ADDIS ABABA UNIVERSITY
SCHOOL OF GRADUATE STUDIES
DEPARTMENT OF BIOLOGY

THE EFFECT OF WATER PHYSICAL QUALITY AND WATER LEVEL CHANGES ON THE ABUNDANCE AND OCCURRENCE OF *Anopheles* LARVAE (DIPTERA:CULICIDAE) AROUND THE SHORELINE OF THE KOKA RESERVOIR, CENTRAL ETHIOPIA.

BY
BERHAN MELLESE

IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF MASTER OF SCIENCE IN BIOLOGY (INSECT SCIENCES).

Addis Ababa, Ethiopia
July 2008

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The effect of water physical quality and water level changes on the abundance and occurrence of *Anopheles* larvae (Diptera: Culicidae) around the shoreline of the Koka Reservoir, Central Ethiopia.

BY
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A Thesis presented to the School of Graduate Studies of the Addis Ababa University in Partial Fulfillment of the Requirements for the degree of Master of Science in Biology (Insect Sciences).

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Acknowledgment

First of all my deepest gratitude goes to my advisors Dr. Habte Tekie from Addis Ababa University and Dr. Matthew McCartney from International Water Management Institute (IWMI), for their valuable support and encouragement. The inputs they provide at various phases of the work and through correcting the thesis were very valuable. My Special thanks go to Dr. McCartney for his support from the start of the project till the end in every aspect.

I am very thankful to Ato Solomon Kibret from IWMI for his support in organizing things for the field and lab work. The assistance obtained from Ato Aydefer Negash from Computer Solutions for Professionals (CSP) training center during the statistical analysis of the data is greatly valued.

The cooperation of Nazareth Malaria Control Center technicians especially Ato Tesfaye Agonafer for the assistance in the field and lab work is greatly acknowledged. And my friend Nebiyu Alemu for his support in the field work needs much recognition.

I would also like to give many thanks for my mother Fana Kidane, my sister Eleni Mellese and her family, Eyob Mellese, especially Nuria Seid for the provision of Laptop computer, and all my relatives and friends, especially the hospitable friend of mine at Nazareth Abel Debebe that were my wonderful sources of support.

Finally I would like to thank the staff members of Ethiopian Electric Power Corporation (EEPCo) and National Meteorological Agency (NMA) for providing me the up to date data that I used in the preparation of my thesis.

The International Water Management Institute (IWMI) Challenge Program for Water and Food (CPWF, Project 36), and the Addis Ababa University (Department of Civil Engineering) funded this project.

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List of Abbreviations

AAU	Addis Ababa University
CDC	Center for Disease Control and Prevention
CI	Confidence Interval
DDT	Dichlorodiphenyl trichloroethane
DF	Degree of Freedom
EEPCo	Ethiopian Electric Power Corporation
ITN	Insecticide Treated Nets
IWMI	International Water management Institute.
MoH	Ministry of Health
MoWR	Ministry of Water Resource
NMA	National Meteorological Agency
RBM	Roll Back Malaria
SE	Standard Error
WHO	World Health Organization
X^2	Chi square tests

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Abstract

Entomological studies on the effect of water physical quality and water level change on the occurrence of *Anopheles* mosquito larvae and the formation of larval breeding habitats were conducted in two villages (Ejersa and Kuma) at the Koka Reservoir in Central Ethiopia between August and December 2007. Data on the type and number and physical characteristics of *Anopheles* larval breeding habitats, species composition and densities were recorded. Meteorological and reservoir water level data were compared with availability of *Anopheles* larval breeding sites and densities. Four-year retrospective clinical data indicated that the pattern of malaria transmission in the village at close proximity of the Koka reservoir is strongly associated with reservoir water level change during the peak malaria transmission season. Data from adult and larval collection showed that *Anopheles pharoensis, An. gambiae* s.l., *An. coustani* and *An. squamosus* were found in the study area in different proportions. *An. pharoensis* larvae were dominant at the village close by the reservoir while *An. gambiae s.l.* more common at the second village away from reservoir in the short breeding interval during the study periods. The total count of *An. pharoensis* larvae at the reservoir site was significantly higher than at the control village ($X^2 = 942.8$, df = 1, $p < 0.05$). This indicates that this species prefers breeding sites created in association with shoreline puddles that provide ideal turbid breeding pools with much floating aquatic vegetation. The total count of *An. gambiae* s.l. at the reservoir site was also significantly higher (X^2=200.5, df=1, $p < 0.05$) than at a the nearby control village. Generally, mean larval density of *An. gambiae* s.l. was higher in slightly turbid and shallow aquatic habitats (F=16.97, $p<0.05$ and F=6.03, $p<0.05$ respectively) than that of turbid and deep aquatic habitats. The density of *An. pharoensis* in breeding habitats with floating vegetation and with relatively shady condition was significantly higher than that of the aquatic habitats with much light and greater emergent vegetation (F=15.75, $p < 0.05$ and F=10.56, $p < 0.05$ respectively). There was also a positive correlation between the occurrence of larvae, water temperature of the breeding habitats and daily minimum atmospheric temperature (r= 0.541 and, r= .0.604, respectively $p <0.05$). Similar comparison indicated a positive correlation between water level changes resulting in subsequent recession of the reservoir and the number of positive breeding habitats during the sampling period in the reservoir village ($r = 0.605$, $p < 0.05$). Results in this study clearly showed that water physical characteristics such as water temperature, turbidity, depth and vegetation cover play an important role in the species composition, total count and density of *Anopheles* mosquitoes in the vicinity. Reservoir water level change is also associated with the proliferation of ideal mosquito breeding habitats.

1.1 *Anopheles* mosquitoes as malaria vectors in Africa

Mosquitoes are found through out the world except in places that are permanently frozen. There are about 3,500 species of mosquitoes (Diptera: Culicidae) grouped in to 41 genera of which nearly three quarters are native to the humid tropics and subtropics. However, only females of genus *Anopheles* transmit human malaria. Of the 430 species of *Anopheles* recognized, some 60 are vectors of human malaria. However in any given geographical area there are usually not more than three or four species of *Anopheles* mosquitoes that are important vectors responsible for the transmission of malaria (Gilles and Warrell, 1993).

In Africa five very efficient vector species transmit malaria: *An. gambiae* s.s. Gilles, *An. arabiensis* Patton, *An. funestus* Patton, *An. nili* Theobald and *An. moucheti* (Fontenille and Lochouarn, 1999). In most parts of Africa, several vectors transmit malaria in each location, in some cases at the same time and in other cases during different seasons, much variation in species composition can be observed between villages a few kilometers away from each other (Fontenille and Lochouarn, 1999).

Of the total 142 *Anopheles* species recorded in Sub Saharan Africa, to date less than 20 species have so far been reported to transmit human malaria under natural conditions (Antonio-Nkondjio *et al*., 2006). Of these *Anopheles gambiae* s.s., *An. arabiensis* and *An. funestus* are the most widely distributed malaria vectors throughout tropical Africa (Gillies and De Meillon, 1968; Fontenille and Lochouarn, 1999).

The *Anopheles gambiae* complex is the most important malaria vector in Africa comprising six morphologically indistinguishable sibling species, cytogenetically identified as *An. gambiae* sensu stricto, *An. arabiensis*, *An. quadriannulatus* A & B, *An. merus*, *An. melas* and *An. bwambae*. Among these *An. gambiae* s.s. and *An. arabiensis* are the most widely distributed (White, 1974; Coetzee *et al*., 2002).

An. arabiensis is also an efficient vector of malaria in many parts of tropical Africa (Gillies and De Meillon, 1968; Hunt *et al.*, 1998; Boyoh and Lindsay, 2003). It is capable of enduring droughts (White, 1974). Geographically, the distribution of *An. arabiensis* is more concentrated in the low lands with lower annual rain fall (<1000mm), which represents the drier region of the Savannah belt (Coetzee *et al*., 2002). *An. gambiae* s.s. is the predominant species in more humid areas (annual rain fall greater than 1000mm) and is widely spread throughout the tropical and equatorial Africa (Gillies and Coetzee, 1987).

An. funestus is a widespread malaria vector composed of a group of several sibling species closely resembling each other that can only be differentiated by minor characteristics of their larvae or adults. These sibling specie are *An. parensis*, *An. rivolurm*, *An. vaneeden* of which only *An. funestus* is anthropophilic while the rest are zoophilic (Fontenille and Lochouarn, 1999).

An. nili and *An. moucheti* are vectors of malaria that haven't been given much consideration interms of malaria transmission in Africa (Foutenille and Lochouarn, 1999). *An nili* is the main vector of malaria among populations that live along rivers since it breeds along the edges of rivers (Carnevale *et al*., 1992), while *An. moucheti* is prevalent in forest region and is a main vector in such villages having thousands of inhabitants (Service, 1976).

An. pharoensis Theobald is primarily a species which breeds in swamps with vegetation and along lake shores among floating plants (Gillies and De Mellion, 1968). It is distributed throughout Africa including Ethiopia (Abose *et al.*, 1998 Nigatu *et al.,* 1994; O'Connor, 1967). In South West Ethiopia (Gambela region) *An. pharoensis* was found to be the second important vector in the area followed by *An. gambiae* s.l. (Nigatu *et al*., 1994). *An. pharoensis* is reported as a secondary malaria vector around the Ziway area (Abose *et al.,* 1998; Kibret, 2008).

1.2 Ecological factors affecting malaria transmission

Malaria transmission involves complex interactions between *Plasmodium* parasites, *Anopheles* mosquitoes and humans. These features include the interaction between environmental variables, socioeconomic factors and biological factors (Lindsay and Martens, 1998, Alemu, 2007). A recent resurgence of malaria is largely associated with man made changes resulting in hospitable conditions for the breeding of anophelines. Malaria, principally a rural disease has diversified into various ecosystems as a consequence of the creation of new vector habitats created implementation of developmental plans (Sharma, 1996).

Temperature, rain fall and humidity, are the most important environmental factors that affect malaria vector abundance and survival as well as parasitic maturation regarded as key determinants of malaria transmission (Martens, 1995, Lindsay and Birley, 1996). For instance temperature affects malaria transmission, initially by affecting the development of larvae of anophelines. This determines the timing and abundance of mosquitoes following adequate rain fall. For example, at 16^0C, larval development may take more than 45 days compared to only 10 days at 30 ^{0}C. Hence at 16^0C the number of mosquito generations is reduced and the larvae are at increased risk of predators (MaCdonald, 1957).

The period of larval development under laboratory observations increases with an increase in water temperature reaching maximum at 28^0C. Adult development period was greatest between $28\text{-}32^0$C and no adults emerged below 18^0C or above 34^0C indicating the effect of temperature fluctuations on the abundance and development of the aquatic stages (Boyoh and Lindsay, 2003).

Temperature also affects the duration of gonotrophic and sporogonic cycle female *Anopheles* mosquitoes (Cook, 1992). The extrinsic incubation period or sporogonic cycle, which refers to the development period taken by the parasite in the adult mosquito to infective stages of the four *Plasmodium* species of human malaria and a gonotrophic cycle that refers to an interval between blood meals of *Anopheles* mosquitoes varies inversely with ambient temperature. This explains why malaria is unusual or rare in cold climates (Boyoh and Lindsay, 2003, Rua *et al.,* 2005; Petz and Olson, 2006)

The intensity and distribution of rainfall is another important factor that determines the distribution of malaria. It not only provides the medium for the aquatic stages of the malaria mosquitoes but also increases the relative humidity and hence longevity of the adult mosquito. Following the rainy season is the time at which peak malaria transmission is recorded in many countries. Exceptionally high rainfall in combination with unusual high temperature in the highlands is often the cause for an increased malaria transmission (Lindsay and Birley, 1996; Lindsay and Martens, 1996, Zhou *et al*., 2004).

Climate change is considered as a cause for the change in the distribution and abundance of malaria around the globe (Cook, 1992). The disturbance in the climatic set up of the earth is expected to bring about a change between 1-4 ^{0}C of the global atmospheric temperature with in the coming century (Lindsay and Birley 1996). It is possible these changes are the main causes for the present unexpected change in the climatic patterns of the globe which result in sudden and unexpected flooding, fluctuation in the maximum and minimum temperatures and rain fall of an area. It is expected that the highlands of the tropical areas and most of the temperate world will be the most affected by climate change (Martens, 1995; Reiter, 2001).

