Sveriges lantbruksuniversitet
Swedish University of Agricultural Sciences

Faculty of Natural Resources and Agricultural Sciences
Department of Ecology

The effect of abiotic and landscape features on abundance of *Anopheles* larvae

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ABSTRACT

Mosquitoes are medically the most important group of insects, transmitting a number of deadly diseases, including malaria. Female mosquitoes transmit them while feeding on human blood, which is required to mature eggs. It is important to understand vector population dynamics in order to effectively control them. Sampling larval populations is one of the methods to estimate mosquito requirements for site selection for oviposition and survival.

Our survey took place from 10th of June to 1st July in the area of village Chano, located in the Southern Nations, Nationalities and Peoples Region in Southern Ethiopia. Mosquito larvae were collected using standard dipping method once a week in three different land cover categories. Water physical parameters were recorded on site and the chemical analysis was performed at the laboratory of The Institute of Health and Nutrition in Addis Ababa.

We found *Anopheles arabiensis* to be the only anopheline species present at the end of the rainy season in the surveyed area. There was no significant association detected between presence of *Anopheles* mosquito larvae and abiotic and landscape characteristics. However, we found that the density of early instar stages was increasing with increasing conductivity (p=0.01) and dissolved oxygen (p=0.02) and when habitats contained aquatic vegetation. Such habitats were associated with pastures. Late larval instars were positively associated with turbidity (p=0.003) and such habitats were more numerous in the settlement. We conclude that *Anopheles arabiensis* females were ovipositing more intensively in habitats that were more stable, but the survival in such habitats was lower. We suggest that water quality may be not the main factor influencing site selection for oviposition when the climatic conditions are not favorable for the survival of the adult and immature stages. This knowledge could be applied in the development of vector control strategies, aiming at the mosquito populations when they are mostly vulnerable.

Keywords: Anopheles arabiensis; larvae; density; breeding habitats; dipping; landscape; Ethiopia;

POPULAR SCIENCE SUMMARY

MOSQUITOES BREED IN UNFAVORABLE SITES TO SURVIVE DRY SEASON

Malaria remains the most serious and widespread vector borne disease, responsible for the death of nearly a million people annually. It is caused by protozoan which is transmitted through the bite of female *Anopheles* mosquitoes.

There has been a great effort to control malaria prevalence using different approaches focused on vector control. The most frequently used and effective methods include indoor spraying with insecticides and the use of insecticide impregnated bed nets. However, mosquitoes show a tendency to change their biting and resting behaviour, as well as to develop resistance to widely used insecticides. Another challenge is climate change, which may allow mosquitoes to spread into wider areas and higher altitudes due to increased temperatures and precipitation rates. These alarming processes increase the pressure to develop alternative vector control strategies. For this matter we need to deepen our knowledge of mosquito ecology, to better understand the factors which influence the size and distribution of vector populations.

Sampling mosquito larvae is one of the methods to estimate mosquito requirements for site selection and survival, because mosquitoes actively select suitable habitats rather than randomly colonize them. Choice of the site will determine distribution pattern, density, development time, body size, survival and influence the performance of the emerging adults.

To better understand larval density dependence on environmental factors, we conducted a study around Chano, a malarious village located in Southern Ethiopia. Mosquito larvae were collected with a dipper from standing water bodies, such as puddles, tire tracks, roadside ditches and ponds which were distributed within three different land cover categories: settlement, agricultural land and the pastures along the lake shore. The physical and chemical properties of the water, such as temperature, pH, salinity and ion content were also tested. While carrying out statistical analysis, we divided the four larval stages into two groups: early and late instars, as density of early instar larvae might indicate the rate of egg-laying, while the density of late instar larvae may represent the rate of survival.

We found only one anopheline species, *Anopheles arabiensis*, present in the surveyed area. The density of early instar stages was increasing with increasing conductivity and dissolved oxygen content and when habitats contained aquatic vegetation. Such habitats were bigger in size, more permanent and located in pastures closer to the lake. Surprisingly, such sites have been previously reported as less favorable. On the other hand, late larval instars were positively associated with turbidity and such habitats were small and more numerous in the settlement.

We conclude that female mosquitoes were ovipositing more intensively in habitats that were less prone to desiccation, but the survival in such habitats was lower, most likely due predation. This phenomenon most likely occurred as a strategy of mosquitoes to survive dry season. Firstly, the preferred small habitats were rapidly desiccating, giving too little time for the immature stages to complete their cycle. Secondly, adult mosquitoes might have been retreating from the settlement, because the area around the lake provided the stable source of water needed to survive desiccation stress. We suggest that water quality may be not the main factor influencing site selection for oviposition when the climatic conditions are not favorable for the survival of the adult and immature stages.

Our survey is a small contribution to a larger effort to understand vector ecology in Southern Ethiopia. Further research will provide an improved understanding of how larval populations vary in space and time. This knowledge could be implemented into developing vector control strategies, aiming at the mosquito populations when they are mostly vulnerable.

