evolutionary history was inferred using the Neighbor-Joining method(Saitou and Nei, 1987). The bootstrap consensus tree inferred from 500 replicates (Felsenstein, 1985) and percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (500 replicates) are shown next to the branches (Felsenstein, 1985). The evolutionary distances were computed using Tamura-Kumar method (Tamura and Kumar, 2002). This analysis involved 11 nucleotide sequences. All positions containing gaps and missing data were eliminated. There was a total of 554 positions in the final dataset. Evolutionary analyses were conducted in MEGA11(Tamura et al., 2021).

Similarly, 25 *Ae. vittatus* samples were identified based on to their morphologically characterstics and also confirm molecularly using PCR targeting the internal transcribed spacer 1 (ITS1) gene (Fig, 19). All samples were confirmed *Ae. vittatus* of which three of the *Ae. vittatus* that showed better bands sent to macro gene company for purification and sequenced. After that it received, it was edited, and aligned using the basic local alignment search tool (BLAST) analysis. Finally, accession code of *Ae. vittatus* isolate AEVIW2 (PQ288671.1 got from BLAST company. The result was revealed to *Ae. vittatus* isolated in the study site had 0.0 E-value, query coverage 100% and per identity 100% when compared with *Ae. vittatus* isolated in India but E-value 0.0, query coverage 94%, and per identity 96.68% Ae. vittatus isolated from France.

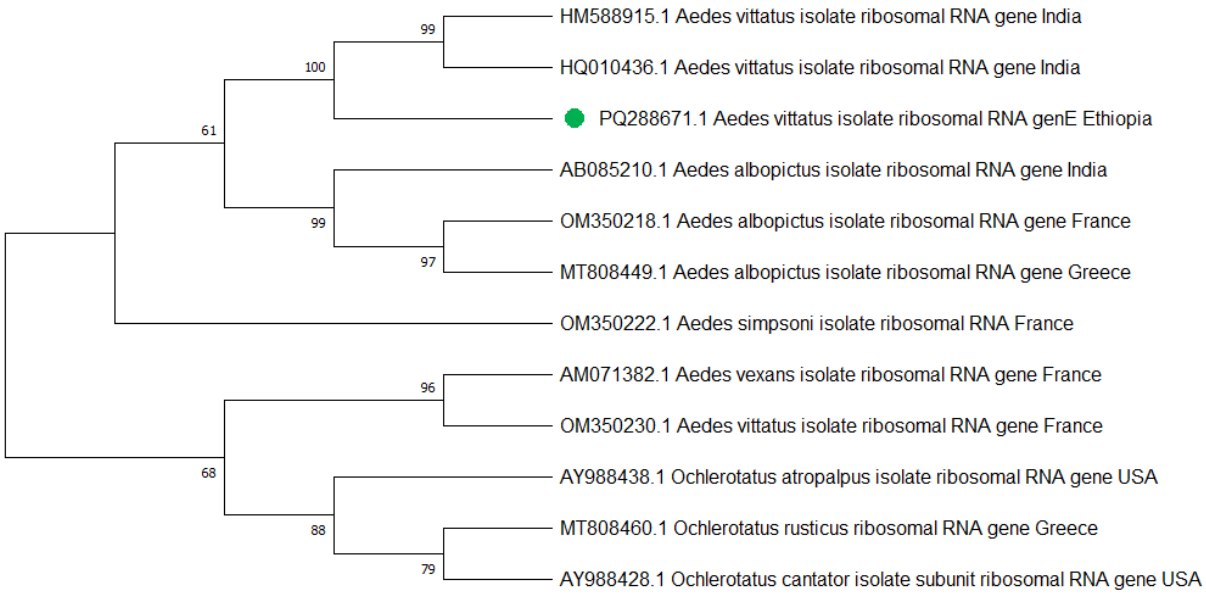


Figure 19: Phylogenetic tree representing of *Aedes vittatus* ITS1 gene of Ethiopian sequence is indicated by green color. The evolutionary history was inferred using the Neighbor-Joining method(Saitou and Nei, 1987). The bootstrap consensus tree inferred from 500 replicates (Felsenstein, 1985) and percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (500 replicates) are shown next to the branches (Felsenstein, 1985). The evolutionary distances were computed using Tamura-Kumar method (Tamura and Kumar, 2002).

This analysis involved 13 nucleotide sequences. All positions containing gaps and missing data were eliminated. There was a total of 554 positions in the final dataset. Evolutionary analyses were conducted in MEGA11(Tamura et al., 2021).

Additionally, 25 *Cs. longiareolata* samples were identified based on to their morphologically characterstics and also confirm molecularly using PCR targeting the internal transcribed spacer 2 (ITS2) genes. All samples were confirmed *Cs. longiareolata* of which three of the *Cs. longiareolata* that showed better bands sent to macro gene company for purification and sequenced. After that it received, it was edited, and aligned using the basic local alignment search tool (BLAST) analysis (Fig. 20). Finally, accession code of *Cs. longiareolata* isolate (PQ309040.1) got from BLAST company. The result was revealed to *Cs. longiareolata* isolated in the study site had 0.0 E-value, query coverage 100% and per identity 100% when compared with *Cs. longiareolata* isolated in Iran and Netherland countries.

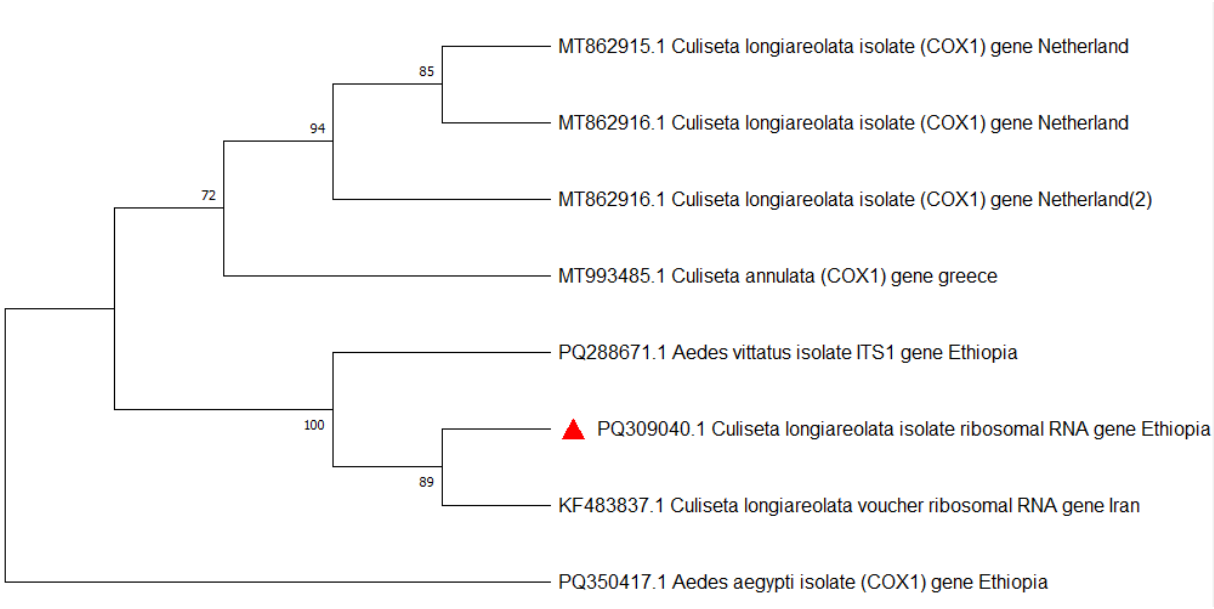


Figure 20: Molecular phylogenetic analysis of ITS2 of *Cs. longiareolata*, by maximum likelihood method. The percentage of trees in which the associated taxa clustered together is shown next to the branches. Initial tree(s) for the heuristic search were obtained automatically by applying

Neighbor-Join and BioNJ algorithms to a matrix of pairwise distances estimated using the Maximum Composite Likelihood (MCL) approach, and then selecting the topology with superior log likelihood value. The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. The analysis involved 8 nucleotide sequences. All positions containing gaps and missing data were eliminated. Internal transcribed spacer 2.

Chapter Five: Discussion

Aedes larvae were abundant in Metema-Yohannes, Kokit and Genda-Wuha towns of Metema Woreda, Northwestern, Ethiopia. The house index (HI), container index (CI) and breteau index (BI) were much higher than the cutoff points of WHO which were 10%, 10% and 20%, respectively. The indices suggest a high risk of arbovirus transmission in the towns (WHO, 2009). Physical and chemical characterstices of larval habitats influnced the distribution, abundance and species compostion of *Aedes* species. Five *Aedes* species namely *Ae*. *aegypti*. *Ae. vittatus, Ae. communis, Ae, albopictus and Ae. opok* were obseved in the towns. *Ae. aegypti* and *Ae. vittatus* had two peak biting time at eatly morning and late afternoon aligned with human active time. Resting place distribution of *Aedes* mosquitoes were influnced by season variation and preference to suitable habitat that indicate to ecolgical adaptation. DENV, CHIKV and ZIKV infectious status of *Ae. aegypti* was no detected from field collected larvae and rearing under laboratory condition. Interstingly, wMel strain of Wolbachia was detected from the first 21 pools and in the same as to second generation but not detected in the third generation.

Aedes larvae were distributed in artificial breeding containers including discared tyres, plastics, metals, clay pots and uncovered water tank. They also occurred in natural habitats such as in rain pools, hoof prints, river banks and tree holes. High larval densities of *Ae. aegypti, Ae. vittatus* and *Ae. albopictus* were found in the discared tyres during the rainy seasons compared to the rest of the containers. These results indicated to environmental adaptaion of *Aedes* species to their visual cue of camouflage to hide from predators, avoid sunlight intensity and also reduce desiccation of laid eggs to keep mostuire (Jeffrey Gutiérrez *et al.*, 2020). Similarly, in a privous reported showed that tyres serve as to suitable breeding containers for *Aedes* mosquitoes in urban areas(Selvan et al., 2015). In Cote d'Ivoire, Kenya and Mozambique, tyres were observed to be the most

predominant water containers and the highest *Aedes* larvae positive habitats (Medeiros-Sousa *et al.,* 2020). Tyres were used for carts and discarded in the towns thereby contributing to the proliferation of the mosquitoes. Several reports showed high *Aedes* larvae density in urban and semi-urban localities that were associated with discarded artificial containers (Badolo *et al*., 2022; Mereta *et al.*, 2013; Saleh *et al.*, 2018).

A number of larvae were recoreded at shrink rain pools, hoof prints and tree hole near to housholds (Avramov *et al*., 2024; Mangudo *et al.*, 2015). Small number of *Aedes* larvae were observed along river banks as their volume decreased during the dry seasons in the study sites. The previous reported showed that *Ae. aegypti* larvae located in the natural breeding habitats at the river bank (Avramov *et al*., 2024; Chukwuekezie, 2010). The highest number of *Ae. opok* were observed in natural habitat especially in the river pool and tree holes to compared with rest of breeding habitats. Similarly, the previous reported showed that *Ae. opok* distributed in natural breeding habittas especially in the tree hole (Sang *et al.*, 2022)

Generally, distribution, abundance and species composition of *Aedes* larvae were influenced by availability of discared materials in the study sites. Similarliy, in a pervious reportd, *Ae. aegypti* and *Ae. vitatus* had short development in the water-holding coconut shells, followed by tyres, glass jars and plastic cups (Awang and Dom, 2020). Almost all positive artificial habitats were located outdoors than indoors. This could be partly due to the fact that indoor containers might commonly be used for hygiene, cooking and drinking and are subject to frequent emptying and cleaning which interrupt larval development but outdoor container management could be less frequent. This is in agreement with previous study (Chareonviriyaphap *et al.,* 2003; Nikookar *et al.*, 2017).

Aedes larvae/pupae preferred to not exposed sunlight than the exposed. This could result from the tendency of gravid females to lay their eggs in shaded habitats in order to aviod deccication of deposited egges from sunlight intensity until the next rain and seeking better relative humidity. Previous studies reported that, *Ae. albopictus* and *Ae. aegpti were* preferred shaded areas for oviposition (Musunzaji *et al.,* 2023; Rey and O'Connell, 2014). Habitats free of emergent vegetation had relatively larger number of *Aedes* larvae compared to those with vegetation. This could potentially result from the release of chemicals and oils by vegetations that deplete dissolved oxygen and cause anoxic formation in the water body (Shah *et al.,* 2022).

Most of adult *Aedes* species have black scales that created assemble themselves with background color and also intrruption of predators to serve as camouflage. Similarly, a previous study showed that *Ae. aegypti* preferred car tyres for oviposition, a shorter larval development time and higher survivorship compared to other habitat type (Abdulai *et al.*, 2024). High mean numbers of *Ae. aegypti* and *Ae. vitatus* larvae/pupae were found in algae containing habitats than the algae free. Green algae could serve as shelter and nutrition source for the larvae. So, algae containing habitat could suppor growth of larvae. A similar result showed that *Ae. aegypti* and *Ae. albopictus* larvae prefer certain types of microbiota species as food (Ranasinghe and Amarasinghe, 2020). Tadpole containing habitats had less mean number of *Ae. aegypti* and *Ae. vitatus* larvae than the tadpole free. This result is supported by the observation under laboratory condition in which case *Aedes* eggs attracted tadpoles and were predated by tadpoles (Bowatte *et al.*, 2013).