Human-led ecological changes related to settlement patterns, land use changes and agricultural practices, arising as consequence of an increased human population have brought new opportunities for *Anopheles* mosquitoes to breed (Ijumba and Lindsay, 2001; Hey *et al*., 2006). Re-settlement of highland communities in lowland areas (i.e. with endemic malaria) have dramatically increased malaria reservoir size and hence level of transmission (Woube, 1997). Land use changes, on the other hand, have created more mosquito larval habits and have changed water chemistry and temperature of mosquito larval habitats so that mosquito larval development is accelerated and survival increased (Coluzzi, 1994).

Water resource development such as construction of irrigation schemes and dams in areas that support malaria transmissions have also been shown to create more favorable breeding grounds for malaria vector species (Kibret, 2008; Snow, 1983; Ijumba and Lindsay, 2001; Lautze *et al.*, 2007). Over the past 50 years an estimated 40,000 large dams and 800,000 small dams have been built, and 272 million hectares of land are currently under irrigation worldwide (Keiser *et al*., 2005). More dams will soon be constructed in Africa and throughout the world (McCartney *et al.*, 2007). Although it is believed that water resource structures do have an impact on alleviating poverty and promoting economic growth, improving food security and mitigating floods, the adverse effects on the health of the people living in the vicinity reduces the overall benefits (Keiser *et al*., 2005). For instance there are evidences that this activity is becoming the major factor for the change in ecological factors suitable for malaria transmission (Keiser *et al*., 2005; Ghebreyesus *et al.,* 1999). Such developmental activities have increased malaria case rates in areas proximal to dams (Lautze *et al*., 2007; Ghebreyesus *et al.,*1999, Yohannes *et al*., 2005). For example, dams in Cameroon and Kenya have resulted in an increased malaria burden (Atangana *et al.,* 1979; Oomen, 1981).

Availability and distribution of *Anopheles* larval habitats are affected by natural or man-made ecological changes in potential malaria areas (Keiser *et al*., 2005). Larval habitat characteristics include both the physical and chemical state of the larval habitat. Physical habitat characters chiefly includes the temperature, turbidity and substrate type of the aquatic habitat, while the chemical characters include the pH, electric conductivity, the presence or absence of some ions and minerals in the breeding water (Sharma, 1990). Spatial distribution of anopheline species is mainly affected by the characteristics of the local habitat (Minakawa *et al*., 1999). Spatial distribution refers to distribution in place while temporal refers to distribution variation in time (Martin, 1999). Knowledge on these characteristics of the aquatic habitats helps to target potential vector breeding sites during larval control operations (Riberio *et al*., 1996; Minakawa *et al*., 1999).

1.3 Epidemiology of malaria and vector control in Ethiopia

Ethiopia is found in the tropical zone of Africa. Its diverse terrain comprises both uplifted ground and the low-lying rift systems of the Great Rift Valley. Various climatic and physiographic conditions and population movement, makes it an interesting country to test the applicability of various models of malaria geography, theories and hypothesis (Woube, 1997). Malaria is a significant public health problem in Ethiopia (Taye *et al*., 2006). It occurs in most parts of the country in an unstable form due to the country's topographical and climatic features (Abose *et al*., 1998, Ghebreyesus *et al*., 2006). Ethiopia has five altitudinal seasons and three geo-climatic zones. They are *Kolla* or hot zone (46%), *wayna daga* or temperate zone (46%) and *dega* or malaria free zone (8%) (Woube, 1997). The altitude in Kolla is < 1500 (m.a.s.l.) and the mean annual temperatures vary between 20-30^{o}C with mean annual rainfall ranging between 100-1500mm and malaria incidence varies from moderately to high endemic. The altitude for *woyna dega* zone is 1500-2500 m.a.s.l. with annual rainfall ranging from 400-2400mm. with mean temperature around 20^{0}C and malaria incidence varies from low endemicity to epidemic (Tulu, 1993).

Transmission of malaria usually occurs at altitudes < 2000 m.a.s.l. The two main transmission seasons for malaria in Ethiopia are September-December, after the heavy summer rains, and March – May, after the light rains. *Plasmodium falciparum* and *P. vivax* are the dominant human malaria parasites, which account for about 60% and 40% of cases, respectively (Woube, 1997). Although the two epidemiologically important malaria parasite species *P. falciparum* and *P. vivax* occur, the two species, *P. malariae* and *P. ovale*, are also reported to occur in Ethiopia (Ghebreyesus *et al*., 2006) .

Malaria is found in about 75% of the total area of the country, and 40-50 million (>65%) of the total population is at risk of infection. In a normal transmission year approximately 2-5 million cases of malaria are reported annually in Ethiopia (WHO, 2005). It is estimated that only 20 percent of children less than five years of age that contract malaria are treated at existing health facilities. Large scale epidemics occur every 5-8 years in certain areas due to climatic fluctuations and drought-related emergencies. There are also areas of stable transmission in some low-lying western regions of the country. The social and economic consequences of the disease are sobering, with a large number of people being kept off work by debilitating illness, resulting in low productivity (WHO, 2005).

Quantification of malaria burden in Ethiopia is problematic since the rural majority has limited access to health institutions. Although reliable communities based information is limited, the magnitude of malaria burden can be referred from the few published studies available. A study in the northern part of Ethiopia revealed that the average number of workdays reported lost during malaria episode was 18 for an adult, and the cost of illness per clinical episode ranges from 46 to 151 Birr, which is a large portion of the average income (58-196 Birr) of the villagers (Ghebreyesus *et al*., 2006).

Vector abundance is the major determinant factor of malaria distribution in any country (O'Connor, 1967). In Ethiopia so far, a total of 42 *Anopheles* species have been recorded with a distribution controlled by altitudinal zone and microhabitats (Ghebreyesus *et al*., 2006). However only four species, namely, *An. gambiae* s.l. (cytogenetically *An. arabiensis*), *An. pharoensis*, *An. funestus* and *An. nilli* have been incriminated as vectors of malaria in different parts of the country (O'Connor, 1967; Ghebreyesus *et al*., 2006). *An. arabiensis* is the major malaria vector in Ethiopia while *An. pharoensis*, *An. funestus* and *An. nili* are reported as secondary vectors (Abose *et al*., 1998).

Ethiopia is among the few African countries that has implemented a malaria control program for a long period of time (Gebremariam, 1988). Early diagnosis and treatment at a village level by community health workers was introduced in several pilot areas. In Tigray, where this program is currently well established, 65-71% of malaria patients are treated each year by community health workers (Ghebreyesus *et al*., 2006).

Indoor residual spraying (with DDT) has also been another primary strategy for control of malaria in the country, in which DDT was extensively employed and all houses in all malarious localities are targeted. However, due to long term application and household factors, various degree of resistance to commonly used insecticides has been documented in different parts of the country. For example in Ziway area, 82.5% *An. pharoensis* and 30.3% *An. arabiensis* population were found to be resistant to DDT in 1994. (Abose *et al.*, 1998). Recently very high DDT resistance (>92%) has been observed in Gorgora area, northern Ethiopia (Balkew *et al.*, 2006). Similarly high levels of resistance in Gambela, Arbaminch and parts of Tigray has necessitated a change from DDT to malation in the past few years (Ghebreyesus *et al.*, 2006).

In areas with strong community based malaria control programs, environmental management for vector control mainly through source reduction has been found be to successful when undertaken during peak transmission season (Ghebreyesus *et al.*, 2006). An integrated approach to malaria control that relies heavily on community involvement is one that may have the brightest future for the very reason that such method is environmentally friendly and cost effective. The foundations of any such approaches should be source reduction to reduce the level of malaria transmission in an area (Yohannes *et al.*, 2005). This strategy should be based on the local ecology and behavior of the vectors. However, source reduction, through the modification of larval habitat has recently been a neglected area of vector research and control although in the past it was one of the main methods of malaria control and eradication efforts in many countries including the US, Israel and Italy (WHO 1982; Yohannes *et al.*, 2005). Appropriate management of larval habitats in places where there are few vectors helps to suppress malaria transmission, as a proportional reduction in transmission is likely to lead to a similar reduction in clinical episodes of malaria in such areas (MaCdonald, 1957).

Recently the distribution of Insecticide Treated Nets (ITN) is becoming the main activity in the control and prevention of malaria in Ethiopia. Between 2001 and 2003 the number of ITNs distributed at household level throughout the country increases by 40% and there has been a 28% and 76.5% increase in house hold coverage and net re-impregnation in Ethiopia respectively. There is recently a general decrease in malaria prevalence after the distribution of ITN although several factors may have been responsible apart from ITN (RBM, 2005, MOH, Unpublished report).

1.4 Malaria and water resource development in Ethiopia

Water resource development is a major activity throughout Africa. Currently, Ethiopia is undergoing an extensive construction activity all over the country. According to McCartney *et al.* (2007) of the 12 big dams under construction or planned to be constructed in the near future in different countries in Africa, four of them are in Ethiopia.

There is an association between malaria incidence and proximity to dam in Ethiopia. Malaria case rates among people living within 3 km of the Koka reservoir are 1.5 times higher than those living between 3 and 6 km from the reservoir and 2.3 times higher than those living 6-9 km away the reservoir (Lautze *et al.*, 2007). Water harvesting activity in Tigray continues as a source of water for drinking, irrigation and power generation. After extensive study done in these areas, malaria incidence in young children was found to be seven fold higher in communities near dams than those further away (Ghebreyesus *et al.,* 1999). A recent study in the same area showed that, the abundance of adult *Anopheles* mosquitoes in villages near dam sites was 5.9-7.2 times higher than those villages further away from dams (Yohannes *et al.*, 2005).

Villages located near dams or impounded waters are thus subjected to increased malaria incidence mainly due to the presence of abundant and suitable breeding habitats for the proliferation of *Anopheles* mosquitoes which increases the density of the malaria vectors capable of transmitting the disease. Proximity to a dam includes command areas or irrigation water that can serve as *Anopheles* larval development sites. This general expectation is valid only when the habitat provides match ecological requirement for the local vectors. In particular, it requires that the new bodies of standing water have sunlight or shade, surrounding vegetation, turbidity etc. compatible with the larval habitats for at least one local species (Minakawa *et al.*, 1999; Shililu *et al.*, 2003; Keiser *et al.*, 2005). Consequently, the creation of new breeding sites will have an effect on the development and survival of these vector species, and interaction among them in terms of their role in local malaria transmission (Keiser *et al.*, 2005).

The International Water Management Institute is undertaking various researches concerning the malaria situation around the Koka dam in central Ethiopia. While trying to design a decision support system to have an efficient dam management that maximize the benefit gained from the reservoir that incorporate the outputs of various former studies done around the area; there is a gap in the studies that how the physical characteristics of breeding habitats govern the occurrence and abundance of *Anopheles* mosquitoes. Therefore this study is aimed at investigating the effect of physical habitat characteristics of newly formed *Anopheles* larvae breeding habitats in two villages, with one adjacent to the reservoir and the other away from it and the association of the rise and fall of water level of the Koka reservoir in the creation of new potential breeding habitats in the village near the shoreline comparison with that of a village away from the dam. It also tries to determine the species composition and occurrence of *Anopheles* larvae in the vicinity of the Koka dam.

2. Objective of the study

2.1 General objective

To study the impact of physical water characteristics and reservoir water level changes on *Anopheles* larval occurrence and productivity near the shoreline area and its effect on malaria transmission on nearby human communities.

2.2 Specific Objectives

- To study the effect of physical water characteristics on the occurrence and abundance of *Anopheles* larvae

- To determine the species composition of *Anopheles* larvae in the vicinity of the Koka reservoir.

- To investigate the effect of water level changes in the formation of new breeding habitats and larval and adult mosquito productivity close to the shoreline of the reservoir

3. Materials and Methods

3.1 Description of Study Area

Koka dam and its reservoir, is located in the Rift Valley of Ethiopia (Figure 1). It is located in a rural area some 100 kms south east of Addis Ababa found along the Awash Basin (8^0 41' N and 39^0 35' E) at 1,590m above sea level. Residents live in traditional houses or huts called Tukuls.

