ADDIS ABABA UNIVERSITY

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ENVIRONMENTAL FACTORS ASSOCIATED WITH LARVAL HABITATS OF ANOPHELINE MOSQUITOES (DIPTERA: CULICIDAE) IN IRRIGATION AND MAJOR DRAINAGE AREAS BETWEEN ADAMI TULU AND MEKI TOWNS, CENTRAL ETHIOPIA

BY

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DECLARATION

I, the under signed, declare that this thesis is my own original work, has not been presented for a degree in any university and that all sources of materials used for the thesis have been duly acknowledged.

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ABSTRACT

A study was conducted to characterize larval habitats of anopheline mosquitoes in irrigation and major drainage areas between Adami Tulu and Meki towns, in the middle course of the Ethiopian Reft Valley. A total of 22 anopheline larval positive aquatic habitats were sampled fortnightly from late December 2007 to early June 2008 by using dipping techniques. Simultaneously, environmental variables of the larval habitats were also measured/estimated and recorded. A total of 3,439 anopheline and 5,213 culicine larvae were collected. Microscopic identification of the late instars (3rd and 4th) of anopheline larvae yielded 47.6% of *An. pharoensis*, 32.1% *An. gambiae* s.l. larvae (presumably *An. arabiensis*) and only 20.3% other anopheline larval species (*An. squamosus, An. coustani and An. cinereus*). *Anopheles* larvae were sampled predominantly from natural swamps, irrigation canals and sand pools with samples from these habitat types representing 88.0% (3025) of the total anopheline larval collection in the study area. Larvae of the malaria vector species, *An. gambiae* s.l. (*An. arabiensis*) and *An. pharoensis* were most frequently sampled from sand pools and natural swamps, respectively. The habitats were characterized based on water temperature, turbidity, water current, water pH, elevation, aquatic vegetation, habitat permanence, origin of the habitats (natural or human made), distance to the nearest house, presence of mats of algae, water depth, intensity of shade, and substrate type. Logistic regression analysis detected six key environmental variables that were associated with the occurrence/abundance of anopheline larvae: Water temperature, presence of mats of algae, water depth, and origin of the habitats, turbidity and water current. Multiple step-up regression analysis further detected four best predictor variables associated with larval abundance of the malaria vector species. Accordingly, relative abundance of *An. gambiae* s.l. larvae was significantly and inversely associated with aquatic vegetation and water current whereas that of *An. pharoensis* larvae were significantly and positively associated with water temperature and the presence of algae in the water bodies. Dry season anopheline larval habitats that are created and maintained by perenial water bodies such as the Ethiopian Rift Valley lakes and rivers and their associated water development projects need to be considered in vector control operation and further research.

1. INTRODUCTION

1.1. GLOBAL OVERVIEW OF MALARIA

Despite several years of research and concerted efforts at control, the realization of a malaria free world remains a dream (Okenu, 1999). The prevalence of the disease continues to increase in many parts of the world. An estimated two billion people (more than 40% of the world population) live in areas with malaria risk. The global annual incidence ranges between three to five hundred million clinical cases, with a death toll between two to three million (Okenu, 1999). The disease remains the most important cause of morbidity and mortality with enormous medical, economic and social impacts worldwide (WHO, 2005). More than half of the world's population is at risk of acquiring the disease, and the situation is worsening with the deteriorating health systems, growing drug and insecticide resistance, climate change and natural disasters (Martens and Hall, 2000).

The presence of the most efficient parasite, accompanied by the vector species and vulnerable human population are some of the factors that favor the transmission of malaria and the associated risk of disease and death (Gillies and Warrell, 1993). Severe malaria and malaria mortality are caused by *Plasmodium falciparum,* which is the predominant species of *Plasmodium* in tropical Africa, eastern Asia, and the Amazon area (Arrow *et al*., 2004). The most efficient malaria vector mosquitoes *Anopheles gambiae* complex occur exclusively in tropical and sub tropical part of the world, especially in Africa. Tropical areas of the world have the best combination of adequate rainfall, temperature and human host allowing for breeding and survival of malaria vector mosquitoes (WHO, 2006).

Malaria was eliminated from Europe, North America and parts of other continents as a result of deliberate programs of mosquito control and clinical treatment, as well as through generally improved social and living conditions. However, the eradication efforts are not successfully achieved in Africa's highly endemic areas (Breman *et al*., 2001). Today, sub-Saharan Africa remains the area of most malaria concentration, but significant problems also exist in Asia, Latin America, and focally in other areas (WHO, 2005).

Malaria re-emerged in several countries of central Asia with an increased frequency of epidemics and with the re-establishment of stable endemic transmission. Factors contributing to the increase in malaria include resistance of parasite to commonly used anti-malaria drugs, breakdown of control programs, poor local health services, resistance of mosquito vectors to insecticides (WHO, 2006; Howard *et al*., 2007). Other factors that expand malaria endemicity include: deforestation, water resources development projects, swamp drainage and specific crop intensification such as rice cultivation (Okenu, 1999; Keiser *et al*., 2005).

Malaria remains one of the world's greatest causes of child mortality and is an obstacle to social and economic development in Africa (WHO, 2000). The disease threatens the lives and livelihoods of more than 300 to 500 million Africans and exerts such a huge public health burden that it has been incriminated in the continued under development of the continent as the whole (Killeen *et al*., 2004). The overwhelming bulk of the world's malaria burden rests upon the population of sub-Saharan Africa because of the unique coincidence of expanding human populations, weak health systems, the world's most effecient vector mosquito species and environmental conditions ideal for transmission (Killeen *et al*., 2002). In sub-Saharan Africa, *An. gambiae* s.s., *An. arabiensis,* and *An. funestus* are the primary vectors of malaria parasites and show highly anthropopholic tendencies (Keating *et al*., 2004). The two most important members of the *An. gambiae* complex are *An. arabiensis,* with plentifully indoors or outdoors, and *An, gambiae* s.s.

with females more likely to bite humans indoors. Evidently these anophelines have coadapted to human ecosystem in the Afro-tropical savannah where their combined contributions to malaria transmission have apparently facilitated evolution of *falciparum* malaria (Mukabana *et al*., 2006).

The distribution, transmission intensity and clinical pathogenesis of malaria in Africa vary greatly across the continent. Africa experiences a complete spectrum of malaria epidemiology ranging from intense perennial transmission to unstable epidemic prone areas (MARA, 1998). The disease epidemics affect non-immune populations in many highland and semi-arid areas of the continent. It frequently affects highlands and semi-arid areas where populations lack immunity (Abeku, 2007). The control of malaria and its anopheline vectors in Africa is less successful because of the occurrence of drug resistance parasites and insecticide resistant vectors, change in the resting behavior of mosquitoes (from indoor to outdoor) as a result of frequent indoor insecticide sprays, lack of efficient infrastructure, shortage of trained manpower, lack of equipment, financial constraints, lack of appropriate management and inability to integrate several methods of control (Toure, 1999; Howard *et al*., 2007).

1.2. MALARIA IN ETHIOPIA

1.2.1. ANOPHELINE MOSQUITOES IN ETHIOPIA

Anopheles mosquitoes have a world-wide distribution, occurring not only in tropical areas but also in temperate regions. There are about 430 different *Anopheles* species (Service, 2000). From these, 123 species are known to be present in Africa (Coetzee *et al*., 2000) and 42 species have been recorded in Ethiopia (Ghebreyesus *et al*., 2006).

Much of the work on the identification and distribution of the *Anopheles* species in Ethiopia was carried out during the Italian occupation of 1938 to 1943 by the Italian

malariologists, during the latter half of the 1940s by the British and during the Malaria Eradication Service in Ethiopia in the 1960`s and early 1970`s (Mekuria, 1983; Gebremariam, 1984; Verrone, 1962a, b).

Extensive entomological surveys have established a good information base about type and distribution of anopheline mosquitoes. The 42 *Anopheles* species that have been recorded vary in distribution by altitudinal zone and microhabitats. Most species are confined to relatively small geographic areas, with the exception of certain malaria vectors such as *An. gambiae* s.l. (Ghebreyesus *et al*., 2006). The 42 anopheline mosquito species that have so far been recorded in Ethiopia are summarized in Appendex I based on the information obtained in (Verrone, 1962a, b).

1.2.2. MALARIA VECTORS IN ETHIOPIA

Human malaria can be transmitted only by anopheline mosquitoes (Gillies and Warrells, 1993). That is, in nature the only way the malaria organism is passed from person to person is through the bite of the *Anopheles* mosquito. Although *Anopheles* species are the sole vectors of the four parasites (*P. falciprum, P. vivax, P. malariae and P. ovale)* that cause human malaria, some *Anopheles* species are also vectors of filariasis (*Wuchereria bancrofti*, *Brugia malayi* and *B. timori*) and a few species transmit arboviruses worldwide (Kettle, 1995, Service, 2000).

There are over 400 species of *Anopheles* mosquitoes throughout the world however, only about 70 species are known to be malaria vectors under natural conditions (Service, 2000). In Africa, members of *An. gambiae* complex and *Anopheles funestus* are widely distributed and are responsible for the transmission of malaria in the region (Coetzee *et al*., 2000). From the *An. gambiae* complex, *An. melas* and *An. merus* are local vectors in west and east Africa respectively. *An. gambiae* s.s. and *An. arabiensis* are the most important malaria vectors in sub-Saharan Africa (Walker and Lynch, 2007). *Anopheles gambiae* s.s. is the most anthropophagic species in the complex and the most important, probably the world's most efficient malaria vector with characteristic indoor and outdoor

resting habits (Coetzee, *et al*., 2000). However, *An. gambiae* s.s. is not reported to be found in Ethiopia.

In Ethiopia, *An. arabiensis and An. quadriannulatus* species B are among the species of the *An. gambiae* complex that have been reported to occur (White *et al*., 1980; Mekuria *et al*., 1983; Hunt *et al*., 1998). The two species differ in their habits and medical importance. *Anopheles arabiensis* occurs in most areas of tropical Africa and could be considered as a major target for control as a major vector where malaria transmission is stable (WHO, 2006). It is the principal vector of epidemic malaria in all administrative regions of Ethiopia (Abose *et al*., 1998; Ghebreyesus *et al*., 2006).

Anopheles quadriannulatus species B is so far restricted to Ethiopia and is zoophilic with a negligible role in malaria transmission, whereas *An. arabiensis* is both zoophilic and anthropophilic and is the primary malaria vector in Ethiopia (White *et al*., 1980; Abose *et al*., 1998). Apart from *An. arabiensis*, *An. pharoensis, An, funestus* and *An. nili* are regarded as secondary vectors (Gebremariam, 1984; 1988; Ghebreyesus *et al*., 2006).

Anopheles arabiensis is responsible for most epidemics in the country. Detailed bionomic studies of *An. arabiensis* mosquitoes have led the way in designing appropriate vector control strategies. Although its larvae may be found around the shores of lakes and rivers, this mosquito species breeds most commonly in small unshaded temporary pools. Adults feed primarily outdoors but rest indoors (Ghebreyesus *et al*., 2006). *Anopheles pharoensis* on the other hand is widely distributed in localities adjacent to permanent water bodies. It breeds in shaded permanent water bodies such as swamps along lake and river shores and also in rise fields (Jolivet, 1959; Gillies and Demeillon, 1968; Service, 2000).

In endemic lowland areas of south western parts of Ethiopia, *An. nili* was regarded as an important vector of malaria. The species commonly breeds in lowland rivers mainly during the wet season when the rivers become over flooded (Nigatu *et al*., 1994). *Anopheles funestus* breeds in large more or less permanent water bodies shaded with

vegetation. However due to human induced ecological changes, such breeding habitats have been distributed in most parts of the country (Nigatu *et al*., 1994).

1.2.3. ANOPHELINE ECOLOGY AND MALARIA EPIDEMIOLOGY IN ETHIOPIA

Malaria epidemiology is driven by temporal and spatial patterns of vector species of anopheline 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's 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). Transmission is generally unstable and seasonal with its level varying from place to place because of altitude and rainfall patterns. The catastrophic malaria epidemic in the country in 1958 for example, was associated with unusually high rainfall over an extended period as well as with elevated temperatures and relative humidity (Fontaine *et al*., 1961). Unstable malaria occurs in most pats of the country particularly in the highland fringes where climatic conditions are conducive for its transmission (Gebremariam, 1984). The major transmission of malaria follows the June to September rains and occurs between September to December while minor transmission season occurs between April to May following the February to March rains (MOH, 2000). Some localities also experience perennial malaria, because of the conducive environmental and climatological situations that permit continual breeding of vectors (e.g. low land areas with permanent water bodies) (MOH, 2000; Ghebreyesus *et al*, 2006).

The most important determinants of anopheline ecology and malaria epidemiology in the country are altitude and climactic factors (mainly rainfall and temperature) although change in settlement patterns, drought and migrations could contribute a lot for the spread.

Areas below 2000 meters above sea level are classified as malasrious. However, a recent report has shown that malaria occurs in highland fringe areas including in urban sites; the main factor being climatic change (Abose *et al*., 1998; Woyessa, 2001; Jima, 2005).

A study in Bure District of north-western Ethiopia by Kebede *et al*. (2005) showed that there was an epidemiological link between maize agro-ecology (*Zea mays* cultivation) and an increase in malaria incidence. Maize cultivation contributed to the production of vector mosquitoes in diverse ways. Nutritional input into breeding sites close to maize pollen sources and human housing may increase vectorial capacity by allowing more mosquitoes to survive to adulthood, and to develop more quickly into larger and longer lived vectors (Ye-Ebiyo *et al*., 2003). Maize has served as an accelerator for vector density, mosquito longevity and malaria transmission (Kebede *et al*., 2005).

The country experiences three locally known climate zones namely, Dega (cold), Weyna Dega (temperate) and Kola (warm) zones. The Dega zone, at altitudes higher than 2,500m, is malaria free since the mean annual temperature of 10-15^{o}C is too low to support development and survival of the parasite in the mosquito vector. In the Weyana Dega zone, between 1,500 and 2,500 meters, where mean annual temperatures range from 15-20^{o}C, malaria most often occurs below 2,000 meters, with short-lived transmission following the rains. However, malaria epidemics have been recorded up to 2400 meters during periods when increased temperature and adequate precipitation are conducive for both vector survival and parasite development within the vector. These malaria epidemics are associated with significant morbidity and mortality since the population is non-immune. In the Kola zone below 1,500 meters, where mean annual temperatures are 20-25^{0}C, malaria transmission is endemic but limited by low rainfall and humidity to areas around permanent water sources (Ghebreyesus *et al*., 2006)

All four species of *Plasmodium* that cause malaria in man are known to be present in the country. However, the two epidemiologically important species are *P. falciparum* and *P. vivax* (Abose *et al*., 1998). About 60% of infections are borne by *P. falciparum* while

40% are of *P. vivax*. The other parasites *P. malariae* and *P. ovale* have little or focal significance (Ghebreyesus *et al*., 2006)

The distribution of humans and anopheline 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. It is also responsible for loss of earnings, low school attendance and high treatment cost (Ghebreyesus *et al*., 2006).

Traditionally, Ethiopian framers minimized the risk of malaria by living in the highlands. However, environmental degradation and increasing population densities are forcing people to occupy the potentially more productive lowlands, thereby putting themselves at greater risk and exposure to the disease.

The main components of malaria control in Ethiopia include the diagnosis and treatment of cases, the application of selective vector control measures and the strengthening of the information system to facilitate prevention, early detection and control of epidemics. Vector control is carried out mainly by means of environmental and chemical measures; either applied singly or in an integrated manner, and is based on local epidemiological conditions. In some areas, the community is actively participating in source reduction with malaria control and other health workers providing technical guidance. Results have been satisfactory, particularly in urban centers, settlement villages, and army camps and agro-industrial centers (Abose *et al*., 1998; Ghebreyesus *et al*., 2006; Lautze *et al*., 2007). As current challenges to prevention and control, other key factors contributing to the persistent malaria burden include population dynamics, war and social upheavals, poverty, water resources and agricultural development. (Ghebreyesus *et al*., 2006).

1.3. LARVAL ECOLOGY OF ANOPHELES MOSQUITOES

1.3.1. BREEDING SITE SELECTION

The habitat characteristics of larval mosquitoes may be determined by the oviposition behavior of gravid females. This is because breeding site is selected by the ovipositing female *Anopheles* mosquito (Kettle, 1995). The female mosquito commutes between blood meal source (host) and aquatic habitat (water) (Menach *et al*., 2005). That is the female mosquitoes emerge from water sources and fly to a blood-meal host, locating a host using a set of cues, including host movement, odor, carbon dioxide and body temperature (Carter *et al*., 2000). Thus, proximity of blood meal source to larval habitat is one important factor that affects breeding site selection in *Anopheles* mosquitoes (Reiter *et al*., 1995).

Oviposition is one potential factor explaining heterogeneous distribution in a landscape with a heterogeneous distribution of larval habitats. Adult female mosquitoes tend to aggregate around places where they oviposite, thereby increasing the risk of malaria, regardless of the suitability of the habitat for larval development (Menach *et al*., 2005).

Oviposition behaviour of mosquitoes and the cues used by *Anopheles* species to select the sites at which they oviposite between blood-meals remain poorly understood, except in very general terms. For example, *An. arabiensis* and *An. gambiae* s.s. typically breed in very transient habitats like shallow sunlit fresh water pools or human made habitats (Shililu *et al*., 2003), though they may also be common in rice fields (Robert *et al*., 1998; Minakawa *et al*., 1999). In contrast, *An. funestus* breeds mainly in marshes and other types of sheltered habitats that contain vegetation (Gillies ad Coetzee, 1987).

There is a general consensus that female mosquitoes are attracted to by-products of bacterial decomposition in aquatic habitats (McCrae, 1983). Volatile substances released from larval habitats have been implicated as potential olfactory cues mediating oviposition (ICIPE, 2003). The microbial fauna of larval habitats likely release volatiles

that may be used as oviposition cues or deterrents. It is not clear how other factors such as the presence of vegetation or the persistence of the habitat might influence the behavior of ovipositing females. It is unlikely that habitats are selected on the basis of these factors; rather these factors may be correlated with other characteristics that act as cues for ovipositing females (Gimnig *et al*., 2001).

When seeking novel avenues for ecological control of mosquitoes and mosquito-borne diseases, mosquito breeding behavior should receive more attention (Chen *et al*., 2006). Mosquito breeding behavior can play an important role in determining the distribution of malaria risk and its anopheline vectors (Menach *et al*., 2005). The types of aquatic habitat in which the adult female has a choice of laying its eggs differ in a number of ways: in size and appearance, in having still or flowing water, in the salinity of the water, in the degree of organic pollution, and consequent oxygenation of the water (Goma, 1966). Other factors such as habitat type, substrate type, semiochemicals, microbial fauna, predators, and vegetation and land cover types also affect the choice of aquatic breeding site by a female mosquito (Chen *et al*., 2006)

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.1 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 oviosition behavior (Chen *et al*., 2006).

1.3.2. BIOLOGICAL AND PHYSICOCHEMICAL FACTORS ASSOCIATED WITH LARVAL HABITATS OF ANOPHELINE MOSQUITOES

Presence of mosquito larvae in a collection of water is initially the result of the oviposition behavior of gravid females. The cues that influence oviposition seem to be a combination of biological and physical parameters of the water and the characteristics of the habitat in which the water is located (Stoops *et al*., 2007). Each mosquito species has its optimum abiotic and biotic characteristics that act as oviposition cues for gravid female mosquitoes and provide ideal environment for the development of the immature (Muturi *et al*., 2007).

The factors affecting larval survival and the mechanisms controlling adult production are largely unknown for even the most important vector species (ICIPE, 2003). Various environmental factors influence the suitability of aquatic habitat for larval anopheline mosquitoes (Ye-Ebiyo *et al*., 2003). The biotic and abiotic factors that affect life history traits such as growth, development and survival of the immature stages of *Anopheles* mosquitoes require more attention, as they will affect productivity in the breeding site and determine the abundance, distribution and fitness of the resultant adult mosquito populations, which will consequently affect malaria transmission (Paaijmans *et al*., (2006).

The environmental factors which determine the suitability of a potential breeding place fall into biological and physicochemical factors (Goma, 1966; Piyaratne *et al*., 2005). However, separating these factors is inherently difficult because many of the biological factors and physicochemical factors of aquatic habitats are highly correlated. For example, water temperature is correlated with the amount of shade provided by tree canopy and is also influenced by movement and volume of water (Stoops *et al*., 2007).

The biological and physicochemical conditions at the larval habitat affect larval development hence affecting the adult body size (Mwangangi *et al*., 2007). Body size affects factors such as longevity, fecundity, and blood meal volume and all these factors

may influence the fitness of the vector for malaria parasite transmission (Lyimo and Takken, 1993).

1.3.2.1. BIOLOGICAL FACTORS

Biological factors of *Anopheles* larval habitats influence larval survival and development and may affect the competence of the resultant adult anopheline vectors. For example, a study by Okech *et al*. (2007) found that organic substances in soil collected from active larval habitats with sand and clay substrates influenced larval development time, pupation rates and competence of *An. gambiae s.s.* to *P. falciparum* parasite. Results of the study further revealed that autoclaving the soils (that reduces presence of microbes) resulted in the production of significantly smaller sized mosquitoes. From this result they concluded an important nutritional role for organic matter and microbial fauna on mosquito fitness and vector competence.

Likewise, growth of *Anopheles* mosquito larvae on dietary microbiota in aquatic surface microlayers was demonstrated (Wotton *et al*., 1997). Hydrophobic organic matter accumulates under the surface film of water bodies to form the surface microlayers. Heterotrophic microorganisms use this organic matter for growth, and they in turn, are fed upon by *Anopheles* mosquito larvae and other animals. From laboratory experiments Wotton *et al*. (1997) showed that two species of mosquito larvae *An. gambiae* and *An. quadrimaculatus* grew most rapidly where surface microlayers were present and, especially, where labile dissolved organic matter was added to promote growth of microorganisms. Because sub-surface microorganisms are the components of the larval diet that most affect growth. Changes in habitat quality due to microbial transformation or in the organic matter content may directly govern the distribution of *Anopheles* mosquitoes and the risk of malaria transmission in endemic areas (Okech *et al*., 2007).

Other biotic factors that may affect survival and development of anophline mosquito larvae at their breeding sites include presence of algae, presence or absence of aquatic vegetation, presence or absence of predators, parasites, pathogens or cannibalism and

other interactions between species (Goma, 1966; Gimnig *et al*., 2001; Koenraadt and Takken, 2003; Paaijmans *et al*., 2007).

The importance of algal biomass in the surface microlayers of larval habitats has been studied. Rejmankova *et al*. (1993) demonstrated that there was strong association between *Anopheles* larval density and the distribution of filamentous algae. A study in western Kenya by Gimnig *et al*. (2002) found that chlorophyll[a] content and algae were positively correlated with *An. gambiae* density and body size. Chlorophyl[a] content in a habitat is an indication of presence of phytoplankton, which indicates the habitats dietary richness for the mosquito larvae (Kaufman *et al*., 2006; Mwangangi *et al*., 2007). The presence of *Anopheles* larvae in their aquatic habitats has been associated with biotic characteristics such as Plankton, suggesting a contribution by Plankton to the growth and development of their larvae (Gimnig *et al*., 2001).

The presence of floating and emergent vegetation can greatly influence what mosquito species are found in a habitat (Goma, 1966; Gimnig *et al*., 2001). For example, a study by Jacob *et al*. (2005) revealed that vegetation was the best predicator for *An. arabiensis* abundance and distribution. It has been suggested that aquatic vegetation promotes anopheline production because it provides a refuge for larvae from predatory, such as *Gambusia affinis.* Additional hypothesis for the beneficial effects of aquatic vegetation include enhanced food resource in vegetated regions, shelter from physical disturbances and favorable condition for oviposition (Omalley, 1992).

The presence of predators and parasites may influence oviposition site selection and larval survival in permanent habitats (Stoops *et al*., 2007). Robert *et al*. (1998) reported that low populations of larvivorous fish and invertebrate predators notably odonates were associated with abundant *An. arabiensis* larvae.

Competition is another biological factor that affects anopheline larval development and adult body size. Munga *et al*. (2006) found that larvae of *An. gambiae* s.s. responded to

increasing intraspecific competition by extending their development times and subsequently emerging as smaller adults. Koenraadt and Takken (2003) investigated some aspects of both inter and intra-specific interactions for three members of *An. gambiae* complex namely *An. Arabiansis, An. gambiae* s.s. and *An. quadriannulatus* under laboratory conditions. Among the aquatic developmental stages of the *An. gambiae* complex, both inter- and intra-specific interactions influenced the resulting densities of adult mosquito population. First-instar larvae were consumed by fourth instar larvae of the same species (cannibalism) and by fourth-instar larvae of other sibling species (predation). Even when larvae were not consumed, the presence of one fourth-instar larva caused a significant reduction in development rate of first-instar larvae. In general, biological conditions at the larval habitat affect larval development hence affecting the adult body size (Mwangangi *et al*., 2007).

1.3.2.2. PHYSICOCHEMICAL FACTORS

The occurrence and abundance of anopheline larvae is closely associated with physicochemical parameters. The importance of many chemical substances dissolved in the breeding water of anopheline 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).

Anolpheles species seem to be able to exploit specific habitat types with very different physical and chemical characteristics. Several studies have shown that physicochemical parameters of the larval habitats such as dissolved oxygen, water temperature and others influence anopheline larval occurrence, abundance and distributions (Goma, 1966; Robert *et al*., 1998; Gimnig *et al*., 2001; Ye-Ebiyo *et al*., 2003; Mwangangi *et al*., 2007).

Mwangangi *et al*. (2007) reported that dissolved oxygen, water p^H, turbidity, water depth, salinity, conductivity and temperature were associated with abundance of *An. arabiensis* larvae in a rice agro- ecosystem in Mwea, Central Kenya. The role of dissolved oxygen

as potential significant variable influencing abundance of *Anopheles* larvae has been reported by several studies (Grillet, 2000; Piyaratne *et al*., 2005; Muturi *et al*., 2007). However, anophelines respire primarily at the water surface, and this raises the question as to whether it is oxygen per se or an associated physicochemical or biological factor that influences the abundance of anopheline species (Muturi *et al*., 2007). In general, anopheline larvae seem to require more dissolved oxygen in their habitats (Grillet, 2000).

Water turbidity was found to be an important parameter associated with the abundance of anopheline larvae in their habitats. For example, Gimnig *et al*. (2001) found increasing *An. gambiae* s.l. larvae densities with increasing turbidity. Robert *et al*. (1998) found a clear-water preference by *An. arabensis* breeding in wells in urban Dakar, Senegal. A study by Ye-Ebiyo *et al*. (2003) found that the production of *An. arabiensis* was favored in moderately turbid water, although excessive turbidity limited the production of larvae. Water that is turbid from particles not edible by *Anopheles* species larvae could disfavour the production of larvae, whereas, water that is turbid from food particles represent a very suitable habitat. Most anopheline species breed in fresh water habitats and show preference for breeding in clean water and their larvae are seldom found in salt water and those heavily polluted habitats (Service, 2000).

Other important factors for the abundance of anopheline mosquito larvae in the habitats include p^H and conductivity. The effect of p^H is apparently an indirect one via the micro-fauna and micro-flora used as food by the mosquito larvae (Goma, 1966). A study by Mwangangi *et al*. (2007) found that p^H was a key factor associated with an increase in anopheline larval abundance. Various chemical properties of the larval habitat related to vegetation such as pH, ammonia, nitrate and sulphate affect larval development and survival (Mutero *et al*., 2004). Ammonia nitrogen, nitrate nitrogen, sulphate and phosphate are important factors regulating larval abundance (Sunish and Reuben, 2001). Nitrogenous fertilizer application accelerates multiplication of microorganisms which form the main diet for the mosquito larvae and also increase pupation rate (mogi, 1978). Nitrogen may be a limiting resource in anopheline larval environment (Gimnig *et al*., 2002). Agricultural activities enhance productivity of the existing larval habitats by

providing nutrients that enter the water bodies through surface runoff in the larval habitats (Munga *et al*., 2006).

Temperature is an important determinant of the distribution and relative abundance of individual species of mosquitoes (Lindsay and Martens, 1998; Mouchet *et al*., 1998). Several mosquito species are sensitive to temperature changes as immature stages in their aquatic environment and as adults. If water temperature rises, *Anopheles* larvae take shorter time to mature and consequently, there is a greater capacity to produce more offspring, during transmission period (Githeko *et al*., 2000). Below 16^0c, the aquatic stages of tropical anophlines fail to develop or breed (Lindsay and Birley, 1996) whereas, warming above 34^0c generally has a negative impact on the survival of most vectors and parasites (Githeko *et al*., 2000).

Anopheline species larvae seem to have optimum temperature ranges for survival and development. For example, Bayoh and Lindasay (2004) observed that under laboratory condition, *An. gambiae* s.s. larvae developed into adults at temperatures ranging from 16 to 34^{o}C. Larval survival was shortest (less than 7 days) at 10-12^{o}C and 38-40^{o}C and longest (greater than 30 days) at 14-20^{o}C. In India, temperature range of 28-32^0c provided the optimum conditions for egg, larval and pupal development (Piyaratne *et al*., 2005). Robert *et al*. (1998) found that warm temperature (28-30^0c), were associated with *An. anabiensis* abundance in urband Dakar, Senegal. Ameneshewa and Service (1996) reported that important *An. arabiensis* breeding sites in Gergedi area in the Awash River valley of Ethiopia are created by hot-springs that form marshy fields which serve as permanent breeding sites. Shililu *et al*. (2003) found a significant effect of temperature on larval densities and reported that the mean water temperature was variable among the different larval habitats ranging from 19.7^{o}C to 28.8^{o}C and larval density was positively correlated with water temperature. A study by Muturi *et al*. (2007) reported that *An. pharoensis* larvae was significantly associated with water temperature indicating that *Anopheles* species seem to breed at species specific optimum temperature ranges.

Temperature also influences the survival of the aquatic stages and subsequent adult production of malaria vectors. The warm water in sunlit habitats may be important for larval development as it allows more microorganisms to grow which provides food source for the mosquito larvae (Minakawa *et al*., 1999). Deforestation activities for purpose of agricultural expansions, human settlement and other development projects have caused a rise in air temperature that favour mosquito breading (Yasuoka and Levins, 2007). Munga *et al*. (2006) reported that deforestation and cultivation of natural swamps created conditions favorable for breeding and survival of *An. gambiae* larvae and consequently increase the risk of malaria transmission. Land cover type may affect larval survivalship and adult productivity through its effects on water temperature and nutrients in the aquatic habitats.

Precipitation (rainfall) is another abiotic factor that determines the survival and spatial and temporal distribution of *Anopheles* mosquitoes. Precipitation creates new breeding sites and adds water to existing ones. The availability, persistence and dimensions of mosquito larval habitats depend to a large extent on the frequency, duration and intensity of precipitation.

However, a study by Paaijmans *et al*. (2006) showed that rainfall significantly affected *An. gambiae* larval mosquitoes by flushing them out of their aquatic habitat and killed them. The significant loss of larvae due to rainfall will as a result decrease the larval density in a breeding site, which will lead to a lower competitive pressure for food and space. Whether such lower densities are advantageous for the development time and survival of the immature of *An. gambiae* is not clear. This study demonstrated that immature populations of malaria mosquitoes suffer high losses during rainfall events. As these populations are likely to experience several rain showers during their life span, rainfall will have a profound effect on the productivity of mosquito breeding sites and as a result on the transmission of malaria.

Previous works on larval ecology in general and on aquatic habitat factors associated with anopheline larvae in particular, is very limited in Ethiopia. Ye-Ebiyo *et al*. (2003)

found that the production of the principal malaria vector, *An. arabiensis* was favoured in moderately turbid water while excessive turbidity limited the production of larvae. The proximity to flowering maize (*Zea mays*) with pollen as food source compensated for the development failure induced by excessive turbidity. Water which is turbid from particles not edible for *Anopheles* species larvae could disfavor the production of larvae while water turbid from food particles represents a very suitable habitat.

1.3.3. LARVAL HABITATS OF THE MAJOR AFROTROPICAL MALARIA VECTOR SPECIES INCLUDING ETHIOPIA

A major reason for the study of mosquito larval ecology is to glean information on factors that may determine oviposition, survival and the spatial and temporal distribution of important disease vector species (Piyaratne *et al*., 2005). Moreover, such information would be very important for selecting and recommending appropriate larval control measures suited to a specific habitat.

The major malaria vector species in sub-Saharan Africa include two members of the *An. gambiae* complex *(An. gambiae s.s* and *An. arabiensis)* and *An. funestus* (Michel *et al*., 2006; Mutuku *et al*., 2006). The development of polymerase chain reaction methods to distinguish species with in the *An. gamiae* complex has allowed more precise study of the larval ecology of *An. gambiae* s.s. and *An. arabiensis* during the past decade (Shililu *et al*., 2007). Several studies observed temporal variations in abundance of the two species, suggesting that *An. gambiae* s.s. is usually the predominant species in saturated environments whereas An. *arabiensis* is more common in arid areas (Gimnig *et al*, 2001; Koenraadt *et al*., 2004).

An. arabiensis is the principal malaria vector that can adapt to different ecological locations in Ethiopia (Ghebreyesus *et al*., 2006). This species type predominantly exists in small sunlit breeding sites flourishing after cessation of the rainy seasons and known to play a crucial role in epidemic situations in the country (Jima *et al*., 2005).

An. arabiensis larvae is associated with small, sunlit, temporary habitats with algae such as foot prints, rain pools, puddles, tire tracks and garden wells (Robert *et al*., 1998; Gimnig *et al*., 2001). Their distribution is determined by the location of suitable water bodies and immature stages prefer usually still water in which they can stay close to the surface with their breathing orifices open to the air (Walker and Lynch, 2007). *An. arabiensis* is a typical r-strategist, colonizing temporary habitats in which selection favours rapid population increase. In general, larval predation is less prevalent in temporary habitats than in large, permanent habitats (Munga *et al*., 2006).