INTRODUCTION

Mosquitoes (family: Culicidae) are holometabolous insects which undergo a complete metamorphosis in their life history. It includes 4 stages: egg, larva, pupa and adult, of which most are exclusively aquatic (Capinera, 2008). The eggs are oviposited singly (*Anopheles*) or in batches (e.g. *Culex*, *Coquillettidia*) on the surface of water or into the moist from which they hatch (Becker et al., 2010). The following larval stage is restricted to an aquatic environment where it goes through four instars (Smith, Macklin, & Thomas, 2013). The larval stage is followed by short pupation, during which adult structures are developed. Finally, mosquitoes emerge as adults ready to disperse in order to find food and mate. Adult mosquitoes are capable of flying distances of 3 to 5 kilometers (Rozeboom & Stone, 1943; Smith et al., 2013), but most of the flights are within 1 km radius (Le Menach, McKenzie, Flahault, & Smith, 2005).

Female mosquitoes require one or more blood meals every 2-3 days in order to mature eggs after mating (Godfray, 2013; N. Minakawa, Mutero, Githure, Beier, & Yan, 1999). Male mosquitoes do not take blood meals but feed on plant sugars (Smith et al., 2013), while adult females rely on this food source between gonotrophic cycles (Becker et al., 2010; Gary, 2005). The requirement for a blood meal makes the mosquitoes medically the most important group of insects (Okwa et al., 2007; Thielman & Hunter, 2007), transmitting a number of diseases and causing great health problems (Araújo, Gil, & De-Almeida, 2012). Mosquitoes are responsible for spreading human diseases such as Japanese encephalitis, West Nile virus, yellow fever, dengue fever, filariasis and most importantly malaria (Araújo et al., 2012; CDC, 2007; Foote & Cook, 1959; Godfray, 2013).

Malaria is still one of the world’s most serious and widespread human diseases (Becker et al., 2010; Speight, Hunter, & Watt, 2008), causing around 500000-900000 deaths annually (WHO, 2011). Nearly 40% of the world’s population live in regions where malaria is endemic with the highest mortality occurring in the Sub-Saharan region in Africa (Speight et al., 2008; Tesfaye et al., 2011; WHO, 2011). Human malaria is caused by a parasitic protozoan from the genus *Plasmodium* (Bomblies, 2009) which can only be transmitted by mosquitoes that belong to *Anopheles* genus (Muriu et al., 2013; Sinka, 2013). Malaria epidemiology is directly linked to population dynamics of the mosquitoes. Mosquito reproductive success is reflected by the fitness and amount of emerging adults, determining vector density, biting rate and life expectancy (Himeidan & Kweka, 2012). These influence vectorial capacity (Garrett-Jones & Shidrawi, 1969), which determines the stability and intensity of disease transmission (Kiszewski et al., 2004).

Reproductive success

The key processes influencing successful development of mosquito larvae are considered to be site selection of gravid females and the survival of the larvae (Fillinger et al., 2009; Hanafi-Bojd et al., 2012). Both of them depend on the suitability and productivity of the habitat, being a key element for mosquito population dynamics (Kenea, Balkew, & Gebre-Michael, 2011; Rejmánková et al, 2013), which is a function of a complex interaction of a range of ecological, climatic and environmental variables (J. Shililu, Ghebremeskel, Seulu, et al., 2003; Silver, 2008b) (Figure 1).