Aedes larvae distribution and abundance was determined by physio-chemical characteristics habitats. In the wet season, temperature increase by 1.5ºC at Metema-Yohannes than Genda-Wuha town which indicated a strong positive relationship between temperature and *Aedes* larval growth. Contrary to this, in the dry season negative correlation was observed between temperature and

density of larvae under low relative humidity. In the other word, water disappeared from discarded containers due to high temperature and low humidity. Previous studies showed that the development time from hatching to adult emergence was shorter at higher temperatures (30°C) and positively correlated with larval density (Couret *et al*., 2014). Turbid and with sulphate habitats were positively association with *Aedes* larvae density. Artificial habitats with dissolved oxygen were negatively association with larvae density but positively in the natural habitat. This result revealed that *Aedes* larvae could adapt to different adaptation mechanism in artificial and natural habitats due to high turbidity and less dissolved oxygen in artificial containers Vs natural habitat. In a previous report, *Ae. aegypti* occurred in clear water and also in habitats which were turbid and have low dissolved oxygen (Hery *et al*., 2021). This is contrary to the findings from Zanzibar in Tanzania where the presence of immature *Ae. aegypti* was not linked significantly to any physico-chemical characteristic of habitats (Saleh *et al.*, 2018).

The genus *Culex* prevailed followed by *Aedes*, *Anopheles* and *Culiseta* in Genda-Wuha, Kokit and Metema-Yohannes towns of Metema district in northwestern Ethiopia during the period of June to September, 2023. But the genus *Culiseta* was not observed in Metema-Yohannes. Among the 16324 mosquitoes caught by CDC-LT, 79.52% (12492) were collected during the day night hour and 23.41% (3822) during the day light hour. All the *Anopheles, Culex* and *Culiseta* were caught only during the day night hours, whereas, *Aedes* species were caught during both day night and day light hours. This entails that Metema district was at a high risk of several types of mosquito-borne diseases that include arboviral diseases, filariasis and malaria among others. *Anopheles* species were observed biting in the evening although peak biting hours could vary with geographical setting and time such as in the early morning (Odero *et al.*, 2024; Van Dung *et al.*, 2023) while *Culex* and *Culiseta* commonly bite overnight (Rani *et al.*, 2020) and *Cx.*

quinquefasciatus and *Cx. tritaeniorhynchus* exhibited unimodal nocturnal behavior in some instances (Vythilingam *et al.,* 1995).

Aedes aegypti exhibited higher density and higher biting rate outdoors than indoors during both the daylight hour and the night hour. In outdoors, *Ae. aegypti* exhibited two peak biting rates one between 07:00 and 08:00 in the morning and the other from 17:00 to 18:00 early night. The peak biting hours of *Ae. aegypti* matched with the active working hours of residents which could expose them to several viral disease infections. Similarly, its indoor peak biting rate in the morning could also expose households to infections as they carry out indoor activities. However, the indoor night biting rate of *Ae. Aegypti* can be prevented, provided that the households sleep under properly tucked mosquito nets. Similar studies reported two peak indoor biting activities; one in the morning and the other in the afternoon (Chompoosri *et al.*, 2012; Zahid *et al.*, 2023). However, according to another research, *Ae. aegypti* exhibits a trimodal pattern of biting activity in the morning, midday, and afternoon(Chadee and Martinez, 2000). Furthermore, the other authors propose that, *Ae. aegypti* may have adapted to become diurnal by expanding its activity from nocturnal hours to both indoor and outdoor (Captain-Esoah *et al*., 2020).

The morning and early night peak biting activity of *Ae. vittatus* also matched with the active working hours in the area. The peak outdoor biting hours of *Ae. vittatus* that aligned with the active working hours could expose the residents to viral disease infections. In a previous study *Ae. vittatus* was reported to exhibit nocturnal biting rhythm in rural south India (Paramasivan *et al.*, 2015). Speculated *Aedes* mosquitoes exhibit ecological adaptation that allows them to transition from nocturnal to diurnal biting behaviors, behavioral change mechanism to weak sunlight intensity after sunrise and before sunset (Chadee and Martinez, 2000; Githeko *et al.*, 2000). Urban landscapes can influence biting times due to factors like artificial lighting and temperature

variations. For instance, well-lit areas may extend mosquito activity into the night, altering traditional biting patterns.

Aedes aegypti and *Ae. vittatus* exhibited two peak biting activates both outdoors and indoors in common. This observation was similar with earlier peak biting hours (Chadee and Martinez, 2000; Rund *et al.*, 2020). *Ae. aegypti* and *Ae. vittatus* are considered to be the major vectors of arboviruses such as DENV, YFV, CHIKV, and ZIKV and hence they could be responsible vectors for CHIKV (Ferede *et al.*, 2021) and DENV (Ferede *et al.*, 2018b) infections in Metema district. The district is also a borderline with east Sudan where there have been arboviral diseases transmission and outbreaks of dengue fever

Both *Ae. aegypti* and *Ae. vittatus* were observed most abundantly closer to humans followed by cattle, sheep, goat, and donkey. This indicates their preference to feed on humans and also their importance as disease vectors in the area. In a previous report, *Ae. aegypti* was also observed to feed on human blood most commonly (Cebrián-Camisón *et al.*, 2020). The transmission potential of a vector-borne pathogen is influenced to a large extent by the human blood feeding behavior of the vector (Kramer and Ciota, 2015; Wilson and Sevarkodiyone, 2015).

Highest number of *Ae. aegypti* and *Ae. vittatus* rested in the animal shelters compared to inside breeding containers, outdoors, indoors, bridge, and deep groove. Similar studies in Northeastern Thailand reported highest number of female *Ae. aegypti* that rested in bedrooms *(Seang-Arwut et al*., 2023). Mosquitoes were abundant in bedrooms likely because people spend a comparatively long time in this room while sleeping, attracting blood-seeking mosquitoes by their body heat and carbon dioxide (Gillies, 1980). The resting behaviors of *Ae. aegypti* was highly endophilic, whereas *Ae. albopictus* demonstrated exophilic behavior, suggesting that a domestic environment

with high human–vector contact (Dalpadado *et al.,* 2022). Mosquitoes can enter and leave the resting site at any time and their resting behaviour is further influenced by different conditions such as temperature, light (Panella *et al*., 2011) and humidity (Egid *et al*., 2022).

In the rainy seasons, *Aedes* species preferred animal shelters and indoors due to avilable optimun temprature and relative humidity, carbondioxide and blood meal host. *Ae. albopictus* and *Ae. communis* preferred resting in underside of artificial larvae habitats than animal shelter, outdoor, indoor, deep groove, and underside bridge in the rain season. Moreover, relatively, high number of *Ae. opok* was observed in underside bridge in the dry season seeking moisture and minimum temperature as humidity and temperature during the dry season become 38-45% and 35-39^{o}C, respectively. Similarly, the previous reported to several *Anopheles* species as resting in bridges, in hollow trees, culverts, and other similar shelters due to optimum moisture produce resting places intensively throughout the dry time (Durkett-Cadena *et al*., 2008).

of the density of *Ae. aegypti* and *Ae. vittatus* were high in the wet season than in the dry season due to optimum temprature and humidity inside the resting places relative to the outside. This could be due to the the large number of larval habitats present and adult mosquitoes enter to appropirate resting places (Manzambi *et al.*, 2023). In the dry season, there were lower numbers of *Ae. aegypti* and *Ae. vittatus* resting in deep groove and underneath bridge. *Ae. opok* occured during the the last two weeks of June and sharply declined to July and August but *Ae. communis* had higly abundance in September and the first week of October. The large *Aedes* population during the wet season could be associated with the presence of water storage containers in every house serving as larval habitats. In general temperature and humidity play critical roles in the population dynamics of *Aedes* species mortality and, hence, pathogen transmission(Schmidt *et al.*, 2018).The difference in distribution and abundance of *Aedes* species in their resting habitats is

determined by the presence of rainfall and water-containing containers for larval growth. These findings pointed out the potential adult and larval control sites.

The majority of the female *Aedes* mosquitoes were unfed. A previous study reported similar findings with a maximum percentage of unfed female *Aedes* species (Kinyatta *et al.*, 2018). The number of blood-fed *Aedes* species during the wet season was approximately five times higher than during the dry season (Marina *et al.,* 2021). This could be due to the favorable environmental conditions such as optimum temperature and relative humidity. In the towns, the great majority of both *Ae. aegypti* and *Ae. vittatus* was observed to be unfed. These results are supported by a previous report in which case a high number of unfed *Ae. aegypti* were collected outdoors using sticky ovitraps (de Santos *et al.,* 2012). The proportion of *Ae. aegypti* and *Ae. vittatus* with distended abdomens indicated their recent access to diverse blood meal sources, high reproductive potential, and also high-risk arboviral disease transmission in the towns. In the dry season, temperature become over to 35 - 39 ºC and relative humidity sharply reduced to 33%, and also artificial water holding containers became to dry out. However, a small number of *Aedes* species have shifted to moisture-producing resting areas. This result suggested that moisture produces resting spot place throughout the dry season, that serve as to arboviral vectors outbreak when favorable environmental condition becomes.

Among these, *Ae. aegypti, Ae. albopictus, Ae. vittatus and Ae. opok* occurred in Metema district which might be responsible for DENV and CHIKV infections reported previously. However, none of these viruses were detected among the *Ae. aegypti* in the current study. This observation is similar to the previous reports from Humera and Metema districts (Ferede *et al.*, 2018a) and Dire Dawa town in the country (Getachew *et al.*, 2015). Even so, the district is at high risk of arboviral diseases outbreak according (WHO, 2003). Interestingly, wMel strain of Wolbachia bacteria was

detected in the first and second generation of *Ae. aegypti*. A similar result was reported under laboratory condition (Shirozu *et al.,* 2024). Therefore, our result suggested that arbovirus transmission through female *Ae. aegypti* might be inhibited by wMel strain of Wolbachia bacteria in the district. However, it needs detail and further investigation about arbovirus infectious status and to be assessed strain of Wolbachia bacteria in the field *Aedes* species in the areas

In the study site, five *Aedes* species and one *Cs. Longiareolata* species were identified based on to thier distinct morphological characterstices. Similar morphological characterstices of *Ae. aegypti* and *Ae. albopictus* were reported by (Supryono *et al.,* 2023). *Ae. vittatus* morpgological feature similarly with the previous reported by (Díez-Fernández *et al.*, 2018). *Ae. communis* morphological feature also similar with (Brust and Munstermann, 1992). Additionaly, *Cs. Longiareolata* has own distict morpholgical structuret from five *Aedes* species (Khaligh *et al.*, 2020). In general, *Aedes* species distriution in worldwide without geographycal barrair due to human mobility and matrials exchang that induced to escalation of arbovirial vectors become to cosmopolitant through ecological adaptattion.

Mitochondrial cytochrome oxidase subunit 1 (COI), internal transcribed spacer 1 (ITS1) and internal transcribed spacer -2 (ITS2) barcode gene used to identified Ae. *aegypti*, *Ae. vittatus* and *Cs. longiareolata* respectively. The subpopulation of *Ae. aegypti* and *Ae. vittatus* species' nucleotide sequences at Metema-Yohannes, Kokit, and Genda-Wuha towns had no differentiated haplotype diversity. However, more genomic data is needed for deeper phylogeographic analysis. The *Ae. aegypti* haplotype sequences in the present study had highly significant relationships with those in China and the UK as indicated by the Gene Bank database. However, a high level of diversity variation of the *Ae. aegypti* haplotypes in northwest Ethiopia was observed compared to west Africa, south Africa, Saudi Arabia, and Brazil.

Aedes vittatus isolated in Metema District had nearly 100% identity with India's national nucleotide sequence of *Ae. vittatus* as certified by the gene bank database.(Panigrahi *et al.*, 2024). However, *Ae. vittatus* isolated in Metema district showed that phylogenetic tree has significant variation in haplotype diversity when compared to *Ae. vittatus* haplotypes found in United States, Greece, and France. Overall, this phylogenetic tree indicates that the origin of the *Aedes* population in Ethiopia is more likely to be China and the UK for the *Ae. aegypti* species, whereas to *Ae. vittatus* is highly related to India. The differentiation trends among the Ethiopian samples suggested that absence of subpopulations of *Ae. aegypti* and *Ae. vittatus* in Northwest, Ethiopia. Phylogenetic tree of *Cs. longiareolata* isolated in Northwest, Ethiopia has high related with *Cs. longiareolata* isolated in Iran. However, there is a need to further explore the level of differentiation between subpopulations in Ethiopia. Additionally, the detail investigation needs between Ethiopia and neighboring countries.

The availability of green algae, tadpole, grass, weed species and other aquatic organism was not detail recorded for the larval habitats which could limit the availability and diversity of larval habitats, blood meal source of *Aedes* species in the peak biting times, and resting site preferences were not molecularly detected due to financial constraint. Shortage of essential chemicals and reagents for molecular diagnosis forced us to reduce the numbers of mosquito species to be identified using molecular techniques. Absence of primers and reagents also forced us to diagnose *Ae. aegypti* only for dengue, chikungunya and Zika viruses and a few strains of Wolbachia bacteria.

Chapter Six: Conclusions and Recommendations

6.1 Conclusions

The high levels of House Index (HI), Container Index (CI), and Breteau Index (BI) in comparison to the WHO's threshold values indicated a high risk of arboviral disease transmission. The highest numbers of *Aedes* larvae and pupae were found in discard tyres, followed by plastic containers, metals, and clay pots. *Aedes* larvae were also present in natural habitats such as tree holes, rain pools, hoof prints, and river banks. *Aedes aegypti* was the most abundant and distributed species in the study site, followed by *Ae. vitatus*. Arboviral vector control strategies, including the adoption of a specific mosquito collection protocol for arbovirus surveillance during both epidemic and inter-epidemic periods, serve as an early warning system to implement vector control measures before a rise in human cases.