Distribution of larval habitats in Africa is highly dynamic. Meteorological fluctuations combine with underlying hydrology and human activity to generate a constant changing array of potential habitats. This is particularly true for notoriously opportunistic members of the *An. gambiae* s.l. for which the majority of habitats are usually man made (Killeen, 2006). It has been reported that *An. gambiae* s.l. larvae are amphibious. Eggs are more likely to be found outside than inside puddles. Eggs can develop and larvae can emerge on mud. Larvae are capable of terrestrial displacement whereby they can reach standing water (Miller *et al*., 2007).

Studies in urban Dakar, Senegal reported that environmental conditions associated with abundant *An. arabiensis* were warm temperatures (28-30^{0}c), clear and not too deep water (<0.5m), low concentrations of NaCl, low populations of larvivorous fish and invertebrate predators indicating that many contributing factors influence the ecology of the immature stages of *An. arabiensis* (Robert *et al*., 1998). *An. arahbiensis* breeds in residual pools such as the beds of drying streams. It was identified as a major malaria vector in irrigation schemes in Kenya (Ijumba, 1990). Studies in Eritrea showed that *An. arabiensis* production in the ephemeral natural aquatic habitats such as the streambed pools was high throughout the year and negatively associated with rainfall (Shililu *et al*., 2007). Breeding sites in arid areas are highly localized at permanent springs, river edges or irrigational projects, and are not affected by local rainfall (Coetze, 2004). *An. arabiensis* breeds in agro- ecosystem where maize pollen is abundant and readily ingested by their larvae promoting their growth and survival rate. It was noted to develop

readily in turbid water and when crowded, provided that their breeding sites are located where maize pollen is abundant (Ye-Ebiyo *et al*., 2003).

Anopheles funestus, one of the major malaria vector species in sub-Saharan Africa and the most anthropophilic vector known, exploits permanent and semi-permanent breeding sites such as marshes or rice fields. Its population density peaks in the dry season, extending malaria transmission by relay after *An. gambiae and An. arabiensis* population have declined (Michel *et al*., 2006). Gimnig *et al*., (2001) reported that *An. funestus* larvae were associated with larger, semi-permanent bodies of water containing aquatic vegetation and algae in western Kenya.

Anopheles pharoensis is primarily a species of large vegetated swamps and breed along lakeshores and among floating vegetations. It is found distributed in Africa (Gillies and Demeillon, 1968). *Anopheles pharoensis* larvae are associated with abundant grassy or floating vegetation and rice fields (Service, 2000). Abose *et al*., (1998) recorded considerable number of *An. pharoensis* from Lake Ziway with peak densities being in November. Indoor resting density of the vector also coincided with the larval density. On the other hand, Akililu (2008) recorded high densities of *An. pharoensis* in marshy areas of main Koka lakes on the last month of July.

1.4. WATER RESOURCES DEVELOPMENT AND MALARIA IN ETHIOPIA

Africa contains countless basins which together supply water for domestic, livelihood, and irrigation needs of the continent's inhabitants (Lautze and Kirshen, 2007). Ethiopia has risen in the forefront in water resource development in Africa: Gilgel Ghibe dam was completed in the Omo/Ghibe basin in 2004, the Koga dam in the Blue Nile/Abay basin was completed in 2006, the Tendaho and Kessem dams are under construction in the Awash basin and two more large dams are also under construction in the Atbara/Tekeze

basin (Tarekegn, 2006, cited in Lautze *et al*., 2007). Impounded water will soon become a prominent feature of Ethiopia's landscape (Lautze *et al*., 2007).

Water impoundment has frequently been accompanied by serious health hazards in Ethiopia. Increased incidence of malaria and schistomiasis, for example, has been recorded in a number of water projects in various parts of the country. The Birr and Koga irrigation project was expected to exacerbate such health hazards as malaria, schistomiasis and river blindness (Rahmato, 1999).

Malaria transmission is dependent upon many hydro-ecological factors that directly affect the vectorial competence including the presence of suitable habitats for the development of preadult anopheline species (Kengluecha *et al*., 2005). Human intervention in the form of hydro- electric dams, irrigation projects, new settlements and open pit mining, has altered natural ecosystems in many countries and paved the way for the emergence of different malaria vectors with increased vector breeding sites (Robert *at al*., 2003; Surendran and Ramasamy, 2005).

The development of irrigation schemes by dam construction has led to an increased risk of malaria in Tigray, Ethiopia (Yohannes *et al*., 2005). Ghebreyesus *et al*., (1999) found that malaria incidence in young children was seven fold higher in communities near dams than those further away. Yohannes *et al*. (2005) found that seepage pools from microdams, irrigation canal pools and other man-made pools were the major breeding habitats of *An. arabiensis*. The study further indicated that the increased malaria associated with the dams resulted from a rise in mosquito numbers was caused by more breeding habitats in fields irrigated with water from the dams or from water seeping from the foot of the dams.

A recent study on Koka water reservoir (large dam) in the Rift Valley of Ethiopia found that malaria case rates among people living within 3km of the reservoir are about 1.5times as great as for those living between 3 and 6km from the reservoir and 2.3 times

as great for those living 6-9km from the reservoir (Lautze *et al*., 2007). This implies that the Koka reservoir and other water impoundments and irrigation areas are responsible for the proliferation of malaria vectors and the disease in the country.

1.4.1. THE RELATIONSHIP BETWEEN MALARIA VECTORS AND IRRIGATION IN ETHIOPIA

The relationships among hydro-agricultural development, mosquitoes and malaria are particularly close (Robert *et al*., 1998). Anopheline breeding sites are prone to change in accord with agricultural development, deforestation or irrigation (Service, 1991). Irrigation systems in both rural and urban arid regions may create fresh water environments that are suitable for mosquito immature (Burroni *et al*., 2007). Expansion of irrigation is a key development strategy in Ethiopia. In many arid areas, fast population growth and subsistence agriculture dependent entirely on rainfall have resulted in household food insecurity. The government has given priority to changing the agrarian system in such areas from rain fed to small-scale irrigation agriculture, through run-off harvesting in micro dams and ponds (Kassahun, 2007). A study by Kassahun (2007) reported that malaria is the leading health hazard induced by rain water harvesting (RWH) and indicated that a good correlation exists between malaria transmission and pond ownerships in the study areas. However, it would be very controversial to attribute the resurgence of malaria to the expansion of RWH in the country. As witnessed in east African highlands (Munga *et al*., 2006), malaria has shown rapid expansion in highland areas which used to be non-malarious. Therefore, it is worth nothing that the recent malaria resurgence, especially in RWH expansion areas, is compounded with the changes in the climatic and eco-epidemiology (Kassahun, 2007).

In less arid ecological zones, agricultural development has focused on expansion of cash crop farming with further development of large-scale irrigation for sugarcane, cotton, and fruit plantations. Large dams are also under construction for the purposes of hydropower generation to meet growing industrial and domestic electricity needs (Ghebreyesus *et al*.,

2006; Kassahun, 2007). Dams and irrigation schemes transform ecosystems thereby changing malaria risk. Canals and drains create ideal breeding sites for malaria mosquitoes, bringing both the vector and the disease closer to people (Boelee, 2004).

In the irrigated fields, mosquito abundance will increase and if these mosquitoes have enough food sources in the breeding sites, the resulting adults may live longer and allow malaria parasites to complete their development cycle so that they can be passed on to another host. This allows year round transmission of the disease (Keiser *et al*., 2005). Breeding sites created by the construction of thousands of small dams in Tigray, Ethiopia, have been shown to increase the incidence of malaria in communities near the dams by a factor of seven (Ghebreyesus *et al*., 2006). Other irrigation structures, such as wells, may provide permanent breeding sites with few larval predators close to human habitations, as Robert *et al*. (1998) observed in urban Dakar, Senegal.

Intense irrigation based agricultural activity is going on in areas between Koka and Adami Tulu which favor breeding and activity of *Anopheles* mosquitoes (Alemu, 2007; Akililu, 2008; Kibret, 2008). Irrigation agriculture may create new breeding sites or increase the productivity of certain breeding sites. Irrigated rice fields are known to breed *An. gambiae* s.l. particularly before the rice vegetation canopy is well developed (Ijumba and Lindsay, 2001).

Irrigation is the most common means of ensuring sustainable agriculture and coping with periods of inadequate rainfall and drought (Rahmato, 1999). Nonetheless, there is concern that the introduction or expansion of irrigation systems in malaria endemic areas may lead to a risk of malaria transmission by creating more breeding habitats for vectors and extending the length of transmission season (Ijumba an Lindsay, 2001). Important practical and feasible steps in the planning and operational stages can significantly mitigate the adverse effect of irrigation projects and dams on malaria in Ethiopia (Ghebreyesus *et al*., 2006).

1.5. LARVAL CONTROL OF MALARIA VECTORS

Ethiopia is one of the few African countries with a history of malaria control strategies for more than 40 years (Ghebreyesus *et al*., 2006). The current control strategies include: indoor residual spraying (IRS), impregnated bed nets (ITNs), that target adult mosquitoes and environmental management for vector control mainly through source reduction during transmission seasons (MOH, 2000; Jima *et al*., 2005; Ghebreyesus *et al*., 2006). Health authorities throughout the country seek to reduce malaria by IRS with dichlorodiphenyltrichlorethane (DDT) or Malathion in selected villages (Lautze *et al*., 2007). Domestic vector control interventions against adult mosquitoes in the form of ITNs or IRS have enormous potential to reduce vector-human contact and community level malaria transmission (Walker and Lynch, 2007). However, even these highly effective interventions are insufficient to eliminate malaria transmission from most endemic parts of Africa (Gu *et al*., 2003).

Thus, integrated mosquito management (IMM) has been advocated as a critical element to help combat malaria (Gu and Novak, 2005). As integral components of IMM, the importance of larval interventions recently regained the attention in the professionals after a long obsolete status in malaria control (Killeen *et al*., 2002). Larval control is not an entirely new strategy for managing malaria. Historically, many successful campaigns of mosquito eradication had heavily relied on management of larval habitats. For example, source reduction through modification of larval habitats was the key to malaria eradication efforts in the United States, Israel, Italy, Brazil and Egypt (ICIPE, 2003; Killeen *et al*., 2002).

Larval habitat should be a focal point for malaria intervention. Mosquito control methods should aim at intervention during each stage of the mosquito's life cycle. The breeding habitat is crucial for mosquito population dynamics, because it is the location where many important life cycle processes take place; oviposition, larval development, emergence, and mating (Overgaard *et al*., 2001).

One advantage of targeting larvae for control is that they cannot escape from their breeding sites unlike the adult mosquitoes which could easily avoid control measures (Killeen *et al*., 2002). The other advantage of larval control is that while, domestic adulticides such as IRS reduce human-vector contact rather than reduction of the vector population, larval control measures by contrast are intended to reduce vector population density near human habitations (Keiser *et al*., 2004).

Some form of larval control may be a helpful supplement to IRS or ITNs, particularly during the dry season when vector larvae are concentrated in relatively few breeding sites (Fillinger *et al*., 2004). Unlike insecticide based vector control that targets adult mosquitoes, non-chemical measures such as environmental management and biological control, pose virtually no risk of environmental contamination, and human expose to pesticides. Non-chemical larval control may also provide a valuable contribution to resistance management programmes, through the prevention or delay in the onset of vector resistance to insecticides used for ITNs or IRS (Walker and Lynch, 2007).

To control mosquitoes, whether adults or larvae, it is crucial to understand the relevant ecology of the target species. This requires the study of not only the fluctuations of the adult populations, but also the factors affecting larval abundance and distribution (Gimnig *et al*., 2001). Knowledge of the ecological characteristics of the breeding habitat and what environmental factors affect mosquito abundance can help in designing optimal vector control strategies (Overgaard *et al*., 2001; Surendran and Ramasamy, 2005). An integrated approach to malaria control that relies heavily on community involvement is one that may have the bright future. The foundation of any such approach should be source reduction to reduce the level of malaria transmission in an area. This strategy needs to be based on a sound understanding of the local ecology and behavior of the vectors (Yohannes *et al*., 2005).

Although malaria is a major public health problem and larval control is an important component of the malaria control program in Ethiopia, little is known about larval habitats of malaria vectors, their distribution in space and environmental factors that

affect their production that would be important for planning and implementing appropriate larval control strategies. Characterization of larval habitats based on biological and physiochemical factors is important for understanding the complex interactions among immature stages of malaria vectors and the biotic and abiotic components of their aquatic environments.

Although previous entomological studies on anopheline vectors in the area is encouraging (Abose *et al*., 1998; Seyoum *et al*., 2002; Ye-Ebiyo *et al*., 2003; Alemu, 2007; Akililu, 2008; Kibret, 2008), little is known about aquatic habitats of anopheline larvae in irrigation and drainage areas in and around Rift Valley lakes. Information about environmental factors associated with the occurrence and abundance of *Anopheles* larvae in the area is also lacking. The basic question of the current study was therefore, which environmental factors of *Anopheles* larval habitats are significantly associated with the occurrence/abundance of the mosquito larvae?

2. OBJECTIVES OF THE STUDY

2.1. GENERAL OBJECTIVES

To study aquatic habitat characteristics associated with larval habitats of *Anopheles* mosquitoes in the areas between Adami Tulu and Meki towns for effective planning and implementation of anopheline vector larval control.

2.2. SPECIFIC OBJECTIVES

1. To determine the types and diversity of aquatic habitats where anopheline larvae occur (positive breeding sites of anopheline larvae) in the study area.

2. To assess the species of anopheline mosquito larvae occurring in the local habitats and determine their relative abundance in the area under consideration.

3. To determine the effect of environmental variables of the larval habitats on anopheline larval occurrence/abundance in the study area.

3. MATERIALS AND METHODS

3.1 STUDY AREA

The study was undertaken between December 2007 and June 2008 in irrigation and major drainage areas located between Adami Tulu and Meki towns in the Rift valley of Ethiopia. The study area is situated about 150 to 170 km south of Addis Ababa on the main road to Shashemene and Awassa that included part of Lake Ziway and its drainages (Figure 1).

The area is semi-arid and the terrain is relatively flat. Its vegetation mainly consists of scattered *Acacia* trees and thorn bush and subjected to intensive grazing and agriculture. The rainfall pattern is similar to other Ethiopian regions where the big rainy season begins in June and extends up to September while the short rainy season begins in March and extends to April/May, but usually it is very erratic. The area has a fairly warm climate with mean annual temperature of 20.69^0c with annual maximum and minimum temperature of 27.22^0c and 14.16^0c respectively. The mean annual rainfall of the area is 20.69mm and the mean annual relative humidity is 59.10% (Figure 2). Elevation of the study localities range from 1636-1670meters above sea level (m. a.s.l.).

The main topographic feature of the area is Lake Ziway, with an area of 434km^2, a depth of about 4 meters and a length of about 25kms (Abose *et al*., 1998). The lake is a backbone for agriculture and fishing (the main economic activities in the area). It is a major source for large and small scale irrigation and agriculture. It is a source of irrigation water, pure water supply for the urban and rural areas, and a recreation area. The swamps along the shoreline of the lake are also a good pasture land for livestock herding. The other major drainage areas with similar economic importance include Meki and Bulbula Rivers. Both rivers are associated with Lake Ziway in that Meki river drain into the lake from the north while Bulbula River flow out of the lake and flows to south under the eastern foothill of Adami Tulu town and finally enters Lake Abjata, one of the other Rift Valley lakes. Both river systems also serve as active economic activities for

fishing, small and large scale irrigation, sand mines for construction as well as sources of drinking water for people residing near them (Adami Tulu, Meki and Gerbi villagers).

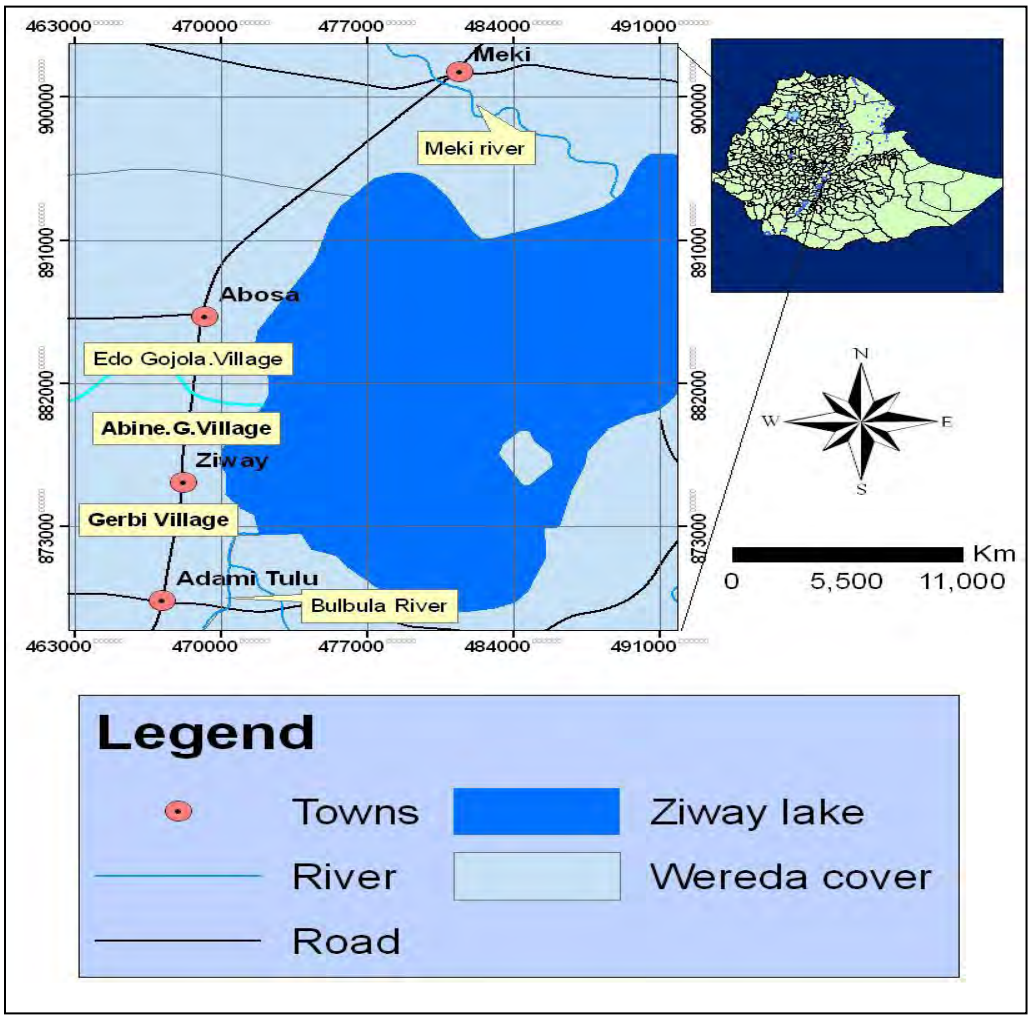

Figure 1. Location of the study area in Ethiopia and map of the study localities

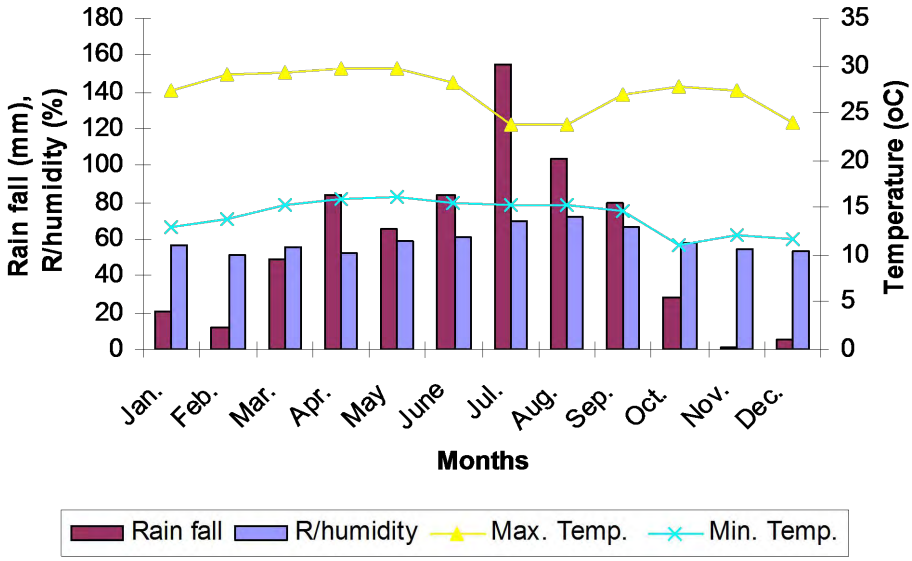

Figure 2. The meteorological pattern of the study area (1998 – 2007)

Source: The National Meteorological Services Agency, Addis Ababa

According to the information obtained from Meki Agricultural office, Lake Ziway, Meki and Bulbula rivers and ground water are the three major sources of irrigation water in the area. The area grows rain-fed maize and other cereal crops during the rainy season (usually June to October) and mainly vegetables such as onions, tomatoes, potatoes, green paper and other vegetables by irrigation during the dry season (November to May).

The present study covered irrigation and major drainage areas in three selected rural towns (urban areas) and three rural farming villages (Kebeles). The urban areas are Adami Tulu, Ziway and Meki towns while the villages included Gerbi Waroma Boromo (formerly Gerbi Gilgile), Abine Gemama and Edo Gojola (Formerly Edo Kontela).

Adami Tulu is situated about 5km south of Ziway town on the road to Awassa at $07^{0}51'$N, $38^{0}42'$E. Its altitude is 1661m. a.s.l. Its population size is about 4460 inhabitants, predominantly of the Oromo ethnicity as informed by Adami Tulu District Administration office (Nura Abaso, Pers. Comm.). Water from Bulbula River has made it possible to irrigate and develop a number of small scale farms owned by local people and private enterprises such as Ethio-flora Farm. They are engaged in cultivating cash

vegetables such as onions, green beans and orchards along Bulbula River. Water is pumped directly from the river bank by irrigation motors planted at intervals along the sides of the river mainly through plastic and metal pipes into irrigation fields. Over flooding of the river during the rainy season and its recession during the dry season has created swamps along both sides of the river that favor mosquitoe breeding.

Gerbi Waromo boramo is a rural village consisting of about 4645 inhabitants (Nura Abasso, Pers. Comm.). It is located between Adami Tulu and Ziway town at 07º53'N, 38^{0}42’E and about 1658m. a. s. l. East of Gerbi village between the road and Bulbula River is found the former Ziway (Gerbi) State farm now owned by Castil Private Limited Company (Castil Vineyard farm). The former state planted a large power house (irrigation motors) on the edge of the river bank. The motors pump water through three large metal pipes uphill and drain into a large cemented primary canal. This large surface canal runs a long distance in the savanna land and drains into secondary and tertiary canals that finally feed the entire vineyard and maize fields. At the junction of secondary and tertiary canals water usually leak out and form pools that serve as breeding grounds for mosquitoes beside interrupted canals and swamps along the river.

Ziway (Batu) is the capital of Adami Tulu district, Eastern shoa zone in Oromia regional state. Ziway is a commercial as well as administrative centre. It is located at 7^{0}56’N, 38^{0}42’E, about 163km south of Addis Ababa. Its elevation is about 1646m. a.s.l. The total population of the town is approximately 41,225 individuals (Nura Abasso, Pers. Comm.). It is also overpopulated by local and migrant laborers from different parts of the country being attracted by job opportunities Intensive large and small scale irrigation farms have been developing on the edges of the town along shore of Lake Ziway. One of the largest agro-industry developments in the area is Sher Ethiopia flower farm, which is situated along the lake on south outskirt of the town. Many town dwellers also grow vegetables and fruits by draining the lake water using surface canals along the lake from Gedam sefer in the south up to Kidanemiheret church in the north. These surface canals and water pools in the swamp, form permanent and semi-permanent breeding sites for mosquitoes. Brick pits that support mosquito breeding are also there largely due to mud

brick making practice of the local people for house construction. It is commonly practiced in the area along the lake during dry season.

Abine Germama is found at the north outskirt of Ziway town by the side of the road from Addis to Awassa. It is located at $7^0$57'N, $38^0$42'E. Its elevation is 1647m. a. s. l. Different ethnic groups live in harmony in this village that consists mainly of Oromo and Silte. They depend on subsistence agriculture and livestock herding and also by fishing on Lake Ziway. The land on both sides of the road along the lake shore is intensively irrigated. In this area, several surface motor canals lead lake water to motors planted at intervals that pump water a long distance through water pipes into fields. Here in addition to lake pools in the swamps, the surface canal pools, canal leakage pools, serve as potential breeding sites for anopheline and other mosquitoes.

The other rural village selected for the study was Edo Gojola. It is found next to Abine Gemama in the north about 4 to 5 kilometers from Ziway town on the main road. It is located at $7^0$58'N, $38^0$43'E. Elevation of the area is 1653m. a.s.l. There are about 2915 inhabitants in this village. Most of the villagers use hand-pumped and Windmill-pumped ground water for domestic consumption and rely on Lake Ziway for agriculture and fishing. The lake water is the major source of irrigation for growing vegetables such as Onions, cabbage and tomatoes in the area. They use surface irrigation and as a result interrupted canals, and lake pools in the swampy land along the lake provide breeding sites for mosquitoes.

Meki town is another study locality situated adjacent to Lake Ziway in the north which is drained by river Meki. It is located at $8^0$90'N, $38^0$49'E. Its elevation is 1670m. a. s. l. The total population of the town was about 34,863 individuals (Kadir Abdu, Pers. comm.). In addition many local laborers from the surrounding rural areas and migrant laborers from different corner of the country inhabit in the town. The laborers are attracted by job opportunities created by vegetable farms and other economic activities in the area.

River Meki flows in the midst of the town from Shoa highlands down steep slop and drain into Lake Ziway. The river flows on sandy substratum that has been deposited since

time in memorial. Intensive sand mining activity takes place in the river bank mainly during the dry season. The sand because of its good quality for large building constructions is directly loaded and transported mainly to Addis Ababa and Adama (Nazhret). Local laborers and farmers mine sand from the river bank usually during dry seasons when the river water dry out (shed) due to evapotransportation and porous nature of the substrates. As a result sand mining pits block the water flow and create pools. These sand pools are ideal breeding sites for mosquitoes.

Just below Meki town botanical nursery site, both sides of the river are also irrigated. Motor pumps are placed at intervals in order to pump water uphill directly from deep in the river bank through plastic pipes into irrigation fields. There were no surface canals between the river bank and irrigation farms. However there were unlined surface canals at uplifted soil mass in the irrigation fields. The surface canals feed the agricultural farms. Water pools were rarely observed in the irrigation fields largely due to porous nature of the soil.

3.2. SAMPLING SITE SELECTION

Three rural towns (urban areas) and three rural villages (kebeles) totally six local study sites were selected based on proximity to major drainage areas and the presence of large and small scale irrigation farms. Selection of the villages and urban areas (sampling sites) was also guided by local guides who had good knowledge about the area. Accordingly, all permanent and semi permanent anopheline positive larval habitats present in and with in 500m radius of the irrigated farm of each selected village/town (Shililu *et al*., 2003) and 700m along the major drainages (lake or river) (Gimnig *et al*., 2001) were sampled for mosquito larvae. All the study localities are situated along the drainage areas.

3.3. ENTOMOLOGICAL STUDIES

3.3.1. LARVAL MOSQUITO COLLECTION AND IDENTIFICATION

Mosquito larvae were sampled at fortnightly interval from December 2007 up to early June 2008 during the dry (December-February) and short rainy seasons (March-June). During each survey a habitat was first inspected for the presence of mosquito larvae by dipping technique (Plate 1). Larvae were collected by using a ladle (11.5cm diameter and 350ml capacity per dip), pipettes, and white plastic pans (Sharma, 1990; Service 1993a).

When mosquito larvae were present 10-30 dips depending on the size of the habitats were taken at intervals along the edge of each larval habitat (Shililu *et al*., 2003). Dipping was done by gently submerging the ladle till just below the water-air interface usually at the edge of the larval habitat (Service, 1993a).

Samples were always taken by the same individuals (myself and a field assistant). During each sampling, the habitat was visited at the same morning (0900-1200hours) or afternoon (1400-1700 hours) hours. Thirty minutes or less was spent at each site sampling for larvae and recording of environmental variables of the larval habitats. We attempted to sample the same sites or sites in the same area. However it was not always possible because some of the sampling sites were inaccessible on some occasions due to over flooding and drying out. A value of 0 was recorded for sites that were dry, flooded with running water and for suitable sites with no larvae per number of dips (Claborn *et al*., 2002).

Larval count was done on all collected mosquito larvae in the field. However, the late instar anopheline larvae (III and IV instars) were preserved and used for species identification. Larval density was initially recorded as per number of dips in the field and later converted to per 100 dips because the number of dips taken was variable depending on the size of the habitats and the number of larvae sampled was also low (Shililu *et al*., 2003). From each habitat larvae were always transferred into large vials with the habitat

water by direct pippeting and labeling and then brought to a room where I used to stay during the field work. The larvae were then killed in warm water (about 60^0c) by gently holding over ethyl alcohol soaked-cotton flame (Balkew, Pers. Comm.). All third and forth instar anopheline larvae were thus preserved in small vials containing 70% ethyl alcohol (Service, 1993a). Vials were labeled with relevant information such as date, site and number of larvae collected and transported to Addis Ababa.

The preserved *Anopheles* larval specimen were brought to the laboratory of Akililu Lema Institute of Pathobiology (ALIPB), Addis Ababa and mounted individually on a microscopic slide in gum chloral mountant by properly arranging its orientation and identified under a light microscope with the objectives x10 and x40 (Plate 2). Larval identification was based on identification keys of Verrone (1962b) and Gellies and Coetzee (1987). Voucher specimens of *Anopheles* larval species for each local site of the study area were deposited at ALIPB Vector Biology and Control Research Unit, Addis Ababa University.

Plate 1. Larval mosquito inspection from a sand pool at Meki River

Plate 2. Larval anopheline mosquito identification at Akililu Lema Institute of Pathobiology

3.3.2. LARVAL HABITAT CHARACTERIZATION AND RECORDING OF ENVIRONMENTAL VARIABLES

Simultaneously with larval mosquito sampling, environmental characteristics of the larval habitat were measured or estimated and recorded at the location for each habitat. The environmental variables recorded were water temperature, pH, water depth, elevation, intensity of shade, turbidity, vegetation type, water current, substrate type, distance to the nearest house, whether the habitat was natural or human made (origin of the habitat) and the presence of algae and permanence of the habitat. Most of the environmental variables are already well established with regard to their associations with mosquito larval development.

Water temperature was measured using LCD portable Digital Multi-stem Thermometer (ST-9269 A/B/C-model). Water pH was measured using pH indicator (Viac. Imbonati 2420159 Milano (Italy). Water depth was measured using a metal ruler (Minakawa *et al*., 1999). Water current was determined by visual inspection and categorized as slow flowing and still (Muturi *et al*., 2007).

Turbidity that was mainly caused by suspended organic matter was estimated by placing water samples in glass test tubes and holding against a white background and categorized as either clear or turbid (Minakawa *et al*., 1999; Mwangangi *et al*., 2007). Intensity of shade was visually categorized as light and shade. The type and presence of aquatic vegetation was observed and recorded as emergent, floating, emergent plus floating and none if no vegetation at all. Emergent plants included both aquatic and immersed terrestrial vegetation.

Substrate types were classified in to mud or soil, and cement or concrete by visual inspection. Distance to the nearest house was measured with a tape when it was shorter than 100m. When the distance exceeded 100m, it was measured by foot step (Gebre-Michael, Pers. Comm.). Then, distance to the nearest house was categorized in to 4 classes (e.g. 1 = 0---100m, 2=100--- 300m, 3=300---500m and 4 for -distances greater

than 500m (Minakawa, *et al*., 1999). It was believed that anthropophilic species would breed near human dwellings so that increasing more anopheline breeding habitats.

The presence or absence of mats of algae (green algae) was visually determined. All visual classifications and physical measurements were done by the same person (myself) to maintain consistency. The coordinates of local sample sites, larval habitats and their elevations were determined using a hand-held geographical positioning system (GPS: Garmin, 12, KS, USA).

3.4. METEOROLOGICAL DATA COLLECTION

Monthly rainfall, minimum and maximum mean temperature, and relative humidity data of the study area for the period of ten years (1998-2007) were kindly obtained from the National Meteorological Services Agency (Addis Ababa).

3.5. DATA ANALYSIS

Data analyses were done using SPSS software (version 13.0 for Windows, SPSS Inc., 2000). Percentile score was used to compare the frequency of occurrence and abundance of *Anopheles* larvae among habitat types and distribution of their species within the habitats. Variations in larval counts (mean densities) among habitat types, variations in mean densities of the collected larvae among environmental factors (characteristics) of the larval habitats were analyzed using one way analysis of variance (ANOVA) test. Larval density was expressed, as number of larvae sampled per 100 dips (Shililu *et al*., 2003). When significant differences were observed in ANOVA, the Tukey test was used to separate the means.

Pearson correlation analysis was used to determine the association among the environmental variables and also to assess the relationship between anopheline larval densities and environmental factors of the larval habitats. That is for each environmental

variable, simple correlations between larval occurrence/abundance and individual parameters were first checked and only significant associations further examined by multiple logistic regression. Logistic regression analysis (Odds ratio) was used to detect key environmental factors associated with anopheline larval occurrence. Step-up multiple regressions were used to determine the best predictor variables associated with relative abundance of the larval species of anopheline mosquitoes including the malaria vectors. Results were considered significant at $p< 0.05$.

