Jimma University

School of Graduate Studies

Department of Biology

Larval Habitat Characterization, Spatial Distribution, and Species Composition of *Anopheles* Mosquitoes in Bambasi Woreda, Benshangul Gumuz Region, Northwestern Ethiopia

By: Hawi keno

Advisors: Eba Alemayehu (PhD)

Professor Delenasaw Yewhalaw

Gadisa Natea (MSc)

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Approval sheet

	Signature	date
Advisors: Eba Alemayehu (PhD)	_____	_____
Delenasaw Yewhalaw (Prof.)	_____	_____
Gadisa Natea (MSc)	_____	_____
Examiner: _____	_____	_____

Declaration

I, the Undersigned, declare that this thesis entitled **Larval habitat characterization, spatial distribution, and species composition of *Anopheles* mosquitoes in Bambasi Woreda, Northwestern Ethiopia** is my original work and that all sources of materials used for the thesis have been correctly acknowledged**.**

Hawi Keno _____ _____

Name Signature Date

Abstract

Malaria is a widespread vector-borne disease in tropics and subtropics causing nearly half a million deaths every year. Malaria vectors control interventions mainly rely on the control of adults using indoor residual sprayings (IRS) and long lasting insecticidal nets (LLINs). However, in regions where the primary malaria vector is exophilic or bites before people are in bed, making indoor residual spraying and impregnated bed nets less effective. It is also important to focus on the breeding sites of mosquitoes as part of malaria vector control interventions. The purpose of this study was to determine the larval composition of Anopheles *mosquitoes in different breeding habitats and to characterize the breeding habitats by assessing the environmental and physicochemical parameters in Bambasi Woreda, Benshangul Gumuz region, Northwestern Ethiopia. Three major* Anopheles *mosquitoes larvae breeding habitats were identified in three Kebeles, namely, drainage ditch (Keshmando), swamp (Amba 46), and stagnant water (Amba 47).* Anopheles *mosquito larvae were collected using a standard dipper. A total of 2185 larvae* Anopheles *mosquitoes were collected and 1786 adults reared from larvae. At Keshmando, the abundance of* An. gambiae s.l. *was found to be 99.80%, and that of* An. coustani *was 0.2%. At Amba 46, the abundance of* An. gambia*e* s.l. *was found to be 99.5% and* An. funestus *was 0.5% whereas a single* An. coustani *larva was not detected. Amba 47 was dominated by* An. gambiae s.l. *(98.85%).* Anopheles *species were abundantly collected from stagnant water in natural habitats. These habitats are temporary with still water in sunlight, making conditions suitable for the development of anopheline mosquitoes. Throughout the study period, low* Anopheles *larval abundance was recorded in the maximum mean EC, salinity, and TDS 18.46±0.05µS/cm, 5.54±1.00 PSU and 12.19±0.26mg/L respectively in a drainage ditch. However, Anopheline larvae were abundantly collected from stagnant water with DO (3.27±0.12mg/L) and high water temperature. This study suggested that environmental and physicochemical factors could have played an important role in the development of mosquito larvae in their habitats. Therefore, targeting and documenting highly productive habitats is important for further implementation of larval control as part of malaria vector control interventions in Ethiopia.*

Key words: *Anopheles* mosquitoes, larval habitats, malaria, Bambasi, Ethiopia

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Table of Contents

List of Acronyms and Abbreviations

An. = *Anopheles*

ANOVA = One way analysis of variance

DO = Dissolved Oxygen

EC = Electrical Conductivity

FMoH = Federal Ministry of Health

ICIPE = International Centre of Insect Physiology and Ecology

IRS = Indoor Residual Spraying

LLIN = Long-lasting Insecticidal Net

LSM = Larval source management

pH = Hydrogen ion concentration

PMI = President's Malaria Initiative

SPSS = Statistical package for social sciences

TDS = Total dissolved solids

WHO = World Health Organization

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CHAPTER ONE

1. Introduction

1.1. Background of the study

Malaria is transmitted to humans by the bite of adult female *Anopheles* mosquitoes (Cox, 2010). In 2019, worldwide there were an estimated 229 million cases of malaria and 409 000 deaths of which 94% of the cases were reported from Africa (WHO, 2020). In Ethiopia, even though there has been steady progress in the reduction of malaria (Deribew *et al*., 2017; Taffese *et al.,* 2018; WHO, 2020) 1.5 million cases of malaria were reported in 2019 (WHO, 2020).

The genus *Anopheles* mosquito is the most studied genera among medically important insects. Of the total 465 *Anopheles* species globally listed, about 70 species are known to transmit human malaria (Sinka *et al*., 2012). Forty one *Anopheles* mosquito species are actively involved in the transmission of human *Plasmodium* parasites, of which about 20 species are the dominant vectors transmitting malaria at a level of main concern to public health in Africa (Sinka *et al*., 2012). The major malaria vectors in Africa include *An. gambiae s.l., An. funestus, An. nili, An. pharoensis* and *An. moucheti* (Sinka *et al*., 2011; Sinka *et al*., 2012).

In Ethiopia, there are more than 45 documented species of *Anopheles* mosquitoes (Gaffigan *et al*., 2018) of which *Anopheles arabiensis, An. funestus, An. pharoensis* and *An. nili* are recognized malaria vectors. *Anopheles arabiensis* is the primary malaria vector in Ethiopia (Abeku *et al.*, 2003) while, *An. funestus, An. pharoensis* and *An. nili* are secondary vectors occurring with varying densities, limited distribution and vector competence (White, 1982; FMoH, 2014). Very recently a new invasive *Anopheles* species, *An. stephensi*, has been documented in the country (Carter *et al*., 2018) which might complicate the malaria elimination effort of the country.

Anopheles mosquitoes undergo egg, larval, pupal and adult stages in their lifetime. The egg, larval and pupal stages are limited to water bodies and have very small spatial dispersion. The level of mosquito breeding activities is significantly higher in the rainy than dry season (Mahgoub *et al*., 2017). In dry seasons, the number and size of larval habitats is generally believed to reduce significantly and contribute to a low population of malaria transmitting adult

Anopheles mosquitoes (Himeidan *et al.*, 2009). Even though, *Anopheles* species exploit a variety of breeding habitats that vary considerably in size, altitude, vegetation cover and topography (Minakawa *et al*., 2012), the majority of vectors may emerge from prolific habitats, which could account only for a small proportion of the habitats. Small sized breeding habitats have many advantages over larger permanent breeding sites that increase the developmental rate or survival probability of the aquatic stage (Gu *et al.,* 2008). Therefore, mosquito larval habitat ecology is important in determining larval densities, the relative importance of breeding habitats and species assemblage as well as designing mosquito control programs(Overgaard *et al*., 2002; Simisek, 2004).

Indoor residual spraying (IRS) and long-lasting insecticidal nets (LLINs) are the main pillars of the malaria vector control strategy in Ethiopia. In the last decade, the contribution of both IRS and LLINs significantly reduced malaria incidence and prevalence in the country (Girum *et al.,* 2019; Taffese *et al*., 2018; WHO, 2020). However, the wide spread of insecticide resistance in the major malaria vector, *An. arabiensis* (Alemayehu *et al.,* 2017; Messenger *et al*., 2017; Yewhalaw *et al.,* 2010) would compromise the effort of malaria vector control strategy with these interventions. Moreover, the change in feeding, biting and resting behavior of *An. arabiensis* contributed for the existing malaria transmission in the country despite intensified malaria intervention, the scale up of IRS, high coverage of LLINs and improved malaria diagnosis and treatment (Degefa *et al.*, 2015; Kenea *et al*., 2016; Yohannes & Boelee, 2012). Therefore, besides targeting adult *Anopheles* populations by the current malaria vector control interventions, it is equally important to control the immature stages of *Anopheles* mosquitoes with appropriate larval control strategies. Different intervention targeting larvae of *Anopheles* mosquitoes successfully suppressed malaria vector density and the risk of malaria infection in some African countries including Ethiopia (Castro *et al*., 2009; Kibret *et al*., 2018; Utzinger *et al*., 2001; Wamae *et al.,* 2010; Yohannes *et al*., 2005).