Aedes aegypti and *Ae. vittatus* exhibited early morning and late afternoon/early evening biting activities. Their biting hours aligned with human activity patterns, increasing the likelihood of human-mosquito interactions and accelerate the risk of disease transmission. The *Aedes* species exhibit diverse resting habitats including indoors, outdoors, cattle shades, inside breeding containers, deep grooves, inside bridges, and tree holes. During the wet season, the highest number of *Aedes aegypti* were collected from cattle shades, compared to other resting sites due to cattle body temperature released, carbondioxid attraction clue and urine. In dry season, the population size declined but the few numbers of mosquitoes shifted to rest inside bridges and in deep grooves. Seasonal variation determined to distribution and abundance of resting place of *Aedes* species in the study towns. Results were not conducted positive infection pools sample DENV, CHIKV and ZIKV of *Aedes aegypti* but wMel strain of *Wolbachia* was detected among the first and second

generations of *Ae. aegypti*. These may suppress arboviral disease transmission by inhibiting viral replication in the local *Aedes* mosquito populations.

6.2 Recommendations

There is a need to properly dispose or recycle used materials such as tyres, plastics, metals and clay pots and natural larval habitats should be modified timely. The biting hours in an urban setting can be affected by late one-hour biting rate observed and reduced the number of entries indoors rather than outdoors due to repellant smoke emitted from indoor, temperature variations and existing of artificial light. It plays a role in the ecology of disease transmission, influencing host populations and dynamics within ecosystems. Mosquitoes develop behavioral adaption to host-seeking patterns that showed to an evolutionary relationship. This result indicated to develop integrate vector control strategies that avoid human-vector interaction. Additionally, *Aedes* species have diverse resting places were observed in wet and dry season in the study sites. Thus, the result suggested to apply residual spray insecticide to the moisture produce resting place in the dry season and heat maintain resting place in the wet season or to design appropriate vector management.

A further study need about pathogenic infectious statues in different *Aedes* species in the study sites is recommended. These results indicated that to combat arboviral disease transmission at the local, national, and international levels.

Reference

Abdulai, A., Owusu-Asenso, C. M., Haizel, C., Mensah, S. K. E., Sraku, I. K., Halou, D., Doe, R. T., Mohammed, A. R., Akuamoah-Boateng, Y., and Forson, A. O. (2024). The role of car tyres in the ecology of *Aedes aegypti* mosquitoes in Ghana. *Current Research in Parasitology & Vector-Borne Diseases* **5**, 100176.

Ajlan, B. A., Alafif, M. M., Alawi, M. M., Akbar, N. A., Aldigs, E. K., and Madani, T. A. (2019). Assessment of the new World Health Organization's dengue classification for predicting severity of illness and level of healthcare required. *PLoS Neglected Tropical Diseases* **13**, e0007144.

Alayu, M., Teshome, T., Amare, H., Kinde, S., Belay, D., and Assefa, Z. (2020). Risk factors for Chikungunya outbreak in Kebridhar City, Somali Ethiopia, 2019. Unmatched case-control study. *Biological Science* **2020**, 21

Amarasinghe, A., Kuritsky, J. N., Letson, G. W., and Margolis, H. S. (2011). Dengue virus infection in Africa. *Emerging Infectious Diseases* **17**, 1349.

Andral, L., Poirier, A., Lindrec, A., and Neri, P. (1968). Studies on yellow fever in Ethiopia. 6. Epidemiologic study. *Bulletin of the World Health Organization* **38**, 879-884.

Andrews, E., Xu, G., and Rich, S. (2014). Microbial communities within field-collected Culiseta melanura and Coquillettidia perturbans. *Medical and Veterinary Vntomology* **28**, 125-132.

Ant, T. H., Herd, C. S., Geoghegan, V., Hoffmann, A. A., and Sinkins, S. P. (2018). The Wolbachia strain w Au provides highly efficient virus transmission blocking in *Aedes aegypti. PLoS Pathogens* **14**, e1006815.

Asebe, G., Michlmayr, D., Mamo, G., Abegaz, W. E., Endale, A., Medhin, G., Larrick, J. W., and Legesse, M. (2021). Seroprevalence of Yellow fever, Chikungunya, and Zika virus at a community level in the Gambella Region, South West Ethiopia. *PloS One* **16**, e0253953.

Avramov, M., Thaivalappil, A., Ludwig, A., Miner, L., Cullingham, C. I., Waddell, L., and Lapen, D. R. (2024). Relationships between water quality and mosquito presence and abundance: a systematic review and meta-analysis. *Journal of Medical Entomology* **61**, 1-33.

Awang, M. F., and Dom, N. C. (2020). The effect of temperature on the development of immature stages of Aedes spp. against breeding containers. *International Journal of Global Warming* **21**, 215-233.

Azari-Hamidian, S., and Harbach, R. E. (2009). Keys to the adult females and fourth-instar larvae of the mosquitoes of Iran (Diptera: Culicidae). *Zootaxa* **2078**, 1-33.

Badolo, A., Sombié, A., Yaméogo, F., Wangrawa, D. W., Sanon, A., Pignatelli, P. M., Sanon, A., Viana, M., Kanuka, H., and Weetman, D. (2022). First comprehensive analysis of *Aedes aegypt*i bionomics during an arbovirus outbreak in west Africa: Dengue in Ouagadougou, Burkina Faso, 2016–2017. *PLoS Neglected Tropical Diseases* **16**, e0010059.

Behura, S. K., and Severson, D. W. (2012). Intrinsic features of *Aedes aegypti* genes affect transcriptional responsiveness of mosquito genes to dengue virus infection. *Infection, Genetics and Evolution* **12**, 1413-1418.

Bian, G., Xu, Y., Lu, P., Xie, Y., and Xi, Z. (2010). The endosymbiotic bacterium Wolbachia induces resistance to dengue virus in *Aedes aegypti. PLoS Pathogens* **6**, e1000833.

Bisanzio, D., Giacobini, M., Bertolotti, L., Mosca, A., Balbo, L., Kitron, U., and Vazquez-Prokopec, G. M. (2011). Spatio-temporal patterns of distribution of West Nile virus vectors in eastern Piedmont Region, Italy. *Parasites & Vectors* **4**, 230.

Blagrove, M. S., Arias-Goeta, C., Failloux, A.-B., and Sinkins, S. P. (2012). Wolbachia strain wMel induces cytoplasmic incompatibility and blocks dengue transmission in *Aedes albopictus. Proceedings of the National Academy of Sciences* **109**, 255-260.

Bowatte, G., Perera, P., Senevirathne, G., Meegaskumbura, S., and Meegaskumbura, M. (2013). Tadpoles as dengue mosquito (*Aedes aegypti*) egg predators. *Biological Control* **67**, 469-474.

Bower, H., El Karsany, M., Adam, A. A. A. H., Idriss, M. I., Alzain, M. a. A., Alfakiyousif, M. E. A., Mohamed, R., Mahmoud, I., Albadri, O., and Mahmoud, S. A. A. (2021). “Kankasha” in Kassala: A prospective observational cohort study of the clinical characteristics, epidemiology, genetic origin, and chronic impact of the 2018 epidemic of Chikungunya virus infection in Kassala, Sudan. *PLoS Neglected Tropical Diseases* **15**, e0009387.

Braack, L., Gouveia de Almeida, A. P., Cornel, A. J., Swanepoel, R., and De Jager, C. (2018). Mosquito-borne arboviruses of African origin: review of key viruses and vectors. *Parasites & Vectors* **11**, 1-26.

Brault, A. C., Powers, A. M., Ortiz, D., Estrada-Franco, J. G., Navarro-Lopez, R., and Weaver, S. C. (2004). Venezuelan equine encephalitis emergence: enhanced vector infection from a single amino acid substitution in the envelope glycoprotein. *Proceedings of the National Academy of Sciences* **101**, 11344-11349.

Brelsfoard, C., and Dobson, S. (2011). Wolbachia effects on host fitness and the influence of male aging on cytoplasmic incompatibility in Aedes polynesiensis (Diptera: Culicidae). *Journal of Medical Entomology* **48**, 1008-1015.

Brust, R. A., and Munstermann, L. E. (1992). Morphological and genetic characterization of the *Aedes* (Ochlerotatus) *communis* complex (Diptera: Culicidae) in North America. *Annals of the Entomological Society of America* **85**, 1-10.

Burkett-Cadena, N. D., Eubanks, M. D., and Unnasch, T. R. (2008). Preference of female mosquitoes for natural and artificial resting sites. *Journal of the American Mosquito Control Association* **24**, 228.

Burkett-Cadena, N., Graham, S. P., and Giovanetto, L. A. (2013). Resting environments of some Costa Rican mosquitoes. *Journal of Vector Ecology* **38**, 12-19.

Byaruhanga, T., Kayiwa, J. T., Nankya, A. M., Ataliba, I. J., McClure, C. P., Ball, J. K., and Lutwama, J. J. (2023). Arbovirus circulation, epidemiology and spatiotemporal distribution in Uganda. *International Society Infectious Disease* **6**, 171-176.

Calle-Prieto, F., Arsuaga, M., Rodríguez-Sevilla, G., Paiz, N. S., and Díaz-Menéndez, M. (2024). The current status of arboviruses with major epidemiological significance in Europe. *Enfermedades Infecciosas Microbiologia Clinica* **42**, 516-526.

Caminade, C., Medlock, J. M., Ducheyne, E., McIntyre, K. M., Leach, S., Baylis, M., and Morse, A. P. (2012). Suitability of European climate for the Asian tiger mosquito *Aedes albopictus:* recent trends and future scenarios. *Journal of the Royal Society Interface* **9**, 2708-2717.

Captain-Esoah, M., Kweku Baidoo, P., Frempong, K. K., Adabie-Gomez, D., Chabi, J., Obuobi, D., Kwame Amlalo, G., Balungnaa Veriegh, F., Donkor, M., and Asoala, V. (2020). Biting behavior and molecular identification of Aedes aegypti (Diptera: Culicidae) subspecies in some selected recent yellow fever outbreak communities in Northern Ghana. *Journal of Medical Entomology* **57**, 1239-1245.

Cebrián-Camisón, S., Martínez-de la Puente, J., and Figuerola, J. (2020). A literature review of host feeding patterns of invasive *Aedes* mosquitoes in Europe. *Insects* **11**, 848.

Chadee, D. D., and Martinez, R. (2000). Landing periodicity of *Aedes aegypti* with implications for dengue transmission in Trinidad, West Indies. *Journal of Vector Ecology* **25**, 158-163.

Chareonviriyaphap, T., Akratanakul, P., Nettanomsak, S., and Huntamai, S. (2003). Larval habitats and distribution patterns of Aedes aegypti (Linnaeus) and *Aedes albopictus* (Skuse), in Thailand. *Southeast Asian Journal of Tropical Medicine and Public Health* **34**, 529-535.

Chen, C. D., Lee, H. L., Lau, K. W., Abdullah, A. G., Tan, S. B., Sa'diyah, I., Norma-Rashid, Y., Oh, P. F., Chan, C. K., and Sofian-Azirun, M. (2014). Biting behavior of Malaysian mosquitoes, Aedes albopictus Skuse, Armigeres kesseli Ramalingam, Culex quinquefasciatus Say, and Culex vishnui Theobald obtained from urban residential areas in Kuala Lumpur. *Asian Biomedicine* **8**, 315-321.

Chepkorir, E., Lutomiah, J., Mutisya, J., Mulwa, F., Limbaso, K., Orindi, B., Ng'ang'a, Z., and Sang, R. (2014). Vector competence of *Aedes aegypti* populations from Kilifi and Nairobi for dengue 2 virus and the influence of temperature. *Parasites & Vectors* **7**, 435.

Chippaux, J.-P., and Chippaux, A. (2018). Yellow fever in Africa and the Americas: a historical and epidemiological perspective. *Journal of Venomous Animals and Toxins including Tropical Diseases* **24**, 20.

Chompoosri, J., Thavara, U., Tawatsin, A., Anantapreecha, S., and Siriyasatien, P. (2012). Seasonal monitoring of dengue infection in *Aedes aegypti* and serological feature of patients with suspected dengue in 4 central provinces of Thailand. *The Thai Journal of Veterinary Medicine* **42**, 185-193.

Chow, V., Chan, Y., Yong, R., Lee, K., Lim, L., Chung, Y., Lam-Phua, S., and Tan, B. (1998). Monitoring of dengue viruses in field-caught *Aedes aegypti* and *Aedes albopictus*

mosquitoes by a type-specific polymerase chain reaction and cycle sequencing. *The American Journal of Tropical Medicine and Hygiene* **58**, 578-586.

Chukwuekezie, O. (2010). Surveillance Studies of Aedes Stegomyia Mosquitoes In Three Ecological Locations Of Enugu, South-Eastern Nigeria. *The Internet Journal of Infectious Diseases*.

Clements, A. (2000). The biology of mosquitoes volume 1: development. In: *Nutrition and Reproduction*, 231-234.

Coetzee, M. (2020). Key to the females of Afrotropical *Anopheles* mosquitoes (Diptera: Culicidae). *Malaria Journal* **19**, 1-20.

Coffey, L. L., Forrester, N., Tsetsarkin, K., Vasilakis, N., and Weaver, S. C. (2013). Factors shaping the adaptive landscape for arboviruses: implications for the emergence of disease. *Future Microbiology* **8**, 155-176.

Costa, E. A. P. d. A., Santos, E. M. d. M., Correia, J. C., and Albuquerque, C. M. R. d. (2010). Impact of small variations in temperature and humidity on the reproductive activity and survival of Aedes aegypti (Diptera, Culicidae). *Revista Brasileira de Entomologia* **54**, 488-493.

Couret, J., Dotson, E., and Benedict, M. Q. (2014). Temperature, larval diet, and density effects on development rate and survival of *Aedes aegypti* (Diptera: Culicidae). *PloS One* **9**, e87468.

Dalpadado, R., Amarasinghe, D., Gunathilaka, N., and Ariyarathna, N. (2022). Bionomic aspects of dengue vectors *Aedes aegypti* and *Aedes albopictus* at domestic settings in urban, suburban and rural areas in Gampaha District, Western Province of Sri Lanka. *Parasites & Vectors* **15**, 148.