4. RESULTS

4.1. SPECIES COMPOSITION OF ANOPHELINE LARVAE

In total, 8,652 mosquito larvae were collected from different aquatic habitats in six local study sites between Adami Tulu and Meki towns in the Rift Valley of Ethiopia (Table 1). Culicine and anophelinae larvae comprised 60.3 and 39.7% of the mosquito fauna, respectively. Both anopheline and culicine larvae were predominantly collected from Ziway. Larvae of the two mosquito sub-families were strongly significantly correlated ($r= 0.48$, 2-tailed, $p< 0.01$) suggesting that larvae of culicine and anopheline coexist in the water bodies currentely examined.

Table 1. Total number of mosquito larvae collected during fortnightly visits from late December 2007 to early June 2008 in irrigation and major drainage areas of six localities between Adami tulu and Meki Towns, Central Ethiopia

	Local sites							
	Adami Tulu	Gerbi	Ziway	Abine Germama	Edo Gojola	Meki	Total	%
No of habitat	1	2	7	6	3	3	22	
Anophelinae	688	420	817	545	375	594	3439	39.7
Culicinae	563	767	2122	760	602	399	5213	60.3
Total	1251	1187	2939	1305	977	993	8652	100.0

From the totat 3,439 anopheline larvae collected, some 62.1% (2134) were late instars (3^{rd} and 4^{th} instars). These were examined microscopically for species identification that

yielded five *Anopheles* species among which *An. pharoensis, An. gambiae* s.l. (Presumably *An. arabiensis)* and *An. squamosus* were the major species whereas *An. coustani* and *An. cinereus* were generally scarce (Table 2). *An. pharoensis* (47.6%, 1015) and *An. gambiae s.l.* (32.1%, 686) were the most abundant species in the study area, respectively. The other *Anopheles* species: *An. squamosus, An. coustani* and *An. cinerues* comprised only 20.3% (433) of the total anopheline larvae collected.

Table 2. Number of late instar (3rd and 4th instars) anopheline mosquito larvae collected from irrigation and major drainage systems in the six localities between Adami Tulu and Meki towns (Dec. 2007-June 2008)

	Local sites							
Anopheles spp.	Adami Tulu	Gerbi	Ziway	Abine Germama	Edo Gojola	Meki	Total	%
An. gambiae s.l.	39	31	130	30	55	401	686	32.1
An. pharoensis	126	117	336	199	187	50	1015	47.6
An. squamosus	59	56	118	68	62	2	365	17.1
An. coustani	3	2	48	4	0	4	61	2.9
An. cinereus	0	0	0	7	0	0	7	0.3
Total	227	206	632	308	304	457	2134	100.0

4.2. ANOPHELINE LARVAL DENSITIES DURING THE STUDY PERIOD

As depicted in Figure 3, *Anopheles* larvae were observed during every month of the study period. Mean densities of the anopheline larval populations underwent marked monthly variations, with their minimum mean density in December and maximum mean density in March.

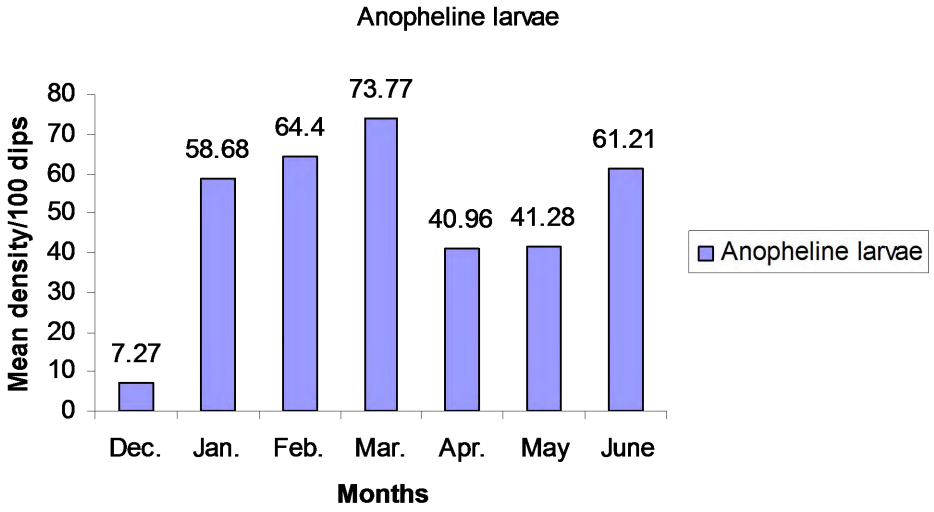

Figure 3. Mean density per 100 dips per month of anopheline larvae collected from irrigation and major drainage areas between Adami Tulu and Meki towns during the study period Dec. 2007- June 2008

As it can be seen from Figure 4, larval densities of both *An. gambie* s. l. and *An. pharoensis* generally rose up from December to March with higher densities of *An. pharoensis* than *An. gambiae* s.l. However, after March through May, larval densities of both species went down, with more densities of *An. gambiae* s. l. than *An. pharoensis*. After the on set of rain in late May, larval density of *An. gambiae* s. l. steeply rose up. Mean larval density of *An. squamosus* tended to increase during the initial larval survellience with peak density in January and thereafter declined with a similar trend with that of *An. pharoensis* larvae.

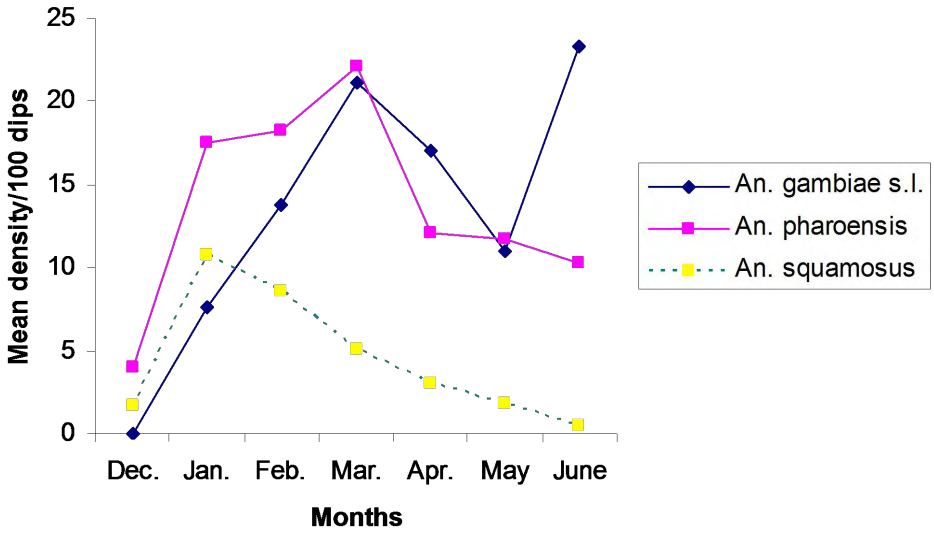

Figure 4. Mean density per 100 dips per month of the major anopheline species in the study area

4.3. HABITAT DIVERSITY AND LARVAL DENSITY

4.3.1. ANOPHELINE LARVAL HABITAT DIVERSITY

In total seven anopheline positive larval habitat types which are mainly permanent and semi-permanent breeding sites were identified in irrigation and major drainage areas between Adami Tulu and Meki towns in the middle course of the Ethiopian Rift Valley (Plates 3). The habitat types included swamps, irrigation canals, canal leakage pools, sand pools, water harvesting pool, brick making pit, and sand pool.

A. Natural swamps.

B. Irrigation canals.

C. Canal leakage pool (left) and sand pools (right).

Plate 3. Typical permanent and semi permanent mosquito breeding habitats in irrigation and major drainage areas between Adami Tulu and Meki towns, Central Ethiopia

Sampling proportion of the habitat types are shown in Figure 5. Irrigation canals (36.4%,96) and swamps (22.7%,60) were the most abundant habitat types most frequently sampled in the study area, respectively, while canal leakage pools and sand pools occurred in the same proportion (13.6%,36) from the total habitat types. Water harvesting pool, brick making pit and rain pool all together comprised only 13.5% (48) of the total anopheline positive habitat types identified. The number of rain pools sampled was few due to unsual extended dry season and even after the on set of rain in late May most of the fresh rain pools observed were negative for anopheline larvae.

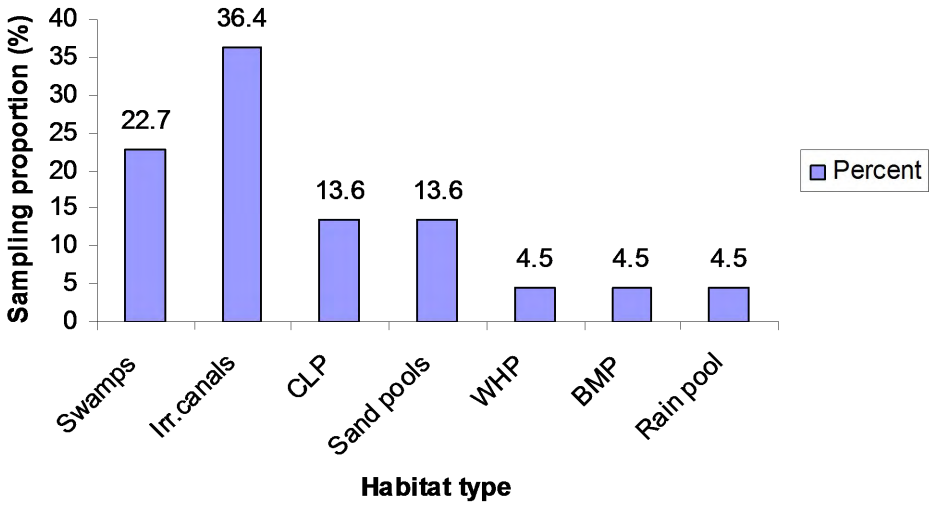

Figure 5. Anopheline larval habitat types in the study area and their sampling proportions during fortnightly survey from December 2007- June 2008.

CLP= Canal leakage pools, WHP= Water harvesting pool, BMP= Brick making pit

4.3.2. OCCURRENCE OF ANOPHELINE LARVAE IN THE AQUATIC HABITATS

The relative importance of the different larval habitats with regard to anopheline larval presence and production is shown in Table 3. Anopheline larvae were sampled predominantly from swamps and irrigation canals. Collections from these habitat types comprised 43.8% (n=1505) and 26.9% (n=926) of the total *Anopheles* larvae collected respectively.

In other words, nearly greater than 70% (n=2431) of the total anopheline larvae collected were from swamps and irrigation canals while the other habitat types such as canal leakage pools, sand pools, brick making pit and rain pool contributed only 29.3%(n=1008) of the total anopheline larvae sampled.

The Brick making pit and rain pool contained anopheline larvae in all of its sampling times. Swamps (68.3%, 41) and sand pools (60.7%, 17) were most frequently contained *Anopheles* larvae respectively whereas canal leakage pools and water harvesting pool are nearly in the same proportion in containing anopheline larvae. On the other hand, 66.7% (8) of the water harvesting pool, 65.6% (21) of the irrigation canals sampled were found negative for anopheline larvae respectively.

Table3. Number of anopheline larvae collected from different larval habitats and proportion of aquatic habitats with larvae sampled during the fortnightly survey (December 2007-June 2008)

Larval habitat type	Total no. of habitats (Frequency)	% larval habitat positive	% larval habitat negative	Total *Anopheles* larvae	% of total *Anopheles* larvae
Swamps	60	68.3	31.7	1505	43.8
Irr. canals	96	38.5	61.5	926	26.9
Sand pools	28	60.7	39.3	594	17.3
CLP	32	34.4	65.6	279	8.1
WHP	12	33.3	66.7	37	1.1
BMP	4	100.0	0.00	62	1.8
Rain pool	2	100.0	0.00	36	1.0

Note: CLP= Canal leakage pools, WHP= Water harvesting pool, BMP= Brick making pit
Negative and positive samples were taken from the same habitats on different occasions

4.3.3. HABITAT TYPES AND MEAN DENSITIES OF ANOPHELINE LARVAE

The mean *Anopheles* larval density over the sampling period was 52.22 larvae per 100 dips. There was variable contribution of each aquatic habitat with regard to larval production (Figure 6). Results of ANOVA and Tukey′s honestly significantly differences showed that mean densities of anopheline larvae collected from sand pools were significantly higher compared with the other habitat types ($F = 3.766$, $df = 6,257$, $P < 0.05$). However, in relation to long-term contribution to larval production swamps and canals were more important because they had water available for anopheline larval development and they were therefore, sampled more frequently for mosquito larvae compared with the other habitat types. Sand pools and swamps were the most productive aquatic habitats for the anopheline larvae sampled in the present study.

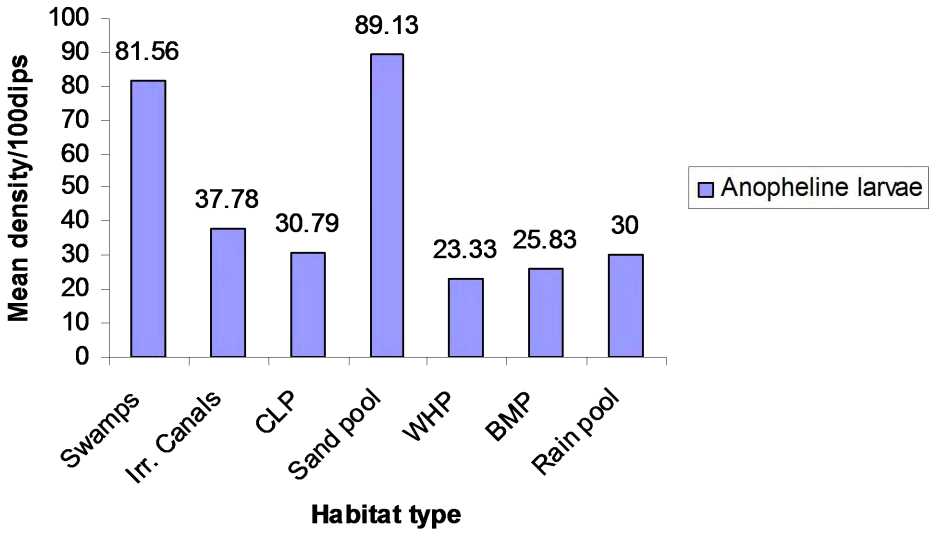

Figure 6. Mean density of anopheline larvae collected from different larval habitats during the study period December 2007 to June 2008

CLP= Canal leakage pool, WHP= Water harvesting pool, BMP= Brick making pit

Likewise, significant variation in the relative importance of the larval habitats at local villages and town level was also observed. Accordingly, mean density of anopheline larvae collected from the swamp habitat in Adami Tulu was significantly higher compared to the other larval habitats in the other local study village and towns ($F = 9.553$, $df = 5258$, $P < 0.05$).

4.3.4. HABITAT DISTRIBUTION OF ANOPHELINE LARVAL SPECIES

Table 4 depicts spatial distribution of *Anopheles* species larvae in different larval habitats during the study period. *An. gambiae* s.l. larvae were collected most abundantly from sand pools (58.5%, 401) and swamps (19.4%, 133) whereas irrigation canals, canal leakage pools, water harvesting pools, brick making pit and rain pool together comprised 22.2% (152) of the total *Anopheles gambiae* s.l. larval collection during the study period. Nearly 80% (803) of the total *an. pharoensis* collected was obtained from swamps and

irrigation canals while only 21.1% (214) of the same species were sampled from other aquatic habitats. Likewise, greater than 85% (311) of the total *An. squamosus* larvae were obtained from swamps and irrigation canals. The rest of *An. squamosus* (14.8%, 54) were obtained from canal leakage pools and sand pools. Water harvesting pool, brick making pit and rain pool didn't harbor *An. squamosus* during the study period. *An. coustani* and *An. cinereus* scarcely occurred in swamps and irrigation canals and generally absent from other habitat types.

Table4. Distribution of *Anopheles* species in different types of larval habitats in irrigation and drainage areas between Adami Tulu and Meki towns during December 2007- June 2008

Larval habitat type	*Anopheles gambiae* s.l. N (%)	*Anopheles pharoensis* N (%)	*Anopheles squamosus* N (%)	*Anopheles coustani* N (%)	*Anopheles cinereus* N (%)	Total N (%)
Swamps	133(19.4)	456(44.8)	181(49.6)	29(47.5)	7(100.0)	806(37.7)
Irr. canals	44(6.4)	347(34.1)	130(35.6)	30(49.2)	0	551(25.8)
Sand pool	401(58.5)	50(4.9)	2(0.5)	0	0	453(21.2)
CLP	10(1.5)	135(13.3)	52(14.2)	2(3.3)	0	199(9.3)
WHP	17(2.5)	14(1.4)	0	0	0	31(1.5)
BMP	57(8.3)	5(0.5)	0	0	0	62(2.9)
Rain pool	24(3.5)	10(1.0)	0	0	0	34(1.6)
Total	686(32.1)	1017(47.6)	365(17.1)	61(2.9)	7(0.3)	100.0

Note: CLP= Canal leakage pools, WHP= Water harvesting pool, BMP= Brick making pit

4.3.5. HABITAT TYPES AND MEAN DENSITIES OF THE LARVAL SPECIES

Expressed as number of larvae per number of dips of sampling, relative abundance of anopheline species in the different larval habitats was also significantly variable (Figure 7). For example, comparing mean densities of *Anopheles* species larvae that were sampled from the different habitat types revealed that *An. pharoensis* ($F= 3.212$, $df= 6, 257$, $p<0.05$) and *An. gambiae* s.l. ($F= 13.370$, $df=6, 257$, $p<0.05$) were the most abundant species in swamps and sand pools, respectively. *An. squamosus* ($F=3.744$, $df= 6, 256$, $p< 0.05$) also more colonized swamps than the other habitat types.

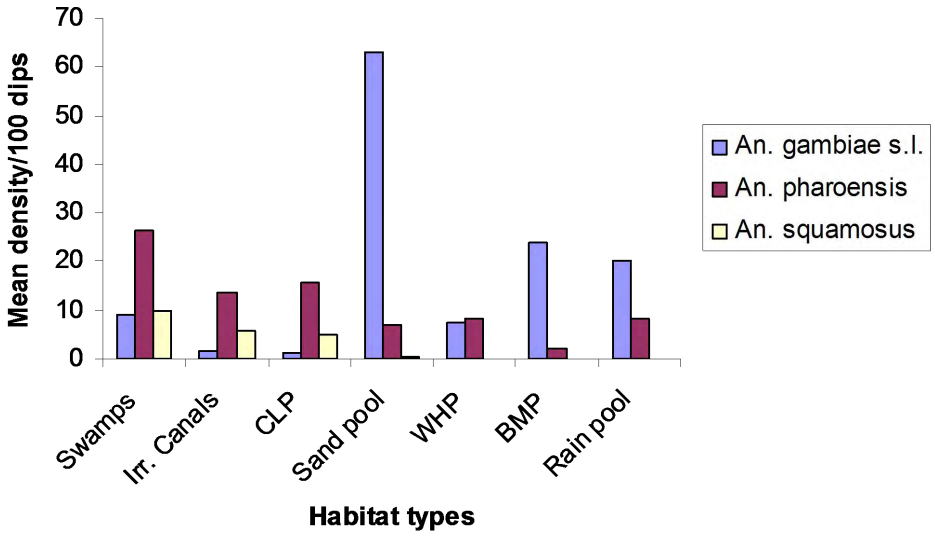

Figure 7. Mean densities of *An. gambiae* s.l., *An. pharoensis* and *An squamosus* larvae collected from different aquatic habitats in the study area (December 2007-June 2008).

4.3.6. RELATIVE MONTHLY CONTRIBUTION OF THE AQUATIC HABITATS TO ANOPHELINE LARVAL PRODUCTION

Relative monthly contribution of the different larval habitats to anopheline larval production over the study period is shown in Table 5. Larval production occurred during all months all over the study period in all the study sites. Larval abundance was generally low during the dry season (December to February) and high during the short rainy season (March to early June). It decreased in the dry season and increased with the onset of the wet season in all the study sites. However, peak densities of larvae were achieved at different months. In Adami Tulu, maximum anopheline larval density was collected in January with its minima in June, while in Gerbi village, the maximum larval density occurred in May with its minimum density in April. In Ziway and Abine Germama village, peak larval activity concentrated between February to April. In Edo Gojola and Meki town, the highest numbers of anopheline larvae were sampled in March and February, respectively.

Table 5. Relative monthly contribution of the different larval habitats to anopheline larval production at six sites over the study period December 2007 to June 2008)

1Local site	Habitat	Dec.	Jan.	Feb.	Mar.	Apr.	May.	June	Total
Adam Tulu	Swamp	50	713.34	90	463.33	133.33	103.36	80	1633.36
Gerbi	CLP	0	306.67	186.67	0	80	-	-	573.34
	Irr. canal	0	0	0	0	80	480	266.67	826.67
Ziway	Swamp	0	60	393.33	390	30	120	110	1103.33
	Irr. canals	0	410	681.66	278.33	150	0	0	1519.99
	BMP	-	-	-	-	135	115	60	310
	Rain pool	-	-	-	-	-	120	240	360
Abine	Swamp	0	340	120	53.33	40	300	140	993.33
Germama	Irr. canals	60	180	175	285	0	0	0	700
	CLP	0	0	245	140	150	0	0	535
Edo Gojola	Swamp	0	50	63.33	100	100	163.33	40	516.66
	Irr. canal	50	153.33	76.67	230	100	0	0	610
	WHP	0	0	0	80	50	120	120	370
Meki	Sand pools	0	368.67	802	12.46	764	168	-	3348.67

Note: CLP= Canal leakage pools, BMP= Brick making pit, WHP= Water harvesting pool

- (minus sign) indicates that no aquatic habitat was present

Larval density was expressed here as number of larvae per 100 dips (Shililu *et al*., 2003)

4.4. FACTORS ASSOCIATED WITH LARVAL OCCURRENCE/ ABUNDANCE IN DIFFERENT AQUATIC HABITATS

4.4.1.. CORRELATION BETWEEN ABUNDANCE OF ANOPHELINE LARVAE AND ENVIRONMENTAL VARIABLES

Before proceeding to regression analysis (regression models) to determine key environmental variables, correlation analysis was made for each environmental variable and abundance of anopheline larvae (Table 6). As it can be seen from Table 6, half of the variables that is 50% (6 of 12) of the environmental variables were significantly correlated with density of anopheline larvae. *An. gambiae* s.l. larvae were significantly correlated with elevation; aquatic vegetation, habitat permanence, water current and distance to the nearest house. *An. pharoensis* larvae were significantly correlated with most of the environmental variables. That is, 66.7% (8 of 12 variables) of the environmental variables examined were significantly correlated with the abundance of *An. pharaensis* larvae. *Anopheles squamosus* larval density was also significantly correlated with half of the environmental variables currentely examined (50%, 6 of 12 correlation coefficients).

Table 6 Correlation coefficients between environmental variables and densities of anopheline larvae and its major species

Environmental variables	Total anopheline	*Anopheles gambiae* s.l.	*Anopheles pharoensis*	*Anopheles squamosus*
Water temperature	0.196**	0.029	0.349**	0.145*
Water depth	-0.129*	-0.067	0.150*	-0.088
Elevation	-0.082	0.131*	-0.106	-0.136
Intensity of shade	0.003	-0.002	-0.007	0.102
Turbidity	-0.214**	-0.047	-0.137*	-0.201
vegetation	0.069	-0.304**	0.284**	0.152
Habitat permanence	-0.035	0.302**	-0.172**	0.262
Water current	-0.264**	-0.180**	-0.207**	-0.261**
Distance to house	-0.043	-0.213**	0.101	0.164*
Natural habitats	-0.147*	-0.078	0.201**	-0.188**
Presence of algae	0.330*	0.084	0.426**	0.421**
Water P^H	0.018	-0.062	0.048	0.198**

* Correlation significant at the 0.05 level

** Correlation significant at the 0.01 level

4.4.2. ASSOCIATION BETWEEN HABITAT CHARACTERISTICS AND DENSITIES OF ANOPHELINE LARVAE

Tables 7, 8, 9 and 10 depict characteristics of larval habitats and mean densities of anopheline larvae. Significantly higher densities of anopheline larvae were collected in larval habitats that were natural and had clear standing water with emergent plus floating vegetation and mats of green algae. Intensity of shade, permanence of larval habitats and the distance between the larval habitats and the nearest house did not significantly affect larval densities among the habitats (Table 7).

Significantly higher mean densities of *An. gambiae* s.l. larvae were obtained from aquatic habitats that had clear and standing water, free of vegetation and also temporary habitats nearest to human dwellings (< 100m). Intensity of shade, origin of the habitats, presence of algae and water turbidity were not siginificantly associated with mean densities of *An. gambiae* s.l. (Table 8).

Significantly higher mean densities of *An. pharoensis* larvae were collected from Permanent and natural habitats that had clear and standing water with mats of algae. Intensity of shade and distance to the nearest house were not significantly associated with mean density of *An. pharoensis* larvae (Table 9).

Likewise, mean densities of *An. squamosus* larvae collected from clear water, permanent habitats with emergent plus floating vegetation, still water, and 100- 300m from human dwellings and that had mats of algae were statistically significant. Whereas, intensity of shade did not significantly affect *An. squamosus* larval densities in the aquatic habitats currently examined (Table 10).

Table 7 Characteristics of larval habitats and mean densities of anopheline larvae collected during Dec. 2007 – June 2008

Habitat characteristics		Mean ± SE	F	P
Intensity of shade	Light	58.9 ± 6.2	0.002	0.966
	Shade	60.0 ± 17.1		
Turbidity	Clear	67.9 ± 6.9	11.134	0.001
	Turbid	17.6 ± 5.5		
Vegetation	Emergent	16.1 ± 6.2		
	Floating	40.6 ± 16.8	3.442	0.018
	Emergent + floating	70.1 ± 8.5		
	None	66.9 ± 13.3		
Permanence	Permanent	63.5 ± 8.1		
	Semi-permanent	50.1 ± 8.5	2.460	0.088
	Temporary	18 ± 9.2		
Water current	Still	71.2 ± 7.1	17.433	0.000
	Slow flowing	12.6 ± 4.7		
Distance to the nearest house	0….100m	55.4 ± 10.2		
	100…300m	71.2 ±10.6	1.932	0.147
	300…500m	42.7 ± 6.9		
Origin of the habitat	Natural	51.1 ± 6.1	5.143	0.024
	Human made	81.6 ±14.4		
Presence of algae	Present	89.2 ± 9.1	28.408	0.000
	Absent	29.6 ± 6.5		

Table 8 Characteristics of larval habitats and mean densities of *An. gambiae* s.l. larvae collected during Dec. 2007 – June 2008

Habitat characteristics		Mean ± SE	F	P
Intensity of shade	Light	15.5 ± 2.9	0.001	0.971
	Shade	15.0 ± 11.5		
Turbidity	Clear	16.4± 3.4	0.516	0.473
	Turbid	10.9 ± 4.2		
Vegetation	Emergent	0.34 ± 0.34		
	Floating	20.9± 11.9	13.012	0.000
	Emergent + floating	5.3 ± 1.4		
	None	45.5± 9.7		
Permanence	Permanent	5.1 ± 1.3		
	Semi-permanent	27.6 ± 6.3	14.353	0.000
	Temporary	120± 40		
Water current	Still	19.5± 3.6	7.739	0.05
	Slow flowing	0.0 ± 0.0		
Distance to the nearest house	0….100m	28.4 ± 7.7		
	100…300m	12.4 ±2.9	5.713	0.004
	300…500m	3.8± 1.7		
Origin of the habitat	Natural	9.1 ±2.7	1.653	0.200
	Human made	17.6 ± 3.7		
Presence of algae	Present	18.1 ± 4.8	0.892	0.346
	Absent	15.5 ± 2.8		

Table 9 Characteristics of larval habitats and mean densities of *An. pharoensis* larvae collected during Dec. 2007 – June 2008

Habitat characteristics		Mean ± SE	F	P
Intensity of shade	Light	16.7 ± 1.8	0.012	0.914
	Shade	15.8 ± 5.8		
Turbidity	Clear	18.4± 1.9	4.444	0.036
	Turbid	8.5 ± 4.5		
Vegetation	Emergent	7.5 ± 3.4		
	Floating	7.4 ± 3.0	8.648	0.000
	Emergent + floating	25 ± 2.9		
	None	7.0 ± 2.3		
Permanence	Permanent	50.0 ± 10		
	Semi-permanent	21.4 ± 2.6	7.023	0.001
	Temporary	9.4 ± 2.0		
Water current	Still	19.6 ± 2.1	10.342	0.001
	Slow flowing	5.5± 2.7		
Distance to the nearest house	0….100m	11.5 ± 2.5		
	100…300m	19.6±3.4	1.963	0.143
	300…500m	18.4 ± 2.9		
Origin of the habitat	Natural	26.2 ±4.7	9.790	0.002
	Human made	13.39 ± 1.7		
Presence of algae	Present	28.7 ± 3.0	51.483	0.000
	Absent	5.0 ± 1.3		

Table 10 Characteristics of larval habitats and mean densities of *An. squamosus* larvae collected during Dec. 2007 – June 2008

Habitat characteristics		Mean ± SE	F	P
Intensity of shade	Light	5.4 ± 0.8	2.455	0. 119
	Shade	11.3± 4.3		
Turbidity	Clear	6.9± 0.9	9.745	0.002
	Turbid	0.2± 0.2		
Vegetation	Emergent	1.6 ± 1.0		
	Floating	0.3 ± 0.3	12.106	0.000
	Emergent + floating	10.1± 1.4		
	None	0.5 ± 0.3		
Permanence	Permanent	8.5 ± 1.2		
	Semi-permanent	1.8 ± 0.8	8.626	0.000
	Temporary	0.0 ± 0.0		
Water current	Still	6.5 ± 1.0	3.800	0.052
	Slow flowing	2.5± 1.0		
Distance to the	0….100m	2.9 ± 0.8		
nearest house	100…300m	6.1 ±1.4	3.205	0.042
	300…500m	8.4 ± 1.9		
Origin of the habitat	Natural	9.7 ± 2.0	8.465	0.004
	Human made	4.3 ± 0.8		
Presence of algae	Present	11.1 ± 1.5	49.9	0.000
	Absent	0.4 ± 0.3		

4.4.3. ENVIRONMENTAL FACTORS ASSOCIATED WITH THE OCCURRENCE OF ANOPHELINE LARVAE

Logistic regression (odds ratio) revealed six key environmental variables associated with anopheline larval occurrence in the different aquatic habitats (Table 11). Table 11 shows the result of logistic analysis between the presence/absence of anopheline larvae and environmental variables in irrigation and major drainage areas between Adami Tulu and Meki towns, Central Ethiopia. Water temperature, water current, turbidity, the presence of algae, origin of the habitats (whether natural or human made) and water depth were significantly associated with the presence /absence of anopheline larvae in the water bodies. Water bodies with algae (odds ratio [OR] =12.88, 95% confidence interval [CI] =6.9-23.9) and warmer temperature (OR=1.52, CI=1.3-1.70) were much more likely to contain anopheline larvae than when algae were absent from the water bodies and when the habitats were cooler. Water depth (OR = 0.9, 95% CI = 0.9 – 0.9) was inversely associated with the occurrence of anopheline larvae as deeper water bodies decreased the chance of finding anopheline larvae.

Similarly, origin of the habitats (OR = 0.4, CI= 0.2-0.6), turbidity (OR = 0.2, CI = 0.1-0.5) and water current (OR = 0.1, CI = 0.1-0.3) were negatively associated with the presence/absence of anopheline larvae. The results indicated that natural habitats, clear and still water bodies more likely contained anopheline larvae whereas human made habitats, turbid and fast flowing water bodies decreased the chance of anopheline larval occurrence in the water bodies during the study period.

Table 11 Repeated measures logistic regression models for the associations between occurrence of anopheline larvae and environmental variables

Parameter	Odds ratio	Lower CI	Upper CI	P
		Anopheline larvae		
Water depth	0.99	0.97	0.99	0.031
Presence of algae	12.88	6.92	23.96	<0.001
Origin of the habitats	0.35	0.19	0.65	0.001
Water temeperature	1.52	1.30	1.77	<0.001
Turbidity	0.22	0.09	0.48	<0.001
Water current	0.14	0.06	0.31	<0.001

For OR > 1, a positive association is said to exist.

For OR < 1, there is a negative association (Gimnig *et al*., 2001).

Multiple step-up regression analysis further detected the best predictor variables associated with the occurrence and abundance of anopheline larval species (Table 12). Accordingly, the top predictors for *An. gambiae* s.l. (*An. arabiensis)* larval abundance were aquatic vegetation and water current while that of An. *pharoensis* and *An. squamosus* were presence of mats of algae. Water temperature and distance to the nearest house were also signfciant predictors of *An. pharoensis* and *An. squamosus* larval abundance in the aquatic habitats, respectively.

As depicted in Table 9, relative abundance of *An. gambiae* s.l. larvae was negatively associated with aquatic vegetation and water current while that of *An. pharoensis* was positively associated with the presence of mats of algae and water temperature. *An.squamosus* larval abundance was also positively associated with presence of algae in the water bodies and distance to the nearest house.