The dam was the first hydropower plant in Ethiopia, originally constructed to generate electricity for Addis Ababa and other urban centers. However, it is now multipurpose and is also used for down stream irrigation, of the 6000 ha Wonji sugarcane project (Lautze *et al.*, 2007). The capacity of Koka reservoir has been reduced from 1650Mm^3 in 1959 to 1186 Mm^3 due to sedimentation. The loss on total storage capacity over the last 39 years is estimated to be 464 Mm^3, which is 28.1% of the total storage volume of the reservoir (Zewdu, 2005).

The area is characterized by its lowland nature since found in the rift valley, with a wide and open plain land suitable for cultivation. Vegetables are grown around the reservoir using the wetland created by the reservoir recession and with the help of water pumps to pump out the shallow ground water. Sparsely distributed *Acacia* trees are common in the area.

The study was conducted at two sites located close to the Koka reservoir. Site 1 is the village of Ejersa where the shoreline of the Koka dam reaches the middle of the village during the peak rainy season; it has a population of 6,500. Site 2 is Kuma, which is located five kilometers away from Ejersa and always at least 5 kilometers from the shoreline of the Koka reservoir with a population 4, 300 according to the respective villages' Agricultural Offices.

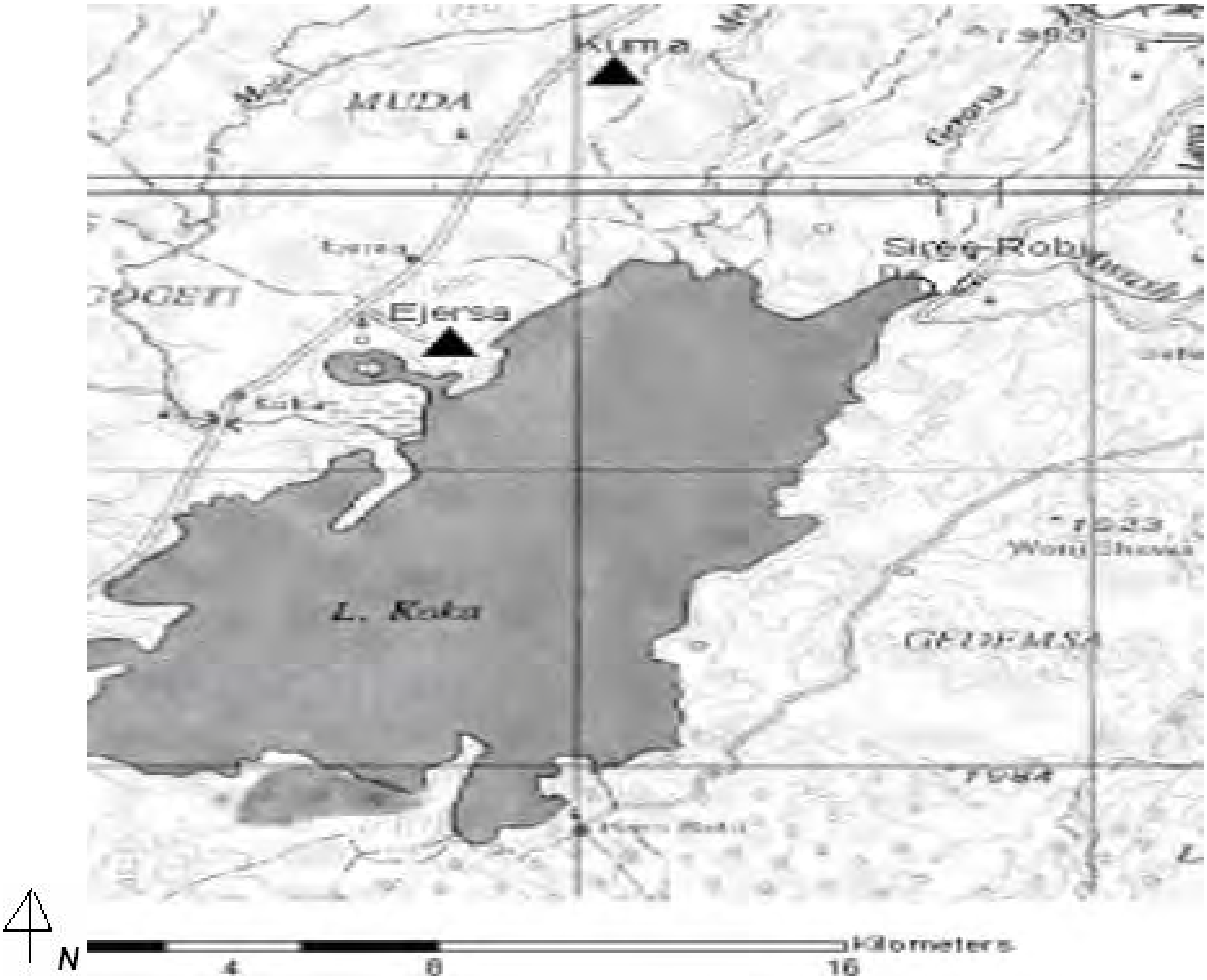

Figure 1: Map of the Koka area in the middle course of the Ethiopian Rift Valley (Triangles show the study villages). (Source: International Water Management Institute (IWMI)).

In Ejersa, there are households that are located only a few meters away from the shore during the peak rainy season when the water levels rise and the dam creates a temporary lake. Farmers in the area are known for the production of vegetables and some cereals especially between the months July and February using the rising ground water near the shoreline. Residents also use the temporary lake as a main site of fishing (Plate 1). Then they cultivate this wet and fertile land following the recessing water (Plate 2).

The village was selected based on its proximity to the dam where the effect of the recession of reservoir water and the formation of new *Anopheles* larvae breeding sites in the form of turbid shoreline puddle is observable (Plate 3); its distance from the control village based on the likely flight range of female *Anopheles* mosquitoes, and with a similar elevation and topography and comparable agricultural system was also used for comparison.

Kuma, is located 5 kms away from the reservoir where the effect of the dam is not apparent (Lautze *et al*., 2007). In this area the breeding sites for *Anopheles* mosquitoes are temporary rain pools created by the effect of the summer rain and Agricultural puddles created by over flow of irrigation ditches in agricultural fields (Plate 4).

Plate 1: Fishermen in Ejersa on the seasonal lake created by the rise in water level of the Koka dam during the rainy season

Plate 2: Farmers cultivating following the recessing shoreline for growing vegetables

Plate 3: Turbid shoreline puddle with floating aquatic vegetation: an ideal breeding site for *An. pharoensis* in Ejersa, a village around the Koka reservoir.

Plate 4: Agricultural puddle formed during the rainy season: Ideal breeding site at Kuma village, located 5 km away from the shoreline of the Koka dam

3.2. Meteorological data and reservoir water level

Ten years of monthly climate data and the recent two years data of daily mean minimum and maximum temperature; rainfall and relative humidity were taken from National Meteorological Agency (NMA). The main rainy season in the area starts in June and ends at the end of August/September, while the short rainy season begins in March and extends to April/May. The location has a warm climate with an annual mean maximum and minimum temperature of 30.4 and 14^0C, respectively (Figure 2).

Secondary data on daily water level change for the study period were obtained from Ethiopian Electric Power Corporation (EEPCo). Weekly water level records during the study period indicated that the water level has an increasing trend during the first weeks of the study period then decreased afterwards (Figure 3).

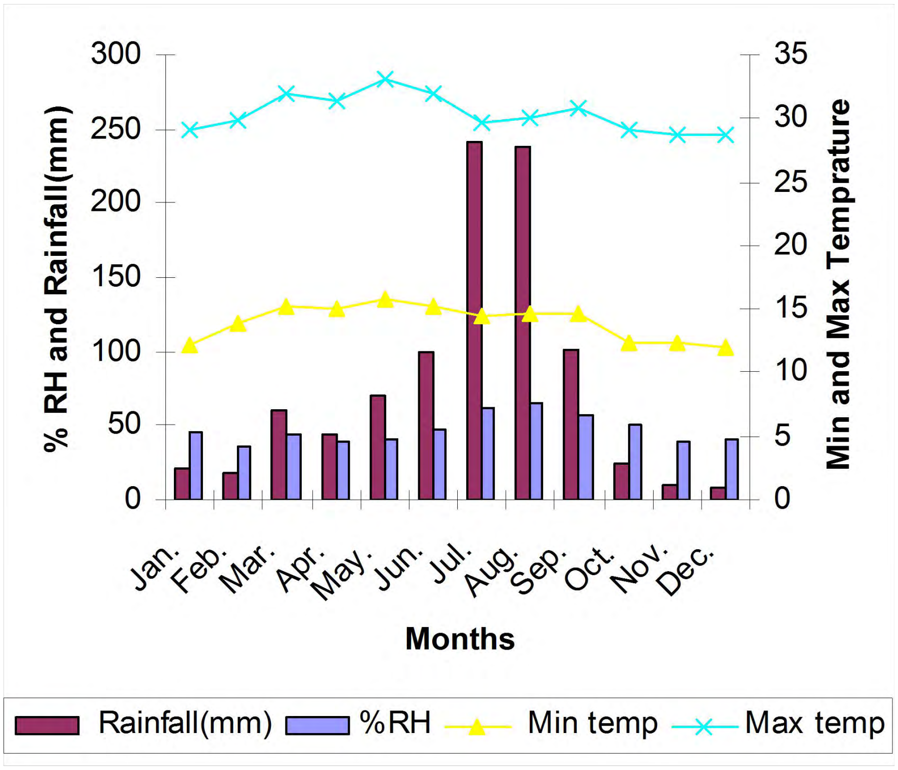

Figure 2: Monthly mean maximum and minimum temperature (°C), relative humidity and rainfall (mm) of the Koka Dam (1998-2007). Source: National Meteorological Agency (NMA)

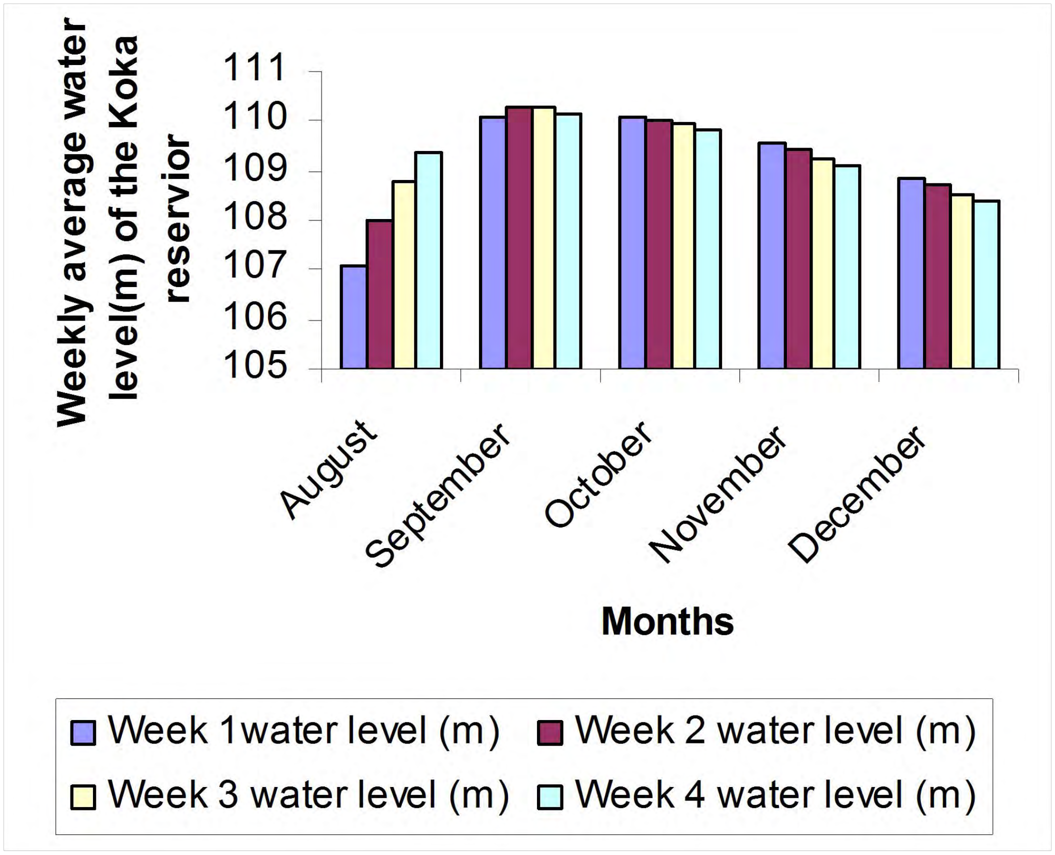

Figure 3. Weekly mean water Level of the Koka Dam for the Months (August-December, 2007)
Source: (Ethiopian Electric Power Corporation (EEPCo)).