Damiani, C., Ricci, I., Crotti, E., Rossi, P., Rizzi, A., Scuppa, P., Esposito, F., Bandi, C., Daffonchio, D., and Favia, G. (2008). Paternal transmission of symbiotic bacteria in malaria vectors. *Current Biology* **18**, R1087-R1088.

David, M. R., Dantas, E. S., Maciel-de-Freitas, R., Codeço, C. T., Prast, A. E., and Lourenço-de-Oliveira, R. (2021). Influence of larval habitat environmental characteristics on Culicidae immature abundance and body size of adult *Aedes aegypti*. *Frontiers in Ecology and Evolution* **9**, 626757.

de Santos, E. M. M., de Melo-Santos, M. A. V., de Oliveira, C. M. F., Correia, J. C., and de Albuquerque, C. M. R. (2012). Evaluation of a sticky trap (AedesTraP), made from disposable plastic bottles, as a monitoring tool for *Aedes aegypti* populations. *Parasites & Vectors* **5**, 195.

Degife, L. H., Worku, Y., Belay, D., Bekele, A., and Hailemariam, Z. (2019). Factors associated with dengue fever outbreak in Dire Dawa administration city, October, 2015, Ethiopia-case control study. *BMC Public Health* **19**, 1-7.

Delatte, H., Desvars, A., Bouétard, A., Bord, S., Gimonneau, G., Vourc'h, G., and Fontenille, D. (2010). Blood-feeding behavior of *Aedes albopictus*, a vector of Chikungunya on La Réunion. *Vector-Borne and Zoonotic Diseases* **10**, 249-258.

Delatte, H., Gimonneau, G., Triboire, A., and Fontenille, D. (2009). Influence of temperature on immature development, survival, longevity, fecundity, and gonotrophic cycles of Aedes albopictus, vector of chikungunya and dengue in the Indian Ocean. *Journal of Medical Entomology* **46**, 33-41.

Diallo, M., Thonnon, J., Traore-Lamizana, M., and Fontenille, D. (1999). Vectors of Chikungunya virus in Senegal: current data and transmission cycles. *The American Journal of Tropical Medicine and Hygiene* **60**, 281-286.

Diaz, L. A., Flores, F. S., Quaglia, A., and Contigiani, M. S. (2013). Intertwined arbovirus transmission activity: reassessing the transmission cycle paradigm. *Frontiers in Physiology* **3**, 493.

Dida, G. O., Anyona, D. N., Abuom, P. O., Akoko, D., Adoka, S. O., Matano, A.-S., Owuor, P. O., and Ouma, C. (2018). Spatial distribution and habitat characterization of mosquito species during the dry season along the Mara River and its tributaries, in Kenya and Tanzania. *Infectious Diseases of Poverty* **7**, 2.

Die, J. V., and Román, B. (2012). RNA quality assessment: a view from plant qPCR studies. *Journal of Experimental Botany* **63**, 6069-6077.

Díez-Fernández, A., Martínez-de la Puente, J., Ruiz, S., Gutiérrez-López, R., Soriguer, R., and Figuerola, J. (2018). *Aedes vittatus* in Spain: current distribution, barcoding characterization and potential role as a vector of human diseases. *Parasites & Vectors* **11**, 297.

Diniz, D. F. A., de Albuquerque, C. M. R., Oliva, L. O., de Melo-Santos, M. A. V., and Ayres, C. F. J. (2017). Diapause and quiescence: dormancy mechanisms that contribute to the geographical expansion of mosquitoes and their evolutionary success. *Parasites & Vectors* **10**, 1-13.

Diouf, B., Dia, I., Sene, N. M., Ndiaye, E. H., Diallo, M., and Diallo, D. (2020). Morphology and taxonomic status of Aedes aegypti populations across Senegal. *PLoS One* **15**, e0242576.

Dissanayake, D., Wijekoon, C., and Wegiriya, H. (2021). The effect of breeding habitat characteristics on the larval abundance of *Aedes* vector mosquitoes (Diptera: Culicidae) in three localities, Galle District, Sri Lanka. *Psyche: A Journal of Entomology* **2021**, 9911571.

Dodson, B. L., Hughes, G. L., Paul, O., Matacchiero, A. C., Kramer, L. D., and Rasgon, J. L. (2014). Wolbachia enhances West Nile virus (WNV) infection in the mosquito *Culex tarsalis*. *PLoS Neglected Tropical Diseases* **8**, e2965.

Durkett-Cadena, D EUBANKS, M., and Unnasch, T. R. (2008). Preference of female mosquitoes for natural and artificial resting sites. *Journal of the American Mosquito Control Association* **24**, 228.

Ebert, D. (2013). The epidemiology and evolution of symbionts with mixed-mode transmission. *Annual Review of Ecology, Evolution, and Systematics* **44**, 623-643.

Egid, B. R., Coulibaly, M., Dadzie, S. K., Kamgang, B., McCall, P. J., Sedda, L., Toe, K. H., and Wilson, A. L. (2022). Review of the ecology and behaviour of *Aedes aegypti* and *Aedes albopictus* in Western Africa and implications for vector control. *Current Research in Parasitology & Vector-Borne Diseases* **2**, 100074.

Elduma, A. H., and Osman, W. M. (2014). Dengue and hepatitis E virus infection in pregnant women in Eastern Sudan, a challenge for diagnosis in an endemic area. *The Pan African Medical Journal* **19**.

Faull, K., Webb, C., and Williams, C. (2016). Desiccation survival time for eggs of a widespread and invasive Australian mosquito species, *Aedes* (Finlaya) *notoscriptus* (Skuse). *Journal of Vector Ecology* **41**, 55-62.

Felsenstein, J. (1985). Confidence limits on phylogenies: an approach using the bootstrap. *Evolution* **39**, 783-791.

Ferede, G., Tiruneh, M., Abate, E., Kassa, W., Wondimeneh, Y., Damtie, D., and Tessema, B. (2018a). Distribution and larval breeding habitats of *Aedes* mosquito species in residential areas of northwest Ethiopia. *Epidemiol Health* **40**: e2018015.

Ferede, G., Tiruneh, M., Abate, E., Wondimeneh, Y., Damtie, D., Gadisa, E., Howe, R., Aseffa, A., and Tessema, B. (2018b). A serologic study of dengue in northwest Ethiopia: Suggesting preventive and control measures. *PLoS Neglected Tropical Diseases* **12**, e0006430.

Ferede, G., Tiruneh, M., Abate, E., Wondimeneh, Y., Gadisa, E., Howe, R., Aseffa, A., and Tessema, B. (2021). Evidence of chikungunya virus infection among febrile patients in northwest Ethiopia. *International Journal of Infectious Diseases* **104**, 183-188.

Flores, H., and O'Neill, S. L. (2018). Controlling vector-borne diseases by releasing modified mosquitoes. *Nature Reviews Microbiology* **16**, 508-518.

Fontenille, D., and Powell, J. R. (2020). From anonymous to public enemy: how does a mosquito become a feared arbovirus vector? *Pathogens* **9**, 265.

Foster, W. A., and Walker, E. D. (2019). mosquitoes (Culicidae). *In*: *Medical and Veterinary Entomology*, pp. 261-325. Elsevier.

Fraser, J. E., De Bruyne, J. T., Iturbe-Ormaetxe, I., Stepnell, J., Burns, R. L., Flores, H. A., and O'Neill, S. L. (2017). Novel Wolbachia-transinfected *Aedes aegypti* mosquitoes possess diverse fitness and vector competence phenotypes. *PLoS Pathogens* **13**, e1006751.

García-Betancourt, T., Higuera-Mendieta, D. R., González-Uribe, C., Cortés, S., and Quintero, J. (2015). Understanding water storage practices of urban residents of an endemic dengue area in Colombia: perceptions, rationale and socio-demographic characteristics. *PLoS One* **10**, e0129054.

Garishah, F. M., Boahen, C. K., Vadaq, N., Pramudo, S. G., Tunjungputri, R. N., Riswari, S. F., van Rij, R. P., Alisjahbana, B., Gasem, M. H., and van der Ven, A. J. (2023). Longitudinal proteomic profiling of the inflammatory response in dengue patients. *PLOS Neglected Tropical Diseases* **17**, e0011041.

Garske, T., Van Kerkhove, M. D., Yactayo, S., Ronveaux, O., Lewis, R. F., Staples, J. E., Perea, W., Ferguson, N. M., and Committee, Y. F. E. (2014). Yellow fever in Africa: estimating the burden of disease and impact of mass vaccination from outbreak and serological data. *PLoS Medicine* **11**, e1001638.

Gashaw, T., Wubaye, G. B., Worqlul, A. W., Dile, Y. T., Mohammed, J. A., Birhan, D. A., Tefera, G. W., van Oel, P. R., Haileslassie, A., and Chukalla, A. D. (2023). Local and regional climate trends and variabilities in Ethiopia: Implications for climate change adaptations. *Environmental Challenges* **13**, 100794.

Gedefie, A., Debash, H., Kassaw, A. B., Mankelkl, G., Metaferia, Y., Belete, M. A., Desale, S., Sebsibe, S., Tilahun, M., and Eshetu, B. (2025). Epidemiology of arboviruses in humans and livestock in Ethiopia: a systematic review and meta-analysis. *BMC Infectious Diseases* **25**, 458.

Geleta, D., Tesfaye, N., Ayigegn, H., Waldetensai, A., Gemechu, F., and Amare, H. (2020). Epidemiological description of chikungunya virus outbreak in Dire Dawa Administrative City, western Ethiopia, 2019. *International Journal of Clinical and Experimental Medical Sciences* **6**, 41.

Geleta, E. N. (2019). Serological evidence of dengue fever and its associated factors in health facilities in the Borena Zone, South Ethiopia. *Research and Reports in Tropical Medicine*, 129-136.

Getachew, D., Tekie, H., Gebre-Michael, T., Balkew, M., and Mesfin, A. (2015). Breeding sites of *Aedes aegypti:* potential dengue vectors in Dire Dawa, East Ethiopia. *Interdisciplinary Perspectives on Infectious Diseases* **2015**.

Gillies, M. (1980). The role of carbon dioxide in host-finding by mosquitoes (Diptera: Culicidae): a review. *Bulletin of Entomological Research* **70**, 525-532.

Githeko, A. K., Lindsay, S. W., Confalonieri, U. E., and Patz, J. A. (2000). Climate change and vector-borne diseases: a regional analysis. *Bulletin of the World Health Organization* **78**, 1136-1147.

Gómez, M., Martínez, D., Hernández, C., Luna, N., Patiño, L. H., Bohórquez Melo, R., Suarez, L. A., Palma-Cuero, M., Murcia, L. M., and González Páez, L. (2022). Arbovirus infection in Aedes aegypti from different departments of Colombia. *Frontiers in Ecology and Evolution* **10**, 999169.

Gupta, K., Dhawan, R., Kajla, M., Kumar, S., Jnanasiddhy, B., Singh, N. K., Dixit, R., Bihani, A., and Gupta, L. (2016). Molecular identification of *Aedes aegypti* mosquitoes from Pilani region of Rajasthan, India. *Journal of Vector Borne Diseases* **53**, 149-155.

Gupta, S., and Preet, S. (2012). Protocol optimization for genomic DNA extraction and RAPD-PCR in mosquito larvae (Diptera: Culicidae). *Annals of Biological Research* **3**, 1553-1561.

Haider, N., Kirkeby, C., Kristensen, B., Kjær, L. J., Sørensen, J. H., and Bødker, R. (2017). Microclimatic temperatures increase the potential for vector-borne disease transmission in the Scandinavian climate. *Scientific Reports* **7**, 8175.

Haidy Massa, M., Ould Lemrabott, M. A., Abdillahi Guedi, O., Briolant, S., and Ould Mohamed Salem Boukhary, A. (2025). Physical and Chemical Characteristics of *Aedes aegypti* Larval Habitats in Nouakchott, Mauritania. *Tropical Medicine and Infectious Disease* **10**, 147.

Halstead, S., and Wilder-Smith, A. (2019). Severe dengue in travellers: pathogenesis, risk and clinical management. *Journal of Travel Medicine* **26**, taz062.

Harbach, R. E. (2007). The Culicidae (Diptera): a review of taxonomy, classification and phylogeny. *Zootaxa* **1668**, 591–638.

Hebbale, C. N. (2015). The Merger Of Thermo Fisher Scientific Inc. & Life Technologies™ And Its Effect On Consumer Surplus A Cournot Model Approach, University of Georgia Athens.

Hery, L., Guidez, A., Durand, A.-A., Delannay, C., Normandeau-Guimond, J., Reynaud, Y., Issaly, J., Goindin, D., Legrave, G., and Gustave, J. (2021). Natural variation in physicochemical profiles and bacterial communities associated with *Aedes aegypti* breeding sites and larvae on Guadeloupe and French Guiana. *Microbial Ecology* **81**, 93-109.

Huang, Y.-M. (2004). The subgenus Stegomyia of Aedes in the Afrotropical Region with keys to the species (Diptera: Culicidae). *Zootaxa* **700**, 1–120-1–120.

Hughes, G. L., and Rasgon, J. L. (2014). Transinfection: a method to investigate W olbachia–host interactions and control arthropod-borne disease. *Insect Molecular Biology* **23**, 141-151.

Imam, A. A., and Deeni, Y. (2015). Larval productivity and detoxification enzymes profile in response to physico-chemical environmental factors of *Anopheles gambiae* breeding ecologies in Nigeria. *British Journal of Applied Science & Technology* **5**, 595-612.

Jeffrey Gutiérrez, E. H., Walker, K. R., Ernst, K. C., Riehle, M. A., and Davidowitz, G. (2020). Size as a proxy for survival in *Aedes aegypti* (Diptera: Culicidae) mosquitoes. *Journal of Medical Entomology* **57**, 1228-1238.