Table 12 Multiple step–up regression for the three common anopheline species larvae in relation to habitat characteristics

		R^2	Coefficient	SE	Standard coefficient	t	P
	(constant)		74.82	10.39		7.19	0.00
An. gambiae s.l	Vegetation	9.3	-12.92	2.22	-0.36	-5.82	0.00
	Water current	15.6	-28.06	6.74	-0.26	-4.17	0.00
	(constant)		-58.28	18.27		-3.14	0.00
An. pharoensis	Presence of algae	18.2	19.09	3.49	0.34	5.47	0.00
	Water temp.	22.3	2.64	0.76	0.22	3.49	0.00
An. squamosus	(constant)		-3.45	2.13		-1.62	0.11
	Presence of algae	17.7	10.37	1.51	0.41	6.89	0.00
	Distance to house	19.3	2.09	0.99	0.13	2.10	0.04

4.4.5. CORRELATION BETWEEN METEOROLOGICAL PATTERN AND ABUNDANCE OF ANOPHELES LARVAE

Due to lack of current meteorological data of the study area, average data of the 1998 up to 2007 that fit the study period was extrapolated. Accordingly, the mean monthly rain fall for the study period (December-June) was 45.58mm while the mean monthly air temperature was 21.33oC. Mean monthly temperature varied among the study months with peak in March and its minima in December. Average relative humidity was 55.66 %.

As it can be seen from Table 13, mean monthly air temperature of the study period had a positive and significant correlation with density of anopheline larvae ($r= 0.132$, $p< 0.05$). However, larval densities of the malaria vector species were not significantly correlated with the average weather variables of the study period. Larval densities of both *An. gambiae* s. l. and *An. pharoensis* were positively correlated with air temperature. Moreover, larval densities of *An. pharoensis* was negatively correlated with both rain fall and relative humidity while that of *An. gambiae* s. l. was positively associated with the two weather variables. Larval abundance of *An. squamosu* was significantly and inversely associated with average monthly rain fall of the study period ($r= -0.203$, $P< 0.01$).

Table 13. Correlation coefficients between relative abundance of anopheline larvae and average Meteorological variables of the study period extrapolated from December 1998- June 2007)

Mosquito larvae	Rain fall (mm)	Temperature (oC)	R/humidity (%)
Anophelinae	0.007	0.132*	0.032
An. gambiae s.l.	0.109	0.120	0.023
An. pharoensis	-0.034	0.118	-0.019
An. squamosus	-0.203**	-0.005	-0.080

*Correlation is significant at the 0.05, ** Correlation is significant at the 0.01 level.

5. DISCUSSION

Five anopheline species were identified in the irrigation and major drainage areas between Adami Tulu and Meki towns of Central Ethiopia. These included *An. pharoensis, An. gambiae* s.l. (*An. arabiensis), An. squamosus, An. coustani* and *An. cinereus.* The first two were the predominant species that were found in the area. Cytogenetic studies have previousely confirmed that *An. arabiensis* is the sole known member of *An. gambiae* complex present in Ziway area (Abose *et al*., 1998; Ye-Ebiyo *et al*., 2003). All the species presently identified have previously been documented (Abose *et al*., 1998) and consistent with a recent report by Kibret (2008) who has worked on the impacts of a small scale irrigation scheme on malaria transmission around Ziway (in Abine Germama) one of the local sites in the present study.

Seven larval habitat types were identified in this study, namely swamps, irrigation canals, canal leakage pools, sand pools, water harvesting pool, brick making pit and rain pool. The first two habitats were the most common breeding sites in the area. The availability, persistence and dimensions of all the larval habitats except rain pool are dependent on water from the major drainage systems namely, Lake Ziway, Meki and Bulbula rivers.

Some of these habitat types were previously reported from Ziway area and elsewhere in the country. For example, Abose *et al*. (1998) found that anopheline larvae occur in permanent main lake shore water with vegetation (swamp) and in temporary rain-pools either in the villages or around the shore of Lake Ziway. Likewise, very recently, Kibret (2008) reported that irrigation canals and canal leakage pools were the most important prolific *Anopheles* larval habitats in Ziway area. Else where in the country, Yohannes *et al*. (2005) also reported that in the dam villages of Tigray, irrigation canals, pools that formed along the bed of streams from the dam and man made pools were the major anopheline breeding habitats. Balkew (2001) also reported similar habitat types from Metehara, Ethiopia.

Brick making pit was another anopheline larval habitat type currently observed in the study area though its occurrence was rare. This would be expected because brick making either for sale or domestic use is a common practice in Ziway area especially during the dry season along Lake Ziway. The latest report from Koka area, (Akililu, 2008) revealed that among the different breeding habitats examined brick making pits were the most favoured sites by larvae of *An. gambiae* s. l. Brick making pits are also common anopheline mosqitos breeding sites else where (Surendran and Ramasamy, 2005; Sogoba *et al*., 2007).

Water harvesting pools (WHP) were another potential mosquito breeding sites currently observed in the study area. These included collection of lake water by surface canals in to human-made wells (Pits) usually at botanical nursery sites and collection of rain water. This habitat type rarely supported anopheline larval development because most of the water harvesting pools observed during the study period were used regularly that could remove unknown proportion of mosquito immature and negatively impact adult oviposition there. The contribution of WHP for anopheline breeding and malaria transmission was previously reported from the other parts of the country (Kassahun, 2007).

Reverine sand mining pits block water flow and create pools which would offer ideal habitats for the proliferation of anopheline mosquitoes. To my knowledge, this habitat type had not been reported so far from the country although it is a common anopheline mosquito habitat elsewhere outside Ethiopia. For example, Robert *et al*. (2003) and Surendran and Ramasamy (2005) reported that open pit mining has altered natural ecosystem in many countries and paved the way for the emergence of different malaria vectors, with increased vector breeding sites. Similar to the present observation, *An. culicifcies* larvae in Sri Lanka were also observed to breed abundantly in rock pools and sand pools along river margins (Surendran and Ramasamy, 2005).

Results of this study also revealed that anopheline larvae were abundantly found in swamps and irrigation canals during the study period. This would be expected because the swamps and canals had water available for anopheline larval development most of the time compared with the other habitat types in relation to long-term contribution to larval productivity. Similar to the present study, Balkew (2001) reported that the existence of permanent anopheline breeding habitats associated with irrigation practices in Metehara supported the survival of vector species throughout the year and anophelines survived the dry months by colonizing permanent breeding sites. Likewise the present result was also similar to a study by Coetze (2004) who reported that anopheline breeding sites in arid areas are highly localized at permanent habitats, river edges or irrigation projects.

This study has documented the occurrence of five species of *Anopheles* larvae. Of all the five anopheline species larvae, *An. pharoensis* was found in appreciable density in herbaceous swamps in the major drainage systems and irrigation area. In contrast, *An.gambiae* s.l. larvae preferred semi-permanent (seasonal) habitats such as sand pools, brick making pits and rain pools. This was in agreement with the study by Abose *et al*. (1998) who found that the most important type of breeding sites for *An. arabiensis* were temporary rain pools whereas *An. pharoensis* preferred the permanent main lakeshore water with vegetation for breeding. Studies elsewhere also revealed that An. *pharoensis* breeds in large vegetated swamps and along lakeshores among floating plants (Gillies and Demeillon, 1968). However, *An. gambiae* s.l. is a typical r-strategiest, colonizing temporary habitats in which selection favours rapid population increase because larval predation is less prevalent in temporary habitats than in large permanent habitats (Munga *et al*., 2006). It was observed that the principal malaria vector in the country: *An. gambiae* s.l. *(An. arabiensis)* larvae were most abundant in sand pools along the edge of Meki river before the on set of the rainy season. This would be expected because sand mining activity in the area coincided with the dry and irrigation seasons where rain dependent anopheline larval habitats dry out elsewhere and the species limit themselves to permanent water bodies such as rivers and lakes. Similar idea was shared by Muturi *et*

al. (2006) who reported that in Africa, *An. arabiensis* breeding sites have been associated with rivers and irrigation systems in drier parts of the continent. The other anopheline species larvae, *An. squamosus* and *An. coustani* were also found in swamps and irrigation canals just like that of *An. pharoensis.* This observation was also in agreement with a study by Abose *et al*. (1998) who collected the same species some 10 years back from swampy area of Edo Kontela (now Edo Gojola) on the shoreline of Lake Ziway, and Kibret (2007) who recently reported the same anopheline larval species from irrigation canals at Abine Germama Village around Ziway town.

Anopheline larval abundance generally tended to increase during the short rainy season (late May to June) than during the dry season (December to April). This would be attributed to the appearance of some anopheline positive rain water dependent breeding sites such as brick making pit and rain pool. Mean densities of *An. gambiae* s.l. and *An. pharoensis* generally increased from December to March with higher abundance of *An. pharoensis* larvae than *An. gambiae* s.l. larvae. This is similar with previous reports by Abose *et al*. (1998) who observed that during rainy season larval densities of both species increased with higher abundance of *An. gambiae* s.l. than *An. pharoensis*. However, after the rainy season in November, larval density of *An. gambiae* s.l. (*An. arabiensis*) started to decline while larval density of *An. pharoensis* steeply rose up. After March and through April, larval densities of both species went down with more abundance of *An. gambiae* s.l. larvae than that of *An. pharoensis*. During this period, anopheline larval density generally declined due to prolonged dry season and as a result lake and river water regressed, more parts of swamps dried, irrigation canals were regularly renewed to deliver more water for irrigation. On the other hand this period coincided with the drying out of Meki river water, intensive sand mining activities, formation of river water residual pools as sand pools and proliferation of *An. gambiae* s.l. larvae. When the river driedout, more sandy ground of the river bed was exposed, transportation was facilitated as vehicles got into the river bank and loaded the sand piles easily (Appendix 8). These all helped formation of more sand pools and mosquito larval proliferations. This

observation was consistent with reports by Ijumba (1990) who pointed out that *An. gambiae* s.l. larvae breed in residual pools such as beds of drying stream and Coetzee (2004) who reported that breeding sites of *An. arabiensis* in arid areas are highly limited to river edges. These habitats are generally with clear water and sunlit which are consistent with *An. arabiensis* larval habitats (Service, 2000).

Although anopheline larval control is an important component of malaria control program in Ethiopia, usually by source reduction through management of larval habitats integrated with adult vector control (Abose *et al*., 1998; Yohannes *et al*., 2005; Gebreyesus *et al*., 2006), little is known about larval ecology of *Anopheles* mosquitoes. For example, the microhabitat factors that influence the occurrence and abundance of *Anopheles* larvae are not well characterized even for the malaria vector species. Reports of larval habitats has been usually given in more general terms as "Marshes", "Rain pools", "man-made pools" and the like. Although somewhat informative, these habitat categories are not specific enough to define local environmental factors associated with specific anopheline species.

The present study was undertaken to assess some environmental factors of anopheline larval habitats in irrigation and major drainage areas between Adami Tulu and Meki towns to determine their association with the occurrence and abundance of the mosquito larvae.

In this study, a number of environmental factors were used to characterize the larval habitats, including water temperature, pH, elevation, water depth, water current, turbidity, presence of mats of algae, distance to the nearest house, permanence of the habitats, origin of the habitat (whether natural or human made), aquatic vegetation, intensity of shade, and substrate types and others.

The environmental variables that we examined were not independent of each other (Appendix 7). Therefore, to examine the association between occurrence and abundance of anophline larvae and environmental variables, multiple linear or multiple logistic regressions analysis is more appropriate than simple linear or logistic regressions (Robert *et al*., 1998; Minakawa *et al*., 1999). Accordingly, in the present study, the association of anopheline larval occurrence and relative abundance with environmental variables was elucidated with different statistical models as depicted in Tables 7- 12.

Logistic regression analysis (odds ratio) and multiple step-up regression altogether detected at least six environmental variables significantly associated with anopheline larval occurrence/abundance. Of these, water temperature and presence of mats of algae were positively associated, while water depth, origin of the habitats, turbidity and water current were negatively associated with the larval occurrence/ abundance in the water bodies. In addition, others being the same one way analysis of variance (ANOVA) detected significantly higher mean densities of anopheline larvae from aquatic habitats with emergent plus floating vegetation suggesting that anopheline larvae prefer this type of aquatic flora.

The positive association between occurrence/abundance of anopheline larvae and water temperature in this study was consistent with previous findings in the country and elsewhere. For example, previous studies collected anopheline species larvae from hot springs in Gergedi and Koka areas in the Awash River Valley of Ethiopia (Ameneshewa and Service, 1996; Aklilu, 2008). Robert *et al*. (1998) reported that *An. arabiensis* abundance was associated with warm temperature (28-30^{0}c). Similarly Shililu *et al*. (2003) found that anopheline larval density was positively correlated with water temperature in Eritrea. A study on habitat characteristics of mosquitoes in Accra, Ghana, identified that water temperature was determinant factor in the anopheline larval occurrence, abundance and distribution (Opoku *et al*., 2000). This view is also shared by Gimnig *et al*., (2002). Piyaratne *et al*. (2005) found that *An. culicifacies* abundance was

associated with water temperature. According to Gillies and Demeillon (1968), warm temperatures shorten larva-pupa development and therefore hasten adult mosquito emergence. There is a critical relationship between temperature and the life cycle of the insects (Bayoh and Lindsay, 2004).

The present investigation also showed positive association between the presence of mats of algae in the aquatic habitats and the occurrence/abundance of anopheline larvae as previously reported by different authors from similar study area and elsewhere. Ye-Ebiyo *et al* (2003) reported that presence of algal density promotes development of anopheline larvae similar to maize pollen and alters feeding strategies of *An. gambiae* s.l. larvae. Likewise, the positive association of mats of algae with anopheline larval occurrence/abundance is also similar to the findings of Gimnig *et al*. (2002) who found that algal food plays a key role in *Anopheles* habitat and contradicts the conclusion of Fillinger *et al*. (2004) who reported negative association between *Anopheles* density and non-matted algal content. The reason for negative association of anopheline larvae with non-matted algal content in the study of Fillinger *et al*. (2004) was attributed to abiotic factors (chemical water quality) and biotic factors (predatory fauna) that were not included in the present study and remains to be explored. Another study in Kenya also demonstrated the importance of algal biomass for the development of anopheline mosquitoes in their larval habitats (Kaufman *et al*., 2006).

The higher abundance of anopheline larvae in aquatic habitats with emergent and floating vegetation revealed by the ANOVA model is also consistent with previous findings. There are a number of papers on the relationships between vegetation and anopheline larvae. (Rejmankova *et al*., 1999; Claborn *et al*., 2002; Fillinger *et al*., 2004; Devi and Jauhari, 2007).

Anopheline larvae were inversely associated with water depth and turbidity. Such observations have been made elsewhere. For example, Rober *et al*. (1998) reported that

An. arabiensis abundance was associated with clear and not to deep water (<0.5m). Similarly, Shililu *et al*. (2003) found more anopheline larval densities from shallow and clear aquatic habitats. The latter observation contradicts previous findings from the study area by Ye-Ebyo *et al*. (2000; 2003) who found that anopheline larvae (*An. arabiensis* larvae) exploit turbid water. It should be noted that the previous studies were specific to *An. gambiae* s.l. (*An. arabiensis larvae)* the opportunistic members of *An. gambiae* complex mosquitoes unlike the present study that included all anopheline species in the area. The other difference is that the former studies were conducted during the rainy season on rain water dependent puddles formed by surface run off and simulated puddles which as a result may be turbid due to soil erosion and pollen from flowering maize as the study period coincided with maize anthesis. Whereas, the current study was carried out during dry and irrigation season on permanent and semi-permanent habitats and as a result transient and rain water dependent habitats as well as flowering maizes were rarely observed.

The present investigation also revealed negative association between anopheline larval abundance and water current. This result was in contrast with the findings of Shililu *et al*. (2003) who reported that anopheline larvae were abundant in slow flowing habitats. This may be because in the former study, *Anopheles* larvae were sampled predominantly from stream edge and stream bed pools with samples from these habitat types comprised 91.2% (n=9481) unlike the present study that were collected mainly from stagnant swamps and sand pools.

It was also observed that anopheline larval occurrence/abundance was more associated with natural habitats compared to human made habitats. This world be expected because, natural habitats (swamps) contained water during the study period and sampled most frequently for anopheline larvae unlike the human-made habitats. On top of this, the human made habitats such as functional irrigation canals were regularly cleared of all

excess vegetation and renewed during the study period to irrigate crops which displaced or removed unknown proportion of larvae.

Moreover, it was observed that the characteristic substrate type for anopheline larval habitats in the study area was soil/mud as no anopheline larvae was observed to occur in larval habitats with any other substrate types such as cement or concrete, plastic or rubber, and others. 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 with out soil substrates. Soil may provide nutrients for the enrichment of bacteria that serve as food source for larvae and possibly oviposition attractants.

Furthermore, step-up multiple regression results demonstrated a strong interaction between some of the environmental factors and the relative abundance of the three major anophleine species: *An. gambiae* s.l., *An. pharoensis* and *An. squamosus* and detected key environmental factors associated with them. Some four key environmental variables were significant predictors of the larval stages of the malaria vectors, *An. gambiae* s.l. and *An. pahraensis*. For example, vegetation and water current were negatively associated with *An. gambiae* s.l. larval abundance. This observation was also described by Ye-Ebiyo *et al*. (2003) who reported that in the relatively dry Ethiopian environment, larval *An. arabiensis* are present mainly in small, temporary rain pools that are free of vegetation. Munga *et al*. (2006) also reported that deforestation of natural swamps created conditions favourable for *An. gambiae* breeding. Similar findings were also observed elsewhere (Killeen, 2006, Miller *et al*., 2007). The negative association between *An. gambiae* s.l. larval abundance and water current is also consistent with previous reports by Walker and Lynch (2007) who pointed out that *An. gambiae* s.l. prefers usually still water on which they can stay close to the surface with their orifice open to the air for breathing. Miller *et al*., (2007) also demonstrated that *An. gambiae* s.l. larvae are capable of terrestrial displacement whereby they can reach standing water. As far as *An. gambiae* s.l. is

concerned, as previously reported it does not lend itself to generalization, it can be found in a wide variety of water bodies (Fillinger, 2004).

The present study also revealed that the presence of mats of algae was key environmental factor positively associated with the abundance of *An. pharoensis.* This would be because as the results of this study indicated, *An. pharoensis* larvae were most frequently sampled (above 85%) from swamps and irrigation canals where algae are well established and grow during most of the study period. *An. pharoensis* larvae were mostly collected in habitats with stagnant water: however, larvae were also observed in flowing water where the presence of algae seemed to drastically reduce water current velocity within the habitat. Previous studies showed that algal growth is a key factor for the growth of anopheline species. For example, Manguin *et al*. (1996) reported that abundant algal growth was a key factor for the presence of *An. pseudopunctipennis* in fresh water stream pools. *An. pharoensis* larval abundance was also positively associated with water temeperature. This view is shared by Muturi *et al*. (2007) who reported that *An. pharoensis* larvae were significantly associated with water temperature.

Anopheles squamosus larval abundance was also significantly and positively associated with the presence of algae similar to that of *An. pharoensis*. This would be expected because significantly higher mean density of *An. squamosus* larvae was obtained from stable habitats specifically; natural swamps where algae are more established and grow. On the otherhand, larval abundance of *An. squamosus* was significantly associated with distance to the nearest house. This finding was surprising, as information on the medical importance of the species is lacking except that Abose *et al*. (1998) reported the occurrence of An. *squamosus* in their indoor and outdoor resting adult anopheline mosquito collection. As far as *An. squamosus* is concerned the present observation contradicts previous reports by Kibret (2008) who did not observe the occurrence of *An. squamosus* in his indoor and outdoor resting collection. Such variations would be expected because; the current study did not include adult mosquito survey unlike the

previous study. On the otherhand, unlike the present study the previous study did not include the most stable dry season anopheline larval habitats such as the swamps which are created and maintained by the perennial water bodies. The medical importance of this anopheline species remains to be explored.

Results of the present study also revealed that meteorological pattern of the study period and seasonal abundance of anopheline larval species were not significantly correlated. However, it was observed that *An. pharoensis* larval abundance was negatively correlated with average relative humidity and rain fall of the study period unlike larval abundance of *An. gambiae* s.l. This observation is consistent with seasonal larval abundance of the malaria vector species (Figure 4). Similar seasonal trend was also previousely reported by Abose *et al*. (1998) who observed that *An gambiae* s.l. density dominated *An. pharoensis* during the wet season unlike the dry season.

To this end, the other anopheline species larvae, *An. coustani* and *An. cinereus* were rarely sampled during the study period and as a result excluded from the statistical models as it is difficult to draw conclusions with limited data available.

6. CONCLUSION AND RECOMMENDATIONS

6.1. CONCLUSIONS

Drains and canals create and maintain dry season mosquito larval habitats in the study area among which natural swamps, sand pools, irrigation canals, and canal leakage pools are the most important. Five anopheline species were found in the study area. Namely, *An. pharoensis, An. gambiae* s.l. (presumably *An. arabiensis)*, *An. squamosus, An. coustani* and *An. cinereus.* Larvae of *An. pharoensis* were the most predominant species found in the area during the study period followed by larvae of *An. gambiae* s.l. Larval abundance of both *An. pharoensis* and *An. gambiae* s.l. were significantly positively correlated with each other. Larvae of *An. pharoensis* were most abundantly found in natural swamps and irrigation canals whereas larvae of *An. gambiae* s.l. were most abundantly found in sand pools during the study period.

At least six key environmental factors of the larval habitats were significantly associated with the occurrence of anopheline larvae in the study area. These included water temperature, presence of mats of algae, origin of the habitats, water depth, turbidity and water current. Water temperature and presence of mats of algae were positively associated with anopheline larval occurrence. Whereaswater current, turbidity and water depth were inversely associated with the larval occurrence

Four key environmental factors were significantly associated with the larvae of the two most abundant malaria vector species *An. gambiae* s.l. and *An. pharoensis*. These were water current, water temperature, aquatic vegetation and the presence of mats of algae. *An. gambiae* s.l. larval abundance was inversely associated with vegetation and water current. *An. pharoensis* larval abundance was positively associated with the presence of mats of algae and water temperature.

6.2. RECOMMENDATIONS

- The importance of dry season anopheline larval habitats that are created and maintained by perennial water bodies and irrigation waters and their role for continuous production of adult vectors and perennial malaria transmission needs to be considered in vector control operations and further research.
- Sand mining pits that are created for construction and other economic purposes were productive breeding sites for larval malaria mosquitoes in the study area. Therefore, it is essential to take suitable public health measures targeting these sites. These habitats are usually discrete and limited in number, so that anti-larval measures are very well suited to targeting these sites during the dry seasons, thus reducing the overall mosquitoes before the increase of habitat availability during the rainy season.
- Intersectorial collaboration between agriculture and health bureaus while planning and implementing water resources development projects in the area and continuous follow up is essential.
- To my knowledge this study is the first attempt to analyze the complex environmental variables that determine anopheline larval occurrence/abundance especially in drainage and irrigation areas in Ethiopia. There fore, further research that examine detailed analysis of water chemistry, ecology of mosquito larval predators and adult mosquito oviposition behaviour is needed.
- The present study examined the aquatic habitats that contained anopheline larvae, thus unsuitable habitats where larvae do not develop where not studied. Inclusion of such habitats may provide more information for sound understanding of anopheline larval ecology.

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APPENDICES

Appendix 1. List of current anopheline species found in Ethiopia.

An. arabiensis	*An. quadriannulatus* species B
An. funestus	*An. garnhami*
An. nili	*An. obscurus*
An. ardensis	*An. dancalicus*
An. gibbinsi	*An. longipalpus*
An. paludis	*An. rufipes*
An. christyi	*An. demeilloni*
An. hargreavsi	*An. maculipapis*
An. pharoensis	*An. salbii*
An. cinereus	*An. domiculus*
An. harperi	*An. marshalli*
An. pretoriensis	*An. sergentimacmahoni*
An. confusus	*An. dthali*
An. implexus	*An. natalensis*
An. rhodesiensis rhodesiensis	*An. seydeli*
An. coustani	*An. squamosus*
An. kingi	*An. turkuhudi*
An. rhodesiensis rupicolus	*An. tenobrosus*
An. cydippis	*An. wellcomi*
An. leesoni	*An. theilleri*
An. rivolurum	*An. ziemanni*

Source: (Verrone, 1962a, b).

Appendix 2. Number of anopheline larvae collected from different aquatic habitats per number of dips per month at six local sites over the study period /Dec. 2007 – June 2008/

Local site	Habitat type	No. of habitat	No. of dips per habitat	Dec.	Jan.	Feb.	Mar.	Apr.	May	June	Total
Adami Tulu	Swamp	1	30	15	214	27	139	40	139	114	688
Gerbi	CLP	1	30	0	92	56	0	24	-	-	172
	Irr.canal	1	30	0	0	0	0	24	144	80	248
Ziway	Irr. Canal	1	30	0	66	73	64	8	0	0	211
	Irr. Canal	1	15	0	0	32	0	0	0	0	32
	Irr. Canal	1	20	0	38	45	13	16	0	0	112
	Swamps	2	30	0	18	118	117	9	69	33	364
	BMP	1	20	-	-	-	-	27	23	12	62
	Rain pool	1	10	-	-	-	-	-	12	24	36
Abine	Swamp	1	30	0	102	36	16	12	90	42	298
Germama	Irr. Canals	3	20	12	36	35	57	0	0	0	140
	CLPs	2	20	0	0	49	28	30	0	0	107
Edo Gojola	Irr. Canals	1	30	15	46	23	69	30	0	0	183
	Swamp	1	30	0	15	19	30	30	49	12	155
	WHP	1	10	0	0	0	8	5	12	12	37
Meki	Sand pools	1	25	0	92	78	74	61	7	-.	312
	Sand pools	1	20	0	0	0	60	34	16	-	110
	Sand pools	1	10	0	0	66	65	35	6	-	172

CLP= Canal leakage pools, WHP= Water harvesting pool, BMP= Brick making pit

- (minus sign) indicates that no aquatic habitat was present.

Appendix 3. Number of *An. gambiae* s.l. larvae collected from different aquatic habitats per number of dips per month at six sites over the study period (Dec. 2007-June 2008)

Local site	Habitat type	No. of habitats	No. of dips perhabitat	Dec.	Jan.	Feb.	Mar.	Apr.	May	June	Total
Adami Tulu	Swamp	1	30	0	2	0	0	0	22	15	39
Gerbi	CLP	1	30	0	0	0	0	5	-	-	5
	Irr. canal	1	30	0	0	0	0	0	14	12	26
Ziway	Irr. Canal	1	30	0	0	3	3	0	0	0	6
	Irr. Canal	1	15	0	0	0	0	0	0	0	0
	Irr. Canal	1	20	0	0	0	0	0	0	0	0
	Swamps	2	30	0	0	2	0	0	22	19	43
	BMP	1	20	-	-	-	-	27	18	12	57
	Rain pool	1	10	-	-	-	-	-	8	16	24
Abine	Swamp	1	30	0	0	0	0	0	13	12	25
Germama	Irr. Canals	3	20	0	0	0	0	0	0	0	0
	CLPs	2	20	0	0	5	0	0	0	0	5
Edo Gojola	Irr. Canals	1	30	0	0	2	8	2	0	0	12
	Swamp	1	30	0	0	0	0	4	16	6	26
	WHP	1	10	0	0	0	1	0	8	8	17
Meki	Sand pools	1	25	0	53	57	50	26	7	-	193
	Sand pools	1	20	0	0	0	41	31	12	-	84
	Sand pools	1	10	0	0	41	48	32	3	-	124

Appendix 4. Number of *An. pharoensis* larvae collected from different habitats per number of dips per-month at six sites over the study period (Dec. 2007- June 2008)

Local site	Habitat type	No. of habitat	No. of dips perhabitat	Dec.	Jan.	Feb.	Mar.	Apr.	May	June	Total
Adami Tulu	Swamp	1	30	5	49	10	17	16	23	6	126
Gerbi	CLP	1	30	0	30	24	0	12	-	-	66
	Irr. canal	1	30	0	0	0	0	15	27	9	51
Ziway	Irr. Canal	1	30	0	35	19	15	6	0	0	75
	Irr. Canal	1	15	0	0	12	0	0	0	0	12
	Irr. Canal	1	20	0	35	15	3	8	0	0	61
	Swamps	2	30	0	12	57	76	9	10	9	173
	BMP	1	20	-	-	-	-	0	5	0	5
	Rain pool	1	10	-	-	-	-	-	4	6	10
Abine	Swamp	1	30	0	40	5	5	8	11	8	77
Germama	Irr. Canals	3	20	8	24	6	15	0	0	0	53
	CLPs	2	20	0	0	29	22	18	0	0	69
Edo Gojola	Irr. Canal	1	30	9	21	14	35	14	0	0	93
	Swamp	1	30	0	9	12	19	15	19	6	80
	WHP	1	10	0	0	0	2	4	4	4	14
Meki	Sand pools	1	25	0	11	1	8	4	0	-	24
	Sand pools	1	20	0	0	0	10	0	4	-	14
	Sand pools	1	10	0	0	0	8	3	1	-	12

Note: CLP= Canal leakage pool, BMP = Brick making pits, WHP = Water harvesting pool

- (minus sign) indicates that no aquatic habitat was present

Appendix 5. Number of *An. squamosus* larvae collected from different aquatic habitats per number of dips per month at six sites over the study period (Dec. 2007 – June 2008

Local site	Habitat type	No. of habitat	No. of dips perhabitat	Dec.	Jan.	Feb.	Mar.	Apr.	May	June	Total
Adami Tulu	Swamp	1	30	2	29	5	10	6	4	3	59
Gerbi	CLP	1	30	0	32	12	0	4	-	-	48
	Irr. canal	1	30	0	0	0	0	0	8	0	8
Ziway	Irr. Canal	1	30	0	7	14	3	2	0	0	26
	Irr. Canal	1	15	0	0	10	0	0	0	0	10
	Irr. Canal	1	20	0	15	10	3	6	0	0	34
	Swamps	2	30	0	9	16	23	0	0	0	48
	BMP	1	20	-	-	-	-	0	0	0	0
	Rain pool	1	10	-	-	-	-	-	0	0	0
Abine	Swamp	1	30	0	24	10	2	0	5	0	41
Germama	Irr. Canals	3	20	4	7	9	3	0	0	0	23
	CLPs	2	20	0	0	0	2	2	0	0	4
Edo Gojola	Irr. Canals	1	30	3	11	0	7	8	0	0	29
	Swamp	1	30	0	6	7	5	8	7	0	33
	WHP	1	10	0	0	0	0	0	0	0	0
Meki	Sand pools	1	25	0	2	0	0	0	0	-	2
	Sand pools	1	20	0	0	0	0	0	0	-	0
	Sand pools	1	10	0	0	0	0	0	0	-	0

Note: CLP= Canal leakage pool, BMP = Brick making pits, WHP = Water harvesting pool

- (minus sign) indicates that no aquatic habitat was present

Appendix 6. Number of *An. coustani* larvae collected from different aquatic habitats per number of dips per month at six local sites over the study period (Dec. 2007- June 2008)

Local site	Habitat type	No. of habitat	No. of dips perhabitat	Dec.	Jan.	Feb.	Mar.	Apr.	May	June	Total
Adami Tulu	Swamp	1	30	0	3	0	0	0	0	0	3
Gerbi	CLP	1	30	0	0	0	0	2	-	-	2
	Irr. canal	1	30	0	0	0	0	0	0	0	0
Ziway	Irr. Canal	1	30	0	2	14	6	4	0	0	26
	Irr. Canal	1	15	0	0	2	0	0	0	0	2
	Irr. Canal	1	20	0	0	1	0	0	0	0	1
	Swamps	2	30	0	0	6	13	0	0	0	19
	BMP	1	20	-	-	-	-	0	0	0	0
	Rain pool	1	10	-	-	-	-	-	0	0	0
Abine	Swamp	1	30	0	0	0	0	4	0	0	4
Germama	Irr. Canals	3	20	0	0	0	0	0	0	0	0
	CLPs	2	20	0	0	0	0	0	0	0	0
Edo Gojola	Irr. Canals	1	30	0	0	0	0	1	0	0	1
	Swamp	1	30	0	0	0	0	3	0	0	3
	WHP	1	10	0	0	0	0	0	0	0	0
Meki	Sand pools	1	25	0	0	0	0	0	0	-	0
	Sand pools	1	20	0	0	0	0	0	0	-	0
	Sand pools	1	10	0	0	0	0	0	0	-	0

Note: CLP= Canal leakage pool, BMP = Brick making pits, WHP = Water harvesting pool

- (minus sign) indicates that no aquatic habitat was present

Appendix 7. Correlation coefficients among the environmental variables of anopheline larval habitats sampled in irrigation and major drainage areas between Adami Tulu and Meki towns, Central Ethiopia.

	Intensity of shade	Turbidity	Vegetat.	Habitat perman.	Water current	Distance to nearest house	Water pH	Water temp.	Water depth	Elevatin	Origin of habitats
Intensity of shade											
Turbidity	0.093										
Vegetation	0.068	- 0.106									
Hab. permanence	-0.082	0.019	-0.696**								
Water current	-0.120	0.060	-0.217**	-0.003							
Distance to house	-0.285**	-0.147**	0.236**	-0.305**	0.161**						
Water pH	0.119	-0.019	0.076	-0.076	-0.063	-0.044					
Water temp.	0.135*	0.149*	0.264**	-0.292**	-0.220**	0.074	0.128				
Water depth	-0.030	0.152*	-0.081	-0.112	0.312**	0.014	0.162*	0.097			
Elevation	-0.086	-0.157*	-0.472**	0.576**	0.064	-0.025	0.040	0.145*	0.147*		
Origin of habitats	0.137*	0.020	-0.442**	0.495**	0.302**	-0.160*	0.030	0.247**	0.122	0.297*	
Presence of algae	0.081	-0.304**	0.564**	-0.461**	-0.359**	0.094	0.194*	0.379**	-0.139*	0.203**	-0.499**

* Correlation significant at the 0.05 level (2 tailed), ** correlation significant at the 0.01 level (2 tailed)

Apendix 8. Some economic activities associated with major drainage systems between Adami Tulu and Meki towns, Centeral Ethiopia

Plate 4. Meki town dwellers fetching residual pools as the river water dried due to prolonged dry season, April, 2008

Plate 5. Intensive sand mining activities at Meki River, April, 2008.