3.3 Entomological studies and larval habitat characteristics

3.3.1 Larval collection and identification

Larval collection was done at the two study villages on a weekly interval over a period of for four months (August- December, 2007). Sampling was carried out between 11:00 and 16:00 hours of the day. At each sampling period, all the available breeding habitats within 500m^2 area of the study sites were visited and checked for the presence of *Anopheles* mosquito larvae by taking three dips using a standard (350 ml) dipper (Yohannes *et al.*, 2005; Shillilu *et al.,* 2003; Minakawa *et al.,* 1999) (Plate 5). Breeding sites were then sampled by taking of 6 dips/m^2 (4 dips at the margin and 2 from the middle). The number of dips taken varied depending on the area (m^2) of the specific positive breeding sites. A maximum of 60 dips were made for small pools less than 10m^2 (Herrel *et al.*, 2004). In positive breeding sites the number of *Anopheles* larvae were counted and recorded.

The larvae collected from different breeding habitats were pipetted into separate vials and taken to the Nazreth Insectary (Oromia Malaria Control Center) for identification. The water in each vial was heated gently to kill the larvae. Excess water was drained and poured from each vial and a syringe with a needle was used to replace it with 70% alcohol (Yohannes *et al.,* 2005; Shillilu *et al.,* 2003).

The third and fourth instar larvae were visually sorted and used for identification (Yohannes *et al.,* 2005; Minakawa *et al.,* 1999). The larvae were first mounted (using gum chloral) on a dissecting microscope and later identified to the species level using morphological characteristics identification key (Verrone, 1962b).

3.3.2 Physical characteristics of larval breeding habitats

Along with the collection of larvae; data on physical habitat characteristics that affect the abundance and distribution of *Anopheles* larvae were collected. Water temperature was measured for each positive habitat identified using a thermometer (Plate 6). The thermometer was lowered below the water surface and left for two minutes for the thermometer reading to stabilize. It was then withdrawn with out touching the bulb end and the temperature was recorded (Yohannes *et al*., 2005; Shillilu *et al.*, 2003; Minakawa *et al.,* 1999).

Turbidity of the water of the aquatic habitat was categorized by taking a small sample of water in a glass test tube and comparing it with a white background and recorded as turbid or slightly turbid (Shillilu *et al*., 2003; Minakawa *et al*., 1999). Depth of positive breeding habitat was measured with a small-labeled stick. Other parameters like the size (area), exposure to sunlight; presence and types of vegetation, substratum or bottom of the breeding habitats and type of breeding habitat were observed and recorded (Yohannes *et al.*, 2005; Shillilu *et al*., 2003; Minakawa *et al*., 1999).

The breeding habitats in the study sites were classified in to three groups:

a) Agricultural puddles that are puddles created by over flow and flooding of irrigation cannels in agricultural fields or any man-made pools around the village.
b) Rain pools that refer to pools that are formed naturally from rain and
c) Shoreline puddles which are puddles formed after recession of the reservoir shoreline.

3.3.3 Adult *Anopheles* collection and identification

Collection of adult mosquitoes was concurrently carried out fortnightly as part of a parallel project by International Water management Institute (IWMI) in the two villages during the study period. CDC Light traps (Model 512; WaFlock Co., Atlanta, GA) were used in six houses not formerly been using bed nets in each of the study villages. Four traps were placed indoors (one trap per house and within an occupied sleeping room) and two light traps were operated outdoors in each village. Light traps were operated between 18:00 and 07:00 hours the following morning. Each light trap was placed in a bedroom, near a wall; with the bulb about 45 cm above the head of a person sleeping under an untreated bed net (Yohannes *et al.*, 2005). Collected mosquitoes were transported to Nazareth Insectary (Oromiya Malaria Control Center) counted and identified using identification keys by Verrone (1962a).

Plate 5: Larval sampling done by the standard 350 ml dipper.

Plate 6: Water temperature measured using a water sampling thermometer.

3.4 Data Analysis

All the data collected on larval density, physical characteristics of breeding habitats, adult female mosquitoes, and relevant meteorological and reservoir water level was entered in Microsoft Excel database system and analyzed using SPSS version 13 statistical software. X^2 test were used to compare total number of larvae in different aquatic habitats and an independent samples t test was used to compare mean larval densities in different physical habitat parameters. Weekly change in water levels is computed by subtracting the value of water level in meters prior to the given sampling week i.e. Weekly water level change (meters/week) Week (n) = Water level week (n) – water level week (n-1). Descriptive statistics and bivariate correlation were also used to determine the strength of association between larval density and the independent variables including atmospheric temperature (min and max), water temperature and water level.

4. Results

4.1. Malaria prevalence in relation to the Koka dam in the study area

Retrospective clinical data on the prevalence of malaria indicated that peak malaria transmission in the study area occurs between July and October (Figure 3). Comparison of average monthly malaria prevalence and the state of gain or lose of water of the Koka reservoir at Ejersa for the years 2004-07 shows that there was an increase in the prevalence of malaria corresponding to water level change observed right after the maximum monthly gain in water level of the Koka reservoir after Mid-August – December (Figure 4).

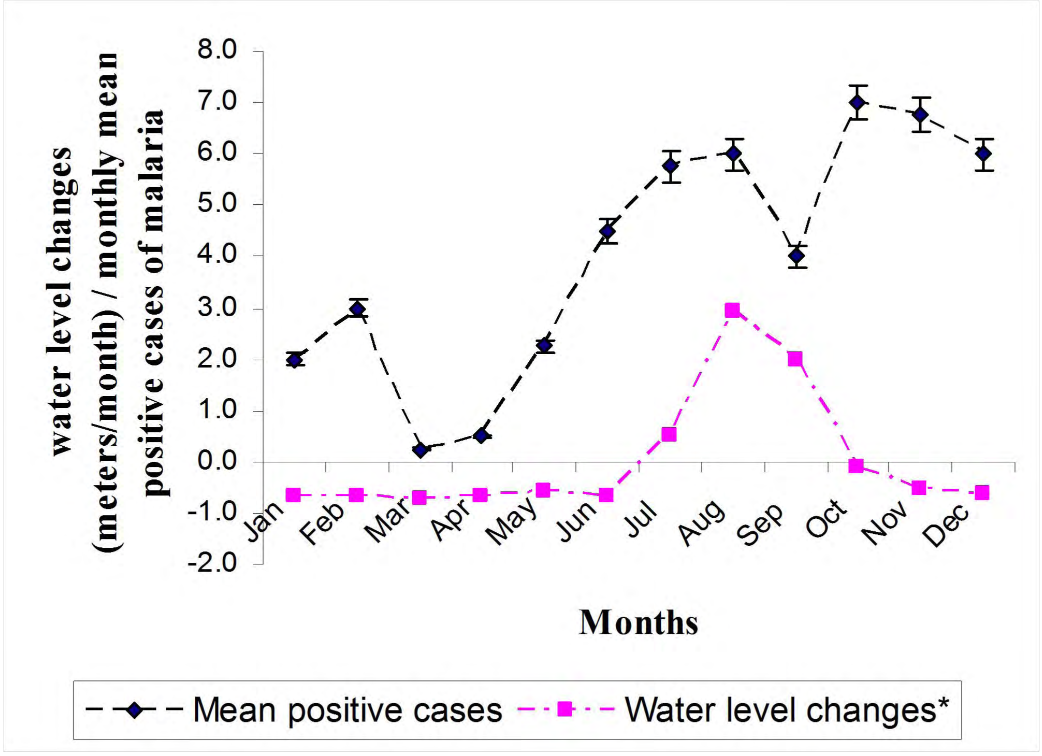

Figure 4: Monthly mean water level changes (meters/month) and average malaria prevalence at Ejersa for the years 2004-2007.

* Negative values indicate reservoir-losing water and positive values indicate reservoir-gaining water.

4.2 Species composition of *Anopheles* mosquitoes based on larval and adult collections

A total of 1797 3rd and 4th instar larvae of *Anopheles* mosquitoes were collected from both study villages during the study period of which 1645 (91.4%) were from Ejersa and 152(8.6%) were from Kuma. Identification of 3rd and 4th instar larvae and adult *Anopheles* mosquitoes from light trap revealed that *Anopheles pharoensis* Theobald *An. gambiae* s.l. Giles*, An. coustani* Grunberg and *An. squamosus* Theobald constitute the anopheline fauna in the study area (Table 1 and Table 2).

Significantly higher number of larvae of *An. gambiae* s.l. was counted throughout the sampling period at the reservoir site Ejersa as compared to Kuma (X^2 = 200.5, df = 1, $p<0.05$). Similar statistical comparison also indicates that *An. pharoensis* larval count was significantly higher at Ejersa than at Kuma (X^2 = 942.8, df =1, $p<0.05$). The number of adult *An. gambiae* s.l. collection from indoor CDC light traps was significantly higher than outdoors at both villages (X^2=30.4, df=1, $p<0.05$ and X^2 14.3, df=1, $p<0.05$ respectively).

Table 1: Species composition *Anopheles* mosquitoes identified from larvae collected during the study period at Ejersa and Kuma (Augst- December. 2007).

Site	*An. pharoensis* no. (%)	*An. gambiae* s.l. no. (%)	*An. coustani* no. (%)	*An. squamosus* no. (%)	Total
Ejersa	1075 (65.3)	476 (28.9)	56 (3.4)	38 (2.3)	1645
Kuma	22 (14.5)	128 (84.2)	0 (0)	2 (1.3)	152
Total	1097 (61.1)	604 (33.6)	56 (3.1)	40 (2.2)	1797

Table 2: Species composition and abundance based on indoor and outdoor CDC adult collections at Ejersa and Kuma for the sampling period (August- December. 2007).

	no. of adult *Anopheles* catches per trap per night			
Sites	*An. pharoensis* no. (%)	*An. gambiae* s.l. no. (%)	*An. coustanie* no. (%)	Total
Ejersa				
Indoor	45 (22.9)	142(72.4)	9 (4.5)	196
Outdoor	40 (33.8)	63(53.4)	15(12.7)	118
Total	85 (27.1)	205 (65.3)	24(7.6)	314
Kuma				
Indoor	12(21.8)	39 (70.9)	4 (7.3)	55
Outdoor	9(39.1)	12 (52.7)	2 (8.6)	23
Total	21 (26.9)	51 (65.4)	6 (7.7)	78

4. 3. Density and types of larval breeding habitats

4.3.1 Density of *Anopheles* larvae at different sampling periods

At Ejersa, peak larval density of *An. pharoensis* was recorded by mid October (50 larvae/ 100 dips) (Figure 5). On the other hand, peak larval density of *An. gambiae* s.l. was recorded at the end of September (21 larvae/ 100 dips). At Ejersa, *An. pharoensis* was with the highest mean larval density for the study period than *An. gambiae* s.l. (F=22.9, df =119, $p < 0.05$) (Table 3).

At Kuma, the highest larval density of *An. gambiae* s.l. was recorded by mid October (33.5 larvae/ 100 dips) (Figure 6). In this site mean larval density were zero for approximately half of the sampling period. (Mid August- Mid September) and (End of October –Mid December). For the remaining sampling period in which larval habitats were found positive, *An. gambiae* s.l. had a higher density than *An. pharoensis* in the area (F=0.019, df =119, $p<0.05$) (Table 3).