In Ethiopia, there are few studies conducted to assess the habitat of mosquito larvae and some of the environmental factors that affect mosquito abundance in designing optimal vector control strategies (Dejenie *et al*., 2011; Getachew *et al.,* 2020; Hawaria *et al.,* 2020; Mereta *et al.,* 2013). However, to our knowledge, there is no study conducted in Bambasi Woreda, one of the malaria sentinel sites in Ethiopia, to characterize and assess larval habitats of *Anopheles* mosquitoes.

Hence, this study aimed to determine the larval composition of *Anopheles* mosquitoes in different breeding habitat and assess the effect of the environmental and physicochemical parameters of the breeding habitats.

1.2. Statement of the problem

Benishangul-Gumuz Region is one of such area in Ethiopia where the threat due to malaria infection is so high. In Benishangul-Gumuz Region few studies reported the prevalence, surveillance, transmission and control methods of malaria (Alemayehu *et al*., 2017; Legesse *et al*., 2007; Seid & Yaregal , 2019; PIM, 2020). These indicate that there is a gap of information about environmental factors associated with the occurrence and abundance of *Anopheles* larvae that can help in designing optimal vector control strategies. According to the annual report Bambasi Woreda, most of the diseases affecting the people of the Woreda are by the case of malaria. This could be associated with the availability of mosquito breeding sites and suitable climatic conditions for the survival of *Anopheles* mosquitoes. Larval control could be cost-effective strategy for integrated vector management if productive larval habitats are identified and when their distribution is limited to specific areas. The larval habitats and the physicochemical parameters that influence *Anopheles* mosquitoes in their breeding habitats on Bambasi Woreda not yet studied. Therefore, this aimed to characterize *Anopheles* mosquito breeding habitat, spatial distribution and species composition of *Anopheles* mosquito in Bambasi Woreda, Northwestern Ethiopia.

1.3. Objectives of the study

1.3.1. General objective

- To characterize and assess larval habitats, distribution, and species composition of *Anopheles* mosquitoes in Bambasi Woreda, Northwestern Ethiopia.

1.3.2. Specific objectives

- To identify breeding sites of anopheline mosquitoes in the study area
- To determine the species composition and abundance of *Anopheles* mosquitoes
- To characterize physical characteristics of larval breeding habitats
- To assess water quality parameters (water temperature, TDS, dissolved oxygen, electric conductivity, and salinity) of breeding sites

1.4. Significance of the study

The present study will provide information on mosquitoes in terms of distribution, breeding sites, to detect the water quality status of each breeding habitat and species composition of *Anopheles* mosquitoes in Bambasi Woreda. Identification of *Anopheles* malaria vectors is essential for the identification of areas that are at risk of malaria and for the formulation of strategies for effective control. Information on the spatial distribution of *Anopheles* vectors will facilitate the targeting of the prolific larval habitats thereby improving the preciseness of the control strategy. Location of larval habitats will provide information on *Anopheles* mosquito feeding strategies and population structure which are essential for control measures distribution in Bambasi. Knowledge of the ecological characteristics of the breeding habitats and the environmental factors affecting mosquito abundance can help in designing optimal vector control strategies.

CHAPTER TWO

2. Literature review

2.1. Malaria vectors

Anopheles mosquitoes in Africa consist of two groups that have been incriminated in malaria transmission. These two broad groups include *An. gambiae s.l.* complex group and *An. funestus* group (Coetzee, 2004). The *An. gambiae s.l.* complex consists of morphologically indistinguishable sibling species namely *An. gambiae s.l., An. arabiensis, An. bwambae, An. merus, An. melas, An. quadriannulatus* species A and B (Coetzee *et al*., 2000). Two species within the complex namely *An. gambiae s.l.* and *An. arabiensis* are responsible for malaria transmission in Africa (Coetzee *et al*., 2000). *An. funestus* group on the other hand consists of *An. funestus Giles, An. rivulorum, An. parensis Gillies, An. brucei Service* and *An. leesoni Evans* (Gillies & Coetzee, 1987). *An. funestus* which is endophilic and anthropophilic, and *An. rivulorum* are the only members of *An. funestus* group involved in malaria transmission (Cohuet *et al*., 2004). In sub-Saharan Africa, *Plasmodium falciparum* is mainly transmitted by *An. gambiae s.l.* and *An. funestus* species complexes. *An. gambiae s.l.* is recognized as the world most important vector of *Plasmodium falciparum* followed by *An. arabiensis* (Fanello *et al*., 2003).

Within the group of *Anopheles* mosquitoes, there are several sibling species that have more or less significance in regards to the transmission of malaria (Becker *et al*., 2010). The major *Anopheles* species transmitting human malaria in Ethiopia consists of *An. Arabiensis, An. pharaoensis, An. nili* and *An. funestus* (WHO, 2014). The capability of mosquitoes to function as disease vectors is characterized by variables such as life expectancy, density and competence. The latter variable includes behavioural, biochemical and environmental factors, which may in return have an impact on the connection between a vector, the pathogen transmitted by the vector and the vertebrate host being infected (Okwa *et al*., 2007). *An. pharaoensis* have been shown to have similar behaviour and suggests that a malaria control measure such as using insecticide-treated mosquito nets as a malaria control measure is less effective (Kibret *et al.,* 2010).

2.2. Life cycle of *Anopheles* mosquitoes

Anopheles mosquitoes develop through four stages of life cycle; egg, larva, pupa, and adult. The first three stages are mainly aquatic and the cycle from egg to adult stage may last for 5-14 days but this is dependent on species, humidity and ambient temperature (Clement, 1992). In tropical climates, development is rapid and therefore the egg-adult cycle may be completed in 6 days (Gillies & De Meillon, 1968). After emergence, the adult mosquito takes at least one day to reach sexual maturity. The adult stage is when the female *Anopheles* mosquito acts as a vector and is capable of transmitting malaria parasites. They are highly anthropophilic and females take more than 90% of their blood meal from human hosts. Blood is needed for egg development which takes about two days (Clement, 1992). After mating and blood feeding, a gravid female mosquito lays about 50-500 eggs the second day after blood feeding (Clement, 1992). Eggs hatch into larvae approximately two days after oviposition and this is largely dependent on ambient temperature and humidity (Clement, 2000). Larvae spend most of their time feeding on algae, bacteria and other micro-organisms in the surface micro layer of water. It takes less than seven days to develop through four larval instars. Duration of the larval stage is however influenced by environmental temperature and availability of food (Gillies & De Meillon, 1968). The pupal stage duration varies from 1-3 days to develop into adult and this also depends on the environmental conditions such as ambient temperature and humidity which may affect duration in this developmental stage. Pupae are also visible upon the surface of the breeding site. After a mosquito is fully developed, it will emerge as an adult from its pupal case. At this time, the new adult stands upon the water and dries its wings to prepare for flight.