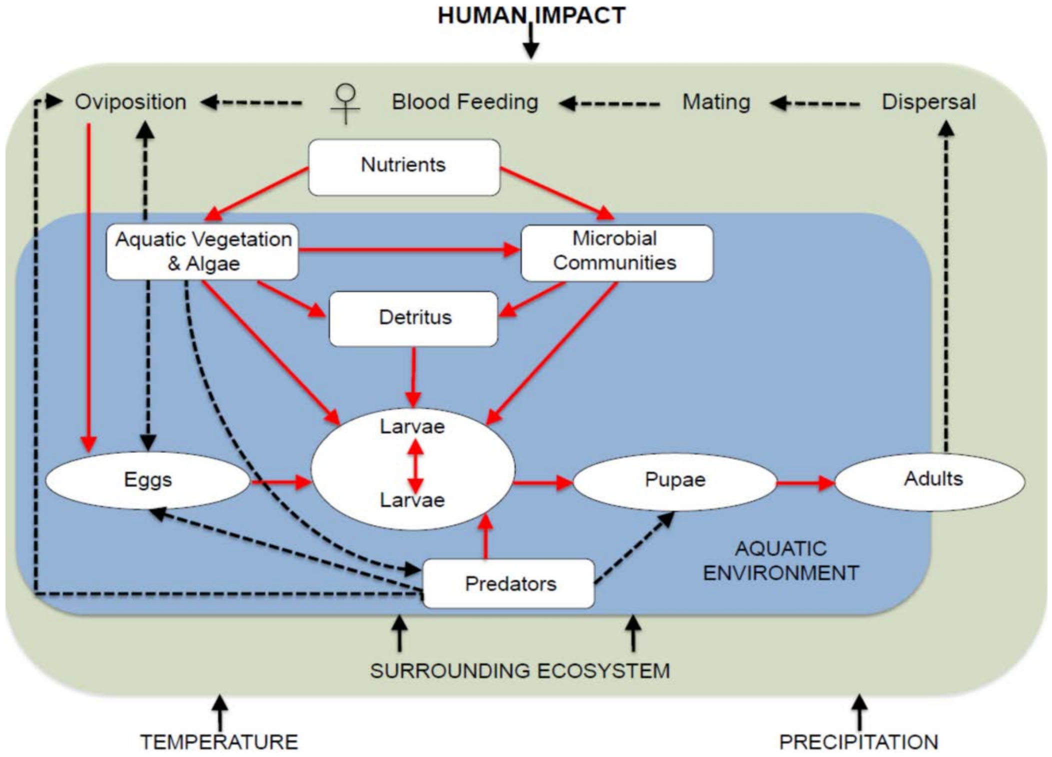

Figure 1. Relationships between larval development and environmental factors on both habitat and ecosystem level (Rejmánková et al., 2013).

Oviposition

Mosquitoes are thought to actively select suitable habitats rather than randomly colonize them (Minakawa et al., 2004) in order to maximize offspring survival and fitness (Yoshioka et al., 2012). Oviposition plays a crucial part in population dynamics of the mosquitoes, since choice of the site will determine distribution pattern, density, development time, body size, survival and further performance of the emerging adults (Adebote, Oniye, & Muhammed, 2008; Animut, Gebre-Michael, Balkew, & Lindtjørn, 2012; Fillinger et al., 2009; Hanafi-Bojd et al., 2012; Le Menach et al., 2005; Munga, Minakawa, Zhou, Githeko, et al., 2006; Yoshioka et al., 2012) with significant demographic consequences carried over to the community level (Kiflawi, Blaustein, & Mangel, 2003; Spencer, Blaustein, & Cohen, 2002).

The key components involved in location and selection of an oviposition site include visual, olfactory, and tactile cues (Bentley & Day, 1989). Visual cues may allow female mosquitoes to identify available habitats and their characteristics on a landscape level, while olfactory and tactical cues become more important as mosquitoes approach the oviposition site (Bentley & Day, 1989). Olfactory cues involve volatile substances released by microorganisms (Sumba, Ogbunugafor, Deng, & Hassanali, 2008), larval habitat materials, larvae themselves as well as predators (Munga, Minakawa, Zhou, Githeko, et al., 2006; Vonesh & Blaustein, 2010), which may attract or deter the oviposition. Experiments in the laboratory have revealed that gravid females avoid laying eggs in habitats that contain predators and competitors (Adebote et al., 2008; Kiflawi et al., 2003; Munga, Minakawa, Zhou, Githeko, et al., 2006; Ogbunugafor & Sumba, 2008; Van Dam & Walton, 2008; Warburg et al., 2011; Vonesh & Blaustein, 2010). Physical factors recognized to be extremely important in site choice include color and optical density of the site, site texture, temperature, and reflectance (Bentley & Day, 1989). Selection of the breeding site is species specific (Hanafi-Bojd et al., 2012) as mosquitoes differ in their preference for the type, size, turbidity, algal cover and stability of the habitat (Animut et al., 2012). For example, *Anopheles plumbeus* breeds in tree holes (Foote & Cook, 1959), *Anopheles arabiensis* from the *gambiae* s.l. complex prefers small and temporary breeding sites, while *A. melas* from the same complex prefers saline flooded areas (Smith et al., 2013). The occurrence of *A. funestus* larvae is restricted to larger, semipermanent or permanent habitats with aquatic vegetation (Noboru Minakawa et al., 2005).

Survival

Another important aspect in population dynamics is the survival of immature stages which depends on the quality of the environment in which they develop (Ye-Ebiyo, Pollack, Kiszewski, & Spielman, 2003). The quality of the habitat is represented by a number of biotic and abiotic factors: nutrient content, physicochemical environment, intra- and interspecific competition and predation (Araújo et al., 2012; Depinay et al., 2004; Mala & Irungu, 2011; Yoshioka et al., 2012). A complex interaction between these factors determines the survival and, therefore, reproductive success of mosquitoes (Le Menach et al., 2005; Rejmánková et al., 2013).

Larval survival differs among habitats as a result of the presence or absence of predators, parasites and pathogens (Koenraadt, Githeko, & Takken, 2003). Predation has the potential for dramatic effects on mosquito populations (Spencer et al., 2002) by suppressing abundance of mosquito larvae directly (Minakawa et al., 1999) or through competition (Mokany & Shine, 2003). Natural predators of mosquito larvae include the tadpole stages of amphibians, planktivorous fishes and aquatic insects (Rejmánková et al., 2013).