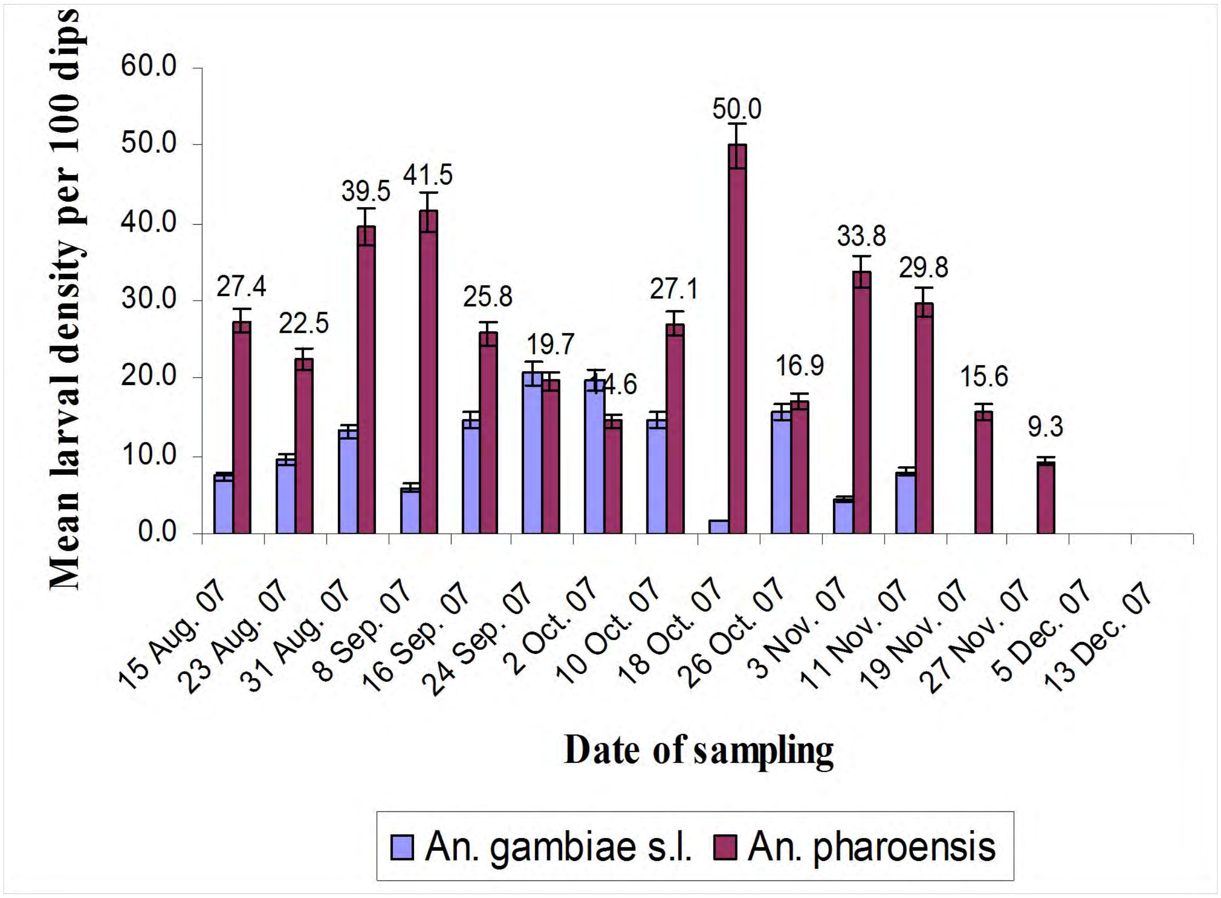

Figure 5: Mean number of larval density (Number of larvae/100dips) of *An. gambiae* s.l. and *An. pharoensis* for the reservoir village Ejersa (n=16 surveys).

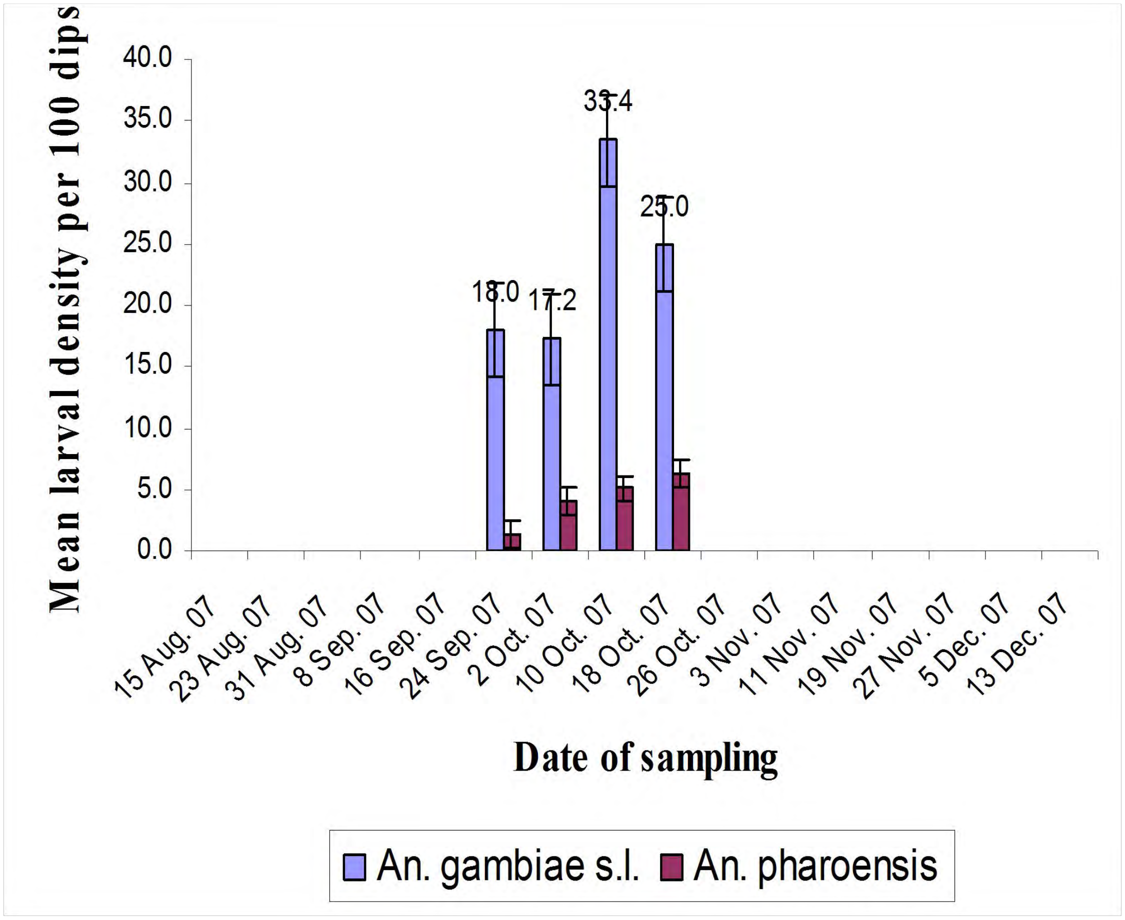

Figure 6: Mean no of larval density (Number of larvae/100dips) of *An. gambiae* s.l. and *An. pharoensis* for the non reservoir village Kuma (n=16 surveys), during the study period (August-December, 2007)

Table 3: Comparison of mean larval density of *An. gambiae* s.l. and *An. pharoensis* in the two study sites

	Mean number of larvae per 100 dips	
Site	*An gambiae s.l.*	*An. pharoensis*
	Mean ± s.e	Mean ± s.e
Ejersa	10.97 ± 0.64	26.96 ± 1.08**
Kuma	17.39± 0.99**	3.10± 0.46

**significantly different at $p<0.05$

4.3.2 Larval density and habitat preference in the two villages

At Ejersa, in addition to the above types of aquatic habitats, shoreline puddles were the main habitats suitable for the breeding of *An. pharoensis.* At Ejersa, of the total 1645 3rd and 4th instar larvae, 74% (1218) were collected in shoreline puddles comprising 17.4% (212) *An. gambiae* s.l., 76% (926) *An. pharoensis*, 4.4% (53) *An. coustani* and 2.2% (27) *An. squamosus* (Table 4). A X^2 analysis done to see association between type of aquatic habitat and type of species revealed that shoreline puddles had the highest proportion of *An. pharoensis* (X^2=671.79,df=1, $P < 0.05$). On the other hand rain pools were also with greater occurrence of *An. gambiae* s.l. than agricultural puddles (X^2 = 87.5, df =1, $P < 0.05$) at Ejersa.

At Ejersa the *An. gambiae* s.l. count in the rain pools was not significantly different from the shoreline puddles (X^2=0.84, df =1, $P > 0.05$). However, both the shoreline puddle and the rain pools had significantly different count of *An. gambiae* s.l. than the agricultural puddles (X^2=106.8, df =1, $p<0.05$) and (X^2=87.5, df=1, $p<0.05$) respectively.

At Kuma, the most common breeding aquatic habitats were seasonal rain pools and agricultural puddles that are created through agricultural activities in the form of man-made pools or overflow and flooding of agricultural fields. At Kuma rain pools there were more *An. gambiae* s.l. than *An. pharoensis* (X^2=28.8, df =1, $p<0.05$).

At the reservoir site, shoreline puddle had the highest mean larval density of *An. pharoensis* (31.3 larvae per 100 dips) than rain pool (F=22.03, df = 87 $p<0.05$). On the other hand rain pools have the higher (28.63) mean density of *An. gambiae* s.l. than shoreline puddles (Figure 7) (F=3.92, df, 87, $p < 0.05$). In the non-reservoir site no shoreline puddles were encountered and the mean larval density of both *An. gambiae* s.l. and *An. pharoensis* in rain pools were not significantly higher than that of the agricultural puddles (F=0.52, df=20, $p>0.05$) and (F=0.30, df=29, $p>0.05$).

Table 4. *Anopheles* larval count in different types of breeding habitats in the two study sites during the study period (August-December 2007).

	Breeding site		Number of *Anopheles* larvae collected*				
Village	Type	no. positive	*An gambiae s.l.*	*An. pharoensis*	*An. coustani*	*An. squamosus*	*Total*
Ejersa	Shoreline puddles	66	212 (17.4)	926 (76.0)	53 (4.4)	27 (2.2)	1218
	Rain pools	23	208 (67.3)	90 (29.1)	2 (0.6)	9 (2.9)	326
	Agricultural puddles	8	56 (47.5)	59 (50.0)	1 (1.0)	2 (2.0)	101
	Total	97	476 (28.9)	1075 (65.3)	56 (3.4)	38 (2.3)	1645
Kuma	Shoreline puddles	NA	NA	NA	NA	NA	NA
	Rain pools	16	95 (83.3)	17 (14.9)	2 (1.8)	0 (0)	114
	Agricultural puddles	6	33 (86.8)	5 (13.2)	0 (0)	0 (0)	38
	Total	22	128 (84.2)	22 (14.5)	2 (0)	0 (0)	152

* Values in parenthesis are percentages of total larvae collected at each type of breeding habitat.

NA= not available.

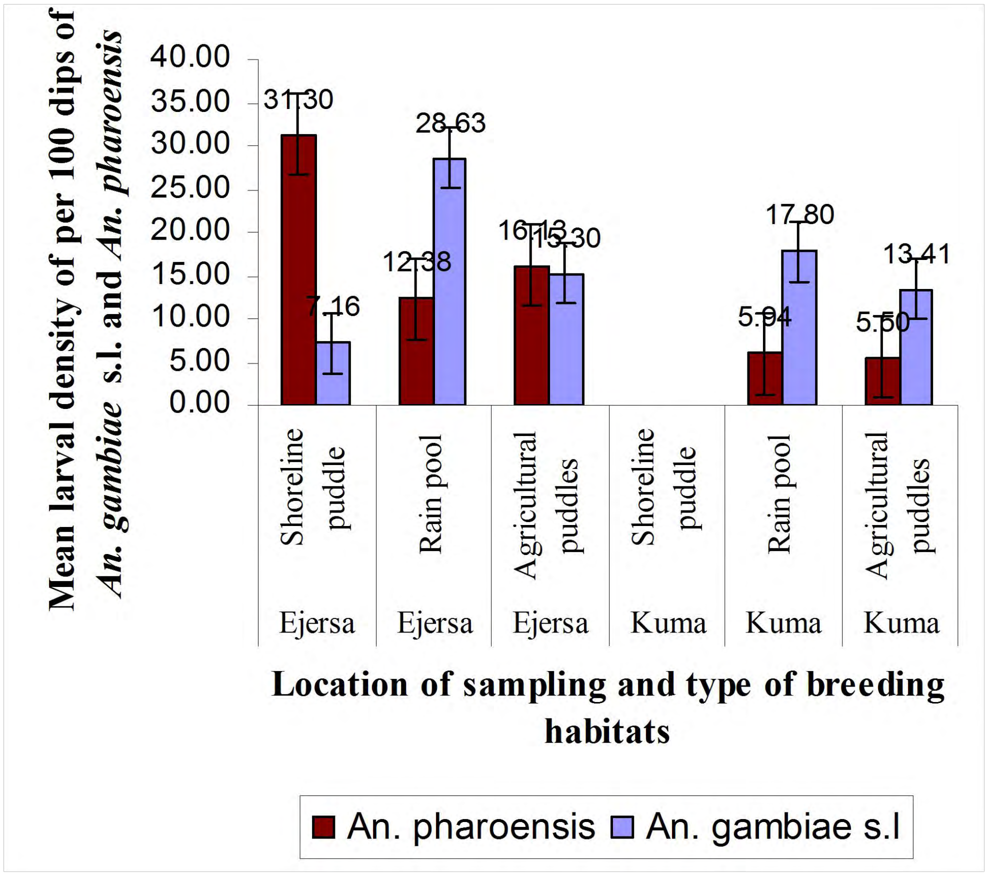

Figure 7: Mean larval density of *An. gambiae* s.l. and *An. pharoensis* in different aquatic habitats at Ejersa and Kuma during the study period (August-December 2007).