2.3. Mosquito breeding habitats

Mosquitoes breed in permanent or any temporary body of water that is present for more than a week. The positive breeding habitats and their quantitative characters (water depth) and qualitative characters (natural or artificial, permanent or temporary, shady or lighted, water movement, vegetation condition and turbidity), determines presence or absence of different mosquitoes species (Rueda *et al.*, 2006). Relatively few mosquito species actually breed in permanent bodies of water such as marshes or swamps and most of the mosquito species associated with marshes or swamps actually breed in temporary pools along the margins of these habitats (Pemola Devi & Jauhari, 2005). From the present study most mosquito species actually

breed in temporary bodies of water such as swamp, drainage ditch and stagnant water. Both quantitative and qualitative characters of the mosquito breeding habitats have contributed to understanding requirements of different larval species of mosquitoes. The choice of oviposition sites of mosquitoes is influenced by myriad environmental factors, which include climatic components such as temperature, rainfall, vegetation, salinity and turbidity of the water, the size of the habitat, and the amount of sunlight (Rejmankova *et al*., 2013).

All the existing 3,000 species of mosquitoes spend part of their life cycle in water (Metzger, 2004). They can breed in virtually any natural or man-made deposit of water, some live and breed deep below the earth in mines, some have been found on top of mountains, others in highly turbid water. *An. gambiae s.l.* is known to proliferate in small temporary rain-dependent water body. From the present study temporary water body was suitable for *An. gambiae s.l. An. funestus* prefer large, permanent or semi-permanent water which contain plants. It increases malaria transmission and is also dominant during the dry season when the population of *An. gambiae s.l.* and *An. arabiensis* are low (Gillies & De Meillon, 1968).

Typical habitats of *An. arabiensis* and *An. gambiae s.l.* are puddles, shallow ponds, tyre tracks, ditches, human foot and animal hoof prints and are often created by activities of humans or domestic animals (Fillinger *et al.,* 2004). These habitats are open, containing no, little or low aquatic vegetation and are often of a transient nature, as their availability corresponds to precipitation. *An. gambiae s.l.* can colonize a breeding habitat within a few days after the site is created. Besides in temporary habitats, *An. arabiensis* is also found in market garden wells and water storage tanks.

2.4. *Anopheles* ecology and malaria epidemiology in Ethiopia

Malaria epidemiology is driven by temporal and spatial patterns of vector species of *Anopheles* mosquitoes (Shililu *et al*., 2003). Temporal and spatial variations in vector ecology across Africa affect the transmission risk and epidemiology of malaria and interventions will have to adopt an approach that allows for the consideration of ecological factors that affect the force of transmission in different geographical zones (Sattler *et al*., 2005). In Ethiopia, malaria transmission varies widely with Ethiopia diverse topography and associated rainfall patterns. About 75% of the landmass is potentially malarious and about two thirds of the population over 40 million people are at risk of infection (Ghebreyesus *et al*., 2006).

The distribution of humans and *Anopheles* mosquitoes is not continuous across the country, but generally clustered on high elevation areas where rainfall is abundant (Nyanjom *et al*., 2003). The disease has contributed to the overcrowding of population to highland areas of the country resulting in destruction of the ecology, reduced productivity and hence famine and poverty. In endemic areas, peak transmission periods coincide with the planting and harvesting seasons reducing productive capacity of agricultural work.

The distribution of mosquito larvae and adult vectors is generally determined by the oviposition sites selected by females. For example, the local dispersal of *An. gambiae s.l.* could be driven by the search for oviposition sites and increased adult dispersal caused by females searching for a suitable breeding site. This may facilitate the spread of malaria parasites (ICIPE, 2003). Direct observation of mosquito oviposition in nature is not feasible because of the untraceable movement, nocturnal activity and tiny size of mosquitoes. However, indirect methods such as genetic approaches can be useful tools for the study of mosquito oviposition behavior (Chen *et al*., 2006).

2.5. Effect of biotic and abiotic factors on larval mosquito population

2.5.1. Environmental Factors

The limiting factors in mosquito breeding are the longevity of the aquatic habitat and the duration of the mosquito species lifecycle (Edillo *et al.,* 2002). In a breeding habitat, biotic factors such as predation and availability of food resources also determine the population of mosquito larvae (Mahesh & Jauhari, 2002). However, the authors also reported that weeds, debris, emergent grasses or some sort of aquatic vegetation shelters the mosquito larvae from fish and other predators thus largely contributing to larval population in breeding habitat.

Other biotic factors that may affect the survival and development of anopheline mosquito larvae at their breeding sites include presence or absence of algae, presence or absence of aquatic vegetation, presence or absence of predators, parasites, pathogens or cannibalism and other interactions between species (Gimnig *et al*., 2001; Koenraadt & Takken, 2003; Paaijmans *et al*., 2007).

Turbid water is preferred by mosquitoes over clear water (Paaijmans, 2008). However, Paaijmans *et al*. (2008) found that the sites with relatively clear water produced more *An.*

Arabiensis is pupae and larger adults than habitats with turbid water. Larvae of *An. gambiae s.l.* are found in both clear and turbid water (Paaijmans *et al*., 2008). However, from the present study, *Anopheles* larvae were collected from turbid water bodies. *An. gambiae s.l.* are seen to breed more prolifically in temporary and turbid water bodies, such as ones formed by rain while, in contrast, *An. funestus* prefers more permanent water bodies. A previous study in western Kenya reported that *An. funestus* prefers to oviposit in large semi-permanent water bodies containing aquatic vegetation and algae (Gimnig *et al*., 2001). A study by Teklu *et al*. (2010) reported that the *An. gambiae s.l.* to exist in greater quantity in slightly turbid aquatic habitat than in turbid aquatic habitat. Also from the present study some *An. gambiae s.l.* larvae were collected from a slightly turbid water body.

2.5.2. Physicochemical parameters

Physicochemical parameters, composed of physical components such as conductivity, temperature, dissolved oxygen and TDS are water associated characteristics that have specific effects on the quality and biological components of the water. The development and survival of the mosquito larvae in their habitats are affected by several physicochemical factors such as optimum temperature, range of pH and the concentrations of sulphate, ammonia and nitrate (Oyewole *et al*., 2009). The occurrence and abundance of *Anopheles* larvae is closely associated with physicochemical parameters. The importance of many chemical substances dissolved in the breeding water of *Anopheles* larvae is still uncertain. However, a few chemicals may combine to limit the breeding of mosquitoes such as dissolved gases and organic pollution, salinity and hydrogen ion concentration (Grillet, 2000).

Temperature has a major effect on several biological processes and physicochemical properties in the natural aquatic environments. It has a magnitude of influence on the rate of photosynthesis by aquatic photosynthetic organisms, amount of dissolved oxygen and the rates of metabolism by aquatic organisms. It also influences the activities of parasites, pathology of diseases, irritability of organisms to toxic wastes and other behavioral activities such as aestivation, migration and periods of reproduction of aquatic organisms (Arroyo Seco Foundation, 2013).

Electrical Conductivity is the property of water that measures the waters ability to conduct electrical currents. EC is dependent on the amount of dissolved ions in the water body and therefore varies with the source of the water such as municipal wastewater and water drained

from agricultural farms, rainfall and groundwater. EC is related to and correlated with the organic compounds and the concentration of total dissolved solids within an aquatic environment. However, EC is regulated by temperature which has a large effect on conductivity.

Dissolved Oxygen is the amount of oxygen dissolved in a water body through the process of diffusion of oxygen from the ambient air, high velocity of the water and also as a by-product of photosynthesis. Characteristically, the solubility of DO in natural surface aquatic environments ranges from 1.5 mg/L to 8 mg/L. Oxygen tensions which can be lethal to mosquito larvae are often associated with breeding habitats with vegetation and therefore most mosquito species prefer open sunlight pools or habitats (Tiimub *et al*., 2012).