Plate 6. Irrigation schemes along Lake Ziway and Bulibula River, April, 2008

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ABSTRACT

A study was conducted to characterize larval habitats of anopheline mosquitoes in irrigation and major drainage areas between Adami Tulu and Meki towns, in the middle course of the Ethiopian Reft Valley. A total of 22 anopheline larval positive aquatic habitats were sampled fortnightly from late December 2007 to early June 2008 by using dipping techniques. Simultaneously, environmental variables of the larval habitats were also measured/estimated and recorded. A total of 3,439 anopheline and 5,213 culicine larvae were collected. Microscopic identification of the late instars (3^{rd} and 4^{th}) of anopheline larvae yielded 47.6% of *An. pharoensis*, 32.1% *An. gambiae* s.l. larvae (presumably *An. arabiensis*) and only 20.3% other anopheline larval species (*An. squamosus, An. coustani and An. cinereus*). *Anopheles* larvae were sampled predominantly from natural swamps, irrigation canals and sand pools with samples from these habitat types representing 88.0% (n= 3025) of the total anopheline larval collection in the study area. Larvae of the malaria vector species, *An. gambiae* s.l. (*An. arabiensis*) and *An. pharoensis* were most frequently sampled from sand pools and natural swamps, respectively. The habitats were characterized based on water temperature, turbidity, water current, water p^H, elevation, aquatic vegetation, habitat permanence, origin of the habitats (natural or human made), distance to the nearest house, presence of mats of algae, water depth, intensity of shade, and substrate type. Logistic regression analysis detected six key environmental variables that were associated with the occurrence/abundance of anopheline larvae: Water temperature, presence of mats of algae, water depth, and origin of the habitats, turbidity and water current. Multiple step-up regression analysis further detected four best predictor variables associated with larval abundance of the malaria vector species. Accordingly, relative abundance of *An. gambiae* s.l. larvae was significantly and inversely associated with aquatic vegetation and water current whereas that of *An. pharoensis* larvae were significantly and positively associated with water temperature and the presence of algae in the water bodies. Dry season anopheline larval habitats that are created and maintained by perenial water bodies such as the Ethiopian Rift Valley lakes and rivers and their associated water development projects need to be considered in vector control operation and further research.

1. INTRODUCTION

1.1. GLOBAL OVERVIEW OF MALARIA

Despite several years of research and concerted efforts at control, the realization of a malaria free world remains a dream (Okenu, 1999). The prevalence of the disease continues to increase in many parts of the world. An estimated two billion people (more than 40% of the world population) live in areas with malaria risk. The global annual incidence ranges between three to five hundred million clinical cases, with a death toll between two to three million (Okenu, 1999). The disease remains the most important cause of morbidity and mortality with enormous medical, economic and social impacts worldwide (WHO, 2005). More than half of the world's population is at risk of acquiring the disease, and the situation is worsening with the deteriorating health systems, growing drug and insecticide resistance, climate change and natural disasters (Martens and Hall, 2000).

The presence of the most efficient parasite, accompanied by the vector species and vulnerable human population are some of the factors that favor the transmission of malaria and the associated risk of disease and death (Gillies and Warrell, 1993). Severe malaria and malaria mortality are caused by *Plasmodium falciparum,* which is the predominant species of *Plasmodium* in tropical Africa, eastern Asia, and the Amazon area (Arrow *et al*., 2004). The most efficient malaria vector mosquitoes *Anopheles gambiae* complex occur exclusively in tropical and sub tropical part of the world, especially in Africa. Tropical areas of the world have the best combination of adequate rainfall, temperature and human host allowing for breeding and survival of malaria vector mosquitoes (WHO, 2006).

Malaria was eliminated from Europe, North America and parts of other continents as a result of deliberate programs of mosquito control and clinical treatment, as well as through generally improved social and living conditions. However, the eradication efforts are not successfully achieved in Africa's highly endemic areas (Breman *et al*., 2001). Today, sub-Saharan Africa remains the area of most malaria concentration, but significant problems also exist in Asia, Latin America, and focally in other areas (WHO, 2005).

Malaria re-emerged in several countries of central Asia with an increased frequency of epidemics and with the re-establishment of stable endemic transmission. Factors contributing to the increase in malaria include resistance of parasite to commonly used anti-malaria drugs, breakdown of control programs, poor local health services, resistance of mosquito vectors to insecticides (WHO, 2006; Howard *et al*., 2007). Other factors that expand malaria endemicity include: deforestation, water resources development projects, swamp drainage and specific crop intensification such as rice cultivation (Okenu, 1999; Keiser *et al*., 2005).

Malaria remains one of the world's greatest causes of child mortality and is an obstacle to social and economic development in Africa (WHO, 2000). The disease threatens the lives and livelihoods of more than 300 to 500 million Africans and exerts such a huge public health burden that it has been incriminated in the continued under development of the continent as the whole (Killeen *et al*., 2004). The overwhelming bulk of the world's malaria burden rests upon the population of sub-Saharan Africa because of the unique coincidence of expanding human populations, weak health systems, the world's most effecient vector mosquito species and environmental conditions ideal for transmission (Killeen *et al*., 2002). In sub-Saharan Africa, *An. gambiae* s.s., *An. arabiensis,* and *An. funestus* are the primary vectors of malaria parasites and show highly anthropopholic tendencies (Keating *et al*., 2004). The two most important members of the *An. gambiae* complex are *An. arabiensis,* with plentifully indoors or outdoors, and *An, gambiae* s.s.

with females more likely to bite humans indoors. Evidently these anophelines have coadapted to human ecosystem in the Afro-tropical savannah where their combined contributions to malaria transmission have apparently facilitated evolution of *falciparum* malaria (Mukabana *et al*., 2006).

The distribution, transmission intensity and clinical pathogenesis of malaria in Africa vary greatly across the continent. Africa experiences a complete spectrum of malaria epidemiology ranging from intense perennial transmission to unstable epidemic prone areas (MARA, 1998). The disease epidemics affect non-immune populations in many highland and semi-arid areas of the continent. It frequently affects highlands and semi-arid areas where populations lack immunity (Abeku, 2007). The control of malaria and its anopheline vectors in Africa is less successful because of the occurrence of drug resistance parasites and insecticide resistant vectors, change in the resting behavior of mosquitoes (from indoor to outdoor) as a result of frequent indoor insecticide sprays, lack of efficient infrastructure, shortage of trained manpower, lack of equipment, financial constraints, lack of appropriate management and inability to integrate several methods of control (Toure, 1999; Howard *et al*., 2007).

1.2. MALARIA IN ETHIOPIA

1.2.1. ANOPHELINE MOSQUITOES IN ETHIOPIA

Anopheles mosquitoes have a world-wide distribution, occurring not only in tropical areas but also in temperate regions. There are about 430 different *Anopheles* species (Service, 2000). From these, 123 species are known to be present in Africa (Coetzee *et al*., 2000) and 42 species have been recorded in Ethiopia (Ghebreyesus *et al*., 2006).

Much of the work on the identification and distribution of the *Anopheles* species in Ethiopia was carried out during the Italian occupation of 1938 to 1943 by the Italian

malariologists, during the latter half of the 1940s by the British and during the Malaria Eradication Service in Ethiopia in the 1960`s and early 1970`s (Mekuria, 1983; Gebremariam, 1984; Verrone, 1962a, b).

Extensive entomological surveys have established a good information base about type and distribution of anopheline mosquitoes. The 42 *Anopheles* species that have been recorded vary in distribution by altitudinal zone and microhabitats. Most species are confined to relatively small geographic areas, with the exception of certain malaria vectors such as *An. gambiae* s.l. (Ghebreyesus *et al*., 2006). The 42 anopheline mosquito species that have so far been recorded in Ethiopia are summarized in Appendex I based on the information obtained in (Verrone, 1962a, b).

1.2.2. MALARIA VECTORS IN ETHIOPIA

Human malaria can be transmitted only by anopheline mosquitoes (Gillies and Warrells, 1993). That is, in nature the only way the malaria organism is passed from person to person is through the bite of the *Anopheles* mosquito. Although *Anopheles* species are the sole vectors of the four parasites (*P. falciprum, P. vivax, P. malariae and P. ovale)* that cause human malaria, some *Anopheles* species are also vectors of filariasis (*Wuchereria bancrofti*, *Brugia malayi* and *B. timori*) and a few species transmit arboviruses worldwide (Kettle, 1995, Service, 2000).

There are over 400 species of *Anopheles* mosquitoes throughout the world however, only about 70 species are known to be malaria vectors under natural conditions (Service, 2000). In Africa, members of *An. gambiae* complex and *Anopheles funestus* are widely distributed and are responsible for the transmission of malaria in the region (Coetzee *et al*., 2000). From the *An. gambiae* complex, *An. melas* and *An. merus* are local vectors in west and east Africa respectively. *An. gambiae* s.s. and *An. arabiensis* are the most important malaria vectors in sub-Saharan Africa (Walker and Lynch, 2007). *Anopheles gambiae* s.s. is the most anthropophagic species in the complex and the most important, probably the world's most efficient malaria vector with characteristic indoor and outdoor

resting habits (Coetzee, *et al*., 2000). However, *An. gambiae* s.s. is not reported to be found in Ethiopia.

In Ethiopia, *An. arabiensis and An. quadriannulatus* species B are among the species of the *An. gambiae* complex that have been reported to occur (White *et al*., 1980; Mekuria *et al*., 1983; Hunt *et al*., 1998). The two species differ in their habits and medical importance. *Anopheles arabiensis* occurs in most areas of tropical Africa and could be considered as a major target for control as a major vector where malaria transmission is stable (WHO, 2006). It is the principal vector of epidemic malaria in all administrative regions of Ethiopia (Abose *et al*., 1998; Ghebreyesus *et al*., 2006).

Anopheles quadriannulatus species B is so far restricted to Ethiopia and is zoophilic with a negligible role in malaria transmission, whereas *An. arabiensis* is both zoophilic and anthropophilic and is the primary malaria vector in Ethiopia (White *et al*., 1980; Abose *et al*., 1998). Apart from *An. arabiensis*, *An. pharoensis, An, funestus* and *An. nili* are regarded as secondary vectors (Gebremariam, 1984; 1988; Ghebreyesus *et al*., 2006).

Anopheles arabiensis is responsible for most epidemics in the country. Detailed bionomic studies of *An. arabiensis* mosquitoes have led the way in designing appropriate vector control strategies. Although its larvae may be found around the shores of lakes and rivers, this mosquito species breeds most commonly in small unshaded temporary pools. Adults feed primarily outdoors but rest indoors (Ghebreyesus *et al*., 2006). *Anopheles pharoensis* on the other hand is widely distributed in localities adjacent to permanent water bodies. It breeds in shaded permanent water bodies such as swamps along lake and river shores and also in rise fields (Jolivet, 1959; Gillies and Demeillon, 1968; Service, 2000).

In endemic lowland areas of south western parts of Ethiopia, *An. nili* was regarded as an important vector of malaria. The species commonly breeds in lowland rivers mainly during the wet season when the rivers become over flooded (Nigatu *et al*., 1994). *Anopheles funestus* breeds in large more or less permanent water bodies shaded with

vegetation. However due to human induced ecological changes, such breeding habitats have been distributed in most parts of the country (Nigatu *et al*., 1994).

1.2.3. ANOPHELINE ECOLOGY AND MALARIA EPIDEMIOLOGY IN ETHIOPIA

Malaria epidemiology is driven by temporal and spatial patterns of vector species of anopheline 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's 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). Transmission is generally unstable and seasonal with its level varying from place to place because of altitude and rainfall patterns. The catastrophic malaria epidemic in the country in 1958 for example, was associated with unusually high rainfall over an extended period as well as with elevated temperatures and relative humidity (Fontaine *et al*., 1961). Unstable malaria occurs in most pats of the country particularly in the highland fringes where climatic conditions are conducive for its transmission (Gebremariam, 1984). The major transmission of malaria follows the June to September rains and occurs between September to December while minor transmission season occurs between April to May following the February to March rains (MOH, 2000). Some localities also experience perennial malaria, because of the conducive environmental and climatological situations that permit continual breeding of vectors (e.g. low land areas with permanent water bodies) (MOH, 2000; Ghebreyesus *et al*, 2006).

The most important determinants of anopheline ecology and malaria epidemiology in the country are altitude and climactic factors (mainly rainfall and temperature) although change in settlement patterns, drought and migrations could contribute a lot for the spread.

Areas below 2000 meters above sea level are classified as malasrious. However, a recent report has shown that malaria occurs in highland fringe areas including in urban sites; the main factor being climatic change (Abose *et al*., 1998; Woyessa, 2001; Jima, 2005).

A study in Bure District of north-western Ethiopia by Kebede *et al*. (2005) showed that there was an epidemiological link between maize agro-ecology (*Zea mays* cultivation) and an increase in malaria incidence. Maize cultivation contributed to the production of vector mosquitoes in diverse ways. Nutritional input into breeding sites close to maize pollen sources and human housing may increase vectorial capacity by allowing more mosquitoes to survive to adulthood, and to develop more quickly into larger and longer lived vectors (Ye-Ebiyo *et al*., 2003). Maize has served as an accelerator for vector density, mosquito longevity and malaria transmission (Kebede *et al*., 2005).

The country experiences three locally known climate zones namely, Dega (cold), Weyna Dega (temperate) and Kola (warm) zones. The Dega zone, at altitudes higher than 2,500m, is malaria free since the mean annual temperature of 10-15^{o}C is too low to support development and survival of the parasite in the mosquito vector. In the Weyana Dega zone, between 1,500 and 2,500 meters, where mean annual temperatures range from 15-20^{o}C, malaria most often occurs below 2,000 meters, with short-lived transmission following the rains. However, malaria epidemics have been recorded up to 2400 meters during periods when increased temperature and adequate precipitation are conducive for both vector survival and parasite development within the vector. These malaria epidemics are associated with significant morbidity and mortality since the population is non-immune. In the Kola zone below 1,500 meters, where mean annual temperatures are 20-25^{0}C, malaria transmission is endemic but limited by low rainfall and humidity to areas around permanent water sources (Ghebreyesus *et al*., 2006)

All four species of *Plasmodium* that cause malaria in man are known to be present in the country. However, the two epidemiologically important species are *P. falciparum* and *P. vivax* (Abose *et al*., 1998). About 60% of infections are borne by *P. falciparum* while

40% are of *P. vivax*. The other parasites *P. malariae* and *P. ovale* have little or focal significance (Ghebreyesus *et al*., 2006)

The distribution of humans and anopheline 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. It is also responsible for loss of earnings, low school attendance and high treatment cost (Ghebreyesus *et al*., 2006).

Traditionally, Ethiopian framers minimized the risk of malaria by living in the highlands. However, environmental degradation and increasing population densities are forcing people to occupy the potentially more productive lowlands, thereby putting themselves at greater risk and exposure to the disease.

The main components of malaria control in Ethiopia include the diagnosis and treatment of cases, the application of selective vector control measures and the strengthening of the information system to facilitate prevention, early detection and control of epidemics. Vector control is carried out mainly by means of environmental and chemical measures; either applied singly or in an integrated manner, and is based on local epidemiological conditions. In some areas, the community is actively participating in source reduction with malaria control and other health workers providing technical guidance. Results have been satisfactory, particularly in urban centers, settlement villages, and army camps and agro-industrial centers (Abose *et al*., 1998; Ghebreyesus *et al*., 2006; Lautze *et al*., 2007). As current challenges to prevention and control, other key factors contributing to the persistent malaria burden include population dynamics, war and social upheavals, poverty, water resources and agricultural development. (Ghebreyesus *et al*., 2006).

1.3. LARVAL ECOLOGY OF ANOPHELES MOSQUITOES

1.3.1. BREEDING SITE SELECTION

The habitat characteristics of larval mosquitoes may be determined by the oviposition behavior of gravid females. This is because breeding site is selected by the ovipositing female *Anopheles* mosquito (Kettle, 1995). The female mosquito commutes between blood meal source (host) and aquatic habitat (water) (Menach *et al*., 2005). That is the female mosquitoes emerge from water sources and fly to a blood-meal host, locating a host using a set of cues, including host movement, odor, carbon dioxide and body temperature (Carter *et al*., 2000). Thus, proximity of blood meal source to larval habitat is one important factor that affects breeding site selection in *Anopheles* mosquitoes (Reiter *et al*., 1995).

Oviposition is one potential factor explaining heterogeneous distribution in a landscape with a heterogeneous distribution of larval habitats. Adult female mosquitoes tend to aggregate around places where they oviposite, thereby increasing the risk of malaria, regardless of the suitability of the habitat for larval development (Menach *et al*., 2005).

Oviposition behaviour of mosquitoes and the cues used by *Anopheles* species to select the sites at which they oviposite between blood-meals remain poorly understood, except in very general terms. For example, *An. arabiensis* and *An. gambiae* s.s. typically breed in very transient habitats like shallow sunlit fresh water pools or human made habitats (Shililu *et al*., 2003), though they may also be common in rice fields (Robert *et al*., 1998; Minakawa *et al*., 1999). In contrast, *An. funestus* breeds mainly in marshes and other types of sheltered habitats that contain vegetation (Gillies ad Coetzee, 1987).

There is a general consensus that female mosquitoes are attracted to by-products of bacterial decomposition in aquatic habitats (McCrae, 1983). Volatile substances released from larval habitats have been implicated as potential olfactory cues mediating oviposition (ICIPE, 2003). The microbial fauna of larval habitats likely release volatiles

that may be used as oviposition cues or deterrents. It is not clear how other factors such as the presence of vegetation or the persistence of the habitat might influence the behavior of ovipositing females. It is unlikely that habitats are selected on the basis of these factors; rather these factors may be correlated with other characteristics that act as cues for ovipositing females (Gimnig *et al*., 2001).

When seeking novel avenues for ecological control of mosquitoes and mosquito-borne diseases, mosquito breeding behavior should receive more attention (Chen *et al*., 2006). Mosquito breeding behavior can play an important role in determining the distribution of malaria risk and its anopheline vectors (Menach *et al*., 2005). The types of aquatic habitat in which the adult female has a choice of laying its eggs differ in a number of ways: in size and appearance, in having still or flowing water, in the salinity of the water, in the degree of organic pollution, and consequent oxygenation of the water (Goma, 1966). Other factors such as habitat type, substrate type, semiochemicals, microbial fauna, predators, and vegetation and land cover types also affect the choice of aquatic breeding site by a female mosquito (Chen *et al*., 2006)

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.1 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 oviosition behavior (Chen *et al*., 2006).

1.3.2. BIOLOGICAL AND PHYSICOCHEMICAL FACTORS ASSOCIATED WITH LARVAL HABITATS OF ANOPHELINE MOSQUITOES

Presence of mosquito larvae in a collection of water is initially the result of the oviposition behavior of gravid females. The cues that influence oviposition seem to be a combination of biological and physical parameters of the water and the characteristics of the habitat in which the water is located (Stoops *et al*., 2007). Each mosquito species has its optimum abiotic and biotic characteristics that act as oviposition cues for gravid female mosquitoes and provide ideal environment for the development of the immature (Muturi *et al*., 2007).

The factors affecting larval survival and the mechanisms controlling adult production are largely unknown for even the most important vector species (ICIPE, 2003). Various environmental factors influence the suitability of aquatic habitat for larval anopheline mosquitoes (Ye-Ebiyo *et al*., 2003). The biotic and abiotic factors that affect life history traits such as growth, development and survival of the immature stages of *Anopheles* mosquitoes require more attention, as they will affect productivity in the breeding site and determine the abundance, distribution and fitness of the resultant adult mosquito populations, which will consequently affect malaria transmission (Paaijmans *et al*., (2006).

The environmental factors which determine the suitability of a potential breeding place fall into biological and physicochemical factors (Goma, 1966; Piyaratne *et al*., 2005). However, separating these factors is inherently difficult because many of the biological factors and physicochemical factors of aquatic habitats are highly correlated. For example, water temperature is correlated with the amount of shade provided by tree canopy and is also influenced by movement and volume of water (Stoops *et al*., 2007).

The biological and physicochemical conditions at the larval habitat affect larval development hence affecting the adult body size (Mwangangi *et al*., 2007). Body size affects factors such as longevity, fecundity, and blood meal volume and all these factors

may influence the fitness of the vector for malaria parasite transmission (Lyimo and Takken, 1993).

1.3.2.1. BIOLOGICAL FACTORS

Biological factors of *Anopheles* larval habitats influence larval survival and development and may affect the competence of the resultant adult anopheline vectors. For example, a study by Okech *et al*. (2007) found that organic substances in soil collected from active larval habitats with sand and clay substrates influenced larval development time, pupation rates and competence of *An. gambiae s.s.* to *P. falciparum* parasite. Results of the study further revealed that autoclaving the soils (that reduces presence of microbes) resulted in the production of significantly smaller sized mosquitoes. From this result they concluded an important nutritional role for organic matter and microbial fauna on mosquito fitness and vector competence.

Likewise, growth of *Anopheles* mosquito larvae on dietary microbiota in aquatic surface microlayers was demonstrated (Wotton *et al*., 1997). Hydrophobic organic matter accumulates under the surface film of water bodies to form the surface microlayers. Heterotrophic microorganisms use this organic matter for growth, and they in turn, are fed upon by *Anopheles* mosquito larvae and other animals. From laboratory experiments Wotton *et al*. (1997) showed that two species of mosquito larvae *An. gambiae* and *An. quadrimaculatus* grew most rapidly where surface microlayers were present and, especially, where labile dissolved organic matter was added to promote growth of microorganisms. Because sub-surface microorganisms are the components of the larval diet that most affect growth. Changes in habitat quality due to microbial transformation or in the organic matter content may directly govern the distribution of *Anopheles* mosquitoes and the risk of malaria transmission in endemic areas (Okech *et al*., 2007).

Other biotic factors that may affect survival and development of anophline mosquito larvae at their breeding sites include presence of algae, presence or absence of aquatic vegetation, presence or absence of predators, parasites, pathogens or cannibalism and

other interactions between species (Goma, 1966; Gimnig *et al*., 2001; Koenraadt and Takken, 2003; Paaijmans *et al*., 2007).

The importance of algal biomass in the surface microlayers of larval habitats has been studied. Rejmankova *et al*. (1993) demonstrated that there was strong association between *Anopheles* larval density and the distribution of filamentous algae. A study in western Kenya by Gimnig *et al*. (2002) found that chlorophyll[a] content and algae were positively correlated with *An. gambiae* density and body size. Chlorophyl[a] content in a habitat is an indication of presence of phytoplankton, which indicates the habitats dietary richness for the mosquito larvae (Kaufman *et al*., 2006; Mwangangi *et al*., 2007). The presence of *Anopheles* larvae in their aquatic habitats has been associated with biotic characteristics such as Plankton, suggesting a contribution by Plankton to the growth and development of their larvae (Gimnig *et al*., 2001).

The presence of floating and emergent vegetation can greatly influence what mosquito species are found in a habitat (Goma, 1966; Gimnig *et al*., 2001). For example, a study by Jacob *et al*. (2005) revealed that vegetation was the best predicator for *An. arabiensis* abundance and distribution. It has been suggested that aquatic vegetation promotes anopheline production because it provides a refuge for larvae from predatory, such as *Gambusia affinis.* Additional hypothesis for the beneficial effects of aquatic vegetation include enhanced food resource in vegetated regions, shelter from physical disturbances and favorable condition for oviposition (Omalley, 1992).

The presence of predators and parasites may influence oviposition site selection and larval survival in permanent habitats (Stoops *et al*., 2007). Robert *et al*. (1998) reported that low populations of larvivorous fish and invertebrate predators notably odonates were associated with abundant *An. arabiensis* larvae.

Competition is another biological factor that affects anopheline larval development and adult body size. Munga *et al*. (2006) found that larvae of *An. gambiae* s.s. responded to

increasing intraspecific competition by extending their development times and subsequently emerging as smaller adults. Koenraadt and Takken (2003) investigated some aspects of both inter and intra-specific interactions for three members of *An. gambiae* complex namely *An. Arabiansis, An. gambiae* s.s. and *An. quadriannulatus* under laboratory conditions. Among the aquatic developmental stages of the *An. gambiae* complex, both inter- and intra-specific interactions influenced the resulting densities of adult mosquito population. First-instar larvae were consumed by fourth instar larvae of the same species (cannibalism) and by fourth-instar larvae of other sibling species (predation). Even when larvae were not consumed, the presence of one fourth-instar larva caused a significant reduction in development rate of first-instar larvae. In general, biological conditions at the larval habitat affect larval development hence affecting the adult body size (Mwangangi *et al*., 2007).

1.3.2.2. PHYSICOCHEMICAL FACTORS

The occurrence and abundance of anopheline larvae is closely associated with physicochemical parameters. The importance of many chemical substances dissolved in the breeding water of anopheline 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).

Anolpheles species seem to be able to exploit specific habitat types with very different physical and chemical characteristics. Several studies have shown that physicochemical parameters of the larval habitats such as dissolved oxygen, water temperature and others influence anopheline larval occurrence, abundance and distributions (Goma, 1966; Robert *et al*., 1998; Gimnig *et al*., 2001; Ye-Ebiyo *et al*., 2003; Mwangangi *et al*., 2007).

Mwangangi *et al*. (2007) reported that dissolved oxygen, water p^H, turbidity, water depth, salinity, conductivity and temperature were associated with abundance of *An. arabiensis* larvae in a rice agro- ecosystem in Mwea, Central Kenya. The role of dissolved oxygen

as potential significant variable influencing abundance of *Anopheles* larvae has been reported by several studies (Grillet, 2000; Piyaratne *et al*., 2005; Muturi *et al*., 2007). However, anophelines respire primarily at the water surface, and this raises the question as to whether it is oxygen per se or an associated physicochemical or biological factor that influences the abundance of anopheline species (Muturi *et al*., 2007). In general, anopheline larvae seem to require more dissolved oxygen in their habitats (Grillet, 2000).

Water turbidity was found to be an important parameter associated with the abundance of anopheline larvae in their habitats. For example, Gimnig *et al*. (2001) found increasing *An. gambiae* s.l. larvae densities with increasing turbidity. Robert *et al*. (1998) found a clear-water preference by *An. arabensis* breeding in wells in urban Dakar, Senegal. A study by Ye-Ebiyo *et al*. (2003) found that the production of *An. arabiensis* was favored in moderately turbid water, although excessive turbidity limited the production of larvae. Water that is turbid from particles not edible by *Anopheles* species larvae could disfavour the production of larvae, whereas, water that is turbid from food particles represent a very suitable habitat. Most anopheline species breed in fresh water habitats and show preference for breeding in clean water and their larvae are seldom found in salt water and those heavily polluted habitats (Service, 2000).

Other important factors for the abundance of anopheline mosquito larvae in the habitats include p^H and conductivity. The effect of p^H is apparently an indirect one via the micro-fauna and micro-flora used as food by the mosquito larvae (Goma, 1966). A study by Mwangangi *et al*. (2007) found that p^H was a key factor associated with an increase in anopheline larval abundance. Various chemical properties of the larval habitat related to vegetation such as pH, ammonia, nitrate and sulphate affect larval development and survival (Mutero *et al*., 2004). Ammonia nitrogen, nitrate nitrogen, sulphate and phosphate are important factors regulating larval abundance (Sunish and Reuben, 2001). Nitrogenous fertilizer application accelerates multiplication of microorganisms which form the main diet for the mosquito larvae and also increase pupation rate (mogi, 1978). Nitrogen may be a limiting resource in anopheline larval environment (Gimnig *et al*., 2002). Agricultural activities enhance productivity of the existing larval habitats by

providing nutrients that enter the water bodies through surface runoff in the larval habitats (Munga *et al*., 2006).

Temperature is an important determinant of the distribution and relative abundance of individual species of mosquitoes (Lindsay and Martens, 1998; Mouchet *et al*., 1998). Several mosquito species are sensitive to temperature changes as immature stages in their aquatic environment and as adults. If water temperature rises, *Anopheles* larvae take shorter time to mature and consequently, there is a greater capacity to produce more offspring, during transmission period (Githeko *et al*., 2000). Below 16^0c, the aquatic stages of tropical anophlines fail to develop or breed (Lindsay and Birley, 1996) whereas, warming above 34^0c generally has a negative impact on the survival of most vectors and parasites (Githeko *et al*., 2000).

Anopheline species larvae seem to have optimum temperature ranges for survival and development. For example, Bayoh and Lindasay (2004) observed that under laboratory condition, *An. gambiae* s.s. larvae developed into adults at temperatures ranging from 16 to 34^{o}C. Larval survival was shortest (less than 7 days) at 10-12^{o}C and 38-40^{o}C and longest (greater than 30 days) at 14-20^{o}C. In India, temperature range of 28-32^0c provided the optimum conditions for egg, larval and pupal development (Piyaratne *et al*., 2005). Robert *et al*. (1998) found that warm temperature (28-30^0c), were associated with *An. anabiensis* abundance in urband Dakar, Senegal. Ameneshewa and Service (1996) reported that important *An. arabiensis* breeding sites in Gergedi area in the Awash River valley of Ethiopia are created by hot-springs that form marshy fields which serve as permanent breeding sites. Shililu *et al*. (2003) found a significant effect of temperature on larval densities and reported that the mean water temperature was variable among the different larval habitats ranging from 19.7^{o}C to 28.8^{o}C and larval density was positively correlated with water temperature. A study by Muturi *et al*. (2007) reported that *An. pharoensis* larvae was significantly associated with water temperature indicating that *Anopheles* species seem to breed at species specific optimum temperature ranges.

Temperature also influences the survival of the aquatic stages and subsequent adult production of malaria vectors. The warm water in sunlit habitats may be important for larval development as it allows more microorganisms to grow which provides food source for the mosquito larvae (Minakawa *et al*., 1999). Deforestation activities for purpose of agricultural expansions, human settlement and other development projects have caused a rise in air temperature that favour mosquito breading (Yasuoka and Levins, 2007). Munga *et al*. (2006) reported that deforestation and cultivation of natural swamps created conditions favorable for breeding and survival of *An. gambiae* larvae and consequently increase the risk of malaria transmission. Land cover type may affect larval survivalship and adult productivity through its effects on water temperature and nutrients in the aquatic habitats.

Precipitation (rainfall) is another abiotic factor that determines the survival and spatial and temporal distribution of *Anopheles* mosquitoes. Precipitation creates new breeding sites and adds water to existing ones. The availability, persistence and dimensions of mosquito larval habitats depend to a large extent on the frequency, duration and intensity of precipitation.

However, a study by Paaijmans *et al*. (2006) showed that rainfall significantly affected *An. gambiae* larval mosquitoes by flushing them out of their aquatic habitat and killed them. The significant loss of larvae due to rainfall will as a result decrease the larval density in a breeding site, which will lead to a lower competitive pressure for food and space. Whether such lower densities are advantageous for the development time and survival of the immature of *An. gambiae* is not clear. This study demonstrated that immature populations of malaria mosquitoes suffer high losses during rainfall events. As these populations are likely to experience several rain showers during their life span, rainfall will have a profound effect on the productivity of mosquito breeding sites and as a result on the transmission of malaria.

Previous works on larval ecology in general and on aquatic habitat factors associated with anopheline larvae in particular, is very limited in Ethiopia. Ye-Ebiyo *et al*. (2003)

found that the production of the principal malaria vector, *An. arabiensis* was favoured in moderately turbid water while excessive turbidity limited the production of larvae. The proximity to flowering maize (*Zea mays*) with pollen as food source compensated for the development failure induced by excessive turbidity. Water which is turbid from particles not edible for *Anopheles* species larvae could disfavor the production of larvae while water turbid from food particles represents a very suitable habitat.

1.3.3. LARVAL HABITATS OF THE MAJOR AFROTROPICAL MALARIA VECTOR SPECIES INCLUDING ETHIOPIA

A major reason for the study of mosquito larval ecology is to glean information on factors that may determine oviposition, survival and the spatial and temporal distribution of important disease vector species (Piyaratne *et al*., 2005). Moreover, such information would be very important for selecting and recommending appropriate larval control measures suited to a specific habitat.

The major malaria vector species in sub-Saharan Africa include two members of the *An. gambiae* complex *(An. gambiae s.s* and *An. arabiensis)* and *An. funestus* (Michel *et al*., 2006; Mutuku *et al*., 2006). The development of polymerase chain reaction methods to distinguish species with in the *An. gamiae* complex has allowed more precise study of the larval ecology of *An. gambiae* s.s. and *An. arabiensis* during the past decade (Shililu *et al*., 2007). Several studies observed temporal variations in abundance of the two species, suggesting that *An. gambiae* s.s. is usually the predominant species in saturated environments whereas An. *arabiensis* is more common in arid areas (Gimnig *et al*, 2001; Koenraadt *et al*., 2004).

An. arabiensis is the principal malaria vector that can adapt to different ecological locations in Ethiopia (Ghebreyesus *et al*., 2006). This species type predominantly exists in small sunlit breeding sites flourishing after cessation of the rainy seasons and known to play a crucial role in epidemic situations in the country (Jima *et al*., 2005).

An. arabiensis larvae is associated with small, sunlit, temporary habitats with algae such as foot prints, rain pools, puddles, tire tracks and garden wells (Robert *et al*., 1998; Gimnig *et al*., 2001). Their distribution is determined by the location of suitable water bodies and immature stages prefer usually still water in which they can stay close to the surface with their breathing orifices open to the air (Walker and Lynch, 2007). *An. arabiensis* is a typical r-strategist, colonizing temporary habitats in which selection favours rapid population increase. In general, larval predation is less prevalent in temporary habitats than in large, permanent habitats (Munga *et al*., 2006).