Joubert, D. A., Walker, T., Carrington, L. B., De Bruyne, J. T., Kien, D. H. T., Hoang, N. L. T., Chau, N. V. V., Iturbe-Ormaetxe, I., Simmons, C. P., and O'Neill, S. L. (2016). Establishment of a Wolbachia superinfection in *Aedes aegypti* mosquitoes as a potential approach for future resistance management. *PLoS Pathogens* **12**, e1005434.

Joy, T. K., Arik, A. J., Corby-Harris, V., Johnson, A. A., and Riehle, M. A. (2010). The impact of larval and adult dietary restriction on lifespan, reproduction and growth in the mosquito *Aedes aegypti. Experimental Gerontology* **45**, 685-690.

Kendie, F. A., Mekuriaw, S. A., and Dagnew, M. A. (2018). Ethnozoological study of traditional medicinal appreciation of animals and their products among the indigenous people of Metema Woreda, North-Western Ethiopia. *Journal of Ethnobiology and Ethnomedicine* **14**, 1-12.

Ketkar, H., Herman, D., and Wang, P. (2019). Genetic determinants of the re-emergence of arboviral diseases. *Viruses* **11**, 150.

Khaligh, F. G., Naghian, A., Soltanbeiglou, S., and Gholizadeh, S. (2020). Autogeny in *Culiseta longiareolata* (Culicidae: Diptera) mosquitoes in laboratory conditions in Iran. *BMC Research Notes* **13**, 81.

Khongwichit, S., Chansaenroj, J., Chirathaworn, C., and Poovorawan, Y. (2021). Chikungunya virus infection: molecular biology, clinical characteristics, and epidemiology in Asian countries. *Journal of Biomedical Science* **28**, 84.

Kinyatta, N. M., Wangui Ng'ang'a, Z., Kamau, L., and Kagai, J. M. (2018). Comparison of indoor mosquito collection methods in the assessment of lymphatic filariasis transmission dynamics in mosquito vectors in Tana River County, Kenya. *The East African Health Research Journal* **2**, 58.

Korgaonkar, N. S., Kumar, A., Yadav, R. S., Kabadi, D., and Dash, A. P. (2012). Mosquito biting activity on humans & detection of Plasmodium falciparum infection in *Anopheles* stephensi in Goa, India. *Indian Journal of Medical Research* **135**, 120-126.

Kraemer, M. U., Faria, N. R., Reiner, R. C., Golding, N., Nikolay, B., Stasse, S., Johansson, M. A., Salje, H., Faye, O., and Wint, G. W. (2017). Spread of yellow fever virus outbreak in Angola and the Democratic Republic of the Congo 2015–16: a modelling study. *The Lancet Infectious Diseases* **17**, 330-338.

Kraemer, M. U., Sinka, M. E., Duda, K. A., Mylne, A. Q., Shearer, F. M., Barker, C. M., Moore, C. G., Carvalho, R. G., Coelho, G. E., and Van Bortel, W. (2015). The global distribution of the arbovirus vectors *Ae. aegypti* and *Ae. albopictus*. *Elife* **4**, e08347.

Kramer, L. D., and Ciota, A. T. (2015). Dissecting vectorial capacity for mosquito-borne viruses. *Current Opinion in Virology* **15**, 112-118.

Kuldeep Gupta, K. G., Rini Dhawan, R. D., Mithilesh Kajla, M. K., Sanjeev Kumar, S. K., Jnanasiddhy, B., Singh, N. K., Rajnikant Dixit, R. D., Ashish Bihani, A. B., and Lalita Gupta, L. G. (2016). Molecular identification of *Aedes aegypti* mosquitoes from Pilani region of Rajasthan, India. *Jurnal Vector Borne Disease* **53**, 149–155

Kumar, N. P., Kalimuthu, M., Kumar, M. S., Govindrajan, R., Venkatesh, A., Paramasivan, R., Kumar, A., and Gupta, B. (2022). Morphological and molecular characterization of Aedes aegypti variant collected from Tamil Nadu, India. *Journal of Vector Borne Diseases* **59**, 22-28.

LaBeaud, A., Bashir, F., and King, C. H. (2011). Measuring the burden of arboviral diseases: the spectrum of morbidity and mortality from four prevalent infections. *Population Health Metrics* **9**, 1-11.

Lande, R. (1996). Statistics and partitioning of species diversity, and similarity among multiple communities. *Oikos*, 5-13.

Laporta, G. Z., Potter, A. M., Oliveira, J. F., Bourke, B. P., Pecor, D. B., and Linton, Y.-M. (2023). Global distribution of *Ae. aegypti* and *Ae. albopictus* in a climate change scenario of regional rivalry. *Insects* **14**, 49.

Leparc-Goffart, I., Nougairede, A., Cassadou, S., Prat, C., and De Lamballerie, X. (2014). Chikungunya in the Americas. *The Lancet* **383**, 514.

Lequime, S., Paul, R. E., and Lambrechts, L. (2016). Determinants of arbovirus vertical transmission in mosquitoes. *PLoS Pathogens* **12**, e1005548.

Li, X., Stringer, L. C., and Dallimer, M. (2022). The impacts of urbanisation and climate change on the urban thermal environment in Africa. *Climate* **10**, 164.

Li, Y., Kamara, F., Zhou, G., Puthiyakunnon, S., Li, C., Liu, Y., Zhou, Y., Yao, L., Yan, G., and Chen, X.-G. (2014). Urbanization increases *Aedes albopictus* larval habitats and accelerates mosquito development and survivorship. *PLoS Neglected Tropical Diseases* **8**, e3301.

Lilay, A., Asamene, N., Bekele, A., Mengesha, M., Wendabeku, M., Tareke, I., Girmay, A., Wuletaw, Y., Adossa, A., and Ba, Y. (2017). Reemergence of yellow fever in Ethiopia after 50 years, 2013: epidemiological and entomological investigations. *BMC Infectious Diseases* **17**, 1-6.

Lima-Camara, T. N. (2010). Activity patterns of *Aedes aegypti* and *Aedes albopictus* (Diptera: Culicidae) under natural and artificial conditions. *Oecologia Australis* **14**, 737-744.

Liu, X., Yang, S., Yao, Y., Wu, S., Wu, P., and Zhai, Z. (2022). Opsin1 regulates light-evoked avoidance behavior in *Aedes albopictus*. *BMC Biology* **20**, 110.

Lope Choque, Y. Y. (2020). Efecto del filgrastim sobre la activación de CD32 y M-CSFR en macrófagos murinos como tratamiento de la Proteinosis Alveolar Pulmonar–Arequipa, 2020.

Ma, M., Huang, M., and Leng, P. (2016). Abundance and distribution of immature mosquitoes in urban rivers proximate to their larval habitats. *Acta Tropica* **163**, 121-129.

Mamai, W., Maiga, H., Bimbilé Somda, N. S., Wallner, T., Masso, O. B., Resch, C., Yamada, H., and Bouyer, J. (2021). Does tap water quality compromise the production of *Aedes* mosquitoes in genetic control projects? *Insects* **12**, 57.

Mangudo, C., Aparicio, J. P., and Gleiser, R. M. (2015). Tree holes as larval habitats for *Aedes aegypti* in urban, suburban and forest habitats in a dengue affected area. *Bulletin of Entomological Research* **105**, 679-684.

Manouana, G. P., Sarah-Matio, E.-M., Hellhammer, F., Zahouli, J. Z. B., Tapé, A. S. B., Biré, Y. N., Dibo, J.-D. K., Houriaaidji, G. E., Maganga, G. D., and Koumba, J. P. (2024). Ecology of arboviruses and their potential mosquito vectors in Benin, Côte d'Ivoire and Gabon: a mini review. *Frontiers in Tropical Diseases* **5**, 1355778.

Manzambi, E. Z., Mbuka, G. B., Ilombe, G., Takasongo, R. M., Tezzo, F. W. s., del Carmen Marquetti, M., Metelo, E., Vanlerberghe, V., and Bortel, W. V. (2023). Behavior of Adult *Aedes aegypti* and *Aedes albopictus* in Kinshasa, DRC, and the Implications for Control. *Tropical Medicine and Infectious Disease* **8**, 207.

Marchi, S., Trombetta, C. M., and Montomoli, E. (2018). Emerging and re-emerging arboviral diseases as a global health problem. *In* "Public Health-Emerging and Re-emerging Issues". IntechOpen.

Marcos-Marcos, J., Olry de Labry-Lima, A., Toro-Cardenas, S., Lacasaña, M., Degroote, S., Ridde, V., and Bermudez-Tamayo, C. (2018). Impact, economic evaluation, and sustainability of integrated vector management in urban settings to prevent vector-borne diseases: a scoping review. *Infectious Diseases of Poverty* **7**, 1-14.

Mariconti, M., Obadia, T., Mousson, L., Malacrida, A., Gasperi, G., Failloux, A.-B., and Yen, P.-S. (2019). Estimating the risk of arbovirus transmission in Southern Europe using vector competence data. *Scientific Reports* **9**, 17852.

Marina, C. F., Bond, J. G., Hernández-Arriaga, K., Valle, J., Ulloa, A., Fernández-Salas, I., Carvalho, D. O., Bourtzis, K., Dor, A., and Williams, T. (2021). Population dynamics of *Aedes aegypti* and *Aedes albopictus* in two rural villages in southern Mexico: Baseline data for an evaluation of the sterile insect technique. *Insects* **12**, 58.

Massengo, N. R. B., Tinto, B., and Simonin, Y. (2023). One health approach to arbovirus control in Africa: interests, challenges, and difficulties. *Microorganisms* **11**, 1496.

Mattah, P. A. D., Futagbi, G., Amekudzi, L. K., Mattah, M. M., de Souza, D. K., Kartey-Attipoe, W. D., Bimi, L., and Wilson, M. D. (2017). Diversity in breeding sites and distribution of *Anopheles* mosquitoes in selected urban areas of southern Ghana. *Parasites & Vectors* **10**, 1-15.

Mbanzulu, K. M., Mboera, L. E., Wumba, R., Engbu, D., Bojabwa, M. M., Zanga, J., Mitashi, P. M., Misinzo, G., and Kimera, S. I. (2022). Physicochemical characteristics of *Aedes* mosquito

breeding habitats in suburban and urban areas of Kinshasa, Democratic Republic of the Congo. *Frontiers in Tropical Diseases* **2**, 789273.

McLean-Cooper, N., Achee, N., Foggie, T., Grieco, J., and Williams, J. (2008). Space optimizing methods for laboratory rearing of *Aedes aegypti*. *Journal of the American Mosquito Control Association* **24**, 460-462.

McMeniman, C. J., and O'Neill, S. L. (2010). A virulent Wolbachia infection decreases the viability of the dengue vector *Aedes aegypti* during periods of embryonic quiescence. *PLoS Neglected Tropical Diseases* **4**, e748.

Medeiros-Sousa, A. R., de Oliveira-Christe, R., Camargo, A. A., Scinachi, C. A., Milani, G. M., Urbinatti, P. R., Natal, D., Ceretti-Junior, W., and Marrelli, M. T. (2020). Influence of water's physical and chemical parameters on mosquito (Diptera: Culicidae) assemblages in larval habitats in urban parks of Sao Paulo, Brazil. *Acta Tropica* **205**, 105394.

Mekonnen, M., and Kloos, H. (2019). Yellow fever and other arboviral diseases. *In* "The Ecology of Health and Disease in Ethiopia", pp. 389-397. Routledge.

Mereta, S. T., Yewhalaw, D., Boets, P., Ahmed, A., Duchateau, L., Speybroeck, N., Vanwambeke, S. O., Legesse, W., De Meester, L., and Goethals, P. L. (2013). Physico-chemical and biological characterization of anopheline mosquito larval habitats (Diptera: Culicidae): implications for malaria control. *Parasites & Vectors* **6**, 1-16.

Mesfin, Z., Ali, A., Abagero, A., and Asefa, Z. (2022). Dengue Fever Outbreak Investigation in Werder Town, Dollo Zone, Somali Region, Ethiopia. *Infection and Drug Resistance*, 7207-7217.

Minakawa, N., Mutero, C. M., Githure, J. I., Beier, J. C., and Yan, G. (1999). Spatial distribution and habitat characterization of anopheline mosquito larvae in Western Kenya. *The American Journal of Tropical Medicine and Hygiene* **61**, 1010-1016.

Mohamed, M. A., Hassan, N. Y., Osman, M. M., Gedi, S., Maalin, B. A. A., Sultan, K. M., Garba, B., Osman, A. A., Osman, A. Y., and Ahmed, A. D. (2024). Epidemiological investigation of dengue fever outbreak and its socioeconomic determinants in Banadir region, Somalia. *BMC Infectious Diseases* **24**, 393.

Mordecai, E. A., Cohen, J. M., Evans, M. V., Gudapati, P., Johnson, L. R., Lippi, C. A., Miazgowicz, K., Murdock, C. C., Rohr, J. R., and Ryan, S. J. (2017). Detecting the impact of temperature on transmission of Zika, dengue, and chikungunya using mechanistic models. *PLoS Neglected Tropical Diseases* **11**, e0005568.

Mousson, L., Dauga, C., Garrigues, T., Schaffner, F., Vazeille, M., and Failloux, A.-B. (2005). Phylogeography of *Aedes* (Stegomyia) *aegypti* (L.) and *Aedes* (Stegomyia) *albopictus* (Skuse)(Diptera: Culicidae) based on mitochondrial DNA variations. *Genetics Research* **86**, 1-11.

Mousson, L., Zouache, K., Arias-Goeta, C., Raquin, V., Mavingui, P., and Failloux, A.-B. (2012). The native Wolbachia symbionts limit transmission of dengue virus in *Aedes albopictus*. *PLoS Neglected Tropical Diseases* **6**, e1989.