Another known major regulator of vector populations is nutrient competition, usually occurring at both inter- and intraspecific level (Depinay et al., 2004; Munga, Minakawa, Zhou, Githeko, et al., 2006). Both quantitative and qualitative aspects of larval nutrition are important to mosquitoes (Araújo et al., 2012) with the impact to development success, rapidity of development, and the body size of emerging adults (Aboagye-Antwi & Tripet, 2010; Adebote et al., 2008; Araújo et al., 2012; C J M Koenraadt et al., 2003; Ye-Ebiyo et al., 2003). Interspecific competition lengthens the time mosquitoes require to reach the pupal stage and reduces the size of the resulting adult mosquitoes (Gimnig et al., 2002; Munga et al., 2006a).

Mosquito larvae are filter feeders (Godfray, 2013) that rely on few sources of food. The most important are bacteria and algae within surface microlayers (Gimnig et al., 2002; Gimnig, Ombok, Kamau, & Hawley, 2001; Tuno et al., 2005) and the soil (Okech et al., 2007). Mosquitoe larvae are also known to feed on protozoa, invertebrates and suspended organic material, such as maize pollen (Araújo et al., 2012; Capinera, 2008; Gimnig et al., 2002; Munga, Minakawa, Zhou, Mushinzimana, et al., 2006; Tuno et al., 2005; Ye-Ebiyo et al., 2003).

AIMS AND OBJECTIVES

Since survival rate of the vector is one of the key factors for transmission dynamics, it is important to understand mosquito ecology and population dynamics (Smith et al., 2013). Despite intensively ongoing research on mosquito ecology, relatively little is known about the environmental factors determining mosquito population size. Anopheline mosquitoes have broad habitat tolerances and can be found in many different types of water bodies, while equally can be absent from some apparently suitable pools (Muriu et al., 2013). Moreover, mosquitoes from different geographic locations might differ significantly in their behaviors (Ogbunugafor & Sumba, 2008).

A thorough understanding of larval population dynamics and spatio-temporal variation requires deep knowledge on ecological parameters involved in habitat selection by proliferating species (Kenea et al., 2011; Mala & Irungu, 2011; Sérandour et al., 2010). This understanding is needed to effectively model and map vector populations and develop sustainable and cost-effective insect management strategies (Bentley & Day, 1989; Overgaard, Ekbom, Suwonkerd, & Takagi, 2003; Thwing, Fillinger, Gimnig, Newman, & Lindsay, 2011). It can improve epidemiological understanding and control capabilities (Adebote et al., 2008).

Such analyses may allow us to make predictions about how anopheline fauna will change as a result of landscape changes and it may give us clues how landscapes could be managed to suppress populations of disease vectors (Overgaard et al., 2003). Accurate identification of the habitat requirements of the immature stages of mosquitoes is desirable as it can assist in designing focused and cost-efficient control programmes within a resource-limited environment

(Silver, 2008a; Vanwambeke et al., 2007). Larval abundance and density may indicate the suitability and productivity of the habitat, as a result of oviposition preference and the survival rate. It is important to analyze mosquito population dynamics in landscape level not only because landscape might influence physicochemical environment of the habitats, but also may contain characteristics in the landscapes themselves.

We hypothesize that the quality of the aquatic habitat, which is a result of the processes at landscape level, affects oviposition and survival rates hence, the occurrence and abundance of *Anopheles* mosquitoes.

The aim of the study was to investigate habitat preferences and density dependence of *Anopheles* mosquitoes on abiotic parameters and landscape features in a malarious village in Southern Ethiopia.

Objectives:

1. Identify species composition of *Anopheles* mosquitoes in the area.
2. Determine if the habitats are heterogeneous in regards to physicochemical environment through landscape structure and physical characteristics of the habitats.
3. Identify which environmental parameters are important for the presence of mosquito larvae in the habitat.
4. Identify which environmental parameters contribute to higher larval densities as a result of increased oviposition rate and survival

MATERIALS AND METHODS

Malaria in Ethiopia

Ethiopia is among the most affected countries, as malaria is unstable and occurs as intraanual and interannual epidemics (Olana et al., 2011). Approximately 70% of Ethiopia's population (84.3 million) lives in areas at risk of malaria (FMOH, 2006; Loha & Lindtjørn, 2012; PMI, 2013). The intensity of malaria transmission is heterogeneous across the continent (Kelly-Hope, Hemingway, & McKenzie, 2009). It is influenced by mosquito species' compositions, vector competence and demographic and environmental factors, such as topography and climate (Abeku, Oortmarssen, Borsboom, Vlas, & Habbema, 2003; Kelly-Hope et al., 2009; Olana et al., 2011). The diverse eco-climatic condition in the country makes the malaria transmission pattern seasonal and unstable with frequent focal and cyclic widespread epidemics (FMOH, 2006) (Figure 2). As the western, central and eastern highlands and highland-fringe areas along the Rift Valley are especially prone to periodic malaria epidemics (Abeku et al., 2003), malaria remains the leading cause of outpatient morbidity in the southern part of the country (Loha & Lindtjørn, 2012).