Distribution of larval habitats in Africa is highly dynamic. Meteorological fluctuations combine with underlying hydrology and human activity to generate a constant changing array of potential habitats. This is particularly true for notoriously opportunistic members of the *An. gambiae* s.l. for which the majority of habitats are usually man made (Killeen, 2006). It has been reported that *An. gambiae* s.l. larvae are amphibious. Eggs are more likely to be found outside than inside puddles. Eggs can develop and larvae can emerge on mud. Larvae are capable of terrestrial displacement whereby they can reach standing water (Miller *et al*., 2007).

Studies in urban Dakar, Senegal reported that environmental conditions associated with abundant *An. arabiensis* were warm temperatures (28-30^{0}c), clear and not too deep water (<0.5m), low concentrations of NaCl, low populations of larvivorous fish and invertebrate predators indicating that many contributing factors influence the ecology of the immature stages of *An. arabiensis* (Robert *et al*., 1998). *An. arahbiensis* breeds in residual pools such as the beds of drying streams. It was identified as a major malaria vector in irrigation schemes in Kenya (Ijumba, 1990). Studies in Eritrea showed that *An. arabiensis* production in the ephemeral natural aquatic habitats such as the streambed pools was high throughout the year and negatively associated with rainfall (Shililu *et al*., 2007). Breeding sites in arid areas are highly localized at permanent springs, river edges or irrigational projects, and are not affected by local rainfall (Coetze, 2004). *An. arabiensis* breeds in agro- ecosystem where maize pollen is abundant and readily ingested by their larvae promoting their growth and survival rate. It was noted to develop

readily in turbid water and when crowded, provided that their breeding sites are located where maize pollen is abundant (Ye-Ebiyo *et al*., 2003).

Anopheles funestus, one of the major malaria vector species in sub-Saharan Africa and the most anthropophilic vector known, exploits permanent and semi-permanent breeding sites such as marshes or rice fields. Its population density peaks in the dry season, extending malaria transmission by relay after *An. gambiae and An. arabiensis* population have declined (Michel *et al*., 2006). Gimnig *et al*., (2001) reported that *An. funestus* larvae were associated with larger, semi-permanent bodies of water containing aquatic vegetation and algae in western Kenya.

Anopheles pharoensis is primarily a species of large vegetated swamps and breed along lakeshores and among floating vegetations. It is found distributed in Africa (Gillies and Demeillon, 1968). *Anopheles pharoensis* larvae are associated with abundant grassy or floating vegetation and rice fields (Service, 2000). Abose *et al*., (1998) recorded considerable number of *An. pharoensis* from Lake Ziway with peak densities being in November. Indoor resting density of the vector also coincided with the larval density. On the other hand, Akililu (2008) recorded high densities of *An. pharoensis* in marshy areas of main Koka lakes on the last month of July.

1.4. WATER RESOURCES DEVELOPMENT AND MALARIA IN ETHIOPIA

Africa contains countless basins which together supply water for domestic, livelihood, and irrigation needs of the continent's inhabitants (Lautze and Kirshen, 2007). Ethiopia has risen in the forefront in water resource development in Africa: Gilgel Ghibe dam was completed in the Omo/Ghibe basin in 2004, the Koga dam in the Blue Nile/Abay basin was completed in 2006, the Tendaho and Kessem dams are under construction in the Awash basin and two more large dams are also under construction in the Atbara/Tekeze

basin (Tarekegn, 2006, cited in Lautze *et al*., 2007). Impounded water will soon become a prominent feature of Ethiopia's landscape (Lautze *et al*., 2007).

Water impoundment has frequently been accompanied by serious health hazards in Ethiopia. Increased incidence of malaria and schistomiasis, for example, has been recorded in a number of water projects in various parts of the country. The Birr and Koga irrigation project was expected to exacerbate such health hazards as malaria, schistomiasis and river blindness (Rahmato, 1999).

Malaria transmission is dependent upon many hydro-ecological factors that directly affect the vectorial competence including the presence of suitable habitats for the development of preadult anopheline species (Kengluecha *et al*., 2005). Human intervention in the form of hydro- electric dams, irrigation projects, new settlements and open pit mining, has altered natural ecosystems in many countries and paved the way for the emergence of different malaria vectors with increased vector breeding sites (Robert *at al*., 2003; Surendran and Ramasamy, 2005).

The development of irrigation schemes by dam construction has led to an increased risk of malaria in Tigray, Ethiopia (Yohannes *et al*., 2005). Ghebreyesus *et al*., (1999) found that malaria incidence in young children was seven fold higher in communities near dams than those further away. Yohannes *et al*. (2005) found that seepage pools from microdams, irrigation canal pools and other man-made pools were the major breeding habitats of *An. arabiensis*. The study further indicated that the increased malaria associated with the dams resulted from a rise in mosquito numbers was caused by more breeding habitats in fields irrigated with water from the dams or from water seeping from the foot of the dams.

A recent study on Koka water reservoir (large dam) in the Rift Valley of Ethiopia found that malaria case rates among people living within 3km of the reservoir are about 1.5times as great as for those living between 3 and 6km from the reservoir and 2.3 times

as great for those living 6-9km from the reservoir (Lautze *et al*., 2007). This implies that the Koka reservoir and other water impoundments and irrigation areas are responsible for the proliferation of malaria vectors and the disease in the country.

1.4.1. THE RELATIONSHIP BETWEEN MALARIA VECTORS AND IRRIGATION IN ETHIOPIA

The relationships among hydro-agricultural development, mosquitoes and malaria are particularly close (Robert *et al*., 1998). Anopheline breeding sites are prone to change in accord with agricultural development, deforestation or irrigation (Service, 1991). Irrigation systems in both rural and urban arid regions may create fresh water environments that are suitable for mosquito immature (Burroni *et al*., 2007). Expansion of irrigation is a key development strategy in Ethiopia. In many arid areas, fast population growth and subsistence agriculture dependent entirely on rainfall have resulted in household food insecurity. The government has given priority to changing the agrarian system in such areas from rain fed to small-scale irrigation agriculture, through run-off harvesting in micro dams and ponds (Kassahun, 2007). A study by Kassahun (2007) reported that malaria is the leading health hazard induced by rain water harvesting (RWH) and indicated that a good correlation exists between malaria transmission and pond ownerships in the study areas. However, it would be very controversial to attribute the resurgence of malaria to the expansion of RWH in the country. As witnessed in east African highlands (Munga *et al*., 2006), malaria has shown rapid expansion in highland areas which used to be non-malarious. Therefore, it is worth nothing that the recent malaria resurgence, especially in RWH expansion areas, is compounded with the changes in the climatic and eco-epidemiology (Kassahun, 2007).

In less arid ecological zones, agricultural development has focused on expansion of cash crop farming with further development of large-scale irrigation for sugarcane, cotton, and fruit plantations. Large dams are also under construction for the purposes of hydropower generation to meet growing industrial and domestic electricity needs (Ghebreyesus *et al*.,

2006; Kassahun, 2007). Dams and irrigation schemes transform ecosystems thereby changing malaria risk. Canals and drains create ideal breeding sites for malaria mosquitoes, bringing both the vector and the disease closer to people (Boelee, 2004).

In the irrigated fields, mosquito abundance will increase and if these mosquitoes have enough food sources in the breeding sites, the resulting adults may live longer and allow malaria parasites to complete their development cycle so that they can be passed on to another host. This allows year round transmission of the disease (Keiser *et al*., 2005). Breeding sites created by the construction of thousands of small dams in Tigray, Ethiopia, have been shown to increase the incidence of malaria in communities near the dams by a factor of seven (Ghebreyesus *et al*., 2006). Other irrigation structures, such as wells, may provide permanent breeding sites with few larval predators close to human habitations, as Robert *et al*. (1998) observed in urban Dakar, Senegal.

Intense irrigation based agricultural activity is going on in areas between Koka and Adami Tulu which favor breeding and activity of *Anopheles* mosquitoes (Alemu, 2007; Akililu, 2008; Kibret, 2008). Irrigation agriculture may create new breeding sites or increase the productivity of certain breeding sites. Irrigated rice fields are known to breed *An. gambiae* s.l. particularly before the rice vegetation canopy is well developed (Ijumba and Lindsay, 2001).

Irrigation is the most common means of ensuring sustainable agriculture and coping with periods of inadequate rainfall and drought (Rahmato, 1999). Nonetheless, there is concern that the introduction or expansion of irrigation systems in malaria endemic areas may lead to a risk of malaria transmission by creating more breeding habitats for vectors and extending the length of transmission season (Ijumba an Lindsay, 2001). Important practical and feasible steps in the planning and operational stages can significantly mitigate the adverse effect of irrigation projects and dams on malaria in Ethiopia (Ghebreyesus *et al*., 2006).

1.5. LARVAL CONTROL OF MALARIA VECTORS

Ethiopia is one of the few African countries with a history of malaria control strategies for more than 40 years (Ghebreyesus *et al*., 2006). The current control strategies include: indoor residual spraying (IRS), impregnated bed nets (ITNs), that target adult mosquitoes and environmental management for vector control mainly through source reduction during transmission seasons (MOH, 2000; Jima *et al*., 2005; Ghebreyesus *et al*., 2006). Health authorities throughout the country seek to reduce malaria by IRS with dichlorodiphenyltrichlorethane (DDT) or Malathion in selected villages (Lautze *et al*., 2007). Domestic vector control interventions against adult mosquitoes in the form of ITNs or IRS have enormous potential to reduce vector-human contact and community level malaria transmission (Walker and Lynch, 2007). However, even these highly effective interventions are insufficient to eliminate malaria transmission from most endemic parts of Africa (Gu *et al*., 2003).

Thus, integrated mosquito management (IMM) has been advocated as a critical element to help combat malaria (Gu and Novak, 2005). As integral components of IMM, the importance of larval interventions recently regained the attention in the professionals after a long obsolete status in malaria control (Killeen *et al*., 2002). Larval control is not an entirely new strategy for managing malaria. Historically, many successful campaigns of mosquito eradication had heavily relied on management of larval habitats. For example, source reduction through modification of larval habitats was the key to malaria eradication efforts in the United States, Israel, Italy, Brazil and Egypt (ICIPE, 2003; Killeen *et al*., 2002).

Larval habitat should be a focal point for malaria intervention. Mosquito control methods should aim at intervention during each stage of the mosquito's life cycle. The breeding habitat is crucial for mosquito population dynamics, because it is the location where many important life cycle processes take place; oviposition, larval development, emergence, and mating (Overgaard *et al*., 2001).

One advantage of targeting larvae for control is that they cannot escape from their breeding sites unlike the adult mosquitoes which could easily avoid control measures (Killeen *et al*., 2002). The other advantage of larval control is that while, domestic adulticides such as IRS reduce human-vector contact rather than reduction of the vector population, larval control measures by contrast are intended to reduce vector population density near human habitations (Keiser *et al*., 2004).

Some form of larval control may be a helpful supplement to IRS or ITNs, particularly during the dry season when vector larvae are concentrated in relatively few breeding sites (Fillinger *et al*., 2004). Unlike insecticide based vector control that targets adult mosquitoes, non-chemical measures such as environmental management and biological control, pose virtually no risk of environmental contamination, and human expose to pesticides. Non-chemical larval control may also provide a valuable contribution to resistance management programmes, through the prevention or delay in the onset of vector resistance to insecticides used for ITNs or IRS (Walker and Lynch, 2007).

To control mosquitoes, whether adults or larvae, it is crucial to understand the relevant ecology of the target species. This requires the study of not only the fluctuations of the adult populations, but also the factors affecting larval abundance and distribution (Gimnig *et al*., 2001). Knowledge of the ecological characteristics of the breeding habitat and what environmental factors affect mosquito abundance can help in designing optimal vector control strategies (Overgaard *et al*., 2001; Surendran and Ramasamy, 2005). An integrated approach to malaria control that relies heavily on community involvement is one that may have the bright future. The foundation of any such approach should be source reduction to reduce the level of malaria transmission in an area. This strategy needs to be based on a sound understanding of the local ecology and behavior of the vectors (Yohannes *et al*., 2005).

Although malaria is a major public health problem and larval control is an important component of the malaria control program in Ethiopia, little is known about larval habitats of malaria vectors, their distribution in space and environmental factors that

affect their production that would be important for planning and implementing appropriate larval control strategies. Characterization of larval habitats based on biological and physiochemical factors is important for understanding the complex interactions among immature stages of malaria vectors and the biotic and abiotic components of their aquatic environments.

Although previous entomological studies on anopheline vectors in the area is encouraging (Abose *et al*., 1998; Seyoum *et al*., 2002; Ye-Ebiyo *et al*., 2003; Alemu, 2007; Akililu, 2008; Kibret, 2008), little is known about aquatic habitats of anopheline larvae in irrigation and drainage areas in and around Rift Valley lakes. Information about environmental factors associated with the occurrence and abundance of *Anopheles* larvae in the area is also lacking. The basic question of the current study was therefore, which environmental factors of *Anopheles* larval habitats are significantly associated with the occurrence/abundance of the mosquito larvae?

2. OBJECTIVES OF THE STUDY

2.1. GENERAL OBJECTIVES

To study aquatic habitat characteristics associated with larval habitats of *Anopheles* mosquitoes in the areas between Adami Tulu and Meki towns for effective planning and implementation of anopheline vector larval control.

2.2. SPECIFIC OBJECTIVES

1. To determine the types and diversity of aquatic habitats where anopheline larvae occur (positive breeding sites of anopheline larvae) in the study area.

2. To assess the species of anopheline mosquito larvae occurring in the local habitats and determine their relative abundance in the area under consideration.

3. To determine the effect of environmental variables of the larval habitats on anopheline larval occurrence/abundance in the study area.

3. MATERIALS AND METHODS

3.1 STUDY AREA

The study was undertaken between December 2007 and June 2008 in irrigation and major drainage areas located between Adami Tulu and Meki towns in the Rift valley of Ethiopia. The study area is situated about 150 to 170 km south of Addis Ababa on the main road to Shashemene and Awassa that included part of Lake Ziway and its drainages (Figure 1).

The area is semi-arid and the terrain is relatively flat. Its vegetation mainly consists of scattered *Acacia* trees and thorn bush and subjected to intensive grazing and agriculture. The rainfall pattern is similar to other Ethiopian regions where the big rainy season begins in June and extends up to September while the short rainy season begins in March and extends to April/May, but usually it is very erratic. The area has a fairly warm climate with mean annual temperature of 20.69^0c with annual maximum and minimum temperature of 27.22^0c and 14.16^0c respectively. The mean annual rainfall of the area is 20.69mm and the mean annual relative humidity is 59.10% (Figure 2). Elevation of the study localities range from 1636-1670meters above sea level (m. a.s.l.).

The main topographic feature of the area is Lake Ziway, with an area of 434km^2, a depth of about 4 meters and a length of about 25kms (Abose *et al*., 1998). The lake is a backbone for agriculture and fishing (the main economic activities in the area). It is a major source for large and small scale irrigation and agriculture. It is a source of irrigation water, pure water supply for the urban and rural areas, and a recreation area. The swamps along the shoreline of the lake are also a good pasture land for livestock herding. The other major drainage areas with similar economic importance include Meki and Bulbula Rivers. Both rivers are associated with Lake Ziway in that Meki river drain into the lake from the north while Bulbula River flow out of the lake and flows to south under the eastern foothill of Adami Tulu town and finally enters Lake Abjata, one of the other Rift Valley lakes. Both river systems also serve as active economic activities for

fishing, small and large scale irrigation, sand mines for construction as well as sources of drinking water for people residing near them (Adami Tulu, Meki and Gerbi villagers).

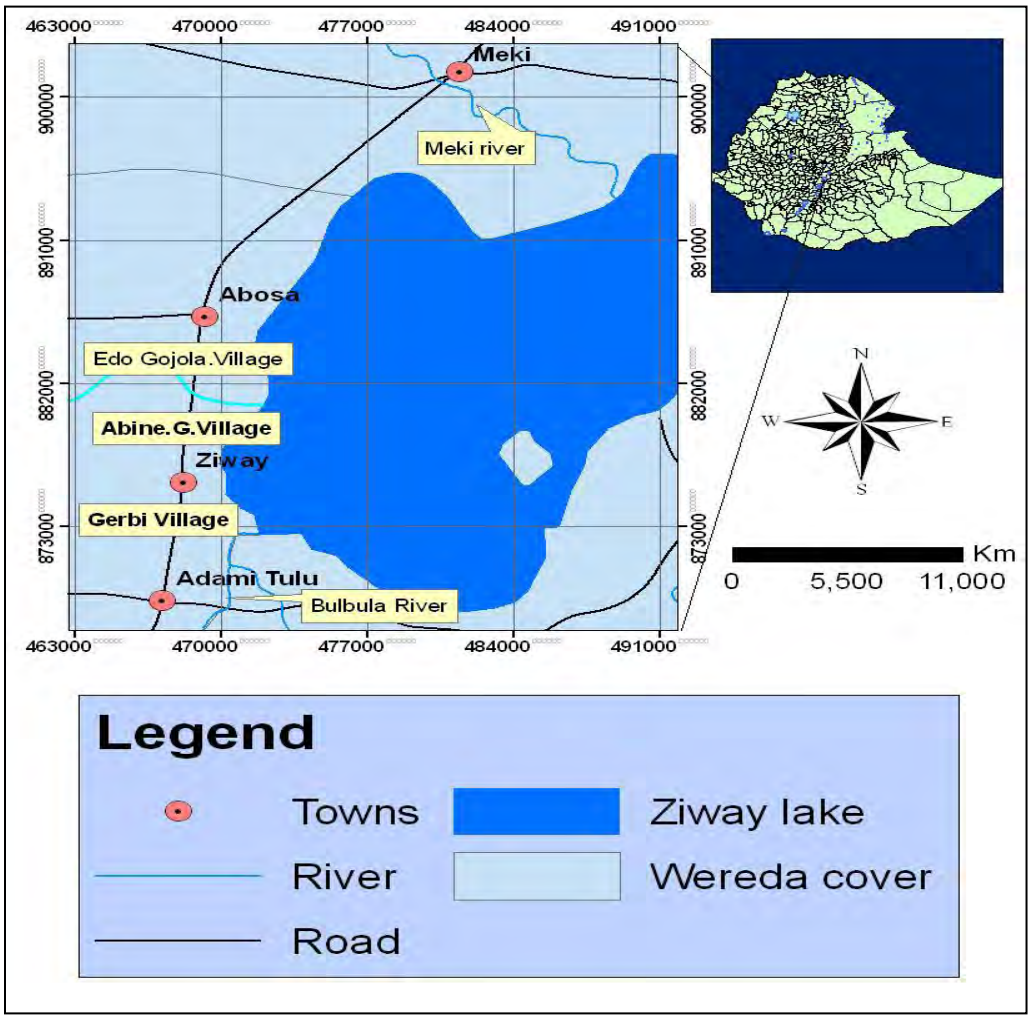

Figure 1. Location of the study area in Ethiopia and map of the study localities

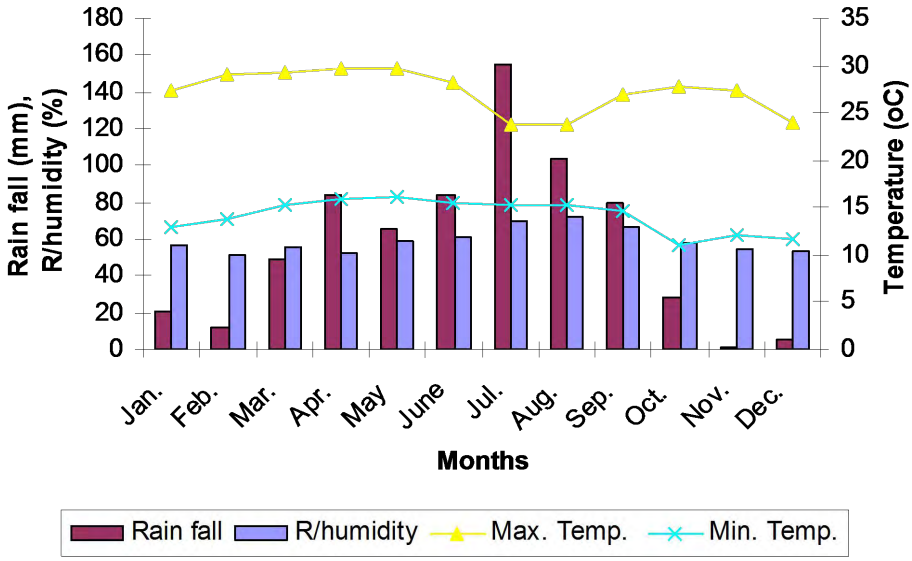

Figure 2. The meteorological pattern of the study area (1998 – 2007)

Source: The National Meteorological Services Agency, Addis Ababa

According to the information obtained from Meki Agricultural office, Lake Ziway, Meki and Bulbula rivers and ground water are the three major sources of irrigation water in the area. The area grows rain-fed maize and other cereal crops during the rainy season (usually June to October) and mainly vegetables such as onions, tomatoes, potatoes, green paper and other vegetables by irrigation during the dry season (November to May).

The present study covered irrigation and major drainage areas in three selected rural towns (urban areas) and three rural farming villages (Kebeles). The urban areas are Adami Tulu, Ziway and Meki towns while the villages included Gerbi Waroma Boromo (formerly Gerbi Gilgile), Abine Gemama and Edo Gojola (Formerly Edo Kontela).

1. Adami Tulu is situated about 5km south of Ziway town on the road to Awassa at $07^{0}51'$N, $38^{0}42'$E. Its altitude is 1661m. a.s.l. Its population size is about 4460 inhabitants, predominantly of the Oromo ethnicity as informed by Adami Tulu District Administration office (Nura Abaso, Pers. Comm.). Water from Bulbula River has made it possible to irrigate and develop a number of small scale farms owned by local people and private enterprises such as Ethio-flora Farm. They are engaged in cultivating cash

vegetables such as onions, green beans and orchards along Bulbula River. Water is pumped directly from the river bank by irrigation motors planted at intervals along the sides of the river mainly through plastic and metal pipes into irrigation fields. Over flooding of the river during the rainy season and its recession during the dry season has created swamps along both sides of the river that favor mosquitoe breeding.

2. Gerbi Waromo boramo is a rural village consisting of about 4645 inhabitants (Nura Abasso, Pers. Comm.). It is located between Adami Tulu and Ziway town at 07º53'N, $38^0$42’E and about 1658m. a. s. l. East of Gerbi village between the road and Bulbula River is found the former Ziway (Gerbi) State farm now owned by Castil Private Limited Company (Castil Vineyard farm). The former state planted a large power house (irrigation motors) on the edge of the river bank. The motors pump water through three large metal pipes uphill and drain into a large cemented primary canal. This large surface canal runs a long distance in the savanna land and drains into secondary and tertiary canals that finally feed the entire vineyard and maize fields. At the junction of secondary and tertiary canals water usually leak out and form pools that serve as breeding grounds for mosquitoes beside interrupted canals and swamps along the river.

3. Ziway (Batu) is the capital of Adami Tulu district, Eastern shoa zone in Oromia regional state. Ziway is a commercial as well as administrative centre. It is located at $7^0$56’N, $38^0$42’E, about 163km south of Addis Ababa. Its elevation is about 1646m. a.s.l. The total population of the town is approximately 41,225 individuals (Nura Abasso, Pers. Comm.). It is also overpopulated by local and migrant laborers from different parts of the country being attracted by job opportunities Intensive large and small scale irrigation farms have been developing on the edges of the town along shore of Lake Ziway. One of the largest agro-industry developments in the area is Sher Ethiopia flower farm, which is situated along the lake on south outskirt of the town. Many town dwellers also grow vegetables and fruits by draining the lake water using surface canals along the lake from Gedam sefer in the south up to Kidanemiheret church in the north. These surface canals and water pools in the swamp, form permanent and semi-permanent breeding sites for mosquitoes. Brick pits that support mosquito breeding are also there largely due to mud

brick making practice of the local people for house construction. It is commonly practiced in the area along the lake during dry season.

4. Abine Germama is found at the north outskirt of Ziway town by the side of the road from Addis to Awassa. It is located at $7^0$57'N, $38^0$42'E. Its elevation is 1647m. a. s. l. Different ethnic groups live in harmony in this village that consists mainly of Oromo and Silte. They depend on subsistence agriculture and livestock herding and also by fishing on Lake Ziway. The land on both sides of the road along the lake shore is intensively irrigated. In this area, several surface motor canals lead lake water to motors planted at intervals that pump water a long distance through water pipes into fields. Here in addition to lake pools in the swamps, the surface canal pools, canal leakage pools, serve as potential breeding sites for anopheline and other mosquitoes.

5. The other rural village selected for the study was Edo Gojola. It is found next to Abine Gemama in the north about 4 to 5 kilometers from Ziway town on the main road. It is located at $7^0$58'N, $38^0$43'E. Elevation of the area is 1653m. a.s.l. There are about 2915 inhabitants in this village. Most of the villagers use hand-pumped and Windmill-pumped ground water for domestic consumption and rely on Lake Ziway for agriculture and fishing. The lake water is the major source of irrigation for growing vegetables such as Onions, cabbage and tomatoes in the area. They use surface irrigation and as a result interrupted canals, and lake pools in the swampy land along the lake provide breeding sites for mosquitoes.

6. Meki town is another study locality situated adjacent to Lake Ziway in the north which is drained by river Meki. It is located at $8^0$90'N, $38^0$49'E. Its elevation is 1670m. a. s. l. The total population of the town was about 34,863 individuals (Kadir Abdu, Pers. comm.). In addition many local laborers from the surrounding rural areas and migrant laborers from different corner of the country inhabit in the town. The laborers are attracted by job opportunities created by vegetable farms and other economic activities in the area.

River Meki flows in the midst of the town from Shoa highlands down steep slop and drain into Lake Ziway. The river flows on sandy substratum that has been deposited since time in memorial. Intensive sand mining activity takes place in the river bank mainly during the dry season. The sand because of its good quality for large building constructions is directly loaded and transported mainly to Addis Ababa and Adama (Nazhret). Local laborers and farmers mine sand from the river bank usually during dry seasons when the river water dry out (shed) due to evapotransportation and porous nature of the substrates. As a result sand mining pits block the water flow and create pools. These sand pools are ideal breeding sites for mosquitoes.

Just below Meki town botanical nursery site, both sides of the river are also irrigated. Motor pumps are placed at intervals in order to pump water uphill directly from deep in the river bank through plastic pipes into irrigation fields. There were no surface canals between the river bank and irrigation farms. However there were unlined surface canals at uplifted soil mass in the irrigation fields. The surface canals feed the agricultural farms. Water pools were rarely observed in the irrigation fields largely due to porous nature of the soil.

3.2. SAMPLING SITE SELECTION

Three rural towns (urban areas) and three rural villages (kebeles) totally six local study sites were selected based on proximity to major drainage areas and the presence of large and small scale irrigation farms. Selection of the villages and urban areas (sampling sites) was also guided by local guides who had good knowledge about the area. Accordingly, all permanent and semi permanent anopheline positive larval habitats present in and with in 500m radius of the irrigated farm of each selected village/town (Shililu *et al*., 2003) and 700m along the major drainages (lake or river) (Gimnig *et al*., 2001) were sampled for mosquito larvae. All the study localities are situated along the drainage areas.

3.3. ENTOMOLOGICAL STUDIES

3.3.1. LARVAL MOSQUITO COLLECTION AND IDENTIFICATION

Mosquito larvae were sampled at fortnightly interval from December 2007 up to early June 2008 during the dry (December-February) and short rainy seasons (March-June). During each survey a habitat was first inspected for the presence of mosquito larvae by dipping technique (Plate 1). Larvae were collected by using a ladle (11.5cm diameter and 350ml capacity per dip), pipettes, and white plastic pans (Sharma, 1990; Service 1993a).

When mosquito larvae were present 10-30 dips depending on the size of the habitats were taken at intervals along the edge of each larval habitat (Shililu *et al*., 2003). Dipping was done by gently submerging the ladle till just below the water-air interface usually at the edge of the larval habitat (Service, 1993a).

Samples were always taken by the same individuals (myself and a field assistant). During each sampling, the habitat was visited at the same morning (0900-1200hours) or afternoon (1400-1700 hours) hours. Thirty minutes or less was spent at each site sampling for larvae and recording of environmental variables of the larval habitats. We attempted to sample the same sites or sites in the same area. However it was not always possible because some of the sampling sites were inaccessible on some occasions due to over flooding and drying out. A value of 0 was recorded for sites that were dry, flooded with running water and for suitable sites with no larvae per number of dips (Claborn *et al*., 2002).

Larval count was done on all collected mosquito larvae in the field. However, the late instar anopheline larvae (III and IV instars) were preserved and used for species identification. Larval density was initially recorded as per number of dips in the field and later converted to per 100 dips because the number of dips taken was variable depending on the size of the habitats and the number of larvae sampled was also low (Shililu *et al*., 2003). From each habitat larvae were always transferred into large vials with the habitat

water by direct pippeting and labeling and then brought to a room where I used to stay during the field work. The larvae were then killed in warm water (about 60^0c) by gently holding over ethyl alcohol soaked-cotton flame (Balkew, Pers. Comm.). All third and forth instar anopheline larvae were thus preserved in small vials containing 70% ethyl alcohol (Service, 1993a). Vials were labeled with relevant information such as date, site and number of larvae collected and transported to Addis Ababa.

The preserved *Anopheles* larval specimen were brought to the laboratory of Akililu Lema Institute of Pathobiology (ALIPB), Addis Ababa and mounted individually on a microscopic slide in gum chloral mountant by properly arranging its orientation and identified under a light microscope with the objectives x10 and x40 (Plate 2). Larval identification was based on identification keys of Verrone (1962b) and Gellies and Coetzee (1987). Voucher specimens of *Anopheles* larval species for each local site of the study area were deposited at ALIPB Vector Biology and Control Research Unit, Addis Ababa University.

Plate 1. Larval mosquito inspection from a sand pool at Meki River

Plate 2. Larval anopheline mosquito identification at Akililu Lema Institute of Pathobiology

3.3.2. LARVAL HABITAT CHARACTERIZATION AND RECORDING OF ENVIRONMENTAL VARIABLES

Simultaneously with larval mosquito sampling, environmental characteristics of the larval habitat were measured or estimated and recorded at the location for each habitat. The environmental variables recorded were water temperature, pH, water depth, elevation, intensity of shade, turbidity, vegetation type, water current, substrate type, distance to the nearest house, whether the habitat was natural or human made (origin of the habitat) and the presence of algae and permanence of the habitat. Most of the environmental variables are already well established with regard to their associations with mosquito larval development.

Water temperature was measured using LCD portable Digital Multi-stem Thermometer (ST-9269 A/B/C-model). Water pH was measured using p^H indicator (Viac. Imbonati 2420159 Milano (Italy). Water depth was measured using a metal ruler (Minakawa *et al*., 1999). Water current was determined by visual inspection and categorized as slow flowing and still (Muturi *et al*., 2007).

Turbidity that was mainly caused by suspended organic matter was estimated by placing water samples in glass test tubes and holding against a white background and categorized as either clear or turbid (Minakawa *et al*., 1999; Mwangangi *et al*., 2007). Intensity of shade was visually categorized as light and shade. The type and presence of aquatic vegetation was observed and recorded as emergent, floating, emergent plus floating and none if no vegetation at all. Emergent plants included both aquatic and immersed terrestrial vegetation.

Substrate types were classified in to mud or soil, and cement or concrete by visual inspection. Distance to the nearest house was measured with a tape when it was shorter than 100m. When the distance exceeded 100m, it was measured by foot step (Gebre-Michael, Pers. Comm.). Then, distance to the nearest house was categorized in to 4 classes (e.g. 1 = 0---100m, 2=100--- 300m, 3=300---500m and 4 for -distances greater

than 500m (Minakawa, *et al*., 1999). It was believed that anthropophilic species would breed near human dwellings so that increasing more anopheline breeding habitats.

The presence or absence of mats of algae (green algae) was visually determined. All visual classifications and physical measurements were done by the same person (myself) to maintain consistency. The coordinates of local sample sites, larval habitats and their elevations were determined using a hand-held geographical positioning system (GPS: Garmin, 12, KS, USA).

3.4. METEOROLOGICAL DATA COLLECTION

Monthly rainfall, minimum and maximum mean temperature, and relative humidity data of the study area for the period of ten years (1998-2007) were kindly obtained from the National Meteorological Services Agency (Addis Ababa).

3.5. DATA ANALYSIS

Data analyses were done using SPSS software (version 13.0 for Windows, SPSS Inc., 2000). Percentile score was used to compare the frequency of occurrence and abundance of *Anopheles* larvae among habitat types and distribution of their species within the habitats. Variations in larval counts (mean densities) among habitat types, variations in mean densities of the collected larvae among environmental factors (characteristics) of the larval habitats were analyzed using one way analysis of variance (ANOVA) test. Larval density was expressed, as number of larvae sampled per 100 dips (Shililu *et al*., 2003). When significant differences were observed in ANOVA, the Tukey test was used to separate the means.

Pearson correlation analysis was used to determine the association among the environmental variables and also to assess the relationship between anopheline larval densities and environmental factors of the larval habitats. That is for each environmental

variable, simple correlations between larval occurrence/abundance and individual parameters were first checked and only significant associations further examined by multiple logistic regression. Logistic regression analysis (Odds ratio) was used to detect key environmental factors associated with anopheline larval occurrence. Step-up multiple regressions were used to determine the best predictor variables associated with relative abundance of the larval species of anopheline mosquitoes including the malaria vectors. Results were considered significant at $p< 0.05$.