4.4. Physical characteristics of larval habitats, occurrence and mean densities of *An. pharoensis* and *An. gambiae* s.l.

The combined data on mean larval density from Ejersa and Kuma showed that *An. gambiae* s.l. was greater in slightly turbid and shallow aquatic habitats (20 larvae per 100 dips; 15 larvae per 100 dips, respectively) than that of turbid and deep aquatic habitats (F=16.97, $p<0.05$ and F=6.03, $p<0.05$). Similarly *An. gambiae* s.l. was greater in larval density in habitats with emergent aquatic vegetation and with relatively open sunlit condition (F=11.14, $p<0.05$ and F=10.77, $p<0.05$) (Table 5).

The mean larval density of *An. pharoensis* in turbid aquatic habitats **(**32.37 per 100 dips) was found to be significantly higher from that of the slightly turbid aquatic habitats (F=13.82, $P<0.05$). The density of *An. pharoensis* in aquatic habitats with floating vegetation and with relatively shady condition (31.11 and 31.56, respectively) was significantly higher than that of the aquatic habitats with much light and more emergent vegetation (F=15.75, $p<0.05$ and F=10.56, $p <0.05$). Depth of larval habitats had no significant effect on the mean larval density of *An. pharoensis* in the study area (F=0.84, $p>0.05$) (Table 5).

In this study the water temperature recorded throughout the study period ranged between 18 and 27.2^0C for breeding habitats at Ejersa, and between 22.5 and 25.5^0C for those at Kuma (Table 6). Fluctuation in water temperature of the aquatic habitats had a positive correlation with mean larval density of *An. pharoensis* and *An. gambiae* s.l at Ejersa ($r = 0.604$, $p < 0.05$ and $r=0.524$, $p<0.05$ respectively) but not at Kuma during the study period (Appendix 1).

Table 5. Physical habitat characteristics and mean larval density (no. of larvae per 100 dips) of *An. gambiae* s.l. and *An. pharoensis*

			Number of larvae per 100 dips		
Species	Habitat characteristics*		Mean + SE	F- ratio	P- value
An. gambiae **s.l.**	Turbidity	Turbid	5.55±0.25	16.97	0.00
		Slightly turbid	20.13± 0.46**		
	Depth[†]	Deep	8.55±0.5	6.03	0.02
		Shallow	15.54±0.47*		
	Vegetation	Emergent	23.31±0.67* *	11.14	0.001
		Floating	6.93± 0.41		
	Intensity of shade	Light	19.64± 0.46* *	10.77	0.001
		Shade	6.09± 0.29		
An. pharoensis	Turbidity	turbid	32.37±1.11**	13.82	0.00
		Slightly turbid	12.2± 0.85		
	Depth	Deep	21.34±1.46	0.84	0.363
		Shallow	24.03±0.96		
	Vegetation	Emergent	10.08±0.88	15.75	0.00
		Floating	31.11±1.01**		
	Intensity of shade	Light	12.86±09	10.56	0.002
		Shade	31.56± 1.11**		

** Significant at $p < 0.01$
* Significant at $p<0.05$

Table 6: Mean water temperatures of positive aquatic habitats and larval density of *An.gambiae* s.l and *An. pharoensis* in different sampling interval during the study period (August-December 2007).

	Ejersa			**Kuma**		
Sampling		Mean larval Density Per 100 Dips			Mean larval Density Per 100 Dips	
Weeks	Water T^0	*An. gambiae* s.l	*An. pharoensis*	Water T^0	*An. gambiae* s.l	*An. pharoensis*
1	20.22	7.4± 1.0	27.37± 3.7	(-)	(-)	(-)
2	24.44	9.5 ± 1.1	22.54 ± 3.0	(-)	(-)	(-)
3	25.11	13.2 ± 1.3	39.5± 4.7	(-)	(-)	(-)
4	23.11	5.9 ± 0.9	41.5 ± 2.8	(-)	(-)	(-)
5	23.80	14.8± 1.8	25.8± 2.7	22.50	7.7 ± 0.2	2.4 ±0.4
6	25.73	20.7 ± 1.2	19.70 ± 2.5	23.40	18.0 ± 0.8	1.30 ±0.2
7	21.00	19.8 ± 1.4	14.6 ± 2.1	25.50	17.2 ± 1.2	4.0 ±0.6
8	23.00	14.5 ± 0.7	27.1 ± 1.6	23.60	33.4 ± 2.7	5.1 ±0.9
9	26.33	1.7 ± 0.2	50.0 ± 1.3	24.30	25.0 ± 1.4	6.3 ±0.5
10	27.20	15.6± 0.6	16.9 ± 0.8	(-)	(-)	(-)
11	26.20	4.4 ± 0.8	33.8 ± 0.9	(-)	(-)	(-)
12	18.20	7.9 ± 0.2	29.8 ± 0.7	(-)	(-)	(-)
13	18.00	(-)	15.63 ± 0.8	(-)	(-)	(-)
14	18.00	(-)	9.3 ± 0.5	(-)	(-)	(-)
15	(-)	(-)	(-)	(-)	(-)	(-)
16	(-)	(-)	(-)	(-)	(-)	(-)

(-) indicates no temperature reading since there was no positive breeding habitats.

4.5. The effect of reservoir water level change on the availability of *Anopheles* breeding habitats

Water level of the Koka had an increasing trend at the beginning of sampling with the highest water level recorded (110.6 m) by mid September. Subsequently it decreased afterwards attaining (108.8m) at the end of sampling period (Table 7). The number of positive breeding habitats became zero by mid-December while the number of potential breeding habitats had shown an increasing trend by the end of the sampling period (Figure 8).

Weekly change in water level (m) of the Koka reservoir has a positive correlation with the number of positive larval habitats ($r = 0.605$, $p < 0.05$). No correlation existed between weekly change in water level (m) and the number of potential breeding habitats observed ($r = 0.423$, $P > 0.05$). Absolute water level (m) of the Koka reservoir indicated no correlation with the number of neither positive breeding habitats nor with potential breeding habitats ($r = 0.402$, $p > 0.05$ and $r = 0.473$, $p < 0.05$) (Appendix 2).

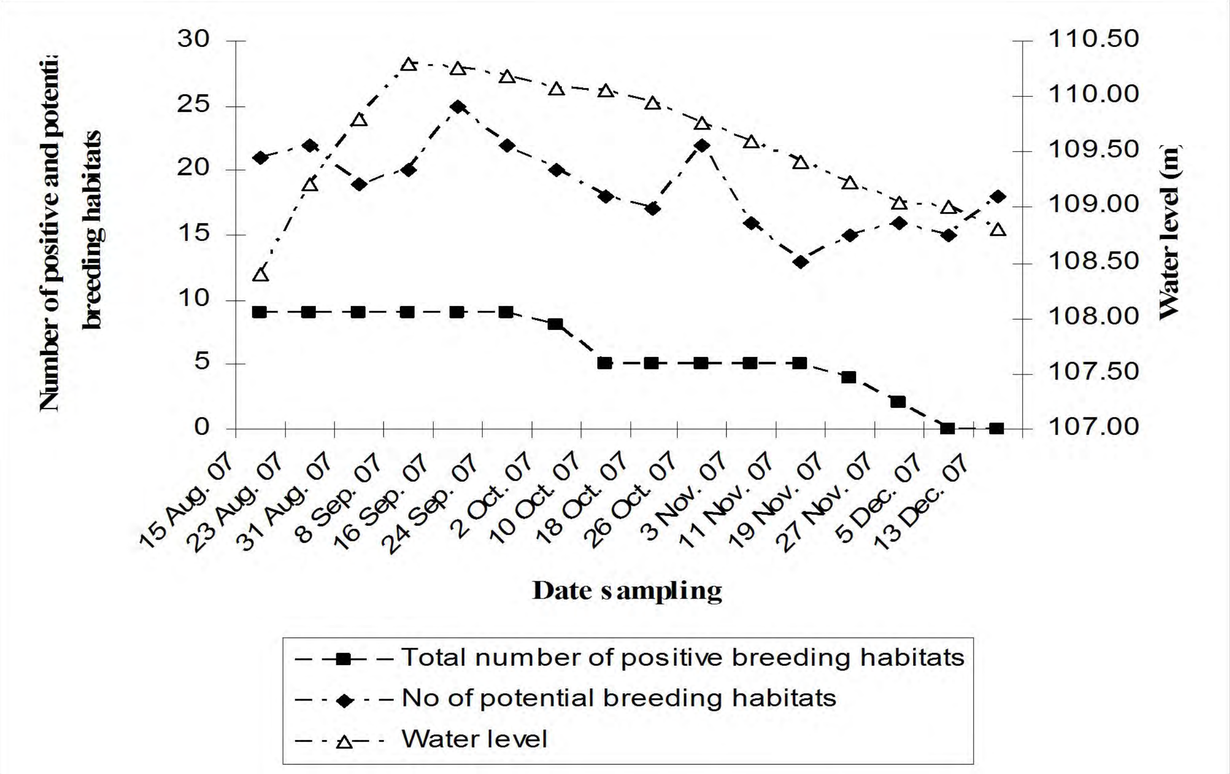

Figure 8. Water Level (m) of the Koka dam, number of positive breeding habitats, and number of potential breeding habitats (Aug 2007-Dec 2007).

Table 7. Water level (m) of the Koka reservoir, weekly change in water level number of positive breeding habitats and number of potential breeding habitats at Ejersa during the sampling period (Mid-August-Mid-December, 2007)

Sampling Week	Water level (m)	weekly change in water level (m/ week)	number of potential habitats	number of +ve breeding habitats	% +ve breeding habitats
Week 1	108.40	0.92	21	9	42.00
Week 2	109.21	0.81	22	9	40.90
Week 3	109.80	0.59	19	9	47.90
Week 4	110.29	0.49	20	9	45.00
Week 5	110.26	-0.03	25	9	36.00
Week 6	110.19	-0.07	22	9	40.90
Week 7	110.07	-0.12	20	8	45.00
Week 8	110.05	-0.02	18	5	44.44
Week 9	109.95	-0.10	17	5	29.41
Week 10	109.77	-0.18	22	5	22.72
Week 11	109.60	-0.17	16	5	31.25
Week 12	109.42	-0.18	13	5	38.46
Week 13	109.22	-0.02	15	4	26.67
Week 14	109.04	-0.18	16	2	12.50
Week 15	109.01	-0.02	15	0	0.00
Week 16	108.80	-0.19	16	0	0.00

4.6 Relationship between meteorological variables and larval breeding activity of *Anopheles* mosquitoes

At Ejersa minimum atmospheric temperature has a slightly positive correlation with both *An. gambiae* s.l and *An. pharoensis* (r= 0.541 and 0.509, respectively, p<0.05) (Table 8). At Kuma only rainfall showed a positive correlation with mean larval density of *An. pharoensis* (r=0.575, p<0.05)*,* while no correlation existed between the remaining meteorological variables and mean larval density of *An. gambiae* s.l. and *An. pharoensis* (Table 8) (Appendix 5). At Ejersa there was an occurrence of *Anopheles* larvae after the end of the rain (Appendix 3) whereas larvae occurrence became none after the last rainfall record at Kuma (Appendix 4).