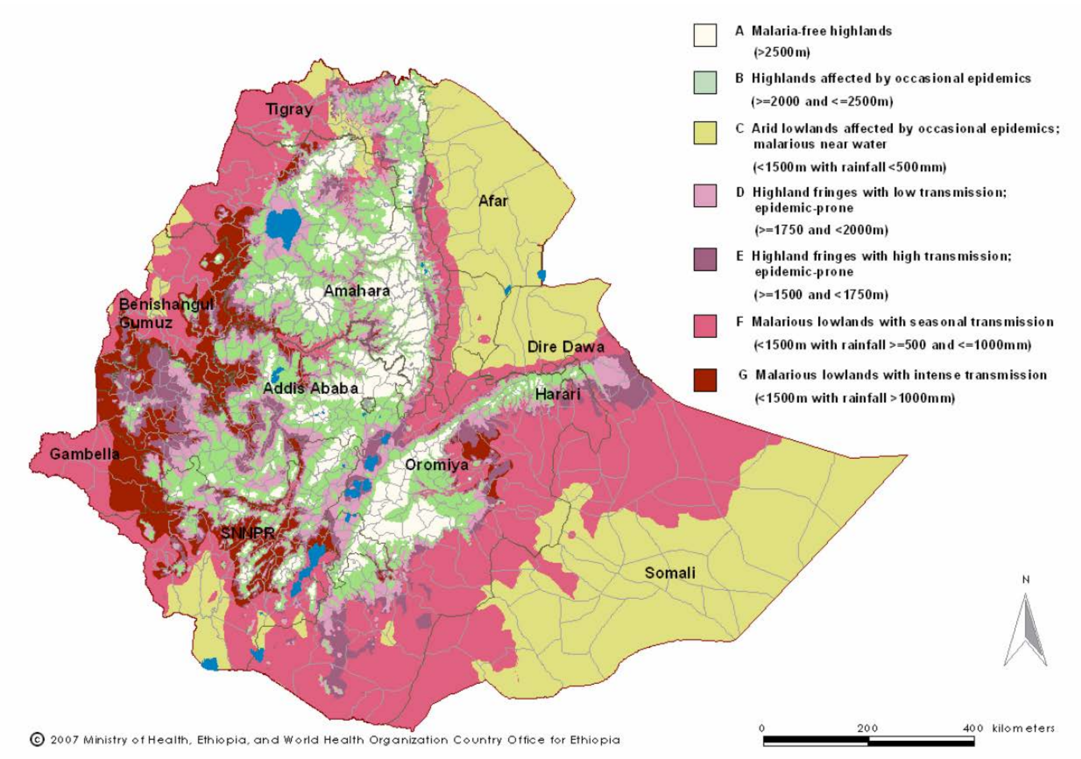

Figure 2. Distribution and seasonality of malaria in Ethiopia (PMI, 2013).

Around 68% of malaria cases in Ethiopia are caused by *Plasmodium falciparum*, the most virulent human parasite in the continent (Lindsay & Martens, 1998; Ndenga, Simbauni, Mbugi, Githeko, & Fillinger, 2011), while remaining are *P. vivax* infections (Abeku et al., 2003; Tesfaye et al., 2011).

There are 43 *Anopheles* species recorded in Ethiopia (Gaffigan et al., 2013), but few are considered as important vectors for malaria transmission. *Anopheles arabiensis* Patton, which belongs to *A. gambiae* s.l. complex, is considered as the primary vector (Kenea et al., 2011; Loha & Lindtjørn, 2012; Olana et al., 2011; PMI, 2013; Tirados, Costantini, Gibson, & Torr, 2006), while *A. funestus* Giles and *A. nili* Theobald as secondary vectors (Krafsur & Armstrong, 1978; PMI, 2013). *Anopheles pharoensis* has also been reported as an important vector in the country (Kibret, Petros, Boelee, & Tekie, 2007; Massebo, Balkew, Gebre-michael, & Lindtjørn, 2013). *Anopheles gambiae* s.l. is one of the most important and best-known vector species in Africa (Afrane, Githeko, & Yan, 2012; Muriu et al., 2013; Sinka et al., 2010). It is a group of seven sibling species, which are morphologically indistinguishable (Godfray, 2013) but have variations in some of the ecological characteristics, such as preference for site selection and feeding behavior. The members of this complex are known to breed in shallow sun-exposed pools that dry up after rains and develop very rapidly (Rozeboom & Stone, 1943). Larvae of *A. gambiae* s.l. have broad tolerance for breeding conditions (Muriu et al., 2013), such as reduced nutritional quality (Okech et al., 2007) and are able to utilize a great variety of locations (Sinka et al., 2010). The majority of larvae are found in fresh waters while several species (*A. melas* and *A. merus* from the gambiae complex) show high salinity tolerance and are associated with coastal malaria transmission (Rejmánková et al., 2013). The larvae of *A. arabiensis* can occur in water bodies reaching extremely high temperature levels. *A. arabiensis* is considered to prefer dry, savannah environments (Kirby & Lindsay, 2009; Lindsay & Martens, 1998; Sogoba et al., 2007), yet occurring in forested areas with recent land disturbance (Muriu et al., 2013). Yohannes et al. (2005) and Fornadel et al. (2010) describe *A. arabiensis* as highly antropophilic mosquito species. Other sources report this mosquito to show both zoophilic and antropophilic biting behaviours, with high proportion of meals from non-human hosts such as cattle, sheep and goats (Mahande, Mosha, Mahande, & Kweka, 2007; Massebo et al., 2013; Prior & Torr, 2002; Tirados et al., 2006).