Musunzaji, P. S., Ndenga, B. A., Mzee, S., Abubakar, L. U., Kitron, U. D., Labeaud, A. D., and Mutuku, F. M. (2023). Oviposition preferences of *Aedes aegypti* in msambweni, kwale county, Kenya. *Journal of the American Mosquito Control Association* **39**, 85-95.

Mutebi, J.-P., and Barrett, A. D. (2002). The epidemiology of yellow fever in Africa. *Microbes and Infection* **4**, 1459-1468.

Mwangangi, J. M., Shililu, J., Muturi, E. J., Muriu, S., Jacob, B., Kabiru, E. W., Mbogo, C. M., Githure, J., and Novak, R. J. (2010). *Anopheles* larval abundance and diversity in three rice agro-village complexes Mwea irrigation scheme, central Kenya. *Malaria Journal* **9**, 1-10.

Mwanyika, G. O., Moir, M., Musa, A. O., Poongavanan, J., Dor, G., Wilkinson, E., Baxter, C., de Oliveira, T., and Tegally, H. (2024). A decade of dengue disease burden in Africa (2013–2023): *A Systematic Review* **10,** 22.24315827.

Neri, P., Andral, L., and Poirier, A. (1968). Studies on yellow fever in Ethiopia. 4. Entomological studies in the locality of Manera. *Bulletin of the World Health Organization* **38**, 863-872.

Ngugi, H. N., Mutuku, F. M., Ndenga, B. A., Musunzaji, P. S., Mbakaya, J. O., Aswani, P., Irungu, L. W., Mukoko, D., Vulule, J., and Kitron, U. (2017). Characterization and productivity profiles of *Aedes aegypti* (L.) breeding habitats across rural and urban landscapes in western and coastal Kenya. *Parasites & Vectors* **10**, 1-12.

Nigatu, W., Asrat, Y., Sisay, C., Gunta, M., Belay, D., Tasew, G., and Waldetensai, A. (2020). Aedes Mosquitoes distribution and risk of Yellow Fever transmission in Gurage Zone, Southwest Ethiopia. *Ethiopian Journal of Public Health and Nutrition* **4**, 21-31.

Nikookar, S. H., Fazeli-Dinan, M., Azari-Hamidian, S., Mousavinasab, S. N., Aarabi, M., Ziapour, S. P., Esfandyari, Y., and Enayati, A. (2017). Correlation between mosquito larval density and their habitat physicochemical characteristics in Mazandaran Province, northern Iran. *PLoS Neglected Tropical Diseases* **11**, e0005835.

Ocampo, C. B., Salazar-Terreros, M. J., Mina, N. J., McAllister, J., and Brogdon, W. (2011). Insecticide resistance status of Aedes aegypti in 10 localities in Colombia. *Acta Tropica* **118**, 37-44.

Odero, J. I., Abong'o, B., Moshi, V., Ekodir, S., Harvey, S. A., Ochomo, E., Gimnig, J. E., Achee, N. L., Grieco, J. P., and Oria, P. A. (2024). Early morning anopheline mosquito biting, a potential driver of malaria transmission in Busia County, western Kenya. *Malaria Journal* **23**, 66.

Ouédraogo, W. M., Toé, K. H., Sombié, A., Viana, M., Bougouma, C., Sanon, A., Weetman, D., McCall, P. J., Kanuka, H., and Badolo, A. (2022). Impact of physicochemical parameters of *Aedes aegypti* breeding habitats on mosquito productivity and the size of emerged adult mosquitoes in Ouagadougou City, Burkina Faso. *Parasites & Vectors* **15**, 478.

Padonou, G. G., Konkon, A. K., Salako, A. S., Zoungbédji, D. M., Osse, R., Sovi, A., Azondekon, R., Sidick, A., Ahouandjinou, J. M., and Adoha, C. J. (2023). Distribution and Abundance of

Aedes aegypti and *Aedes albopictus* (Diptera: Culicidae) in Benin, West Africa. *Tropical Medicine and Infectious Disease* **8**, 439.

Panella, N. A., Crockett, R. J. K., Biggerstaff, B. J., and Komar, N. (2011). The Centers for Disease Control and Prevention resting trap: a novel device for collecting resting mosquitoes. *Journal of the American Mosquito Control Association* **27**, 323.

Panigrahi, S. K., Parida, S. R., Das, J., Behera, R., and Nayak, N. R. (2024). Genetic Characterisation and Molecular Phylogeny of Mosquito *Aedes vittatus* Based on COI Gene from Bhawanipatna, Kalahandi, Odisha, India. *Asian Journal of Research in Zoology* **7**, 77-88.

Panmei, K., Syed, A. H., Okafor, O., Mammen, S., and Abraham, A. M. (2025). Performance evaluation of TaqMan™ Arbovirus Triplex Kit (ZIKV/DENV/CHIKV) for detection and differentiation of Dengue and Chikungunya viral RNA in serum samples of symptomatic patients. *Journal of Virological Methods* **332**, 115072.

Pant, C., and Yasuno, M. (1973). Field studies on the gonotrophic cycle of *Aedes aegypti* in Bangkok, Thailand. *Journal of Medical Entomology* **10**, 219-223.

Paramasivan, R., Philip, S., and Selvaraj, P. (2015). Biting rhythm of vector mosquitoes in a rural ecosystem of South India. *International Journal Mosquito Research* **2**, 106-113.

Pereira, T. N., Rocha, M. N., Sucupira, P. H. F., Carvalho, F. D., and Moreira, L. A. (2018). Wolbachia significantly impacts the vector competence of *Aedes aegypti* for Mayaro virus. *Scientific reports* **8**, 1-9.

Perrins, C. M. (1996). Eggs, egg formation and the timing of breeding. *Ibis* **138**, 2-15.

Pourzangiabadi, M., Najafi, H., Fallah, A., Goudarzi, A., and Pouladi, I. (2025). Dengue virus: Etiology, epidemiology, pathobiology, and developments in diagnosis and control–A comprehensive review. *Infection, Genetics and Evolution* **127**, 105710.

Prasad, A., Sreedharan, S., Bakthavachalu, B., and Laxman, S. (2023). Eggs of the mosquito Aedes aegypti survive desiccation by rewiring their polyamine and lipid metabolism. *PLoS Biology* **21**, e3002342.

Ranasinghe, H., and Amarasinghe, L. (2020). Naturally occurring microbiota in dengue vector mosquito breeding habitats and their use as diet organisms by developing larvae in the Kandy district, Sri Lanka. *BioMedecine Research International* **2020**, 5830604.

Rani, E., Surendran, A., and Thatheyus, A. (2020). Biting Behaviour of The Filarial Vector Mosquito, *Culex quinquefasciatus* Say, in an Urban Area. *Journal of Plant and Animal Ecology* **2**, 1-13.

Rashidi, H. S., Alghamdi, K. M., Al-Otaibi, W. M., Al-Solami, H. M., and Mahyoub, J. A. (2022). Effects of blood meal sources on the biological characteristics of *Aedes aegypti* and *Culex pipiens* (Diptera: Culicidae). *Saudi Journal of Biological Sciences* **29**, 103448.

Rey, J. R., and O'Connell, S. M. (2014). Oviposition by *Aedes aegypti* and *Aedes albopictus*: Influence of congeners and of oviposition site characteristics. *Journal of Vector Ecology* **39**, 190-196.

Ries, M., Bravo, B., Balliana, E., Heisinger, F., and Limame, I. (2024). Molecular Microscopy by Thermo-Fisher-Scientific: FTIR-Imaging of a Tintoretto-Fresco and Raman-Imaging of the Strain-Distribution in Semiconductors. *In*: BIO Web of Conferences, Vol. 129, pp. 06022. EDP Sciences.

Robert, M. A., Stewart-Ibarra, A. M., and Estallo, E. L. (2020). Climate change and viral emergence: evidence from Aedes-borne arboviruses. *Current Opinion in Virology* **40**, 41-47.

Ross, P. A., Endersby, N. M., Yeap, H. L., and Hoffmann, A. A. (2014). Larval competition extends developmental time and decreases adult size of wMelPop Wolbachia-infected *Aedes aegypti*. *The American Journal of Tropical Medicine and Hygiene* **91**, 198.

Rueda, L. M. (2004a). "Pictorial keys for the identification of mosquitoes (Diptera: Culicidae) associated with dengue virus transmission." Walter Reed Army Inst Of Research Washington Dc Department Of Entomology.

Rueda, L. M. (2004b). Pictorial keys for the identification of mosquitoes (Diptera: Culicidae) associated with dengue virus transmission. *Zootaxa* **589**, 1–60-1–60.

Rund, S. S., Labb, L. F., Benefiel, O. M., and Duffield, G. E. (2020). Artificial light at night increases *Aedes aegypti* mosquito biting behavior with implications for arboviral disease transmission. *The American Journal of Tropical Medicine and Hygiene* **103**, 2450.

Saitou, N., and Nei, M. (1987). The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Molecular Biology and Evolution* **4**, 406-425.

Saleh, F., Kitau, J., Konradsen, F., Alifrangis, M., Lin, C.-H., Juma, S., Mchenga, S. S., Saadaty, T., and Schiøler, K. L. (2018). Habitat characteristics for immature stages of *Aedes aegypti* in Zanzibar city, Tanzania. *Journal of the American Mosquito Control Association* **34**, 190-200.

Samuel, G. H., Adelman, Z. N., and Myles, K. M. (2016). Temperature-dependent effects on the replication and transmission of arthropod-borne viruses in their insect hosts. *Current Opinion in Insect Science* **16**, 108-113.

Sang, R., Lutomiah, J., Chepkorir, E., and Tchouassi, D. P. (2022). Evolving dynamics of Aedes-borne diseases in Africa: a cause for concern. *Current Opinion in Insect Science* **53**, 100958.

Sauer, F., Grave, J., Lühken, R., and Kiel, E. (2021). Habitat and microclimate affect the resting site selection of mosquitoes. *Medical and Veterinary Entomology* **35**, 379-388.

Sauer, F. G., Kiel, E., and Lühken, R. (2022). Effects of mosquito resting site temperatures on the estimation of pathogen development rates in near-natural habitats in Germany. *Parasites & Vectors* **15**, 390.

Schmidt, C. A., Comeau, G., Monaghan, A. J., Williamson, D. J., and Ernst, K. C. (2018). Effects of desiccation stress on adult female longevity in *Aedes aegypti* and *Aedes albopictus* (Diptera: Culicidae): results of a systematic review and pooled survival analysis. *Parasites & Vectors* **11**, 1-21.

Schoener, E., Uebleis, S. S., Butter, J., Nawratil, M., Cuk, C., Flechl, E., Kothmayer, M., Obwaller, A. G., Zechmeister, T., and Rubel, F. (2017). Avian Plasmodium in eastern Austrian mosquitoes. *Malaria Journal* **16**, 389.

Seang-Arwut, C., Hanboonsong, Y., Muenworn, V., Rocklöv, J., Haque, U., Ekalaksananan, T., Paul, R. E., and Overgaard, H. J. (2023). Indoor resting behavior of *Aedes aegypti* (Diptera: Culicidae) in northeastern Thailand. *Parasites & Vectors* **16**, 127.

Seid, M., Aklilu, E., and Animut, A. (2024a). Spatio-temporal occurrence and habitat characteristics of *Aedes aegypti* (Diptera: Culicidae) larvae in Southern Afar region, Ethiopia. *Tropical Medicine and Health* **52**, 51.

Seid, M., Aklilu, E., and Animut, A. (2024b). Susceptibility status of *Aedes aegypti* (Diptera: Culicidae) to public health insecticides in Southern Afar Region, Ethiopia. *Plos One* **19**, e0309335.

Selvan, P. S., Jebanesan, A., Divya, G., and Ramesh, V. (2015). Diversity of mosquitoes and larval breeding preference based on physico-chemical parameters in Western Ghats, Tamilnadu, India. *Asian Pacific Journal of Tropical Disease* **5**, S59-S66.

Service, M. (1971). The daytime distribution of mosquitoes resting in vegetation. *Journal of Medical Entomology* **8**, 271-278.

Shah, N. L. M., Dom, N. C., Abdullah, S., Precha, N., and Dapari, R. (2022). Evaluation on the effects of different plant matter in water on *Aedes aegypti* development (Diptera: Culicidae). *International Journal of Tropical Insect Science* **42**, 3831-3838.

Shimelis, T., Mulu, A., Mengesha, M., Alemu, A., Mihret, A., Tadesse, B. T., Bartlett, A. W., Belay, F. W. G., Schierhout, G., and Dittrich, S. (2023). Detection of dengue virus infection in children presenting with fever in Hawassa, southern Ethiopia. *Scientific Reports* **13**, 7997.

Shirozu, T., Regilme, M. A. F., Ote, M., Sasaki, M., Soga, A., Bochimoto, H., Kawabata, H., Umemiya-Shirafuji, R., Kanuka, H., and Fukumoto, S. (2024). Wolbachia infection in Aedes aegypti does not affect its vectorial capacity for Dirofilaria immitis. *Scientific Reports* **14**, 22528.

Shocket, M. S., Verwillow, A. B., Numazu, M. G., Slamani, H., Cohen, J. M., El Moustaid, F., Rohr, J., Johnson, L. R., and Mordecai, E. A. (2020). Transmission of West Nile and five other temperate mosquito-borne viruses peaks at temperatures between 23 C and 26 C. *Elife* **9**, e58511.

Siam, H. A., Elaagip, A., Abrahim, S. A. A., Mohammed, M. H., Noaman, O. M., and Samy, A. M. (2022). A recent chikungunya outbreak associated with the occurrence of *Aedes* vectors (Diptera: Culicidae) in Kassala state, eastern Sudan in 2018. *Parasitology International* **90**, 102613.