The survivability and growth of many aquatic organisms is dependent on the presence of oxygen. Therefore, the absence of oxygen in any aquatic habitat may result in the destruction of mosquito eggs or larvae, death of adults, stunt growth and change in biota (Arroyo Seco Foundation, 2013). A wide spread study on the egg laying preference of mosquito species to aquatic habitats with different levels of DO and salts shows that, on the average, *Anopheles*, *Culex* and *Aedes* prefer DO of 6.6, 2.1 and 6.2 ppm respectively. However, the general tolerable DO required by most mosquito species is 4 ppm or less (Owusu, 2016).

TDS is the sum total of all the substances such as mineral sources, dissolved in a water body. Due to the enormous amount of dissolved salts in natural water, salinity is measured for as TDS content in the water. TDS is primarily composed of potassium, nitrates, phosphates, manganese, carbonates, chlorides, iron, sodium, calcium, sulphates and a few others, but specifically not of gases, sediments or colloids (Amankona, 2010). High concentrations of the constituents of TDS in any water body has some regulatory effects such as reducing the utility and solubility of gases like oxygen, the water density and the osmoregulation of organisms in freshwater.

2.6. Malaria vector control

Vector control is a cornerstone of malaria control and it remains the most generally effective measure to prevent malaria transmission and therefore is one of the strategic approaches to malaria control (Scott & Morrison, 2010).The objectives of malaria vector control are two-fold; to protect individual people against infective malaria mosquito bites, and to reduce the intensity of local malaria transmission at community level by reducing the longevity, density and human-vector contact of the local vector mosquito population. The three major control measures are reducing human vector contact, adult mosquito control and larval control.

Larval control is indicated as the sole method of vector control only if a high proportion of the anopheline breeding sites within the vectors flight range of the community to be protected are few, fixed, findable, and manageable (WHO, 2012). Larval control may be also undertaken to supplement the effects of the core vector control interventions (LLIN and IRS). LSM is the management of aquatic habitats (water bodies) that are potential larval habitats for mosquitoes, in order to prevent the completion of immature stages of mosquito development, the egg, larvae and pupae. There are four categories of LSM, which are habitat modification, habitat manipulation, biological control and larviciding.

Before a larval control intervention can be implemented, the majority of the vector larvae productive breeding sites must be identified (Elimam *et al*., 2009). If the number of breeding sites is extremely large or many sites are inaccessible or ephemeral, larval control may not be feasible. One advantage of targeting larvae is that they cannot escape from their breeding sites until the adult stage and unlike adult mosquitoes, cannot easily avoid control measures (Killeen *et al*., 2002). Larval control may be particularly valuable in regions where the primary malaria vector is exophilic or bites before people are in bed, making indoor residual spraying and impregnated bed nets less effective, as in parts of Eritrea (Shililu *et al*., 2004). In order to be able to design and implement control measures directed to the larva stages such as larva source reduction and larviciding, an understanding of the spatial and temporal distribution of malaria mosquito larva and its determinants in different malaria transmission settings is important (Maheu-Giroux & Castro, 2014).

CHAPTER THREE

3. Materials and Methods

3.1. Description of the study area

Bambasi Woreda is located in Assosa Zone, Benishangul-Gumuz Regional State, Northwestern Ethiopia. Assosa town is the capital of the Assosa Woreda and Benishangul-Gumuz Regional State (BGR), which is located about 600 kilometers West of Addis Ababa. Bambasi town is 45 kilometers East of Assosa town. The study was conducted in three Kebeles namely Keshmando, Amba 46 and Amba 47 (Fig.1). Amba 46 and Amba 47 are located in agricultural fields whereas, Keshmando is mainly a human settlement. Keshmando Kebele is located at 9°36.257′N and 34°41.2008′E geographical coordinates and elevation of 1399 meters above sea level (masl). Amba 46 Kebele is located at 9°50.88′N and 34°41.316′E geographical coordinates and elevation of 1446 meters above sea level (masl). Amba 47 Kebele is located at 9°52.087′N and 34°41.145′E geographical coordinates and elevation of 1423 meters above sea level (masl).

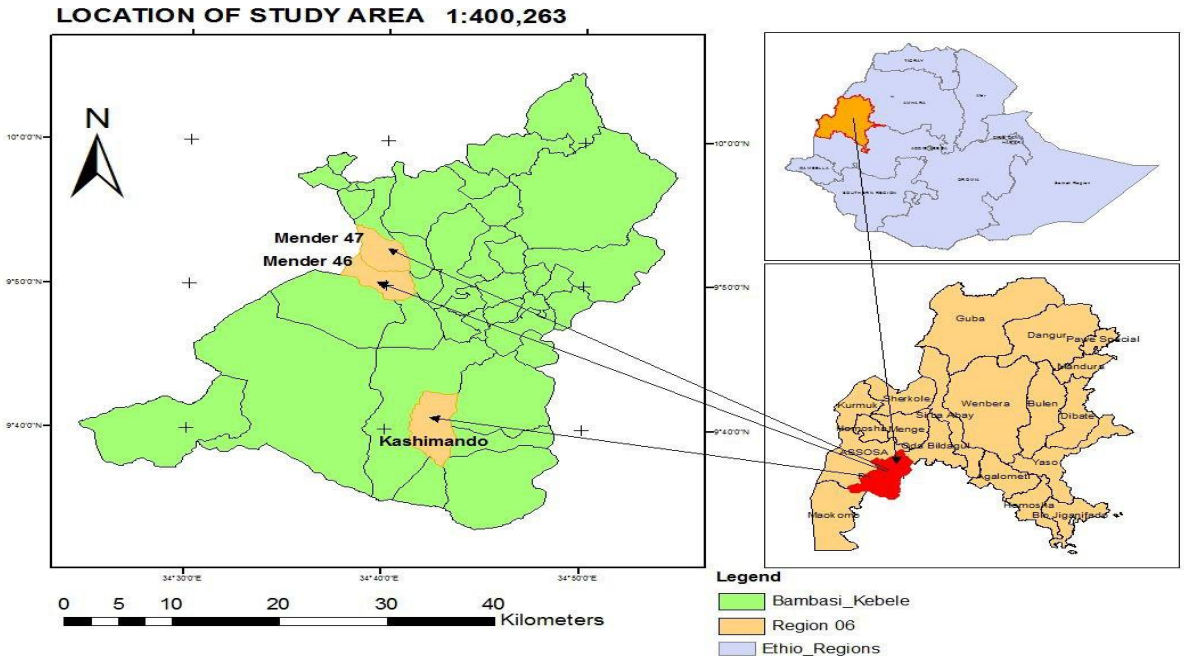

Figure 3.1: Map of study area

3.2. Mosquito larval sampling

Cross-sectional larval survey was conducted on three Kebeles in Bambasi. Mosquito larvae were sampled twice a month from September 2020 up to November 2020 during after long rainy season. Different breeding habitat types were visited and examined for *Anopheles* larvae species productivity. Larval collections were carried out in a variety of aquatic habitats in the study area. Three breeding sites were identified due to the limitation of the palintest instrument used to measure water quality parameters. This instrument was received from Jimma University for five days, from this day four days were spent on the way; I was used only one day. Due to this limitation, only three breeding sites were visited. During each survey, a habitat was first inspected for the presence of mosquito larvae and then larvae were collected using a standard dipper. From each larval habitat, about 20 dips were taken at intervals along the edge of each larval habitat. Sampling was always done by the same individual in the morning (10:00-12:00 hours) or afternoon (2:00-4:00 hours) for about thirty minutes or less at each larval habitat. *Anopheles* larvae were sorted from *Culicine* and transferred to a white tray. Larvae were then searched and identified in the tray by simple visual observation. The larvae below or on the surface of the water in the tray were picked by a pipette and transferred to collecting containers. From each habitat, larvae were always transferred into containers with water from the site of collection. Containers were labeled with relevant information such as date, site and number of larvae collected, and were kept in a room and allowed to rear in to adults. A room also was kept stable, at the temperature of 25ºC-27ºC and 70±10% relative humidity. Larvae were fed with brewery yeast. Feeding was done once per day. Larvae get sunlight twice a day for maximum 30 minutes. Then pupae were collected and transferred into a beaker, placed in cage adults to emerge.