Vector control in Ethiopia

Since 2005 The President's Malaria Initiative (PMI) has been launched in order to reduce malaria-related mortality in 15 high-burden countries in sub-Saharan Africa (PMI, 2013). The most widely used methods for vector control are insecticide treated nets (ITN) and indoor residual spraying (IRS), targeting the host-seeking adult mosquitoes (PMI, 2013).

However, even high demographic coverage with these measures is not likely to completely eliminate malaria. Bed nets are of little advantage, as the peak indoor and outdoor activities of malaria vectors were found to be during the early period of the night, coinciding with the evening activities of the people (Kibret et al., 2010). What is more, adult vectors have been reported to change their behavior by switching to feed on cattle or rest outdoors, therefore evading insecticide contact (Sattler et al., 2005; Thwing et al., 2011; Yohannes et al., 2005). The development of insecticide resistance is also a looming threat to the sustainability of IRS and ITNs (Balkew, Elhassen, Ibrahim, Gebre-Michael, & Engers, 2006; Massebo et al., 2013). Moreover, the warming climate could increase metabolic rate with increasing temperature (Afrane et al., 2012; Chen, Githeko, Zhou, Githure, & Yan, 2006). Highlands are especially vulnerable as warming environment could allow the spread of vector to higher elevations, while increased precipitation could produce more breeding sites. Animut et al. (2012) and Shililu et al. (2003) reported *A. arabiensis* breeding above 2000 m.a.s.l., which was previously reported as the upper limit for malaria incidence (Lindsay & Martens, 1998).

Study Area

The study area is located in the Southern Nations, Nationalities, and People's Region, Gamo Gofa Zone in the Great Rift Valley. It lies north from city Arba Minch, West of the Lake Abaya, the second largest of the Ethiopian rift valley (Clark, 2010) (Figure 3). The area is categorized as malarious lowlands with seasonal transmition (PMI, 2013). The sampling took place in the surrounding area of village Chano, located to the north of town Arba Minch. It lies at 6°6.6660 N and 37°35.7750 E, at altitude of around 1200 m.a.s.l., close to Abaya Lake. The human population size in the village is reported to be 6661 (Massebo et al., 2013).

The area lies within the monsoon region with the Indian Ocean winds bringing the main rains during March-May and the Atlantic Ocean winds bringing more rain during the September-November wet season. Malaria is more prevalent after the end of the rainy season (SAC & ICF International, 2012). The average maximum temperature is 30˚C, while the average minimum is 17˚C. January to March is the hottest time of the year, while the months of November and December are usually the coolest (Clark, 2010).

The natural vegetation in the study area consists of thorn shrubs and bushes such as a commonly found Acacia (Massebo et al., 2013). The major cash crops cultivated in the area include bananas (*Musa acuminata* Coll.), maize (*Zea mays* L.) and mango (*Mangifera sp.*). Domestic animals such as cattle and goats are common to graze in the area.

The water from river Hare is directed via three permanent canals, passing through the village to irrigate the agricultural land. The incidence of falciparum malaria was reported to be 3.57/10,000 person with 29.1% of all falciparum malaria episodes occurring among temporary residents or visitors (Loha & Lindtjørn, 2012).