Simmons, C. P., Farrar, J. J., van Vinh Chau, N., and Wills, B. (2012). Dengue. *New England Journal of Medicine* **366**, 1423-1432.

Sinclair, B. J. (2023). An annotated checklist of the Diptera of the Galápagos Archipelago (Ecuador). *Zootaxa* **5283**, 1-102.

Struchiner, C. J., Rockloev, J., Wilder-Smith, A., and Massad, E. (2015). Increasing dengue incidence in Singapore over the past 40 years: population growth, climate and mobility. *PloS One* **10**, e0136286.

Sudeep, A., Mohandas, S., Bhanarkar, S., Ghodke, Y., and Sonawane, P. (2020). Vector competence of *Aedes vittatus* (Bigot) mosquitoes from India for Japanese encephalitis, West Nile, Chandipura and Chittoor viruses. *Journal of Vector Borne Diseases* **57**, 234-239.

Supriyono, S., Soviana, S., Novianto, D., Musyaffa, M. F., Tan, S., and Hadi, U. K. (2022). Morphological characteristic of malaria vector *Anopheles aconitus* (Family: Culicidae) revealed by advanced light and scanning electron microscope. *Biodiversitas Journal of Biological Diversity* **23**, 17-28

Supryono, S., Soviana, S., Musyaffa, M. F., NOVIANTO, D., and Hadi, U. K. (2023). Morphological characteristic of dengue vectors *Aedes aegypti* and *Ae. albopictus* (Family: Culicidae) using advanced light and scanning electron microscope. *Biodiversitas Journal of Biological Diversity* **24**, 23-31

Tajudeen, Y. A., Oladipo, H. J., Oladunjoye, I. O., Yusuf, R. O., Sodiq, H., Omotosho, A. O., Adesuyi, D. S., Yusuff, S. I., and El-Sherbini, M. S. (2022). Emerging arboviruses of public health concern in Africa: priorities for future research and control strategies. *Challenges* **13**, 60.

Tamura, K., and Kumar, S. (2002). Evolutionary distance estimation under heterogeneous substitution pattern among lineages. *Molecular Biology and Evolution* **19**, 1727-1736.

Tamura, K., Stecher, G., and Kumar, S. (2021). MEGA11: molecular evolutionary genetics analysis version 11. *Molecular Biology and Evolution* **38**, 3022-3027.

Tamura, K., Stecher, G., Peterson, D., Filipski, A., and Kumar, S. (2013). MEGA6: molecular evolutionary genetics analysis version 6.0. *Molecular Biology and Evolution* **30**, 2725-2729.

Thavara, U., Tawatsin, A., Chansang, C., Kong-ngamsuk, W., Paosriwong, S., Boon-Long, J., Rongsriyam, Y., and Komalamisra, N. (2001). Larval occurrence, oviposition behavior and biting activity of potential mosquito vectors of dengue on Samui Island, Thailand. *Journal of Vector Ecology* **26**, 172-180.

Thongsripong, P., Green, A., Kittayapong, P., Kapan, D., Wilcox, B., and Bennett, S. (2013). Mosquito vector diversity across habitats in central Thailand endemic for dengue and other arthropod-borne diseases. *PLoS Neglected Tropical Diseases* **7**, e2507.

Troyo, A., Calderón-Arguedas, O., Fuller, D. O., Solano, M. E., Avendaño, A., Arheart, K. L., Chadee, D. D., and Beier, J. C. (2008). Seasonal profiles of Aedes aegypti (Diptera: Culicidae) larval habitats in an urban area of Costa Rica with a history of mosquito control. *Journal of Vector Ecology: Journal of the Society for Vector Ecology* **33**, 76.

Tufa, Y. A., Fufa, Y., Abebe, M., Habebe, S., Sufa, D., Melese, M., and Doda, Z. (2020). Qualitative research on yellow fever outbreak responses in Wolaita zone of SNNPR region, Ethiopia. *American Journal of Health Research* **8**, 32-41.

Van Dung, N., Thieu, N. Q., Canh, H. D., Le Duy, B., Hung, V. V., Ngoc, N. T. H., Mai, N. V. T., Van Anh, N. T., Son, L. D., and Oo, W. H. (2023). *Anopheles* diversity, biting behaviour and transmission potential in forest and farm environments of Gia Lai province, Vietnam. *Malaria Journal* **22**, 204.

Vasconcelos, P. F., Bryant, J. E., Da Rosa, A. P. T., Tesh, R. B., Rodrigues, S. G., and Barrett, A. D. (2004). Genetic divergence and dispersal of yellow fever virus, Brazil. *Emerging Infectious Diseases* **10**, 1578.

Venter, M. (2018). Assessing the zoonotic potential of arboviruses of African origin. *Current Opinion in Virology* **28**, 74-84.

Vitale, M., Leo, C., Courty, T., Kranjc, N., Connolly, J. B., Morselli, G., Bamikole, C., Haghighat-Khah, R. E., Bernardini, F., and Fuchs, S. (2023). Comprehensive characterization of a transgene insertion in a highly repetitive, centromeric region of *Anopheles* mosquitoes. *Pathogens and Global Health* **117**, 273-283.

Vulu, F., Futami, K., Sunahara, T., Mampuya, P., Bobanga, T. L., Mumba Ngoyi, D., and Minakawa, N. (2024). Geographic expansion of the introduced *Aedes albopictus* and other native Aedes species in the Democratic Republic of the Congo. *Parasites & Vectors* **17**, 35.

Vythilingam, I., Oda, K., Chew, T., Mahadevan, S., Vijayamalar, B., Morita, K., Tsuchie, H., and Igarashi, A. (1995). Isolation of Japanese encephalitis virus from mosquitoes collected in Sabak Bernam, Selangor, Malaysia in 1992. *Journal of the American Mosquito Control Association* **11**, 94-98.

Waldetensai, A., Nigatu, W., Asrat, Y., Sisay, C., Gunta, M., Belay, D., Tasew, G., and Waldetensai, A. (2020). *Aedes* Mosquitoes distribution and risk of Yellow Fever transmission in Gurage Zone, Southwest Ethiopia. *Ethiopia Journal Public HealTH Nutitionr* **4**, 21-31.

Waldetensai, A., Pareyn, M., and Massebo, F. (2023). Human-biting activity, resting behavior and yellow fever virus transmission potential of aedes mosquitoes in southwest Ethiopia. *Parasitologia* **3**, 87-100.

Waldetensai, A., Sisay, C., Asrat, Y., Debebe, Y., Kinde, S., and Alemnesh, H. (2021a). Assessment of the resting behavior of *Aedes aegypti* during dengue fever outbreak of Dire Dawa, eastern Ethiopia. *International Journal Applied Science-Research Review* **8**, 1-6.

Waldetensai, A., Sisay, C., Asrat, Y., Debebe, Y., Kinde, S., and Alemnesh, H. (2021b). Assessment of the resting behavior of *Aedes aegypti* during dengue fever outbreak of Dire Dawa, eastern Ethiopia. *International Journal Applied Science-Research Review* **8**, 25.

Weaver, S. C. (2014). Arrival of chikungunya virus in the new world: prospects for spread and impact on public health. *PLoS Neglected Tropical Diseases* **8**, e2921.

Weetman, D., Kamgang, B., Badolo, A., Moyes, C. L., Shearer, F. M., Coulibaly, M., Pinto, J., Lambrechts, L., and McCall, P. J. (2018). *Aedes* mosquitoes and *Aedes*-borne arboviruses in Africa: current and future threats. *International Journal of Environmental Research and Public Health* **15**, 220.

Were, F. (2012). The dengue situation in Africa. *Paediatrics and International Child Health* **32**, 18-21.

Werren, J. H., Baldo, L., and Clark, M. E. (2008). Wolbachia: master manipulators of invertebrate biology. *Nature Reviews Microbiology* **6**, 741-751.

WHO (1986). World Health Organization: Prevention and control of yellow fever in Africa. *In:* Prevention and control of yellow fever in Africa. Geneva.

WHO (2003). World Health Organization: A review of entomological sampling methods and indicators for dengue vectors. World Health Organization. *TDR/IDE/Den/03.1*, 16-21.

WHO (2009). "World Health Organization: Dengue: guidelines for diagnosis, treatment, prevention and control," World Health Organization. Geneva

WHO (2011). World Health Organization: Manual for indoor residual spraying: application of residual sprays for vector control [electronic resource]. *In:* Manual for indoor residual spraying: application of residual sprays for vector control. World Health Organization. New editing

Wilca-Cepeda, A., López-Rosero, A., Yumiseva, C. A., Grijalva, M. J., and Villacís, A. G. (2025). Arbovirus Detection in *Aedes aegypti* Mosquitoes in Manabí, Ecuador. *Pathogens* **14**, 446.

Wilder-Smith, A., Gubler, D. J., Weaver, S. C., Monath, T. P., Heymann, D. L., and Scott, T. W. (2017). Epidemic arboviral diseases: priorities for research and public health. *The Lancet Infectious Diseases* **17**, e101-e106.

Wilson, A. L., Courtenay, O., Kelly-Hope, L. A., Scott, T. W., Takken, W., Torr, S. J., and Lindsay, S. W. (2020). The importance of vector control for the control and elimination of vector-borne diseases. *PLoS Neglected Tropical Diseases* **14**, e0007831.

Wilson, J. J., and Sevarkodiyone, S. (2015). Host preference of blood feeding mosquitoes in rural areas of southern Tamil Nadu, India. *Academic Journal Entomology* **8**, 80-83.

Xue, R. D., Barnard, D., and Ali, A. (2001). Laboratory and field evaluation of insect repellents as oviposition deterrents against the mosquito *Aedes albopictus*. *Medical and Veterinary Entomology* **15**, 126-131.

Yamany, A., Adham, F., and Abdel-Gaber, R. (2024). Morphological description of the pupa of Aedes aegypti (Diptera: Culicidae) using a scanning electron microscope. *Arquivo Brasileiro de Medicina Veterinária e Zootecnia* **76**, 43-54.

Yared, S., Gebressilasie, A., Worku, A., Mohammed, A., Gunarathna, I., Rajamanickam, D., Waymire, E., Balkew, M., and Carter, T. E. (2024). Breeding habitats, bionomics and phylogenetic analysis of Aedes aegypti and first detection of Culiseta longiareolata, and Ae. hirsutus in Somali Region, eastern Ethiopia. *Plos One* **19**, e0296406.

Yasuno, M., and Tonn, R. J. (1970). A study of biting habits of *Aedes aegypti* in Bangkok, Thailand. *Bulletin of the World Health Organization* **43**, 319.

Zahid, M. H., Van Wyk, H., Morrison, A. C., Coloma, J., Lee, G. O., Cevallos, V., Ponce, P., and Eisenberg, J. N. (2023). The biting rate of *Aedes aegypti* and its variability: A systematic review (1970–2022). *PLoS Neglected Tropical Diseases* **17**, e0010831.

Zhang, D., Zheng, X., Xi, Z., Bourtzis, K., and Gilles, J. R. (2015). Combining the sterile insect technique with the incompatible insect technique: I-impact of Wolbachia infection on the fitness of triple-and double-infected strains of *Aedes albopictus*. *PloS One* **10**, e0121126.

Zhang, W. (2024). Role of Climate and Environmental Changes in Mosquito Population Dynamics. *Journal of Mosquito Research* **14**.

List of publications

Jemberie, W.; Dugassa, S.; Animut, A. Biting Hour and Host Seeking Behavior of Aedes Species in Urban Settings, Metema District, Northwest Ethiopia. Trop. Med. Infect. Dis. 2025, 10, 38. https://doi.org/10.3390/tropi calmed10020038

Jemberie, W., Dugassa, S. & Animut, A. Physical and chemical characteristics of *Aedes* larval habitats in Metema District, Northwest, Ethiopia. *BMC Ecol Evo* **25**, 59 (2025). https://doi.org/10.1186/s12862-025-02368-w

Prepared manuscripts

Effect of seasonal variation on the resting habitat of Aedes species (Diptera: Culicidae) in Metema district, Northwest, Ethiopia. It is under review and manuscript ID No. Manuscript ID: PNTD-D-25-01252

Detection wMel strain of Wolbachia bacteria infectious status of *Aedes aegypti* in Metema district, Northwest, Ethiopia.