A


B

Plate 1: *Anopheles* larval collection from different breeding sites (A) drainage ditch, (B) swamp and (C) stagnant water.

Plate 2: The experimental set up

3.3. Identification of adult *Anopheles* mosquitoes

Adult *Anopheles* mosquitoes were provided 10% sugar solution with imbibed cotton. Adult mosquitoes were picked from the cage by using an aspirator and transferred into cup covered by a net and were killed by Chloroform. Adult mosquitoes were morphologically identified to the species under a light microscope using standard taxonomic key (Coetzee, 2020; Gillies & Coetzee, 1987) in Biology laboratory, Assosa University, Assosa. Afterwards they were individually preserved in silica gel in well-labeled Eppendorf tubes and transported to Jimma University for re-identification.

Plate 3: Identification, preserving and labeling of *Anopheles* mosquitoes

3.4. Larval habitat characterization

Characterization of a larval habitat required data from both environmental and physicochemical variables. Environmental and physicochemical characteristics of each larval habitat were observed, measured and recorded during the larval collection. The environmental variables including intensity of light, origin of habitats, water current, presence/absence of vegetation and

algae, substrate type, turbidity and permanence of the habitats were observed and recorded during the study. The physicochemical variables were measured and recorded during the larval collection were; salinity, dissolved oxygen, conductivity, total solid dissolved and water temperature.

For each habitat identified, algal coverage was recorded as being present or absent based on visual observation. The proportion of the water surface covered by vegetation was estimated visually and expressed as presence or absence of vegetation. Turbidity was observed by placing the water in a clear glass container and placing it against a white background and recorded as either slightly turbid or turbid (Minakawa *et al*., 1999; Shililu *et al*., 2003). The substrate type was categorized as mud, stone if the pool was lined with stones that were large in size (rocks generally larger than 10 cm in diameter) and gravel when the stones were small in size but larger than sand. The proportion of the water surface exposed to sunlight was estimated visually by assessing the proportion of the water surface shadowed at midday (Sattler *et al*., 2005). Light intensity was visually categorized as full sunlight if the habitat received full sunlight that could occur throughout the day; however, if the habitat received partial sunlight, the habitat was described as partial sunlight. Water current was categorized as still or flow. Habitats were categorized as artificial or natural.

The physicochemical variables were measured on-site during collection by using palintest (micro 800) instrument. The machine automatically measured salinity, temperature, dissolved oxygen, total dissolved solids and conductivity only by one probe. After the instrument was configured, the probe was placed in the water for one to two minutes after which it displayed the results of the readings on the screen and recorded.

Plate 4: Measurement of water quality of larval breeding sites

3.5. Data analysis

Anopheles mosquito species composition, spatial distribution and mean larval density difference among the study area were entered into excel computer program and analyzed using SPSS version 20 statistical software. Percentile scores were used to compare the frequency of occurrence and abundance of *Anopheles* larvae among habitat types and distribution of their species within the habitats. One way analysis of variance (ANOVA) was used to determine if there was a significant difference among and between the groups at the significance level of 0.05%. Variations in larval counts (mean densities) among habitat types and variations in mean densities of the collected larvae among environmental factors (characteristics) of the larval habitats were analyzed. Water physicochemical parameters were expressed as mean ± standard deviation. Larval density in all breeding habitats and study villages was calculated as *Anopheles* mosquito larvae per dip (Sattler *et al*., 2005).). In all cases P values < 0.05 were considered significant.

CHAPTER FOUR

4. Results

4.1. Species composition of *Anopheles* mosquitoes

A total of 2185 larvae *Anopheles* mosquitoes were collected from three aquatic habitats during the study period of which 595, 765 and 823 were collected from Keshmando, Amba 46 and Amba 47 respectively. The total numbers of *Anopheles* mosquitoes larvae collected in Amba 47 were significantly higher than *Anopheles* mosquitoes larvae collected from the other two sites ($P < 0.05$).

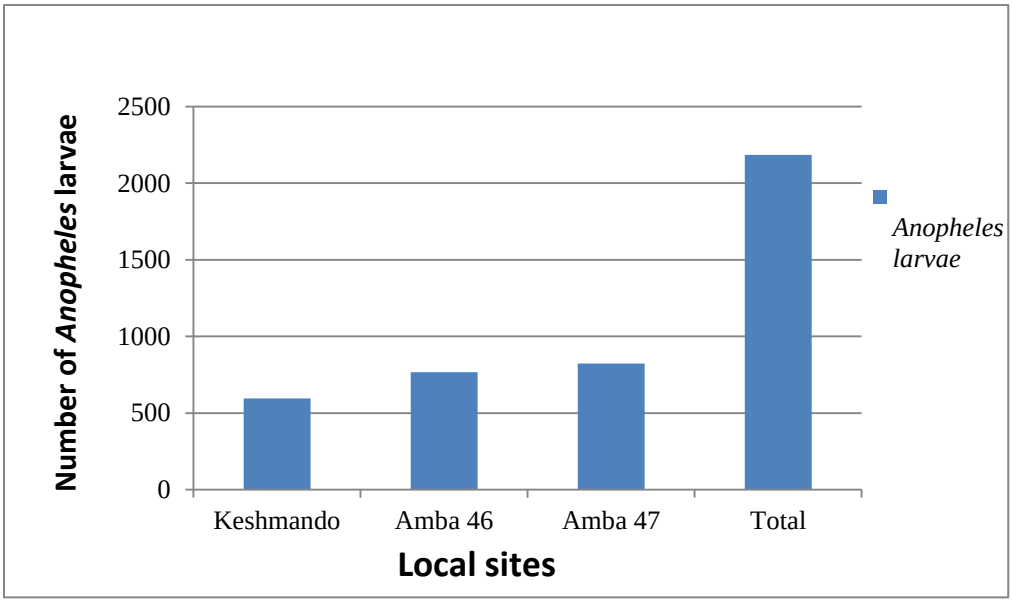

Figure 4.1**:** Abundance of *Anopheles* larvae collected from different localities during the study period.

A total of 1786 adults emerged from larval collections. From these, three malaria species complexes were morphologically identified, namely, *An. gambiae s.l., An. funestus and An. coustani. An. gambiae s.l.* was the highest (1774) followed by *An. funestus* (11) and the least was *An. coustani* (1) (Table 4.1). *Anopheles gambiae s.l.* was found more abundantly in all study sites and breeding habitats. The highest numbers of *An. gambiae s.l. were* collected throughout the study period at Amba 47 as compared to Keshmando and Amba 46. Overall, the densities of adults of the *An. funestus* and *An. coustani* that emerged from the larval collections were low compared to *An. gambiae s.l.*

In Keshmando, the abundance of *An. gambiae s.l.* was found to be 99.80% whereas *An. coustani* was 0.2% and there was no *An. funestus.* At Amba 46, the number of *An. gambiae s.l.* was found to be 99.5% and *An. funestus* was found as 0.5% and there was no *An. coustani.* Amba 47 was dominated by *An. gambiae s.l.* (98.85%) and *An. funestus* was found to be the next abundant 1.15% and there was no *An. coustani* observed.