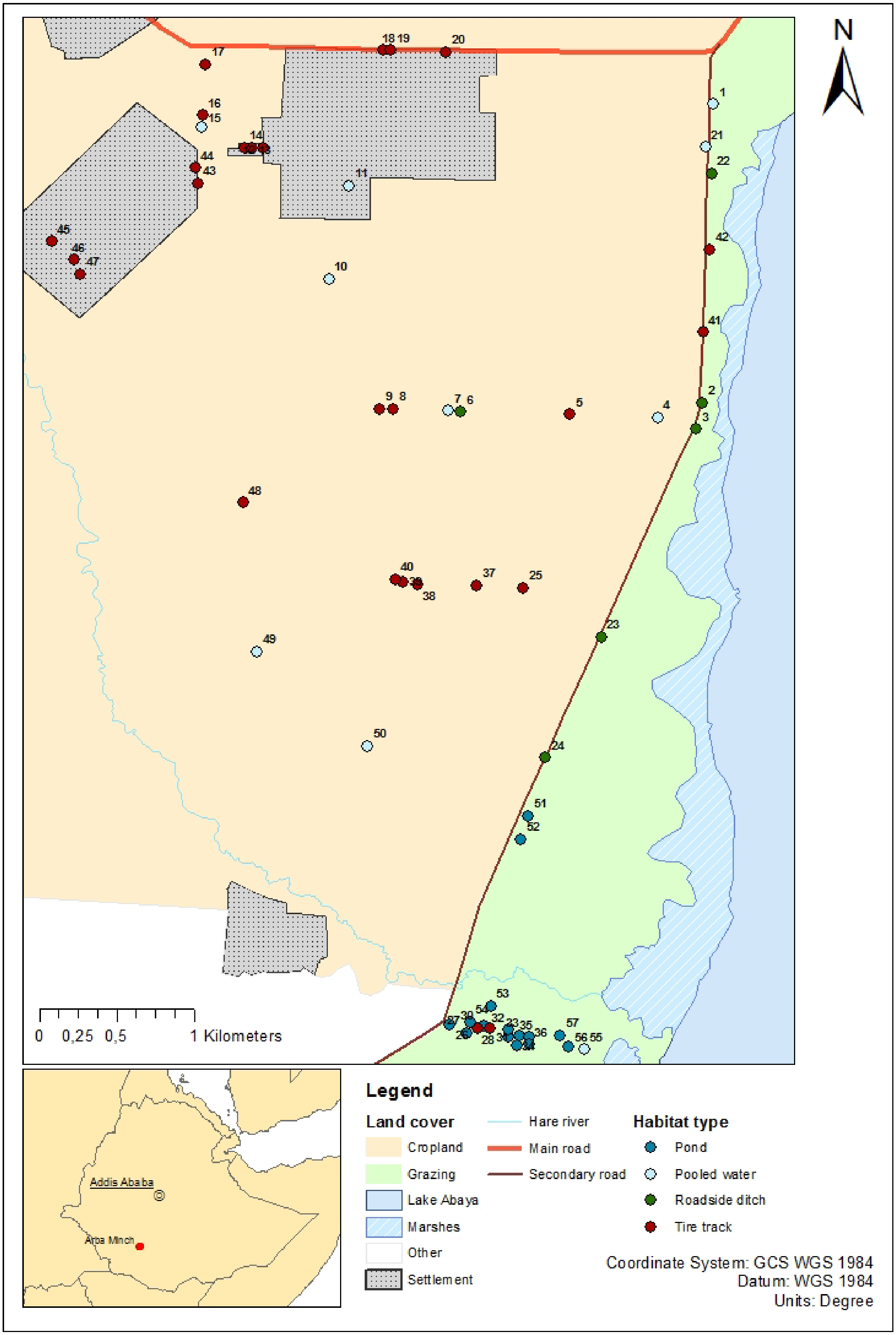

Figure 3. Map of the study area. Sampling sites are labeled with identification numbers that were assigned in chronological order of each visit.

Mosquito sampling

All potential mosquito breeding habitats were identified along accessible roads in the surrounding area of Chano village. The sampling took place from 10th June to 1st July, using a standard dipping method. Dipper is the most commonly used tool for collecting mosquito larvae from a wide variety of habitats (Silver, 2008b). Dippers vary in size and shape depending on local availability. We used a soup ladle with a diameter of 9 cm and approximately 250 ml capacity for larval sampling (Figure 4). The study area was visited once a week for 4 successive weeks and 12 to 17 sites were sampled during each visit. Ten dips were taken from each site in intervals along the edge of ponds and roadside ditches and randomly in pooled water bodies and tire tracks. The distance of at least 30 meters was kept between sampled sites. The larvae from each site were pooled into a plastic container. To avoid predators, the samples were filtered through a plastic sieve. The collected larvae were brought to the laboratory for estimating quantities of each instar. The number of larvae was expressed as counts of larvae per 10 dips.

Figure 4. A soup ladle used for mosquito sampling.

Species identification

The number of larvae was counted in the laboratory of Arba Minch University. Then by using the water from natural habitat, larvae were poured into plastic trays where they developed to pupal stage (Figure 5, left). Pupae were transferred to mosquito cages until they emerged as adults (Figure 5, right). The adults were collected with a suction tube and placed in the freezer for 15 minutes. Individuals were morphologically identified to species level following the key of Gilles & Coetzee (1987). The adults were placed in vials and kept in the freezer before they were transported to the Swedish University of Agricultural Sciences for further identification. Specimens that belonged to *An. gambiae* complex were identified to subspecies level using polymerase chain reaction (PCR) analysis. It was performed for one randomly selected adult from each site.

Figure 5. The setup of mosquito rearing to adults in plastic trays (left) and cages (right).

Larval habitat characterization

The potential *Anopheles* mosquito breeding sites with stagnant water identified were classified according to their nature. These included four habitat types (Figure 6):

Pooled water – ephemeral and shallow water bodies, formed as a result of rainwater or irrigation water pooled on the surface depression.

Tire track – small habitats in the marks of passed trucks or motorbikes, fed by rainwater.

Pond – semi- permanent, bigger in size and deeper habitats, clustered in the grazing area, mostly fed by rainwater.

Roadside ditch – more stable habitats on the side of the road with the main water source deriving from land irrigation.