Table 8. Correlation between abundance of *An. gambiae* s.l. and *An. pharoensis* with daily mean atmospheric temperature (ºC), atmospheric minimum and maximum temperature (ºC) and total rainfall (mm) at Ejersa and Kuma for the sampling period (August-December 2007).

	An. gambiae		*An. pharoensis*	
	r value	p value	r value	P value
<u>Ejresa</u>				
Daily mean temperature	0.451	0.079	0.451	0.079
Min. atm. temperature	0.541*	0.030	0.509*	0.044
Max. atm. temperature	-0.034	0.902	0.145	0.592
Total rainfall	0.316	0.234	0.316	0.234
<u>Kuma</u>				
Dailey mean temperature	0.140	0.604	0.056	0.838
Min. atm. temperature	0.175	0.516	0.187	0.488
Max. atm. temperature	-0.021	0.939	-0.189	0.483
Total rainfall (mm)	0.575*	0.020	0.390	0.135

* Significant at P<0.05

5. Discussion:

The morphological identification of matured larvae in this study revealed four species in the vicinity, namely *An. pharoensis*, *An. gambiae* s.l., *An. coustani* and *An. squamosus*. The proportion of occurrence of these species in the two study sites indicates domination of the two major vectors of malaria *An. pharoensis* 61.1% and *An. gambiae* s.l. 33.6%. Similar study on the vicinity of the Koka dam indicated *An. coustani* and *An. funestus also* occur in the area (Lautze, 2008).

The larger number of *An. pharoensis* at larvae at Ejersa than at Kuma (X^2=942.8, df=1, p<0.05). could be due to shoreline puddles providing suitable breeding habitats for *An. pharoensis*. Previous studies indicated that *Anopheles gambiae* s.l. prefers open, shallow and temporary breeding habitat (Coetezee *et al.*, 2002) while *An. pharoensis* flourish in shaded, permanent water bodies with large vegetated swamps, where the main larval habitat is characterized by floating plants (Gillies and De Mellion, 1968, Carrara *et al*., 1990).

The total *An. gambiae* s.l. count at the reservoir site was also significantly higher than that of Kuma (X^2=200.5, df=1, p<0.05). Since the amount of open sun lit rain pools and the duration of occurrence of positive breeding site were higher Ejersa than that of Kuma, more over these anopheline larvae were also common in some slightly turbid and freshly formed shoreline puddles in the site. For the ephemeral positive breeding habitat encountered at the non-reservoir site the *An. gambiae* s.l. was the one found to be dominating in the area. This situation is presumed to be due to the presence of abundant aquatic habitats that were open sun lit rain pools and man made agricultural puddles. Similar study done on the same site indicated that greater *An. gambiae* s.l. presumably *An. arabiensis* was reported in reservoir site with a conclusion *An. gambiae* s.l. prefers to exploit temporary breeding sites and *An. pharoensis* prefer to utilize permanent sites associated with the reservoir (Lautze *et al*., 2007).

The number of positive habitats encountered throughout the sampling time was 97 for the reservoir site and 22 for the non-reservoir site. At Ejersa 66 (68%) of the breeding habitats were shoreline puddles, whilst rain pools 16 (83.3%) dominate the non-reservoir site. Shoreline puddles are turbid by nature and have plenty of aquatic vegetation floating on them (plate 4). Such habitats may exist for a considerable period of time since they are created in areas where the ground water level is close to surface and this increases the longevity of the puddles (Fillinger *et al*., 2004). The size of such puddles is much greater and typically they are deeper than the shallow breeding habitats like rain pools and agricultural puddles (Keiser *et al*., 2005). This result is consistent with that of Yohannes *et al.* (2005), in which in the dam village a total of 61 positive pools were observed while the control village were restricted to rain pools in the wet season and no anopheline larvae has been collected from the dam itself. Lautze (2008) also indicated that villages associated with the reservoir Koka had much shoreline puddles while control villages were with breeding habitats created mainly in association with rain.

Generally mean larval density of *An. gambiae* s.l. was greater in slightly turbid and shallow aquatic habitats (F=16.97, p<0.05 and F=6.03, p<0.05) than that of turbid and deep aquatic habitats. Similarly *An. gambiae* s.l. was greater in larval density in habitats with emergent aquatic vegetation and with relatively open sunlit condition (F=11.14, p<0.05 and F=10.77, p<0.05). Many of the rain pools and agricultural puddles were under a slightly turbid category with muddy substrate type. These breeding sites are ephemeral with the possibility of drying in a very short period of time. Such a condition gives these breeding habitats a slight turbidity, with much less aquatic vegetation and open sun lit condition. This result is consistent with the observation of Minakawa *et al.* (1999) in that *An. gambiae* s.l. prefers breeding habitat with relatively less shade, muddy substrate and slight turbidity. The soil substrates provide nutrients for the enrichment of bacteria that serve as food source of larvae, and possibly as oviposition attractants.

Mean larval density of *An. pharoensis* in turbid aquatic habitats was **(**32.37) which was found to be significantly different from that of the slightly turbid aquatic habitats (F=13.82, p<0.05). The major reason for this was, naturally *An. pharoensis* prefers an aquatic breeding habitat with much turbid situation (Gillies and De Mellion, 1968; Carrara *et al*., 1990). The density of *An. pharoensis* in aquatic habitats with floating vegetation and with relatively shady condition (31.11 and 31.56) was found higher than that of the aquatic habitats with much light and greater emergent vegetation (F=15.75, $p<0.05$ and F=10.56, $p<0.05$). Depth of larval habitat made no difference in the mean larval density of *An. pharoensis* (F=0.84, $p>0.05$). These results verify the importance of physical characteristic of larval habitat to be colonized by a specific type of local *Anopheles* larvae. Similar studies conducted in Eritrea indicated that turbidity, depth of puddle and water current were the physical aquatic habitat parameters found to have a significant difference in mean larval abundance in the area (F=4.093,$p<0.05$; F=13.79, $p<0.05$ and F=12.95,$p<0.05$) (Shililu *et al*., 2003). These physical characteristics were also reported to bring about a significant variation in mean larval density in different aquatic habitats (Jackob *et al*., 2005).

In this study minimum atmospheric temperature was found to be positively correlated with mean larval density of *An. pharoensis* and *An. gambiae* s.l. at Ejersa (r=0.541 and r=0.509, $p < 0.05$) respectively. Water temperature were found to be strongly positively correlated with mean larval density of the two dominant *Anopheles* species, *An. pharoensis* and *An. gambiae* s.l. at Ejersa (r=0.604 and r = 0.524, $p<0.05$) respectively. According to Shililu *et al*. (2003), water temperature was positively correlated to larval density (r=0.167, $P < 0.01$). Similar studies conducted on the water temperature preference indicated that *An. gambiae* s.l. is tolerant to relatively high water temperatures. Open sunlit rain pools water temperature sometimes reach up to 40 ^{0}C and the larvae of *An. gambiae* s.l. has the capacity of surviving even developing better as the water temperature increases. This might be attributed to higher temperatures encourage better development of eggs or warmer water temperatures allow the more microorganisms to grow in the water that are used as food for larvae (McCrae, 1983, Minakawa *et al*., 1999). In this study the range of water temperature recorded throughout the study period was 17-27.2^{0}C for breeding habitats at Ejersa and 22.5-25.5^0 at Kuma.

The positive correlation of both *An. gambiae* s.l. and *An. pharoensis* with minimum atmospheric temperature might be attributed to the sudden decrease in the atmospheric temperature from the meteorological record that decreased the temperature of the aquatic habitats which then affects occurrence of larvae during that particular sampling period. In hot tropical climatic situations, maximum temperature and minimum temperature exposures can easily be avoided by different adaptation mechanisms like by moving of mosquito larvae to shade or diving to the bottom of the water column for the extreme hot condition or by simple difference in adaptation to cold climate by becoming more cold tolerant than others like *An. quadrimiaculatus* are killed by exposure to 10^0C where as *An. culicifacies* survived a low temperature of 5^0C (Boyoh and Lindsay, 2003). The disappearance of larvae at that particular period of time might be attributed to the response of the larvae to lower water temperatures, remaining at the surface of aquatic habitats.

Different aquatic habitat characteristics influence the abundance and species composition of *Anopheles* mosquitoes in an area. These physical habitat characteristics include temperature of the water, turbidity, depth of puddle and substrate type (Fillinger *et al*., 2004), which are influenced by the external man-made and natural environment in which the particular habitat is located (Robert *et al*., 1998).

By the end of the study period most of the breeding habitats were dry and those newly formed shoreline puddles were fresh. For this reason almost all the breeding habitats visited were negative for anopheline larvae. The sharp decrease in the atmospheric temperature of the areas in association with the windy condition that prevents adults from reaching to the breeding habitats to lay eggs could also be another factor for the absence of anopheline larvae in these breeding sites. Most shoreline puddles which were previously positive dried up and newly formed potential breeding habitats due to recession of reservoir water require some time for oviposition and colonization by *Anopheles* mosquitoes and other pioneer flora and fauna.

In this study, weekly water level changes of the Koka reservoir had positive correlation with the number of positive breeding habitats ($r=0.605$, $P < 0.05$) indicating that higher water level changes result in more potential breeding habitats which increases the amount of positive breeding habitats as well. Similar studies done on dam and larval breeding habitat association indicate that faster rates of reservoir recessions were correlated with fewer breeding sites ($r^2=0.43$); with a strong conclusion that the abundance of *Anopheles* larvae along the reservoir shoreline Koka is affected by water level changes (Lautze *et al*., 2008). It is apparent that the water level change of the Koka dam is responsible for the creation of new breeding habitats this study also showed that the number of potential breeding habitats was at an increasing trend while reservoir keeps on losing water, but difficult to conclude that is has such an association in the present short term study.

Finally, the results in this study showed that the decline in the occurrence and density of *Anopheles* larvae and subsequent decline in total adult count in the presence of potential breeding habitats in the area suggests that climatic factors are important sources of variation in larval density in the vicinity (Appendix 7). This comparison is strengthened by the correlation observed between larval density and minimum temperature at Ejersa during the study period. The grater number of *An. gambie* s.l. catch at both villages in the indoor traps is due to the more indoor biting behavior of *An. gambiae* s.l. than *An. pharoensis* (Ghebreyesus *et al*., 2006; Ameneshewa, 1996).

6. Conclusion and recommendations:

6.1. Conclusions

Four *Anopheles* species were identified in the area during the study period. These are *An. pharoensis*, *An. gambiae* s.l. *An. coustani* and *An. squamosus.* The occurrence of *An. pharoensis* was greater in the reservoir village than in the non-reservoir village mainly because of the availability of shoreline puddles suitable for their breeding. The occurrence of *An. gambiae* s.l. was also greater than that of the non-reservoir village in which the effect of the dam was not observable.

Three major *Anopheles* mosquitoes breeding habitats were identified through out the study period, these are shoreline puddles, rain pools and agricultural puddles. Shoreline puddles are the major type of breeding habitats in the reservoir village. Their creation is directly associated with recession of the Koka dam in the reservoir village.

The occurrence of *An. pharoensis* was greater in turbid aquatic habitats with much floating vegetation. These were the major characteristics of the shoreline puddles. *Anopheles gambiae* s.l. occurrence in shallow slightly turbid habitats with emergent vegetation and open sun lit condition was also higher. Both water and atmospheric temperature fluctuations are important sources of change in larval density. Water level changes are the major reasons for the creation of potential breeding habitats as shoreline puddles in the reservoir village Ejersa.

6.2 Recommendations

- Appropriate reservoir management measures are necessary that aims at breeding habitat reduction in areas near the shoreline of the Koka reservoir. This includes increasing the storage capacity of reservoir by avoiding sedimentation so that reservoir can be prevented coming back to villages during peak rainy season.

- Source reduction of larval habitats through community participation during the peak transmission period through application of larvicides.

- Proper management of the water level changes like keeping reservoir water level constant till the end of peak malaria transmission period that decreases the formation of new potential breeding habitats in the area should be considered.

- Raising the level of awareness in the communities living near by the reservoir shoreline on the use of irrigation methods from the ground water near by since these are the major causes of formation of man made field flood pools in the area that increases the potential breeding habitats.

- Further studies that evaluate the impact of soil water holding capacity on the longevity of breeding aquatic habitats and considering the overall climatic variables and chemical habitat characteristics on the larval productivity of *Anopheles* mosquitoes in the area should be considered.