When compared by sampling area, the abundance of *An. gambiae s.l.* was 27.73% at Keshmando, 33.54% at Amba 46, 38.90% at Amba 47. The abundance of *An. funestus* was 27.27% at Amba 46, 72.73% at Amba 47, and there was no *An. funestus* at Keshmando. *An. coustani* was found only at Keshmando and not present at the Amba 46 and Amba 47.

Table 4-1: Abundance of adult *Anopheles* mosquitoes reared from larvae.

Sites	*An. gambiae s.l.*	*An. funestus*	*An. coustani*	Total
Keshmando	492	0	1	493
Amba 46	592	3	0	595
Amba 47	690	8	0	698
Total	1774	11	1	1786

4.2. *Anopheles* larval productivity in different habitat types

Three habitat types (drainage ditch, swamp and stagnant water) were identified as breeding sites in the study area. *Anopheles* mosquito larvae were collected and identified from three breeding sites as shown in table 4.2. The average number of *Anopheles* larval density over the sampling period was 53.13 larvae per 20 dips. There was variation in each aquatic habitat to the larval productivity. Stagnant water was the most productive for *Anopheles* larvae with mean density of with 19.58 larvae per dip followed by swamp and drainage ditch for *Anopheles* mosquito larvae with mean density of 19.18 and 14.37 larvae per dip, respectively.

Table 4-2: Mean monthly larval densities of *Anopheles* larvae of the three habitats.

Habitat types	Months	Mean ± SE
Drainage ditch	September	8.75±0.18
Swamp	September	7.63±0.17
Stagnant water	September	9.83±0.30
Drainage ditch	October	3.37±0.24
Swamp	October	7.50±0.52
Stagnant water	October	5.25±0.21
Drainage ditch	November	2.55±0.22
Swamp	November	4.05±0.31
Stagnant water	November	4.50±0.17

4.3. Abundance and distribution of *Anopheles* mosquitoes in breeding sites

Table 4.3 depicts the abundance and spatial distribution of *Anopheles* species in different breeding habitats during the study period. *An. gambiae s.l.* adult reared from larvae were obtained most abundantly from stagnant water (690) and swamps (592) whereas drainage ditch (492) of the total *Anopheles gambiae s.l.* adult reared from larvae during the study period. Nearly eight of the total *An. funestus* adults reared from larvae were obtained from stagnant water and three *An. funestus* adult reared from larvae were sampled from swamp. *Anopheles coustani* was the only species found in drainage ditch and generally absent from other two habitat types. Stagnant water and swamps were the most productive aquatic habitats for the anopheline larvae sampled in the present study.

Expressed as number of larvae per number of dips of sampling, the relative abundance of anopheline species in the different larval habitats was also significantly variable. For example, comparing the abundance of *Anopheles* species that were sampled from the different habitat types revealed that *An. gambiae s.l.* were the most abundant species in stagnant water ($p<0.05$).

Table 4-3: Breeding sites and *Anopheles* species.

Anopheles spp.	Drainage ditch	Swamp	Stagnant water	Total
An. gambiae s.l.	492	592	690	1774
An. funestus	0	3	8	11
An. coustani	1	0	0	1
Total	493	595	698	1786

4.4. Distribution of *Anopheles* larvae density during the months of study

In the first month (September) Amba 47 was found to have the highest number of larvae per dip followed by Keshmando. Keshmando was found to be the lowest of the three areas in larval density per dip. In October Amba 46 was found to be the highest in larval density per dip followed by Amba 47. The lowest larval density per dip was observed at Keshmando Kebele in October. Amba 47 was found to be highest in larval density per dip, and Amba 46 was the second when the larval density per dip was compared.

When sampling area was compared by month Keshmando area was found to be highest in September followed by October, and November was the lowest in larval density per dip. September was the month that was found to contain the highest number of larvae per dip followed by October, and the lowest larval density was observed in November month from Keshmando. At Amba 47 in September, there was the highest number of larvae per dip, followed by October and November.

Table 4-4: Comparison of mean larval density of *Anopheles* by area and month

Local sites	Months		
	September	October	November
Keshmando	8.75^{Ab}	3.37^{Bc}	2.55^{Cc}
Amba 46	7.63^{Ac}	7.50^{Ba}	4.05^{Cb}
Amba 47	9.83^{Aa}	5.25^{Bb}	4.50^{Ca}

*Means followed by different superscripts (lowercase) in the same column are significantly different at ($p<0.05$).

*Means followed by different superscripts (uppercase) across the row are significantly different at ($p<0.05$).

4.5. Monthly distribution of *Anopheles* species adults reared from larvae

The highest abundance of *An. gambiae s.l.* (n=828) were recorded in September, while lowest (n=380) *An. gambiae s.l.* were recorded in November. However, highest *An. funestus* were recorded in November and *An. funestus* and *An. coustani* accounting 11 and 1 respectively.

In September, there was only *An. gambiae s.l.* and the two species were not observed. In October 99.15% was *An. gambiae s.l.* and 0.85% was *An. coustani* and there was no *An. funestus* observed. In November there was only *An. gambiae s.l.* and there were no *An. funestus* and *An. coustani.*

Table 4-5: Monthly distributions of *Anopheles* species adults reared from larvae in Keshmando.

Anopheles species	September	October	November	Total
An. gambiae s.l.	280	116	96	492
An. funestus	0	0	0	0
An. coustani	0	1	0	1
Total	280	117	96	493

In September there was only *An. gambiae s.l.* observed at this local site. In October there was only *An. gambiae s.l.* observed. In November 97.62% was *An. gambiae s.l.* and 2.38% was *An. funestus*, and there was no *An. coustani*.

Table 4-6: Monthly distributions of *Anopheles* species adults reared from larvae in Amba 46.

Anopheles species	September	October	November	Total
An. gambiae s.l.	220	249	123	592
An. funestus	0	0	3	3
An. coustani	0	0	0	0
Total	220	249	126	595

In September there was only *An. gambiae s.l.* observed at this local site. In October 98.53% was *An. gambiae s.l.,* 1.47% was *An. funestus,* and there was no *An. coustani.* In November 96.99% was *An. gambiae s.l.,* 3.01% was *An. funestus,* and there was no *An. coustani* observed in this month at this local site.

Table 4-7: Monthly distributions of *Anopheles* species adults reared from larvae in Amba 47.

Anopheles species	September	October	November	Total
An. gambiae s.l.	328	201	161	690
An. funestus	0	3	5	8
An. coustani	0	0	0	0
Total	328	204	166	698

4.6. Relative monthly contribution of the aquatic habitats to anopheline larval production

The relative monthly contribution of the different larval habitats to anopheline larval production over the study period is shown in Table 4-8. Larval production occurred during all months all over the study period in all the study sites. The results of the monthly larval collection showed that the varied densities of the anopheline larvae vary markedly with the minimum in November and maximum in September. In Amba 47, maximum anopheline larval was collected in September with its minimum in November, while in Amba 46 Kebele, the maximum larval occurred in September with its minimum in November. In Keshmando and Amba 47 Kebele, the highest numbers of anopheline larvae were sampled in September**.**

When compared by local sites, in Keshmando, 58.82% was found in September, 22.69% was found in October, and 18.49% was found in November out of the total number of larvae. At

Amba 46, 39.77% was found in September, 39.11% was found in October and 21.12% was found in November out of the total number of larvae. At Amba 47, 47.75% was found in September, 30.38% was found in October, and 21.87% was found in November out of the total number of larvae in this area.

Table 4-8: Monthly contribution of the different larval habitats to anopheline larval production.