4. RESULTS

4.1. SPECIES COMPOSITION OF ANOPHELINE LARVAE

In total, 8,652 mosquito larvae were collected from different aquatic habitats in six local study sites between Adami Tulu and Meki towns in the Rift Valley of Ethiopia (Table 1). Culicine and anophelinae larvae comprised 60.3 and 39.7% of the mosquito fauna, respectively. Both anopheline and culicine larvae were predominantly collected from Ziway. Larvae of the two mosquito sub-families were strongly significantly correlated ($r= 0.48$, 2-tailed, $p< 0.01$) suggesting that larvae of culicine and anopheline coexist in the water bodies currentely examined.

Table 1. Total number of mosquito larvae collected during fortnightly visits from late December 2007 to early June 2008 in irrigation and major drainage areas of six localities between Adami tulu and Meki Towns, Central Ethiopia

	Local sites							
	Adami Tulu	Gerbi	Ziway	Abine Germama	Edo Gojola	Meki	Total	%
No of habitat	1	2	7	6	3	3	22	
Anophelinae	688	420	817	545	375	594	3439	39.7
Culicinae	563	767	2122	760	602	399	5213	60.3
Total	1251	1187	2939	1305	977	993	8652	100.0

From the totat 3,439 anopheline larvae collected, some 62.1% (2134) were late instars (3^{rd} and 4^{th} instars). These were examined microscopically for species identification that

yielded five *Anopheles* species among which *An. pharoensis, An. gambiae* s.l. (Presumably *An. arabiensis)* and *An. squamosus* were the major species whereas *An. coustani* and *An. cinereus* were generally scarce (Table 2). *An. pharoensis* (47.6%, 1015) and *An. gambiae s.l.* (32.1%, 686) were the most abundant species in the study area, respectively. The other *Anopheles* species: *An. squamosus, An. coustani* and *An. cinerues* comprised only 20.3% (433) of the total anopheline larvae collected.

Table 2. Number of late instar (3rd and 4th instars) anopheline mosquito larvae collected from irrigation and major drainage systems in the six localities between Adami Tulu and Meki towns (Dec. 2007-June 2008)

	Local sites							
Anopheles spp.	Adami Tulu	Gerbi	Ziway	Abine Germama	Edo Gojola	Meki	Total	%
An. gambiae s.l.	39	31	130	30	55	401	686	32.1
An. pharoensis	126	117	336	199	187	50	1015	47.6
An. squamosus	59	56	118	68	62	2	365	17.1
An. coustani	3	2	48	4	0	4	61	2.9
An. cinereus	0	0	0	7	0	0	7	0.3
Total	227	206	632	308	304	457	2134	100.0

4.2. ANOPHELINE LARVAL DENSITIES DURING THE STUDY PERIOD

As depicted in Figure 3, *Anopheles* larvae were observed during every month of the study period. Mean densities of the anopheline larval populations underwent marked monthly variations, with their minimum mean density in December and maximum mean density in March.

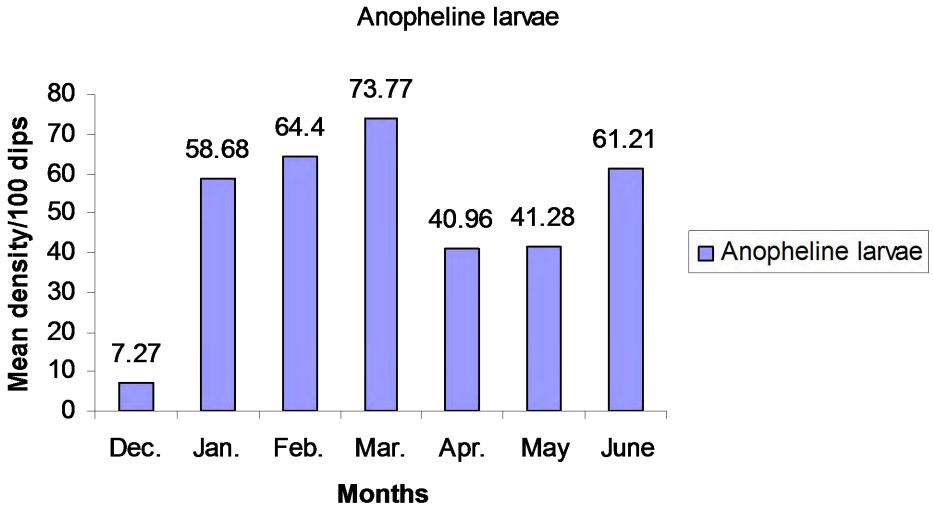

Figure 3. Mean density per 100 dips per month of anopheline larvae collected from irrigation and major drainage areas between Adami Tulu and Meki towns during the study period Dec. 2007- June 2008

As it can be seen from Figure 4, larval densities of both *An. gambie* s. l. and *An. pharoensis* generally rose up from December to March with higher densities of *An. pharoensis* than *An. gambiae* s.l. However, after March through May, larval densities of both species went down, with more densities of *An. gambiae* s. l. than *An. pharoensis*. After the on set of rain in late May, larval density of *An. gambiae* s. l. steeply rose up. Mean larval density of *An. squamosus* tended to increase during the initial larval survellience with peak density in January and thereafter declined with a similar trend with that of *An. pharoensis* larvae.

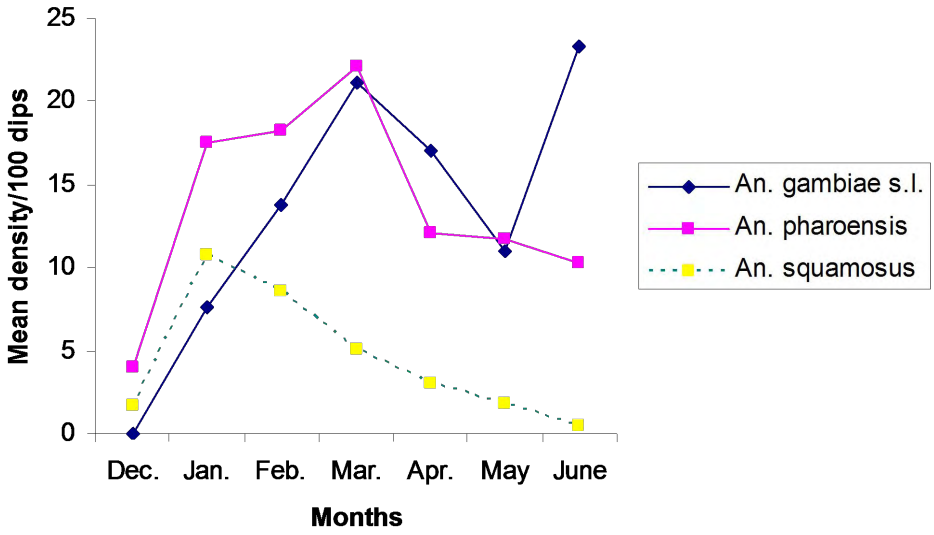

Figure 4. Mean density per 100 dips per month of the major anopheline species in the study area

4.3. HABITAT DIVERSITY AND LARVAL DENSITY

4.3.1. ANOPHELINE LARVAL HABITAT DIVERSITY

In total seven anopheline positive larval habitat types which are mainly permanent and semi-permanent breeding sites were identified in irrigation and major drainage areas between Adami Tulu and Meki towns in the middle course of the Ethiopian Rift Valley (Plates 3). The habitat types included swamps, irrigation canals, canal leakage pools, sand pools, water harvesting pool, brick making pit, and sand pool.

A. Natural swamps.

B. Irrigation canals.

C. Canal leakage pool (left) and sand pools (right).

Plate 3. Typical permanent and semi permanent mosquito breeding habitats in irrigation and major drainage areas between Adami Tulu and Meki towns, Central Ethiopia

Sampling proportion of the habitat types are shown in Figure 5. Irrigation canals (36.4%,96) and swamps (22.7%,60) were the most abundant habitat types most frequently sampled in the study area, respectively, while canal leakage pools and sand pools occurred in the same proportion (13.6%,36) from the total habitat types. Water harvesting pool, brick making pit and rain pool all together comprised only 13.5% (48) of the total anopheline positive habitat types identified. The number of rain pools sampled was few due to unsual extended dry season and even after the on set of rain in late May most of the fresh rain pools observed were negative for anopheline larvae.

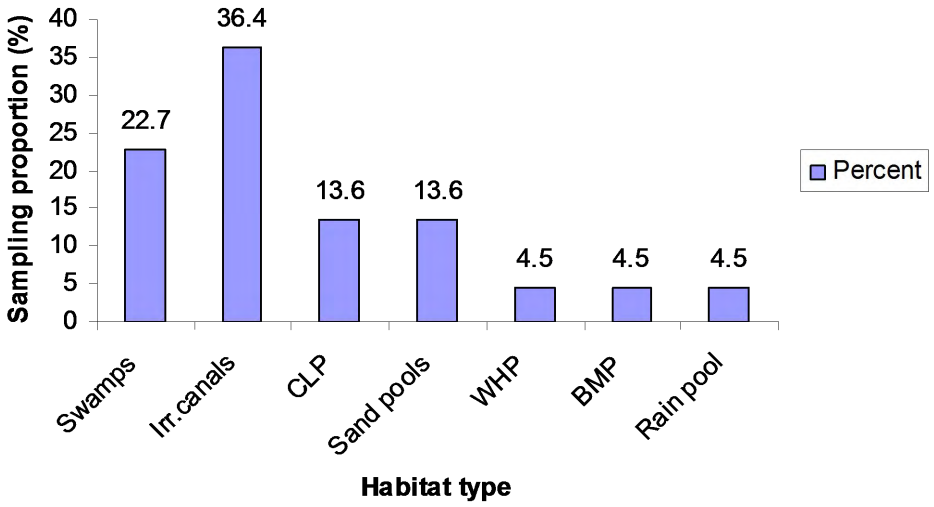

Figure 5. Anopheline larval habitat types in the study area and their sampling proportions during fortnightly survey from December 2007- June 2008.

CLP= Canal leakage pools, WHP= Water harvesting pool, BMP= Brick making pit

4.3.2. OCCURRENCE OF ANOPHELINE LARVAE IN THE AQUATIC HABITATS

The relative importance of the different larval habitats with regard to anopheline larval presence and production is shown in Table 3. Anopheline larvae were sampled predominantly from swamps and irrigation canals. Collections from these habitat types comprised 43.8% (n=1505) and 26.9% (n=926) of the total *Anopheles* larvae collected respectively.

In other words, nearly greater than 70% (n=2431) of the total anopheline larvae collected were from swamps and irrigation canals while the other habitat types such as canal leakage pools, sand pools, brick making pit and rain pool contributed only 29.3%(n=1008) of the total anopheline larvae sampled.

The Brick making pit and rain pool contained anopheline larvae in all of its sampling times. Swamps (68.3%, 41) and sand pools (60.7%, 17) were most frequently contained *Anopheles* larvae respectively whereas canal leakage pools and water harvesting pool are nearly in the same proportion in containing anopheline larvae. On the other hand, 66.7% (8) of the water harvesting pool, 65.6% (21) of the irrigation canals sampled were found negative for anopheline larvae respectively.

Table3. Number of anopheline larvae collected from different larval habitats and proportion of aquatic habitats with larvae sampled during the fortnightly survey (December 2007-June 2008)

Larval habitat type	Total no. of habitats (Frequency)	% larval habitat positive	% larval habitat negative	Total *Anopheles* larvae	% of total *Anopheles* larvae
Swamps	60	68.3	31.7	1505	43.8
Irr. canals	96	38.5	61.5	926	26.9
Sand pools	28	60.7	39.3	594	17.3
CLP	32	34.4	65.6	279	8.1
WHP	12	33.3	66.7	37	1.1
BMP	4	100.0	0.00	62	1.8
Rain pool	2	100.0	0.00	36	1.0

Note: CLP= Canal leakage pools, WHP= Water harvesting pool, BMP= Brick making pit
Negative and positive samples were taken from the same habitats on different occasions

4.3.3. HABITAT TYPES AND MEAN DENSITIES OF ANOPHELINE LARVAE

The mean *Anopheles* larval density over the sampling period was 52.22 larvae per 100 dips. There was variable contribution of each aquatic habitat with regard to larval production (Figure 6). Results of ANOVA and Tukey′s honestly significantly differences showed that mean densities of anopheline larvae collected from sand pools were significantly higher compared with the other habitat types ($F = 3.766$, $df = 6,257$, $P < 0.05$). However, in relation to long-term contribution to larval production swamps and canals were more important because they had water available for anopheline larval development and they were therefore, sampled more frequently for mosquito larvae compared with the other habitat types. Sand pools and swamps were the most productive aquatic habitats for the anopheline larvae sampled in the present study.

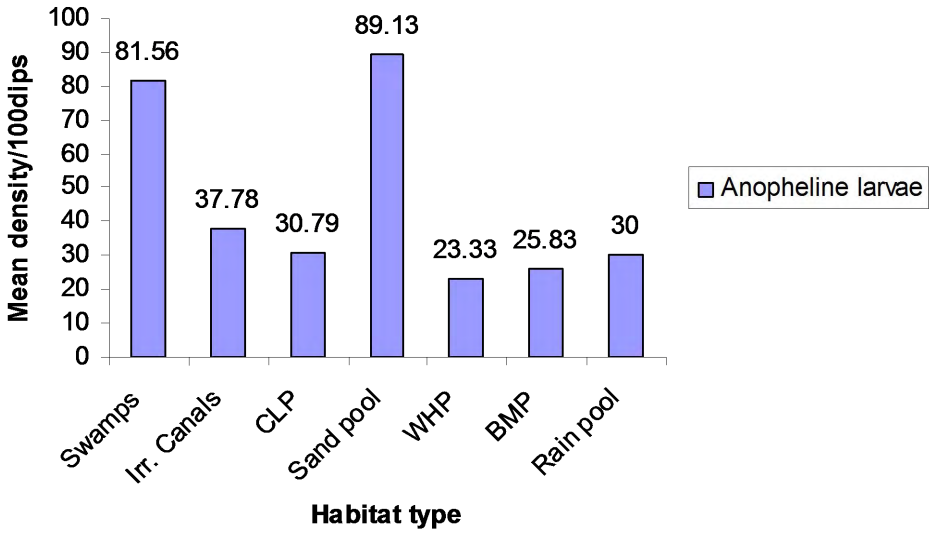

Figure 6. Mean density of anopheline larvae collected from different larval habitats during the study period December 2007 to June 2008

CLP= Canal leakage pool, WHP= Water harvesting pool, BMP= Brick making pit

Likewise, significant variation in the relative importance of the larval habitats at local villages and town level was also observed. Accordingly, mean density of anopheline larvae collected from the swamp habitat in Adami Tulu was significantly higher compared to the other larval habitats in the other local study village and towns ($F = 9.553$, $df = 5258$, $P < 0.05$).

4.3.4. HABITAT DISTRIBUTION OF ANOPHELINE LARVAL SPECIES

Table 4 depicts spatial distribution of *Anopheles* species larvae in different larval habitats during the study period. *An. gambiae* s.l. larvae were collected most abundantly from sand pools (58.5%, 401) and swamps (19.4%, 133) whereas irrigation canals, canal leakage pools, water harvesting pools, brick making pit and rain pool together comprised 22.2% (152) of the total *Anopheles gambiae* s.l. larval collection during the study period. Nearly 80% (803) of the total *an. pharoensis* collected was obtained from swamps and

irrigation canals while only 21.1% (214) of the same species were sampled from other aquatic habitats. Likewise, greater than 85% (311) of the total *An. squamosus* larvae were obtained from swamps and irrigation canals. The rest of *An. squamosus* (14.8%, 54) were obtained from canal leakage pools and sand pools. Water harvesting pool, brick making pit and rain pool didn't harbor *An. squamosus* during the study period. *An. coustani* and *An. cinereus* scarcely occurred in swamps and irrigation canals and generally absent from other habitat types.

Table4. Distribution of *Anopheles* species in different types of larval habitats in irrigation and drainage areas between Adami Tulu and Meki towns during December 2007- June 2008

Larval habitat type	*Anopheles gambiae* s.l. N (%)	*Anopheles pharoensis* N (%)	*Anopheles squamosus* N (%)	*Anopheles coustani* N (%)	*Anopheles cinereus* N (%)	Total N (%)
Swamps	133(19.4)	456(44.8)	181(49.6)	29(47.5)	7(100.0)	806(37.7)
Irr. canals	44(6.4)	347(34.1)	130(35.6)	30(49.2)	0	551(25.8)
Sand pool	401(58.5)	50(4.9)	2(0.5)	0	0	453(21.2)
CLP	10(1.5)	135(13.3)	52(14.2)	2(3.3)	0	199(9.3)
WHP	17(2.5)	14(1.4)	0	0	0	31(1.5)
BMP	57(8.3)	5(0.5)	0	0	0	62(2.9)
Rain pool	24(3.5)	10(1.0)	0	0	0	34(1.6)
Total	686(32.1)	1017(47.6)	365(17.1)	61(2.9)	7(0.3)	100.0

Note: CLP= Canal leakage pools, WHP= Water harvesting pool, BMP= Brick making pit

4.3.5. HABITAT TYPES AND MEAN DENSITIES OF THE LARVAL SPECIES

Expressed as number of larvae per number of dips of sampling, relative abundance of anopheline species in the different larval habitats was also significantly variable (Figure 7). For example, comparing mean densities of *Anopheles* species larvae that were sampled from the different habitat types revealed that *An. pharoensis* ($F= 3.212$, $df= 6, 257$, $p<0.05$) and *An. gambiae* s.l. ($F= 13.370$, $df=6, 257$, $p<0.05$) were the most abundant species in swamps and sand pools, respectively. *An. squamosus* ($F=3.744$, $df= 6, 256$, $p< 0.05$) also more colonized swamps than the other habitat types.

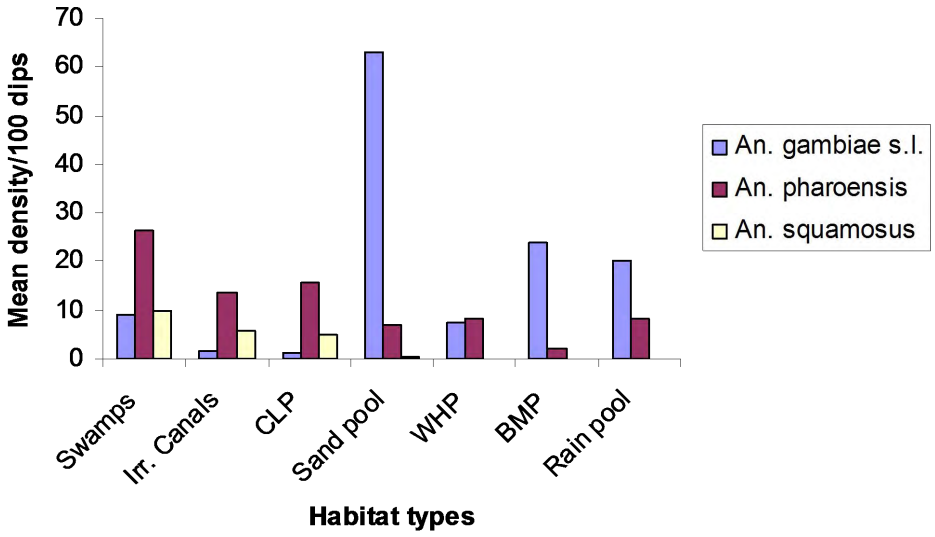

Figure 7. Mean densities of *An. gambiae* s.l., *An. pharoensis* and *An squamosus* larvae collected from different aquatic habitats in the study area (December 2007-June 2008).

4.3.6. RELATIVE MONTHLY CONTRIBUTION OF THE AQUATIC HABITATS TO ANOPHELINE LARVAL PRODUCTION

Relative monthly contribution of the different larval habitats to anopheline larval production over the study period is shown in Table 5. Larval production occurred during all months all over the study period in all the study sites. Larval abundance was generally low during the dry season (December to February) and high during the short rainy season (March to early June). It decreased in the dry season and increased with the onset of the wet season in all the study sites. However, peak densities of larvae were achieved at different months. In Adami Tulu, maximum anopheline larval density was collected in January with its minima in June, while in Gerbi village, the maximum larval density occurred in May with its minimum density in April. In Ziway and Abine Germama village, peak larval activity concentrated between February to April. In Edo Gojola and Meki town, the highest numbers of anopheline larvae were sampled in March and February, respectively.

Table 5. Relative monthly contribution of the different larval habitats to anopheline larval production at six sites over the study period December 2007 to June 2008)

1Local site	Habitat	Dec.	Jan.	Feb.	Mar.	Apr.	May.	June	Total
Adam Tulu	Swamp	50	713.34	90	463.33	133.33	103.36	80	1633.36
Gerbi	CLP	0	306.67	186.67	0	80	-	-	573.34
	Irr. canal	0	0	0	0	80	480	266.67	826.67
Ziway	Swamp	0	60	393.33	390	30	120	110	1103.33
	Irr. canals	0	410	681.66	278.33	150	0	0	1519.99
	BMP	-	-	-	-	135	115	60	310
	Rain pool	-	-	-	-	-	120	240	360
Abine	Swamp	0	340	120	53.33	40	300	140	993.33
Germama	Irr. canals	60	180	175	285	0	0	0	700
	CLP	0	0	245	140	150	0	0	535
Edo Gojola	Swamp	0	50	63.33	100	100	163.33	40	516.66
	Irr. canal	50	153.33	76.67	230	100	0	0	610
	WHP	0	0	0	80	50	120	120	370
Meki	Sand pools	0	368.67	802	12.46	764	168	-	3348.67

Note: CLP= Canal leakage pools, BMP= Brick making pit, WHP= Water harvesting pool

- (minus sign) indicates that no aquatic habitat was present

Larval density was expressed here as number of larvae per 100 dips (Shililu *et al*., 2003)

4.4. FACTORS ASSOCIATED WITH LARVAL OCCURRENCE/ ABUNDANCE IN DIFFERENT AQUATIC HABITATS

4.4.1.. CORRELATION BETWEEN ABUNDANCE OF ANOPHELINE LARVAE AND ENVIRONMENTAL VARIABLES

Before proceeding to regression analysis (regression models) to determine key environmental variables, correlation analysis was made for each environmental variable and abundance of anopheline larvae (Table 6). As it can be seen from Table 6, half of the variables that is 50% (6 of 12) of the environmental variables were significantly correlated with density of anopheline larvae. *An. gambiae* s.l. larvae were significantly correlated with elevation; aquatic vegetation, habitat permanence, water current and distance to the nearest house. *An. pharoensis* larvae were significantly correlated with most of the environmental variables. That is, 66.7% (8 of 12 variables) of the environmental variables examined were significantly correlated with the abundance of *An. pharaensis* larvae. *Anopheles squamosus* larval density was also significantly correlated with half of the environmental variables currentely examined (50%, 6 of 12 correlation coefficients).

Table 6 Correlation coefficients between environmental variables and densities of anopheline larvae and its major species

Environmental variables	Total anopheline	*Anopheles gambiae* s.l.	*Anopheles pharoensis*	*Anopheles squamosus*
Water temperature	0.196**	0.029	0.349**	0.145*
Water depth	-0.129*	-0.067	0.150*	-0.088
Elevation	-0.082	0.131*	-0.106	-0.136
Intensity of shade	0.003	-0.002	-0.007	0.102
Turbidity	-0.214**	-0.047	-0.137*	-0.201
vegetation	0.069	-0.304**	0.284**	0.152
Habitat permanence	-0.035	0.302**	-0.172**	0.262
Water current	-0.264**	-0.180**	-0.207**	-0.261**
Distance to house	-0.043	-0.213**	0.101	0.164*
Natural habitats	-0.147*	-0.078	0.201**	-0.188**
Presence of algae	0.330*	0.084	0.426**	0.421**
Water P^H	0.018	-0.062	0.048	0.198**

* Correlation significant at the 0.05 level

** Correlation significant at the 0.01 level

4.4.2. ASSOCIATION BETWEEN HABITAT CHARACTERISTICS AND DENSITIES OF ANOPHELINE LARVAE

Tables 7, 8, 9 and 10 depict characteristics of larval habitats and mean densities of anopheline larvae. Significantly higher densities of anopheline larvae were collected in larval habitats that were natural and had clear standing water with emergent plus floating vegetation and mats of green algae. Intensity of shade, permanence of larval habitats and the distance between the larval habitats and the nearest house did not significantly affect larval densities among the habitats (Table 7).

Significantly higher mean densities of *An. gambiae* s.l. larvae were obtained from aquatic habitats that had clear and standing water, free of vegetation and also temporary habitats nearest to human dwellings (< 100m). Intensity of shade, origin of the habitats, presence of algae and water turbidity were not siginificantly associated with mean densities of *An. gambiae* s.l. (Table 8).

Significantly higher mean densities of *An. pharoensis* larvae were collected from Permanent and natural habitats that had clear and standing water with mats of algae. Intensity of shade and distance to the nearest house were not significantly associated with mean density of *An. pharoensis* larvae (Table 9).

Likewise, mean densities of *An. squamosus* larvae collected from clear water, permanent habitats with emergent plus floating vegetation, still water, and 100- 300m from human dwellings and that had mats of algae were statistically significant. Whereas, intensity of shade did not significantly affect *An. squamosus* larval densities in the aquatic habitats currently examined (Table 10).

Table 7 Characteristics of larval habitats and mean densities of anopheline larvae collected during Dec. 2007 – June 2008

Habitat characteristics		Mean ± SE	F	P
Intensity of shade	Light	58.9 ± 6.2	0.002	0.966
	Shade	60.0 ± 17.1		
Turbidity	Clear	67.9 ± 6.9	11.134	0.001
	Turbid	17.6 ± 5.5		
Vegetation	Emergent	16.1 ± 6.2		
	Floating	40.6 ± 16.8	3.442	0.018
	Emergent + floating	70.1 ± 8.5		
	None	66.9 ± 13.3		
Permanence	Permanent	63.5 ± 8.1		
	Semi-permanent	50.1 ± 8.5	2.460	0.088
	Temporary	18 ± 9.2		
Water current	Still	71.2 ± 7.1	17.433	0.000
	Slow flowing	12.6 ± 4.7		
Distance to the nearest house	0….100m	55.4 ± 10.2		
	100…300m	71.2 ±10.6	1.932	0.147
	300…500m	42.7 ± 6.9		
Origin of the habitat	Natural	51.1 ± 6.1	5.143	0.024
	Human made	81.6 ±14.4		
Presence of algae	Present	89.2 ± 9.1	28.408	0.000
	Absent	29.6 ± 6.5		

Table 8 Characteristics of larval habitats and mean densities of *An. gambiae* s.l. larvae collected during Dec. 2007 – June 2008

Habitat characteristics		Mean ± SE	F	P
Intensity of shade	Light	15.5 ± 2.9	0.001	0.971
	Shade	15.0 ± 11.5		
Turbidity	Clear	16.4± 3.4	0.516	0.473
	Turbid	10.9 ± 4.2		
Vegetation	Emergent	0.34 ± 0.34		
	Floating	20.9± 11.9	13.012	0.000
	Emergent + floating	5.3 ± 1.4		
	None	45.5± 9.7		
Permanence	Permanent	5.1 ± 1.3		
	Semi-permanent	27.6 ± 6.3	14.353	0.000
	Temporary	120± 40		
Water current	Still	19.5± 3.6	7.739	0.05
	Slow flowing	0.0 ± 0.0		
Distance to the nearest house	0….100m	28.4 ± 7.7		
	100…300m	12.4 ±2.9	5.713	0.004
	300…500m	3.8± 1.7		
Origin of the habitat	Natural	9.1 ±2.7	1.653	0.200
	Human made	17.6 ± 3.7		
Presence of algae	Present	18.1 ± 4.8	0.892	0.346
	Absent	15.5 ± 2.8		

Table 9 Characteristics of larval habitats and mean densities of *An. pharoensis* larvae collected during Dec. 2007 – June 2008

Habitat characteristics		Mean ± SE	F	P
Intensity of shade	Light	16.7 ± 1.8	0.012	0.914
	Shade	15.8 ± 5.8		
Turbidity	Clear	18.4± 1.9	4.444	0.036
	Turbid	8.5 ± 4.5		
Vegetation	Emergent	7.5 ± 3.4		
	Floating	7.4 ± 3.0	8.648	0.000
	Emergent + floating	25 ± 2.9		
	None	7.0 ± 2.3		
Permanence	Permanent	50.0 ± 10		
	Semi-permanent	21.4 ± 2.6	7.023	0.001
	Temporary	9.4 ± 2.0		
Water current	Still	19.6 ± 2.1	10.342	0.001
	Slow flowing	5.5± 2.7		
Distance to the	0….100m	11.5 ± 2.5		
nearest house	100…300m	19.6±3.4	1.963	0.143
	300…500m	18.4 ± 2.9		
Origin of the habitat	Natural	26.2 ±4.7	9.790	0.002
	Human made	13.39 ± 1.7		
Presence of algae	Present	28.7 ± 3.0	51.483	0.000
	Absent	5.0 ± 1.3		

Table 10 Characteristics of larval habitats and mean densities of *An. squamosus* larvae collected during Dec. 2007 – June 2008

Habitat characteristics		Mean ± SE	F	P
Intensity of shade	Light	5.4 ± 0.8	2.455	0. 119
	Shade	11.3± 4.3		
Turbidity	Clear	6.9± 0.9	9.745	0.002
	Turbid	0.2± 0.2		
Vegetation	Emergent	1.6 ± 1.0		
	Floating	0.3 ± 0.3	12.106	0.000
	Emergent + floating	10.1± 1.4		
	None	0.5 ± 0.3		
Permanence	Permanent	8.5 ± 1.2		
	Semi-permanent	1.8 ± 0.8	8.626	0.000
	Temporary	0.0 ± 0.0		
Water current	Still	6.5 ± 1.0	3.800	0.052
	Slow flowing	2.5± 1.0		
Distance to the	0….100m	2.9 ± 0.8		
nearest house	100…300m	6.1 ±1.4	3.205	0.042
	300…500m	8.4 ± 1.9		
Origin of the habitat	Natural	9.7 ± 2.0	8.465	0.004
	Human made	4.3 ± 0.8		
Presence of algae	Present	11.1 ± 1.5	49.9	0.000
	Absent	0.4 ± 0.3		

4.4.3. ENVIRONMENTAL FACTORS ASSOCIATED WITH THE OCCURRENCE OF ANOPHELINE LARVAE

Logistic regression (odds ratio) revealed six key environmental variables associated with anopheline larval occurrence in the different aquatic habitats (Table 11). Table 11 shows the result of logistic analysis between the presence/absence of anopheline larvae and environmental variables in irrigation and major drainage areas between Adami Tulu and Meki towns, Central Ethiopia. Water temperature, water current, turbidity, the presence of algae, origin of the habitats (whether natural or human made) and water depth were significantly associated with the presence /absence of anopheline larvae in the water bodies. Water bodies with algae (odds ratio [OR] =12.88, 95% confidence interval [CI] =6.9-23.9) and warmer temperature (OR=1.52, CI=1.3-1.70) were much more likely to contain anopheline larvae than when algae were absent from the water bodies and when the habitats were cooler. Water depth (OR = 0.9, 95% CI = 0.9 – 0.9) was inversely associated with the occurrence of anopheline larvae as deeper water bodies decreased the chance of finding anopheline larvae.

Similarly, origin of the habitats (OR = 0.4, CI= 0.2-0.6), turbidity (OR = 0.2, CI = 0.1-0.5) and water current (OR = 0.1, CI = 0.1-0.3) were negatively associated with the presence/absence of anopheline larvae. The results indicated that natural habitats, clear and still water bodies more likely contained anopheline larvae whereas human made habitats, turbid and fast flowing water bodies decreased the chance of anopheline larval occurrence in the water bodies during the study period.

Table 11 Repeated measures logistic regression models for the associations between occurrence of anopheline larvae and environmental variables

Parameter	Odds ratio	Lower CI	Upper CI	P
		Anopheline larvae		
Water depth	0.99	0.97	0.99	0.031
Presence of algae	12.88	6.92	23.96	<0.001
Origin of the habitats	0.35	0.19	0.65	0.001
Water temeperature	1.52	1.30	1.77	<0.001
Turbidity	0.22	0.09	0.48	<0.001
Water current	0.14	0.06	0.31	<0.001

For OR > 1, a positive association is said to exist.

For OR < 1, there is a negative association (Gimnig *et al*., 2001).

Multiple step-up regression analysis further detected the best predictor variables associated with the occurrence and abundance of anopheline larval species (Table 12). Accordingly, the top predictors for *An. gambiae* s.l. (*An. arabiensis)* larval abundance were aquatic vegetation and water current while that of An. *pharoensis* and *An. squamosus* were presence of mats of algae. Water temperature and distance to the nearest house were also signfciant predictors of *An. pharoensis* and *An. squamosus* larval abundance in the aquatic habitats, respectively.

As depicted in Table 9, relative abundance of *An. gambiae* s.l. larvae was negatively associated with aquatic vegetation and water current while that of *An. pharoensis* was positively associated with the presence of mats of algae and water temperature. *An.squamosus* larval abundance was also positively associated with presence of algae in the water bodies and distance to the nearest house.

Table 12 Multiple step–up regression for the three common anopheline species larvae in relation to habitat characteristics

		R^2	Coefficient	SE	Standard coefficient	t	P
	(constant)		74.82	10.39		7.19	0.00
An. gambiae s.l	Vegetation	9.3	-12.92	2.22	-0.36	-5.82	0.00
	Water current	15.6	-28.06	6.74	-0.26	-4.17	0.00
	(constant)		-58.28	18.27		-3.14	0.00
An. pharoensis	Presence of algae	18.2	19.09	3.49	0.34	5.47	0.00
	Water temp.	22.3	2.64	0.76	0.22	3.49	0.00
An. squamosus	(constant)		-3.45	2.13		-1.62	0.11
	Presence of algae	17.7	10.37	1.51	0.41	6.89	0.00
	Distance to house	19.3	2.09	0.99	0.13	2.10	0.04

4.4.5. CORRELATION BETWEEN METEOROLOGICAL PATTERN AND ABUNDANCE OF ANOPHELES LARVAE

Due to lack of current meteorological data of the study area, average data of the 1998 up to 2007 that fit the study period was extrapolated. Accordingly, the mean monthly rain fall for the study period (December-June) was 45.58mm while the mean monthly air temperature was 21.33oC. Mean monthly temperature varied among the study months with peak in March and its minima in December. Average relative humidity was 55.66 %.