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Appendix

Appendix 1. Correlation between abundance of *An. gambiae* s.l. and *An. pharoensis* with water temperature (^{o}C)

	An. gambiae s.l.		*An. pharoensis*	
	r value	p value	r value	P value
Ejersa	0.524*	0.037	0.604*	0.013
Kuma	0.273.	0.657	0.462	0.433

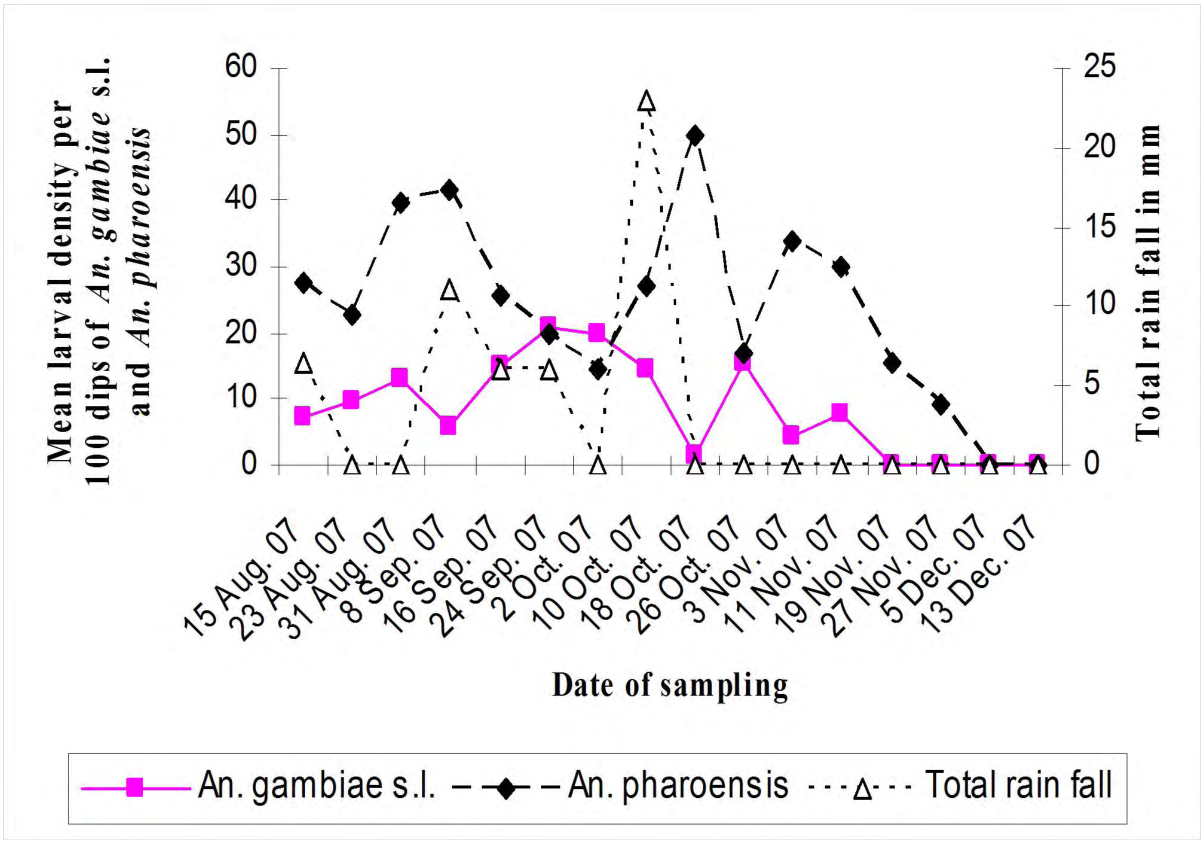
*Significant at $p<0.05$

Appendix 2. Correlation between Water level (m) and weekly water level changes (m) with number of positive larval breeding habitats and potential number of breeding habitat at Ejersa.

	Water level (m)		Weekly change in water level (m)	
	r value	p value	r value	p value
Number of positive breeding Habitats	0.473	0.064	0.605*	0.013
Number of potential breeding Habitats	0.423	0.103	0.402	0.123

- Significant at $p<0.05$

Appendix 3. Total rainfall (mm) and mean larval density of *An. gambiae* s.l. and *An pharoensis* at Ejersa



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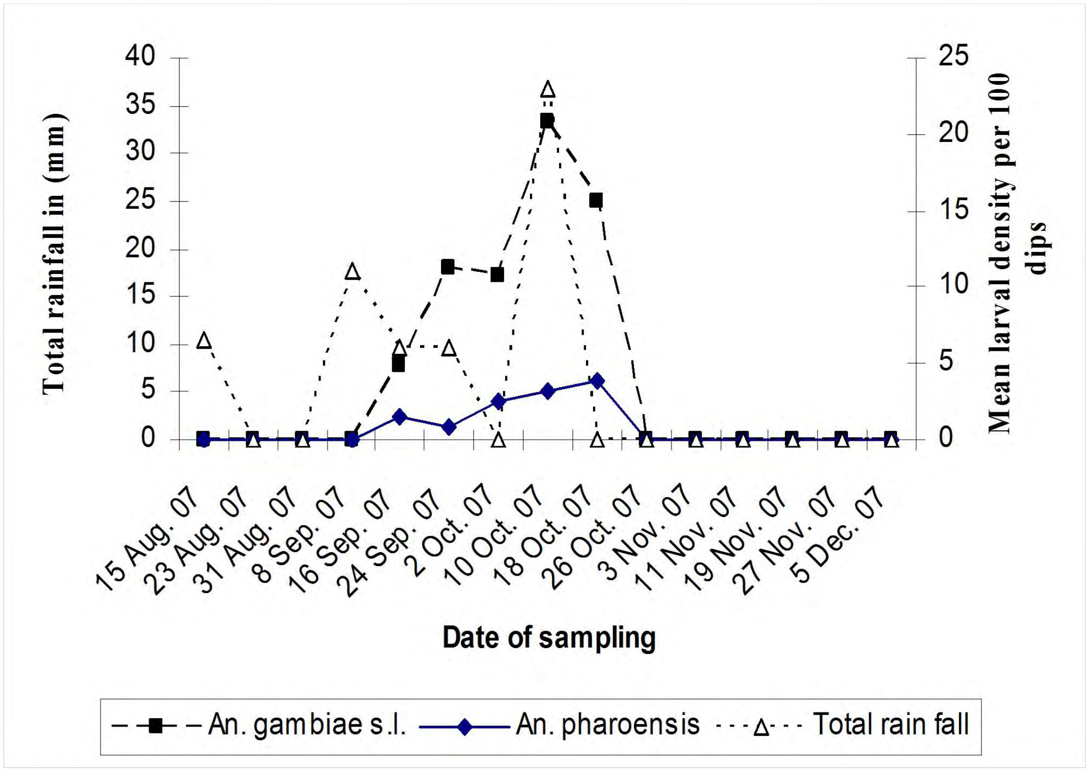
Appendix 4. Total rainfall (mm) and mean larval density of *An. gambiae* s.l. and *An pharoensis* at Kuma.



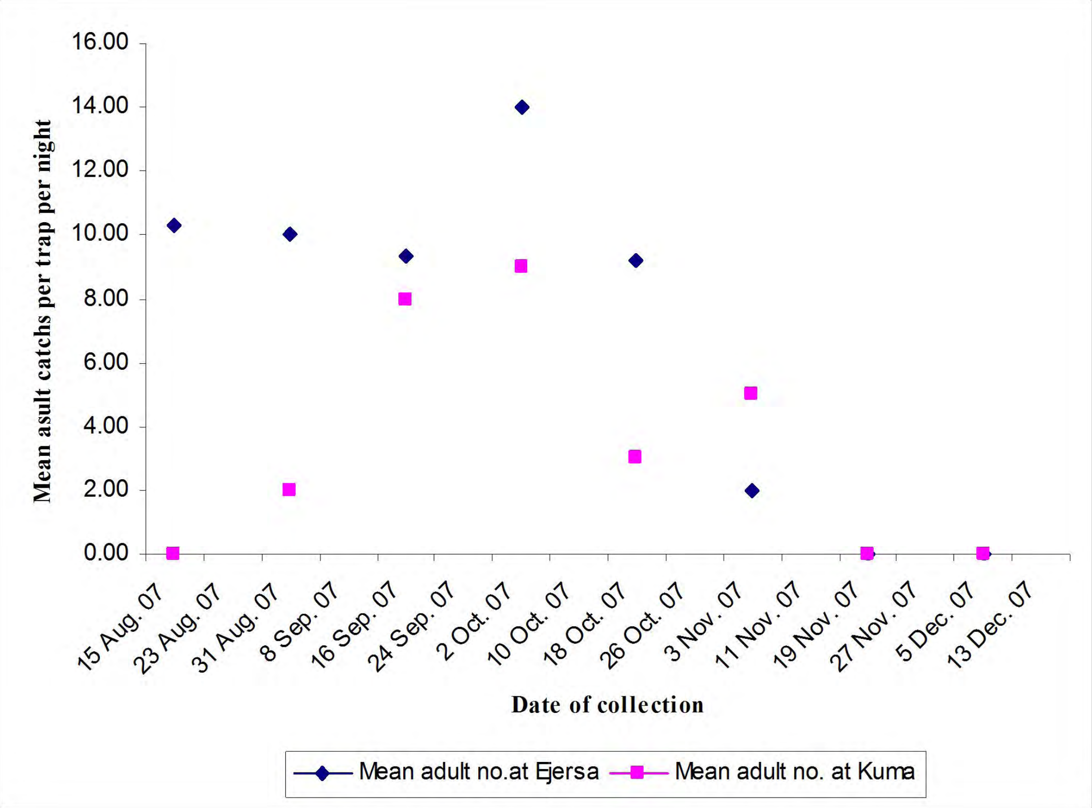
Appendix 5. Meteorological variables and mean larval density of *An. gambiae* s.l. and *An. pharoensis*

Sampling	* Meteorological variables			Mean no Larval Density per 100 Dips			
Weeks	Minimum T ^{0}	Maximum T^0	RF	Ejersa		Kuma	
				An. gambiae s.l	*An. pharoensis*	*An. gambiae* s.l	*An. pharoensis*
1	9.50	25.00	6.80	7.4± 1.0	27.37± 3.7	(-)	(-)
2	11.40	29.00	0.00	9.5 ± 1.1	22.54 ± 3.0	(-)	(-)
3	11.30	30.20	0.00	13.2 ± 1.3	39.5± 4.7	(-)	(-)
4	11.40	29.50	11.00	5.9 ± 0.9	41.5 ± 2.8	(-)	(-)
5	10.50	28.40	6 .00	14.8± 1.8	25.8± 2.7	7.7 ± 0.2	2.4 ±0.4
6	11.20	32.40	6.00	20.7 ± 1.2	19.7 ± 2.5	18.0 ± 0.8	1.3 ±0.2
7	11.00	25.00	0.00	19.8 ± 1.4	14.6 ± 2.1	17.2 ± 1.2	4.0 ±0.6
8	6.50	28.50	23.00	14.5 ± 0.7	27.1 ± 1.6	33.4 ± 2.7	5.1 ±0.9
9	9.00	29.50	0.00	1.7 ± 0.2	50.0 ± 1.3	25.0 ± 1.4	6.3 ±0.5
10	4.90	29.60	0.00	15.6± 0.6	16.9 ± 0.8	(-)	(-)
11	6.50	30.20	0.00	4.4 ± 0.8	33.8 ± 0.9	(-)	(-)
12	8.00	29.00	0.00	7.9 ± 0.2	29.8 ± 0.7	(-)	(-)
13	3.50	29.30	0.00	0.0 ± 0.0	15.6 ± 0.8	(-)	(-)
14	7.00	28.90	0.00	0.0 ± 0.0	9.3 ± 0.5	(-)	(-)
15	5.30	29.50	0.00	0.0 ± 0.0	0.0 ± 0.0	(-)	(-)
16	4.90	28.40	0.00	0.0 ± 0.0	0.0 ± 0.0	(-)	(-)

* All meteorological values indicate the records of the National Meteorological Agency (NMA) at that particular sampling date.

(-) no larval density since no positive breeding habitats available

Appendix 6. Mean adult catches per trap per night at Ejersa and Kuma during the sampling period (mid August-mid December).



DECLARATION

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

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