Local sites	Habitat types	September	October	November	Total
Keshmando	Drainage ditch	350	135	110	595
Amba 46	Swamp	305	300	162	767
Amba 47	Stagnant water	393	250	180	823
Total		1048	685	452	2185

4.7. Physical characteristics of larval habitat and distribution of *Anopheles* larvae

Physical habitat characteristics and abundance of *Anopheles* species in each breeding site are depicted in table 4.9. All of the observed breeding sites were found to be still, temporary and with vegetation and algae. *An. gambiae s.l.* was greater in larval density in turbid and natural habitats than that of slightly turbid and artificial habitat. Also, *An. gambiae s.l.* was greater in larval density in habitats with full sunlight and mud than that of partial sunlight and mud with little gravel habitat. This species preferred temporary, still and turbid water with full sunlight and mud substrates. *An. funestus* were greater in turbid and full sunlight. Similarly, *An. funestus* obtained from temporary and natural habitats that had mud and still water with vegetation and algae. *An. coustani* were obtained from aquatic habitats that had turbid water, temporary, partial sunlight, presences of vegetation and algae, still, mud with little gravel and artificial habitats. *Anopheles coustani* was absent from habitats natural and muddy substrate types.

Overall, three habitats were observed. The drainage ditch located nearest to the house was turbid. They contain a lot of debris from vegetation and plants. They were formed by road construction. They were temporary because dried up during the dry season. Habitats were partial sunlight there were large plants at the edge of habitats. Others were mud with little gravel, still and human-made habitats. From these habitats, 27.61% of the total *Anopheles* species was obtained. Swamp and stagnant water were located in farmlands. They were natural and still habitats. Others were

completely exposed to sunlight. Swamp was slightly turbid water. However, stagnant water was turbid because they contain mud and other organic debris. There was a lot of vegetation and algae. Breeding sites were temporary because they dried off during the dry season. In swamp 33.31% of the total *Anopheles* species and stagnant water 39.08% of the total *Anopheles* species was obtained. Temporary and natural habitats with still and turbid water in full sunlight were the most productive breeding sites for the *An. gambiae s.l.*

Table 4-9: Physical habitat characteristics and abundance of *Anopheles* species in the three breeding sites.

Habitat characteristics		No. of habitats	Number of *Anopheles* species		
Characteristics	Variables		*An. gambiae s.l.*	*An. funestus*	*An. coustani*
Turbidity	Turbid	2	1182	8	1
	Slightly turbid	1	592	3	0
Permanence	Temporary	3	1774	11	1
	Permanent	0	0	0	0
Light intensity	Full sunlight	2	1282	11	0
	Partial sunlight	1	492	0	1
Vegetation & Algae	Absent	0	0	0	0
	Present	3	1774	11	1
Water current	Still	3	1774	11	1
	Flow	0	0	0	0
Substrate type	Mud	2	1282	11	0
	Mud with little gravel	1	492	0	1
Origin of habitat	Natural	2	1282	11	0
	Artificial	1	492	0	1

4.8. Physicochemical characteristics of the habitats

Table 4.10 shows the physicochemical parameters for each of the three different habitats. The three sampling sites vary in levels of physicochemical characteristics. The findings showed that dissolved oxygen (DO) was highest (7.07±0.55mg/L) in swamp and lowest (0.32±0.04 mg/L) in drainage ditch. Conductivity levels across different habitats showed wide variations, 18.46±0.05µS/cm for drainage ditch and (14.13±1.98µS/cm) for swamp. Stagnant water also recorded (12.73± 0.75µS/cm). There were slight variations in temperature between different habitats. Drainage ditch recorded high level of TDS 12.19±0.26mg/L; whereas swamp recorded low level of TDS 9.49±1.62 mg/L. Drainage ditch and stagnant water recorded slight salinity of 5.54±1.00PSU and 3.30±0.97 PSU respectively.

Table 4-10: The Mean ± SD of physicochemical parameters of larval habitat.

Area	Temp. (∘C)	TDS(mg/L)	DO(mg/L)	EC(µS/cm)	Salinity(PSU)
Drainage ditch	29.45±0.93	12.19±0.26	0.32±0.04	18.46±0.05	5.54±1.00
Swamp	29.08±1.36	9.49±1.62	7.07±0.55	14.13±1.98	1.22±1.05
Stagnant water	29.35±0.98	6.05±1.45	3.27±0.12	12.73±0.75	3.30±0.97

CHAPTER FIVE

5. Discussions

Of more than forty known species and subspecies of *Anopheles* mosquitoes in Ethiopia (Gaffigan *et al*., 2018), three of them were documented in the present study. These species were *An. gambiae s.l., An. funestus* and *An. coustani*. The most abundant species was *An. gambiae s.l.* while few *An. funestus* and *An. coustani* were recorded. These differences in larval *Anopheles* species abundance could probably be due to abundance and size of the breeding habitats, as well as there are also larvae that did not reach the adult stage, we are not sure of those on how many are which species might be increased abundance of *Anopheles* species. Thus, the low number of *Anopheles* species might be associated with visited habitats. Unlike our findings large numbers of *An. coustani* was observed from the adult collection in Bambasi in the previous study (PMI, 2020). The variation might be due to differences in the collection, sampling period and also these species rarely feature in larval surveys of *Anopheles* species. The current study revealed differences in the abundance and distribution of *Anopheles* breeding habitats in the different local sites.

Anopheles larvae breed in various types of habitats, varying from large permanent to small temporary water collections (Service, 2008). Three larval habitat types were identified as breeding sites in this study, namely swamps, drainage ditch and stagnant water. Additionally, there are other types of *Anopheles* mosquitoes breeding habitats in this area that might be missed in the present study. All habitats were the most common breeding sites in the area. Hawaria and his co-authors also reported similar habitat types from Arjo-Didessa, Ethiopia (Hawaria *et al*., 2020). In this study, in the different habitats, *Anopheles* species were collected but, the most abundant species was *An. gambiae s.l.* Stagnant water was the most preferred habitat for *Anopheles* larvae and few *Anopheles* larvae were observed in the drainage ditch. This suggests that the area Bambasi district has favorable habitats for the survival and development of *An. gambiae s.l.* larvae.

An. gambiae s.l. was the most predominant in stagnant water 690 and least abundant 492 in the drainage ditch. Variation in the occurrence and abundance of the three larval species might be the differences in the levels of physicochemical parameters among the breeding sites. Also, the suitability, stability and productivity of the larval habitat and rainfall intensity might have affected species abundance and larval habitat productivity of *Anopheles* mosquitoes. *An. coustani* were collected from an only drainage ditch. On the other hand *An. funestus* had its highest number in stagnant water and the lowest was collected from the swamp. This finding disagrees with, mosquitoes of the *An. funestus* group mainly prefers swamps (Dida *et al*., 2018). Habitat type also influences the abundance of *An. funestus*. *An. funestus* mainly prefer to breed permanent water bodies; this could be the reason for very rare in swamps, drainage ditch and stagnant water. The major reason for this was *An. funestus* larvae are associated with larger, semi-permanent bodies of water containing aquatic vegetation and algae (Gimmg *et al*., 2001). This study was conducted during the short rainy season but, for *An. funestus* dry season was favorable than the wet season. Thus, the findings of this study agree with (Umar, 2014), which reported *An. gambiae s.l.* was responsible for malaria transmission during the wet season while *An. funestus* has been confirmed to be responsible for the transmission of malaria during the dry season.