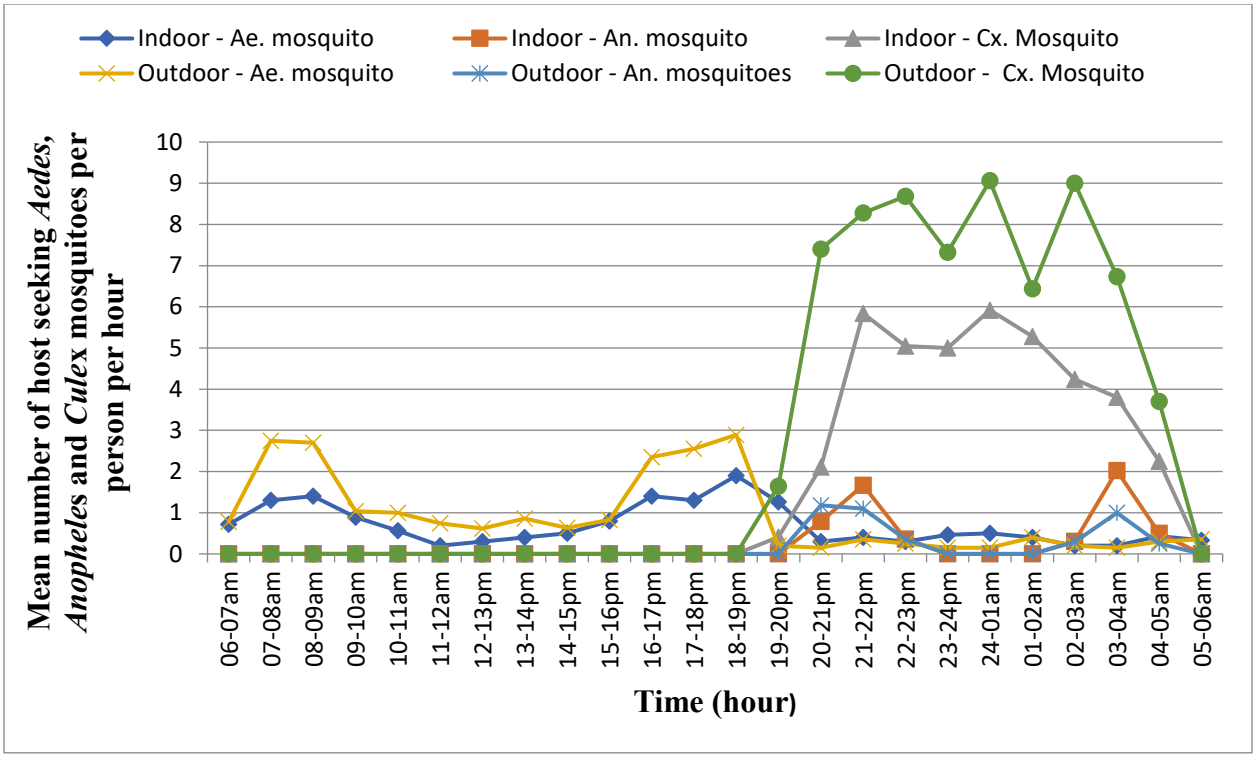
List of Appendices

Appendix 1: Physicochemical parameter of *Aedes* larvae/pupae breeding container in Metema-Yohannes, Kokit and Genda-Wuha, Metema district, Northwest, Ethiopia from January 2022 – December 2023

Physicochemical parametr of breeding habitat																
Study Sites	Breeding (	Tempratu	PH	Disolved Oxygen.	Turbidity	Conductiv	Total disolved substrate	Amonia (N	Amonimum(	Phosphate(PO4)	Sulphate (SO4)	Nitrate (NO3)	Nitric (NO2)	Total hardness	Density of Aedes larvae	Density of Aedes pupae
Metema-Yohannes	Tyre	31.91	7.7	1.6	36.33	331.51	162..32	5.19	5.18	5.8	24.65	1.25	0.37	108.21	45.75	2.87
	Platic	30.83	8.04	4.05	21.62	282.7	131.39	3.75	3.79	8.78	3.33	9.66	0.08	68.81	14.08	3.04
	Metal	31.27	7.61	0.96	0.75	445.05	160.41	4.46	4.42	0.31	1.63	0.75	0.02	128.84	6.72	0.72
	Caly-pot	30.94	8.51	4.9	28.72	581.02	386.53	3.45	3.46	1.37	14.02	3.67	0.47	185.24	11.57	0.89
	Rain pools	29.95	7.6	4.15	19.85	275.08	308.15	2.14	1.04	10.18	12.72	11.87	0.86	147.73	0.73	0.06
	Hoof print	30.15	7.2	3.84	1.75	231.12	103.18	1.07	1.72	1.12	2.12	1.29	0.13	85.27	0.77	0
	River bank	30.65	8.07	4.27	0.62	13.47	404.05	2.71	1.54	7.92	5.65	1.21	0.55	117.09	1.18	0
	Tree hole	29.16	8.12	1.15	25.16	468.28	187.24	3.03	2.16	0.36	1.04	0.91	0.17	67.43	12.22	1.3
Kokit	Tyre	30.1	7.5	0.86	309.41	210.08	207.49	1.52	1.57	1.33	6.43	1.96	0.11	80.28	30.72	2.11
	Platic	28.67	8.04	1.09	10.82	327.71	130.48	0.74	0.79	0.96	2.33	12.66	0.08	76.19	10.14	0.76
	Metal	28.83	7.27	1.07	2.86	445.24	175.89	0.61	0.67	0.3	48.69	0.49	0	180.18	3.61	0.39
	Caly-pot	28.03	8.57	4.1	106	387.34	443.83	1.74	1.87	1.82	10.67	0.54	0.4	136.25	6.84	0.58
	Rain pools	29.95	8.16	4.65	29.15	195.08	207.15	1.74	1.54	9.17	15.22	1.87	0.66	131.01	1.94	0.04
	Hoof print	30.15	8.21	2.81	3.25	251.52	113.38	2.02	2.12	2.32	2.17	2.19	0.23	76.19	0.5	0
	River bank	30.65	7.07	3.37	1.02	11.41	364.08	1.81	1.44	6.92	6.45	1.01	0.85	124.06	0	0
	Tree hole	29.16	7.12	2.95	35.36	368.18	177.54	4.83	3.06	1.36	1.91	1.31	0.37	78.06	4.46	0.46
Genda-Wuha	Tyre	29.99	7.24	0.75	16.44	104.54	98.52	2.14	2.39	0.44	3.6	1.98	0.33	30.99	41.84	2.13
	Platic	28.66	7.6	2.62	6.48	166.48	73.34	3.52	3.85	0.41	0.14	1.57	0.44	64.38	4.44	0.8
	Metal	28.22	7.26	0.95	4.15	474.91	55.47	2.17	1.82	0.5	4.46	6.53	0.57	116.2	4.64	0.61
	Caly-pot	27.57	8.17	3.35	14.14	198.99	234.16	4.97	5.36	0.56	18.28	13.51	0.53	146.32	6.23	1.09
	Rain pools	30.35	7.17	3.65	30.15	181.01	107.15	1.64	2.04	11.07	13.42	1.77	0.76	201.11	1.24	0
	Hoof print	29.65	7.31	3.81	4.15	231.5	103.08	2.22	1.52	1.82	3.07	1.11	0.13	81.09	0.01	0
	River bank	30.05	8.05	4.37	1.42	9.48	261.06	0.92	2.01	5.97	5.85	1.31	0.45	214.26	0.81	0
	Tree hole	29.16	7.19	3.25	33.37	378.21	93.54	3.13	3.17	2.66	1.71	2.08	0.17	98.13	4.46	0.86

Appendix 2: Host seeking behavior of Ae. aegypti and Ae. vittatus per person per hour at outdoor and indoor with human stimulant for 24 hours in Metema-Yohannes, Kokit and Genda-Wuha, Metema district, Northwest, Ethiopia from January 2022 – December 2023

		Number of host seeking Ae. aegypti /person/hour																	
Species	Habitat	06-07 am	07-08 am	08-09 am	09-10 am	10-11 am	11-12 am	12-13 pm	13-14 pm	14-15 pm	15-16 pm	16-17 pm	17-18 pm	18-19 pm	19-20 pm	20-21 pm	21-22 pm	22-23 pm	23-24 pm
Ae. aegypti	outdoor	0.60	4.60	0.83	0.37	0.67	0.37	0.33	0.30	0.47	0.70	0.83	3.77	1.03	0.77	0.68	0.65	0.62	0.72
	indoor	0.2	0.33	1.47	0.77	0.17	0.1	0.13	0.1	0.1	0.2	1.96	0.07	0.17	0.1	0.1	0.2	0.2	0.3
Ae. vittatus	outdoor	0.23	0.47	4	0.63	0.13	0.17	0.17	0.12	0.12	0.13	0.63	0.8	3.2	0.23	0.27	0.23	0.21	0.22
	indoor	0.1	0.1	0.07	1.77	0.07	0.03	0.03	0.01	0.02	0.01	0.2	0.3	0.04	1.43	0.07	0.06	0.05	0.08

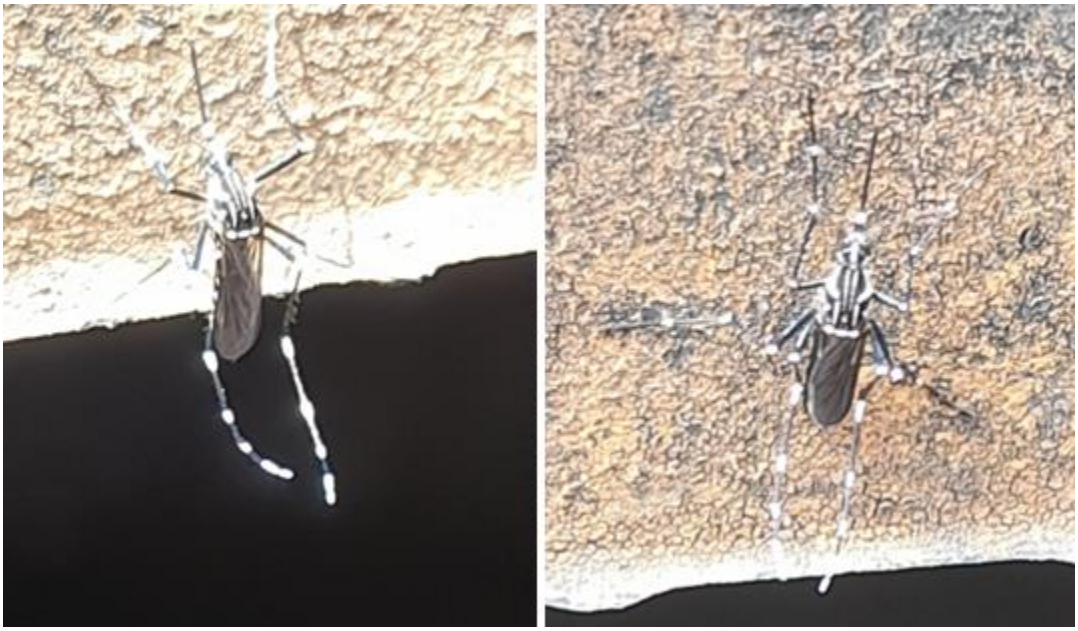


Appendix 3: Host seeking behavior of Aedes, Anopheles and Culex mosquitoes per person per hour at outdoor and indoor with human stimulant for 24 hours in Metema-Yohannes, Kokit and Genda-Wuha, Metema district, Northwest, Ethiopia from January 2022 – December 2023

Appendix 4: Set up of CDC-LT at outdoor and indoor in the field mosquito collection

Appendix 5: Adult *Aedes* species collected from inside larvae/pupae breeding container by prokoparck

Appendix 6**:** Adut *Aedes* species collected from outdoor by protopack

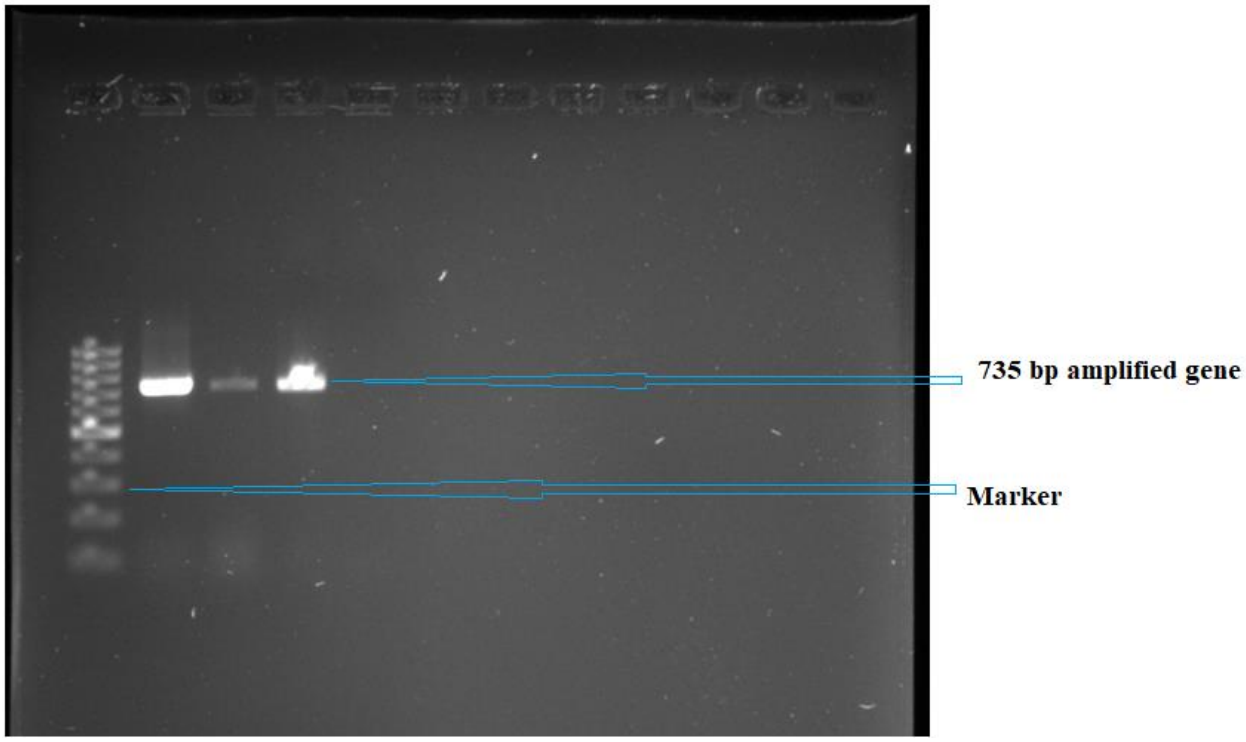


Appendix 7**:** Resting position of Adult female *Aedes aegypti* in the study site

Appendix 8**:** DNA and RNA extraction at virology laboratory room, in Ahari, Ethiopia

Appendix 9**:** Set up of PCR for amplification mosquito genes at molecular laboratory room in Biotechnology Institute, University of Godar, Ethiopia

Appendix 10**:** Preparation of Gel electrophoresis at molecular laboratory room in Biotechnology Institute, University of Godar, Ethiopia



Appendix 11: PCR amplification of the COI gene was achieved positive sample of Ae. aegypti at molecular laboratory room in Biotechnology Institute, University of Godar, Ethiopia