As it can be seen from Table 13, mean monthly air temperature of the study period had a positive and significant correlation with density of anopheline larvae ($r= 0.132$, $p< 0.05$). However, larval densities of the malaria vector species were not significantly correlated with the average weather variables of the study period. Larval densities of both *An. gambiae* s. l. and *An. pharoensis* were positively correlated with air temperature. Moreover, larval densities of *An. pharoensis* was negatively correlated with both rain fall and relative humidity while that of *An. gambiae* s. l. was positively associated with the two weather variables. Larval abundance of *An. squamosu* was significantly and inversely associated with average monthly rain fall of the study period ($r= -0.203$, $P< 0.01$).

Table 13. Correlation coefficients between relative abundance of anopheline larvae and average Meteorological variables of the study period extrapolated from December 1998- June 2007)

Mosquito larvae	Rain fall (mm)	Temperature (oC)	R/humidity (%)
Anophelinae	0.007	0.132*	0.032
An. gambiae s.l.	0.109	0.120	0.023
An. pharoensis	-0.034	0.118	-0.019
An. squamosus	-0.203**	-0.005	-0.080

*Correlation is significant at the 0.05, ** Correlation is significant at the 0.01 level.

5. DISCUSSION

Five anopheline species were identified in the irrigation and major drainage areas between Adami Tulu and Meki towns of Central Ethiopia. These included *An. pharoensis, An. gambiae* s.l. (*An. arabiensis), An. squamosus, An. coustani* and *An. cinereus.* The first two were the predominant species that were found in the area. Cytogenetic studies have previousely confirmed that *An. arabiensis* is the sole known member of *An. gambiae* complex present in Ziway area (Abose *et al*., 1998; Ye-Ebiyo *et al*., 2003). All the species presently identified have previously been documented (Abose *et al*., 1998) and consistent with a recent report by Kibret (2008) who has worked on the impacts of a small scale irrigation scheme on malaria transmission around Ziway (in Abine Germama) one of the local sites in the present study.

Seven larval habitat types were identified in this study, namely swamps, irrigation canals, canal leakage pools, sand pools, water harvesting pool, brick making pit and rain pool. The first two habitats were the most common breeding sites in the area. The availability, persistence and dimensions of all the larval habitats except rain pool are dependent on water from the major drainage systems namely, Lake Ziway, Meki and Bulbula rivers.

Some of these habitat types were previously reported from Ziway area and elsewhere in the country. For example, Abose *et al*. (1998) found that anopheline larvae occur in permanent main lake shore water with vegetation (swamp) and in temporary rain-pools either in the villages or around the shore of Lake Ziway. Likewise, very recently, Kibret (2008) reported that irrigation canals and canal leakage pools were the most important prolific *Anopheles* larval habitats in Ziway area. Else where in the country, Yohannes *et al*. (2005) also reported that in the dam villages of Tigray, irrigation canals, pools that formed along the bed of streams from the dam and man made pools were the major anopheline breeding habitats. Balkew (2001) also reported similar habitat types from Metehara, Ethiopia.

Brick making pit was another anopheline larval habitat type currently observed in the study area though its occurrence was rare. This would be expected because brick making either for sale or domestic use is a common practice in Ziway area especially during the dry season along Lake Ziway. The latest report from Koka area, (Akililu, 2008) revealed that among the different breeding habitats examined brick making pits were the most favoured sites by larvae of *An. gambiae* s. l. Brick making pits are also common anopheline mosqitos breeding sites else where (Surendran and Ramasamy, 2005; Sogoba *et al*., 2007).

Water harvesting pools (WHP) were another potential mosquito breeding sites currently observed in the study area. These included collection of lake water by surface canals in to human-made wells (Pits) usually at botanical nursery sites and collection of rain water. This habitat type rarely supported anopheline larval development because most of the water harvesting pools observed during the study period were used regularly that could remove unknown proportion of mosquito immature and negatively impact adult oviposition there. The contribution of WHP for anopheline breeding and malaria transmission was previously reported from the other parts of the country (Kassahun, 2007).

Reverine sand mining pits block water flow and create pools which would offer ideal habitats for the proliferation of anopheline mosquitoes. To my knowledge, this habitat type had not been reported so far from the country although it is a common anopheline mosquito habitat elsewhere outside Ethiopia. For example, Robert *et al*. (2003) and Surendran and Ramasamy (2005) reported that open pit mining has altered natural ecosystem in many countries and paved the way for the emergence of different malaria vectors, with increased vector breeding sites. Similar to the present observation, *An. culicifcies* larvae in Sri Lanka were also observed to breed abundantly in rock pools and sand pools along river margins (Surendran and Ramasamy, 2005).

Results of this study also revealed that anopheline larvae were abundantly found in swamps and irrigation canals during the study period. This would be expected because the swamps and canals had water available for anopheline larval development most of the time compared with the other habitat types in relation to long-term contribution to larval productivity. Similar to the present study, Balkew (2001) reported that the existence of permanent anopheline breeding habitats associated with irrigation practices in Metehara supported the survival of vector species throughout the year and anophelines survived the dry months by colonizing permanent breeding sites. Likewise the present result was also similar to a study by Coetze (2004) who reported that anopheline breeding sites in arid areas are highly localized at permanent habitats, river edges or irrigation projects.

This study has documented the occurrence of five species of *Anopheles* larvae. Of all the five anopheline species larvae, *An. pharoensis* was found in appreciable density in herbaceous swamps in the major drainage systems and irrigation area. In contrast, *An.gambiae* s.l. larvae preferred semi-permanent (seasonal) habitats such as sand pools, brick making pits and rain pools. This was in agreement with the study by Abose *et al*. (1998) who found that the most important type of breeding sites for *An. arabiensis* were temporary rain pools whereas *An. pharoensis* preferred the permanent main lakeshore water with vegetation for breeding. Studies elsewhere also revealed that An. *pharoensis* breeds in large vegetated swamps and along lakeshores among floating plants (Gillies and Demeillon, 1968). However, *An. gambiae* s.l. is a typical r-strategiest, colonizing temporary habitats in which selection favours rapid population increase because larval predation is less prevalent in temporary habitats than in large permanent habitats (Munga *et al*., 2006). It was observed that the principal malaria vector in the country: *An. gambiae* s.l. *(An. arabiensis)* larvae were most abundant in sand pools along the edge of Meki river before the on set of the rainy season. This would be expected because sand mining activity in the area coincided with the dry and irrigation seasons where rain dependent anopheline larval habitats dry out elsewhere and the species limit themselves to permanent water bodies such as rivers and lakes. Similar idea was shared by Muturi *et*

al. (2006) who reported that in Africa, *An. arabiensis* breeding sites have been associated with rivers and irrigation systems in drier parts of the continent. The other anopheline species larvae, *An. squamosus* and *An. coustani* were also found in swamps and irrigation canals just like that of *An. pharoensis.* This observation was also in agreement with a study by Abose *et al*. (1998) who collected the same species some 10 years back from swampy area of Edo Kontela (now Edo Gojola) on the shoreline of Lake Ziway, and Kibret (2007) who recently reported the same anopheline larval species from irrigation canals at Abine Germama Village around Ziway town.

Anopheline larval abundance generally tended to increase during the short rainy season (late May to June) than during the dry season (December to April). This would be attributed to the appearance of some anopheline positive rain water dependent breeding sites such as brick making pit and rain pool. Mean densities of *An. gambiae* s.l. and *An. pharoensis* generally increased from December to March with higher abundance of *An. pharoensis* larvae than *An. gambiae* s.l. larvae. This is similar with previous reports by Abose *et al*. (1998) who observed that during rainy season larval densities of both species increased with higher abundance of *An. gambiae* s.l. than *An. pharoensis*. However, after the rainy season in November, larval density of *An. gambiae* s.l. (*An. arabiensis*) started to decline while larval density of *An. pharoensis* steeply rose up. After March and through April, larval densities of both species went down with more abundance of *An. gambiae* s.l. larvae than that of *An. pharoensis*. During this period, anopheline larval density generally declined due to prolonged dry season and as a result lake and river water regressed, more parts of swamps dried, irrigation canals were regularly renewed to deliver more water for irrigation. On the other hand this period coincided with the drying out of Meki river water, intensive sand mining activities, formation of river water residual pools as sand pools and proliferation of *An. gambiae* s.l. larvae. When the river driedout, more sandy ground of the river bed was exposed, transportation was facilitated as vehicles got into the river bank and loaded the sand piles easily (Appendix 8). These all helped formation of more sand pools and mosquito larval proliferations. This

observation was consistent with reports by Ijumba (1990) who pointed out that *An. gambiae* s.l. larvae breed in residual pools such as beds of drying stream and Coetzee (2004) who reported that breeding sites of *An. arabiensis* in arid areas are highly limited to river edges. These habitats are generally with clear water and sunlit which are consistent with *An. arabiensis* larval habitats (Service, 2000).

Although anopheline larval control is an important component of malaria control program in Ethiopia, usually by source reduction through management of larval habitats integrated with adult vector control (Abose *et al*., 1998; Yohannes *et al*., 2005; Gebreyesus *et al*., 2006), little is known about larval ecology of *Anopheles* mosquitoes. For example, the microhabitat factors that influence the occurrence and abundance of *Anopheles* larvae are not well characterized even for the malaria vector species. Reports of larval habitats has been usually given in more general terms as "Marshes", "Rain pools", "man-made pools" and the like. Although somewhat informative, these habitat categories are not specific enough to define local environmental factors associated with specific anopheline species.

The present study was undertaken to assess some environmental factors of anopheline larval habitats in irrigation and major drainage areas between Adami Tulu and Meki towns to determine their association with the occurrence and abundance of the mosquito larvae.

In this study, a number of environmental factors were used to characterize the larval habitats, including water temperature, pH, elevation, water depth, water current, turbidity, presence of mats of algae, distance to the nearest house, permanence of the habitats, origin of the habitat (whether natural or human made), aquatic vegetation, intensity of shade, and substrate types and others.

The environmental variables that we examined were not independent of each other (Appendix 7). Therefore, to examine the association between occurrence and abundance of anophline larvae and environmental variables, multiple linear or multiple logistic regressions analysis is more appropriate than simple linear or logistic regressions (Robert *et al*., 1998; Minakawa *et al*., 1999). Accordingly, in the present study, the association of anopheline larval occurrence and relative abundance with environmental variables was elucidated with different statistical models as depicted in Tables 7- 12.

Logistic regression analysis (odds ratio) and multiple step-up regression altogether detected at least six environmental variables significantly associated with anopheline larval occurrence/abundance. Of these, water temperature and presence of mats of algae were positively associated, while water depth, origin of the habitats, turbidity and water current were negatively associated with the larval occurrence/ abundance in the water bodies. In addition, others being the same one way analysis of variance (ANOVA) detected significantly higher mean densities of anopheline larvae from aquatic habitats with emergent plus floating vegetation suggesting that anopheline larvae prefer this type of aquatic flora.

The positive association between occurrence/abundance of anopheline larvae and water temperature in this study was consistent with previous findings in the country and elsewhere. For example, previous studies collected anopheline species larvae from hot springs in Gergedi and Koka areas in the Awash River Valley of Ethiopia (Ameneshewa and Service, 1996; Aklilu, 2008). Robert *et al*. (1998) reported that *An. arabiensis* abundance was associated with warm temperature (28-30^{0}c). Similarly Shililu *et al*. (2003) found that anopheline larval density was positively correlated with water temperature in Eritrea. A study on habitat characteristics of mosquitoes in Accra, Ghana, identified that water temperature was determinant factor in the anopheline larval occurrence, abundance and distribution (Opoku *et al*., 2000). This view is also shared by Gimnig *et al*., (2002). Piyaratne *et al*. (2005) found that *An. culicifacies* abundance was

associated with water temperature. According to Gillies and Demeillon (1968), warm temperatures shorten larva-pupa development and therefore hasten adult mosquito emergence. There is a critical relationship between temperature and the life cycle of the insects (Bayoh and Lindsay, 2004).

The present investigation also showed positive association between the presence of mats of algae in the aquatic habitats and the occurrence/abundance of anopheline larvae as previously reported by different authors from similar study area and elsewhere. Ye-Ebiyo *et al* (2003) reported that presence of algal density promotes development of anopheline larvae similar to maize pollen and alters feeding strategies of *An. gambiae* s.l. larvae. Likewise, the positive association of mats of algae with anopheline larval occurrence/abundance is also similar to the findings of Gimnig *et al*. (2002) who found that algal food plays a key role in *Anopheles* habitat and contradicts the conclusion of Fillinger *et al*. (2004) who reported negative association between *Anopheles* density and non-matted algal content. The reason for negative association of anopheline larvae with non-matted algal content in the study of Fillinger *et al*. (2004) was attributed to abiotic factors (chemical water quality) and biotic factors (predatory fauna) that were not included in the present study and remains to be explored. Another study in Kenya also demonstrated the importance of algal biomass for the development of anopheline mosquitoes in their larval habitats (Kaufman *et al*., 2006).

The higher abundance of anopheline larvae in aquatic habitats with emergent and floating vegetation revealed by the ANOVA model is also consistent with previous findings. There are a number of papers on the relationships between vegetation and anopheline larvae. (Rejmankova *et al*., 1999; Claborn *et al*., 2002; Fillinger *et al*., 2004; Devi and Jauhari, 2007).

Anopheline larvae were inversely associated with water depth and turbidity. Such observations have been made elsewhere. For example, Rober *et al*. (1998) reported that

An. arabiensis abundance was associated with clear and not to deep water (<0.5m). Similarly, Shililu *et al*. (2003) found more anopheline larval densities from shallow and clear aquatic habitats. The latter observation contradicts previous findings from the study area by Ye-Ebyo *et al*. (2000; 2003) who found that anopheline larvae (*An. arabiensis* larvae) exploit turbid water. It should be noted that the previous studies were specific to *An. gambiae* s.l. (*An. arabiensis larvae)* the opportunistic members of *An. gambiae* complex mosquitoes unlike the present study that included all anopheline species in the area. The other difference is that the former studies were conducted during the rainy season on rain water dependent puddles formed by surface run off and simulated puddles which as a result may be turbid due to soil erosion and pollen from flowering maize as the study period coincided with maize anthesis. Whereas, the current study was carried out during dry and irrigation season on permanent and semi-permanent habitats and as a result transient and rain water dependent habitats as well as flowering maizes were rarely observed.

The present investigation also revealed negative association between anopheline larval abundance and water current. This result was in contrast with the findings of Shililu *et al*. (2003) who reported that anopheline larvae were abundant in slow flowing habitats. This may be because in the former study, *Anopheles* larvae were sampled predominantly from stream edge and stream bed pools with samples from these habitat types comprised 91.2% (n=9481) unlike the present study that were collected mainly from stagnant swamps and sand pools.

It was also observed that anopheline larval occurrence/abundance was more associated with natural habitats compared to human made habitats. This world be expected because, natural habitats (swamps) contained water during the study period and sampled most frequently for anopheline larvae unlike the human-made habitats. On top of this, the human made habitats such as functional irrigation canals were regularly cleared of all

excess vegetation and renewed during the study period to irrigate crops which displaced or removed unknown proportion of larvae.

Moreover, it was observed that the characteristic substrate type for anopheline larval habitats in the study area was soil/mud as no anopheline larvae was observed to occur in larval habitats with any other substrate types such as cement or concrete, plastic or rubber, and others. 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 with out soil substrates. Soil may provide nutrients for the enrichment of bacteria that serve as food source for larvae and possibly oviposition attractants.

Furthermore, step-up multiple regression results demonstrated a strong interaction between some of the environmental factors and the relative abundance of the three major anophleine species: *An. gambiae* s.l., *An. pharoensis* and *An. squamosus* and detected key environmental factors associated with them. Some four key environmental variables were significant predictors of the larval stages of the malaria vectors, *An. gambiae* s.l. and *An. pahraensis*. For example, vegetation and water current were negatively associated with *An. gambiae* s.l. larval abundance. This observation was also described by Ye-Ebiyo *et al*. (2003) who reported that in the relatively dry Ethiopian environment, larval *An. arabiensis* are present mainly in small, temporary rain pools that are free of vegetation. Munga *et al*. (2006) also reported that deforestation of natural swamps created conditions favourable for *An. gambiae* breeding. Similar findings were also observed elsewhere (Killeen, 2006, Miller *et al*., 2007). The negative association between *An. gambiae* s.l. larval abundance and water current is also consistent with previous reports by Walker and Lynch (2007) who pointed out that *An. gambiae* s.l. prefers usually still water on which they can stay close to the surface with their orifice open to the air for breathing. Miller *et al*., (2007) also demonstrated that *An. gambiae* s.l. larvae are capable of terrestrial displacement whereby they can reach standing water. As far as *An. gambiae* s.l. is

concerned, as previously reported it does not lend itself to generalization, it can be found in a wide variety of water bodies (Fillinger, 2004).

The present study also revealed that the presence of mats of algae was key environmental factor positively associated with the abundance of *An. pharoensis.* This would be because as the results of this study indicated, *An. pharoensis* larvae were most frequently sampled (above 85%) from swamps and irrigation canals where algae are well established and grow during most of the study period. *An. pharoensis* larvae were mostly collected in habitats with stagnant water: however, larvae were also observed in flowing water where the presence of algae seemed to drastically reduce water current velocity within the habitat. Previous studies showed that algal growth is a key factor for the growth of anopheline species. For example, Manguin *et al*. (1996) reported that abundant algal growth was a key factor for the presence of *An. pseudopunctipennis* in fresh water stream pools. *An. pharoensis* larval abundance was also positively associated with water temeperature. This view is shared by Muturi *et al*. (2007) who reported that *An. pharoensis* larvae were significantly associated with water temperature.

Anopheles squamosus larval abundance was also significantly and positively associated with the presence of algae similar to that of *An. pharoensis*. This would be expected because significantly higher mean density of *An. squamosus* larvae was obtained from stable habitats specifically; natural swamps where algae are more established and grow. On the otherhand, larval abundance of *An. squamosus* was significantly associated with distance to the nearest house. This finding was surprising, as information on the medical importance of the species is lacking except that Abose *et al*. (1998) reported the occurrence of An. *squamosus* in their indoor and outdoor resting adult anopheline mosquito collection. As far as *An. squamosus* is concerned the present observation contradicts previous reports by Kibret (2008) who did not observe the occurrence of *An. squamosus* in his indoor and outdoor resting collection. Such variations would be expected because; the current study did not include adult mosquito survey unlike the

previous study. On the otherhand, unlike the present study the previous study did not include the most stable dry season anopheline larval habitats such as the swamps which are created and maintained by the perennial water bodies. The medical importance of this anopheline species remains to be explored.

Results of the present study also revealed that meteorological pattern of the study period and seasonal abundance of anopheline larval species were not significantly correlated. However, it was observed that *An. pharoensis* larval abundance was negatively correlated with average relative humidity and rain fall of the study period unlike larval abundance of *An. gambiae* s.l. This observation is consistent with seasonal larval abundance of the malaria vector species (Figure 4). Similar seasonal trend was also previousely reported by Abose *et al*. (1998) who observed that *An gambiae* s.l. density dominated *An. pharoensis* during the wet season unlike the dry season.

To this end, the other anopheline species larvae, *An. coustani* and *An. cinereus* were rarely sampled during the study period and as a result excluded from the statistical models as it is difficult to draw conclusions with limited data available.

6. CONCLUSION AND RECOMMENDATIONS

6.1. CONCLUSIONS

Drains and canals create and maintain dry season mosquito larval habitats in the study area among which natural swamps, sand pools, irrigation canals, and canal leakage pools are the most important. Five anopheline species were found in the study area. Namely, *An. pharoensis, An. gambiae* s.l. (presumably *An. arabiensis)*, *An. squamosus, An. coustani* and *An. cinereus.* Larvae of *An. pharoensis* were the most predominant species found in the area during the study period followed by larvae of *An. gambiae* s.l. Larval abundance of both *An. pharoensis* and *An. gambiae* s.l. were significantly positively correlated with each other. Larvae of *An. pharoensis* were most abundantly found in natural swamps and irrigation canals whereas larvae of *An. gambiae* s.l. were most abundantly found in sand pools during the study period.

At least six key environmental factors of the larval habitats were significantly associated with the occurrence of anopheline larvae in the study area. These included water temperature, presence of mats of algae, origin of the habitats, water depth, turbidity and water current. Water temperature and presence of mats of algae were positively associated with anopheline larval occurrence. Whereaswater current, turbidity and water depth were inversely associated with the larval occurrence

Four key environmental factors were significantly associated with the larvae of the two most abundant malaria vector species *An. gambiae* s.l. and *An. pharoensis*. These were water current, water temperature, aquatic vegetation and the presence of mats of algae. *An. gambiae* s.l. larval abundance was inversely associated with vegetation and water current. *An. pharoensis* larval abundance was positively associated with the presence of mats of algae and water temperature.

6.2. RECOMMENDATIONS

- The importance of dry season anopheline larval habitats that are created and maintained by perennial water bodies and irrigation waters and their role for continuous production of adult vectors and perennial malaria transmission needs to be considered in vector control operations and further research.
- Sand mining pits that are created for construction and other economic purposes were productive breeding sites for larval malaria mosquitoes in the study area. Therefore, it is essential to take suitable public health measures targeting these sites. These habitats are usually discrete and limited in number, so that anti-larval measures are very well suited to targeting these sites during the dry seasons, thus reducing the overall mosquitoes before the increase of habitat availability during the rainy season.
- Intersectorial collaboration between agriculture and health bureaus while planning and implementing water resources development projects in the area and continuous follow up is essential.
- To my knowledge this study is the first attempt to analyze the complex environmental variables that determine anopheline larval occurrence/abundance especially in drainage and irrigation areas in Ethiopia. There fore, further research that examine detailed analysis of water chemistry, ecology of mosquito larval predators and adult mosquito oviposition behaviour is needed.
- The present study examined the aquatic habitats that contained anopheline larvae, thus unsuitable habitats where larvae do not develop where not studied. Inclusion of such habitats may provide more information for sound understanding of anopheline larval ecology.

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APPENDICES

Appendix 1. List of current anopheline species found in Ethiopia.

An. arabiensis	*An. quadriannulatus* species B
An. funestus	*An. garnhami*
An. nili	*An. obscurus*
An. ardensis	*An. dancalicus*
An. gibbinsi	*An. longipalpus*
An. paludis	*An. rufipes*
An. christyi	*An. demeilloni*
An. hargreavsi	*An. maculipapis*
An. pharoensis	*An. salbii*
An. cinereus	*An. domiculus*
An. harperi	*An. marshalli*
An. pretoriensis	*An. sergentimacmahoni*
An. confusus	*An. dthali*
An. implexus	*An. natalensis*
An. rhodesiensis rhodesiensis	*An. seydeli*
An. coustani	*An. squamosus*
An. kingi	*An. turkuhudi*
An. rhodesiensis rupicolus	*An. tenobrosus*
An. cydippis	*An. wellcomi*
An. leesoni	*An. theilleri*
An. rivolurum	*An. ziemanni*

Source: (Verrone, 1962a, b).

Appendix 2. Number of anopheline larvae collected from different aquatic habitats per number of dips per month at six local sites over the study period /Dec. 2007 – June 2008/

Local site	Habitat type	No. of habitat	No. of dips per habitat	Dec.	Jan.	Feb.	Mar.	Apr.	May	June	Total
Adami Tulu	Swamp	1	30	15	214	27	139	40	139	114	688
Gerbi	CLP	1	30	0	92	56	0	24	-	-	172
	Irr.canal	1	30	0	0	0	0	24	144	80	248
Ziway	Irr. Canal	1	30	0	66	73	64	8	0	0	211
	Irr. Canal	1	15	0	0	32	0	0	0	0	32
	Irr. Canal	1	20	0	38	45	13	16	0	0	112
	Swamps	2	30	0	18	118	117	9	69	33	364
	BMP	1	20	-	-	-	-	27	23	12	62
	Rain pool	1	10	-	-	-	-	-	12	24	36
Abine	Swamp	1	30	0	102	36	16	12	90	42	298
Germama	Irr. Canals	3	20	12	36	35	57	0	0	0	140
	CLPs	2	20	0	0	49	28	30	0	0	107
Edo Gojola	Irr. Canals	1	30	15	46	23	69	30	0	0	183
	Swamp	1	30	0	15	19	30	30	49	12	155
	WHP	1	10	0	0	0	8	5	12	12	37
Meki	Sand pools	1	25	0	92	78	74	61	7	-.	312
	Sand pools	1	20	0	0	0	60	34	16	-	110
	Sand pools	1	10	0	0	66	65	35	6	-	172

CLP= Canal leakage pools, WHP= Water harvesting pool, BMP= Brick making pit

- (minus sign) indicates that no aquatic habitat was present.

Appendix 3. Number of *An. gambiae* s.l. larvae collected from different aquatic habitats per number of dips per month at six sites over the study period (Dec. 2007-June 2008)

Local site	Habitat type	No. of habitats	No. of dips perhabitat	Dec.	Jan.	Feb.	Mar.	Apr.	May	June	Total
Adami Tulu	Swamp	1	30	0	2	0	0	0	22	15	39
Gerbi	CLP	1	30	0	0	0	0	5	-	-	5
	Irr. canal	1	30	0	0	0	0	0	14	12	26
Ziway	Irr. Canal	1	30	0	0	3	3	0	0	0	6
	Irr. Canal	1	15	0	0	0	0	0	0	0	0
	Irr. Canal	1	20	0	0	0	0	0	0	0	0
	Swamps	2	30	0	0	2	0	0	22	19	43
	BMP	1	20	-	-	-	-	27	18	12	57
	Rain pool	1	10	-	-	-	-	-	8	16	24
Abine	Swamp	1	30	0	0	0	0	0	13	12	25
Germama	Irr. Canals	3	20	0	0	0	0	0	0	0	0
	CLPs	2	20	0	0	5	0	0	0	0	5
Edo Gojola	Irr. Canals	1	30	0	0	2	8	2	0	0	12
	Swamp	1	30	0	0	0	0	4	16	6	26
	WHP	1	10	0	0	0	1	0	8	8	17
Meki	Sand pools	1	25	0	53	57	50	26	7	-	193
	Sand pools	1	20	0	0	0	41	31	12	-	84
	Sand pools	1	10	0	0	41	48	32	3	-	124

Appendix 4. Number of *An. pharoensis* larvae collected from different habitats per number of dips per-month at six sites over the study period (Dec. 2007- June 2008)

Local site	Habitat type	No. of habitat	No. of dips perhabitat	Dec.	Jan.	Feb.	Mar.	Apr.	May	June	Total
Adami Tulu	Swamp	1	30	5	49	10	17	16	23	6	126
Gerbi	CLP	1	30	0	30	24	0	12	-	-	66
	Irr. canal	1	30	0	0	0	0	15	27	9	51
Ziway	Irr. Canal	1	30	0	35	19	15	6	0	0	75
	Irr. Canal	1	15	0	0	12	0	0	0	0	12
	Irr. Canal	1	20	0	35	15	3	8	0	0	61
	Swamps	2	30	0	12	57	76	9	10	9	173
	BMP	1	20	-	-	-	-	0	5	0	5
	Rain pool	1	10	-	-	-	-	-	4	6	10
Abine	Swamp	1	30	0	40	5	5	8	11	8	77
Germama	Irr. Canals	3	20	8	24	6	15	0	0	0	53
	CLPs	2	20	0	0	29	22	18	0	0	69
Edo Gojola	Irr. Canal	1	30	9	21	14	35	14	0	0	93
	Swamp	1	30	0	9	12	19	15	19	6	80
	WHP	1	10	0	0	0	2	4	4	4	14
Meki	Sand pools	1	25	0	11	1	8	4	0	-	24
	Sand pools	1	20	0	0	0	10	0	4	-	14
	Sand pools	1	10	0	0	0	8	3	1	-	12

Note: CLP= Canal leakage pool, BMP = Brick making pits, WHP = Water harvesting pool

- (minus sign) indicates that no aquatic habitat was present

Appendix 5. Number of *An. squamosus* larvae collected from different aquatic habitats per number of dips per month at six sites over the study period (Dec. 2007 – June 2008

Local site	Habitat type	No. of habitat	No. of dips perhabitat	Dec.	Jan.	Feb.	Mar.	Apr.	May	June	Total
Adami Tulu	Swamp	1	30	2	29	5	10	6	4	3	59
Gerbi	CLP	1	30	0	32	12	0	4	-	-	48
	Irr. canal	1	30	0	0	0	0	0	8	0	8
Ziway	Irr. Canal	1	30	0	7	14	3	2	0	0	26
	Irr. Canal	1	15	0	0	10	0	0	0	0	10
	Irr. Canal	1	20	0	15	10	3	6	0	0	34
	Swamps	2	30	0	9	16	23	0	0	0	48
	BMP	1	20	-	-	-	-	0	0	0	0
	Rain pool	1	10	-	-	-	-	-	0	0	0
Abine	Swamp	1	30	0	24	10	2	0	5	0	41
Germama	Irr. Canals	3	20	4	7	9	3	0	0	0	23
	CLPs	2	20	0	0	0	2	2	0	0	4
Edo Gojola	Irr. Canals	1	30	3	11	0	7	8	0	0	29
	Swamp	1	30	0	6	7	5	8	7	0	33
	WHP	1	10	0	0	0	0	0	0	0	0
Meki	Sand pools	1	25	0	2	0	0	0	0	-	2
	Sand pools	1	20	0	0	0	0	0	0	-	0
	Sand pools	1	10	0	0	0	0	0	0	-	0

Note: CLP= Canal leakage pool, BMP = Brick making pits, WHP = Water harvesting pool

- (minus sign) indicates that no aquatic habitat was present

Appendix 6. Number of *An. coustani* larvae collected from different aquatic habitats per number of dips per month at six local sites over the study period (Dec. 2007- June 2008)

Local site	Habitat type	No. of habitat	No. of dips perhabitat	Dec.	Jan.	Feb.	Mar.	Apr.	May	June	Total
Adami Tulu	Swamp	1	30	0	3	0	0	0	0	0	3
Gerbi	CLP	1	30	0	0	0	0	2	-	-	2
	Irr. canal	1	30	0	0	0	0	0	0	0	0
Ziway	Irr. Canal	1	30	0	2	14	6	4	0	0	26
	Irr. Canal	1	15	0	0	2	0	0	0	0	2
	Irr. Canal	1	20	0	0	1	0	0	0	0	1
	Swamps	2	30	0	0	6	13	0	0	0	19
	BMP	1	20	-	-	-	-	0	0	0	0
	Rain pool	1	10	-	-	-	-	-	0	0	0
Abine	Swamp	1	30	0	0	0	0	4	0	0	4
Germama	Irr. Canals	3	20	0	0	0	0	0	0	0	0
	CLPs	2	20	0	0	0	0	0	0	0	0
Edo Gojola	Irr. Canals	1	30	0	0	0	0	1	0	0	1
	Swamp	1	30	0	0	0	0	3	0	0	3
	WHP	1	10	0	0	0	0	0	0	0	0
Meki	Sand pools	1	25	0	0	0	0	0	0	-	0
	Sand pools	1	20	0	0	0	0	0	0	-	0
	Sand pools	1	10	0	0	0	0	0	0	-	0

Note: CLP= Canal leakage pool, BMP = Brick making pits, WHP = Water harvesting pool

- (minus sign) indicates that no aquatic habitat was present

Appendix 7. Correlation coefficients among the environmental variables of anopheline larval habitats sampled in irrigation and major drainage areas between Adami Tulu and Meki towns, Central Ethiopia.

	Intensity of shade	Turbidity	Vegetat.	Habitat perman.	Water current	Distance to nearest house	Water pH	Water temp.	Water depth	Elevatin	Origin of habitats
Intensity of shade											
Turbidity	0.093										
Vegetation	0.068	- 0.106									
Hab. permanence	-0.082	0.019	-0.696**								
Water current	-0.120	0.060	-0.217**	-0.003							
Distance to house	-0.285**	-0.147**	0.236**	-0.305**	0.161**						
Water pH	0.119	-0.019	0.076	-0.076	-0.063	-0.044					
Water temp.	0.135*	0.149*	0.264**	-0.292**	-0.220**	0.074	0.128				
Water depth	-0.030	0.152*	-0.081	-0.112	0.312**	0.014	0.162*	0.097			
Elevation	-0.086	-0.157*	-0.472**	0.576**	0.064	-0.025	0.040	0.145*	0.147*		
Origin of habitats	0.137*	0.020	-0.442**	0.495**	0.302**	-0.160*	0.030	0.247**	0.122	0.297*	
Presence of algae	0.081	-0.304**	0.564**	-0.461**	-0.359**	0.094	0.194*	0.379**	-0.139*	0.203**	-0.499**

* Correlation significant at the 0.05 level (2 tailed), ** correlation significant at the 0.01 level (2 tailed)

Apendix 8. Some economic activities associated with major drainage systems between Adami Tulu and Meki towns, Centeral Ethiopia

Plate 4. Meki town dwellers fetching residual pools as the river water dried due to prolonged dry season, April, 2008

Plate 5. Intensive sand mining activities at Meki River, April, 2008.

Plate 6. Irrigation schemes along Lake Ziway and Bulibula River, April, 2008