The information on physical characteristics of the breeding sites observed during the study was; turbidity, presence and absence of vegetation and algae, habitat permanence, water condition, the origin of habitat and exposure to sunlight. The larvae occur in a wide range of habitats, but most species prefer turbid water. *An. gambiae s.l.* abundance was associated with turbid water. Similarly, the previous findings reported by (Munga *et al.*, 2005; Paaijmans, 2008) suggested that *An. gambiae s.l.* exploit turbid water for oviposition. This indicates during the rainy season, *An. gambiae s.l.* favors turbid water. The other observation contradicts present findings is by (Shililu *et al.,* 2003) who found more anopheline larval densities from clear aquatic habitats. Also, *An. gambiae s.l.* was obtained from a slightly turbid habitat. A similar finding was also reported by (Teklu *et al.,* 2010). *An. gambiae s.l.* was collected from slightly turbid habitats with emergent vegetation and open sunlit conditions. Few *An. funestus* was collected, due to the habitats were turbid. This indicates *An. funestus* prefer clear water than turbid water. A similar finding was reported from Tanzania that *An. funestus* prefer clear waters at depths (Nambunga *et al*., 2020).

In this study permanence of *Anopheles* larval habitat was observed. The three habitats were temporary and at the end of the study period, the three breeding habitats were dry. Highest *An. gambiae s.l.* was collected during the study. This finding agrees with the previous report that *An. gambiae s.l.* prefers temporary breeding sites (Kenea *et al*., 2011).

Moreover, it was observed that the characteristic substrate type for anopheline larva habitat in the study area was mud as high anopheline larvae were observed to occur in larval habitats than other substrate types of mud with little gravel. Soil may provide nutrients for the enrichment of bacteria that serve as food sources for larvae and possibly oviposition attractants. This observation is in agreement with previous reports by (Minakawa *et al.*, 1999) who found that anopheline larvae generally do not like habitats such as water tanks without soil substrates.

Vegetation was also an important predictor for *Anopheles* larvae presence and abundance. A similar finding was reported for *An. gambiae s.l.* larvae (Mwangangi *et al.*, 2007). The presence of vegetation could help the larvae to hide from their predators. Algae was a significant factor in the abundance of *An. gambiae s.l.* larvae in swamp, drainage ditch and stagnant water body; that algae favored the abundance of *An. gambiae s.l.* as it was the main source of its food. The presence of algae with anopheline larval occurrence or abundance is also similar to the finding of Gimnig and his co-workers found that algal food plays a key role in *Anopheles* habitat (Gimnig *et al.*, 2002)

Water movement in habitats was also important in species distribution. The present result showed that anopheline larvae were collected from still waters. They utilize the relatively undisturbed surface tension of still waters and are not found in moving streams. Similar findings were reported in Eritrea, which showed that still waters were the main larval habitats for anopheline mosquitoes (Shililu *et al.*, 2003). The main reason for the high abundance of anopheline larvae in still waters may be those still waters provide favorite situations in which larvae can stay close to the surface with their spiracle open to the air for breathing. Moreover, high water current and flooding are detrimental to *Anopheles* larval survival as a result of the physical harm to the larvae and reduction in their oxygen tension (Okogun, 2005).

It was noted that anopheline larval abundance was more associated with natural habitats compared to artificial habitats. This indicates environmental variables of larval water habitats

regulate the abundance of a mosquito species (Kenawy *et al*., 2013; Okogun *et al*., 2003; Paaijmans *et al*., 2008).

The study results indicated that various physicochemical parameters in mosquito breeding sites at various levels have some influence on mosquito vector hatchability, survival and spatial distribution. High salinity levels were recorded in drainage ditch, due to highly turbid breeding sites. High *An. gambiae s.l.* were collected in stagnant water which had low levels of salinity compared to drainage ditch habitats. This indicates high salinity influences *Anopheles* larval densities. Low salinity levels were observed in swamps, which *An. gambiae s.l.* and *An. funestus* larvae can survive in, this suggests that *An. gambiae s.l.* larvae can survive in high and low salinity of the water.

A maximum mean of EC and TDS 18.46±0.05µS/cm and 12.19±0.26mg/L respectively were recorded in the drainage ditch that might due to high turbid and high salinity recorded in this area. *Anopheles* larvae abundance was low in waters with high levels of salinity and conductivity. A similar study reported that high water conductivity due to high salinity and other dissolved ions have a negative impact on the primary production of mosquito larvae (Closs *et al*., 2009). This finding disagrees with a study conducted in Nigeria reported that conductivity and TDS appeared to have no influence on *Anopheles* larval density (Imam & Deeni, 2014). Also, this finding disagrees with *Anopheles* larvae abundance was higher in waters with high levels of salinity and conductivity (Emidi *et al*., 2017).

Temperature is one of the most important water quality parameters. It affects water chemistry and the functions of aquatic organisms. It influences the amount of oxygen that can be dissolved in water, the rate of photosynthesis by algae and other aquatic plants and the metabolic rates of organisms. In this study slightly varied temperatures were recorded between ranges of 29.08 ºC – 29.45 ºC. It was reported from Ghana that a temperature range between 30ºC – 36.2ºC found the most suitable conditions for the development of the *Anopheles* larval species (Opoku & Ansa-Asare, 2009). Previous studies reported that moderately high temperatures were necessary for the optimum growth of *Anopheles* larvae; they further found that high temperatures usually accelerated their growth (Minakawa *et al.*, 1999). Additionally, other studies observed that warm water also allowed more microorganisms to grow, which provide food sources for mosquito larvae (Sunahara *et al.*, 2002).

Anopheles mosquito larvae were abundantly collected from stagnant water with DO (3.27±0.12mg/L) which is in line with the study of (Owusu, 2016) reported as DO (3.27 mg/L) at the breeding habitats indicates a suitable breeding environment since the general tolerable DO require by most mosquito species is 4 mg/L or less. High DO record in swamp site might be due to the abundance of vegetation that increases photosynthetic activity. The DO of drainage ditch (0.32±0.04mg/L) in which the low number of *Anopheles* larvae collected. Low dissolved oxygen, in drainage ditch habitats, may have been caused by organic pollution resulting from high input of solid and liquid waste from households as the site is in close proximity to human habitation.

This study provides baseline information for designing future surveys and control operations targeting malaria, especially in places such as Bambasi Woreda where *An. gambiae s.l.*, play a major role in malaria transmission. This study has suggested that temporary, still, mud and turbid habitats characterized by the presence of vegetation play a major role in the ecology of *An. gambiae s.l.* Understanding the physicochemical characteristics of mosquito larval habitats is important in understanding their overall ecological needs and assessing options for habitat manipulation to control *Anopheles* mosquitoes.

CHAPTER SIX

6. Conclusions and Recommendations

6.1. Conclusions

Three *Anopheles* species were identified in the study area. *Anopheles gambiae s.l.* is the most abundant species that bred in the three larval habitat types identified in the study area. The density of this species was high in temporary, turbid, mud, still, sunlight, and with vegetation and algae habitats. Each habitat in Bambasi had different physicochemical characteristics that were key determinants of the presence of *Anopheles* larvae. The abundance of the three identified mosquito species larvae was found to vary according to differential environmental and physicochemical factors which determined the quality of water of the breeding habitats. Thus, combinations of these factors might have contributed to the differential abundance of the larval mosquitoes observed at the sampling sites. Therefore, it is highly important to clearly identify the breeding sites of *Anopheles* mosquito and the contributing factors for their productivity so as to implement larval control strategies together with the current IRS and LLINs malaria vector control interventions.

6.2. Recommendations

Depending on the current study the following recommendations were given;

- Further research should be included other physicochemical parameters, such as phosphate, PH, ammonia, and nitrate.
- Malaria vector control intervention strategies should target these temporary water bodies to optimize the efficacy of malaria control.
- Future research should be observing the ecology of mosquito larval predators.
- Local communities should be educated and trained in environmental management and in searching for and identifying mosquito larvae. Since mosquito control officers are trained outside the main transmission season when many larval habitats will be dry, all larval habitats, both new and old must be found and treated during the wet seasons.

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