Figure 6. Four types of habitats that were identified in the study area: a) Pond; b) Pooled water; c) Roadside ditch; d) Tire track.

The sites were also classified according to the land use, based on dominating features in the landscape (Figure 7). These include:

Agricultural area– land used for agricultural purposes. Maize and bananas are most common to grow, with scattered fruit, such as mango, trees;

Pasture – area along the Abaya Lake, dominated by shrubs, where the cattle graze during the day time.

Settlement – the inhabited area in the village.

Figure 7. Different landscapes in the study area: a) Agricultural area; b) Pasture; c) Settlement.

Several additional habitat characteristics were estimated on site:

Exposure to sunlight – each site was identified as exposed, partly exposed or shaded.

Aquatic vegetation – expressed as the presence or absence of aquatic vegetation.

Algae – expressed as the presence or absence of filamentous algae in the aquatic medium.

Water depth – the average depth of water, measured with a plastic ruler at six sampling points of each habitat.

Surface area was calculated from approximate estimations of width and length of the habitat, expressed in m^2.

Water physical analysis

The physical analysis of water in the breeding habitats was performed in situ. The parameters recorded are listed in Table 1. These were obtained using HACH HQ40d portable multi-parameter meter and AquaFluor® Handheld Fluorometer/Turbidimeter after the larval samples were collected.

Table 1. Water physical parameters recorded in the field. * obtained using Hach HQ40d; ** obtained using AquaFluor®.

Parameter	Unit	Range
Temperature*	˚C	10 – 110
pH*	pH units	2 – 14
Salinity*	ppm	0 – 42
Turbidity**	NTU	0.5 – 1000
Conductivity*	µS/cm	0.01 – $2*10^5$
DO (dissolved oxygen)*	mg/l	0.01 – 20
TDS (total dissolved solids)*	mg/l	0.01 – $5*10^4$
Chlorophyll A**	µg/l	0.3 - 300

Water chemical analysis

A water sample of 300 ml was taken from each site and kept in the fridge at 1˚C in the laboratory of Arba Minch University. The samples were later taken to the The Ethiopian Health and Nutrition institute, located in Addis Ababa, where analysis of water chemical composition was carried out from 8th to 17th July. The following ions were estimated: **Mg^+, Ca^{2+}, K^+, Cl^-, Na^{2+}, PO_4^{3-}, NO_2^-, F^-**. **Total hardness** was calculated from the Mg^+ and Ca^{2+} content. All ion quantities were expressed in mg/l.

Data analysis

As the distributions of all recorded continuous variables did not fulfill the requirement of normality, non-parametric Kruskal-Wallis tests were used to compare heterogeneity of the landscape. The median of each continuous variable was compared between the categories of habitat types and land use.

PCA (principal component analysis) was carried out prior to running regression tests to identify correlated variables, which were removed from regression models in order to avoid collinearity. Hardness, Mg^+ and Ca^{2+} were strongly intercorrelated, therefore only hardness was used as the representative variable, as it reflected the content of both magnesium and calcium ions. Strong correlation was also observed between salinity, conductivity, TDS and Cl^-. We chose conductivity as the representative variable for easier comparison with other published research, where conductivity was used to reflect the salinity of the water medium. Dissolved oxygen was strongly related with Chlorophyll A and the former was left in the model.

Sampled sites were categorized into two classes: 1) sites with and 2) sites without *Anopheles* mosquito larvae. Binary logistic regression was ran for these categories to estimate if any of the recorded physicochemical parameter was crucial for mosquito oviposition preference.

The larval density data were further investigated for the sites that were positive of *Anopheles* larvae. Regression of generalized linear model with quasipoisson family was applied for the count data. Regression was performed for total larval counts and for early and late instar counts separately. Early instars included first two, while late instars included last two larval stages. The models were simplified using backward selection.

Larval densities were also compared in regards to landscape features recorded on sites. Kruskal-Wallis tests were used to compare median larval densities between habitat type, land cover and shading category. Mann-Whitney U-test was used to compare median larval densities between sites where aquatic vegetation was present to those that had no aquatic vegetation.

All statistical operations were performed on Minitab 16 Statistical Software, except for quasipoisson regression, which was carried out using R (version 3.0.2).

RESULTS

A total of 57 sites have been sampled during the 4 week data collection, yielding a number of 450 larvae. Out of these sampled sites, 46 were positive of anopheline larvae.
24% (108) of collected larvae were first instars, 34.44% (155) - second instars, 25.12% (113) – third 113 and 16.44% (74) – fourth instar larvae. Early instars comprised 58,4%, late instars 41.56% of total collected larvae.

The temperature in mosquito breeding sites ranged from 24,3 to 35˚C. The lowest pH value was 7.93, while the highest was 10.25. Turbidity ranged from 4.251 to 314.4 NTU. Salinity [0.04; 9.73] ppm, DO [1.05 to 40] mg/l, Chlorophyll A [57.19; 3174] µg/l, TDS [0.508; 9390] mg/l, hardness [100; 1670], Mg [0; 255.36] mg/l, Ca [16.032; 252.500] mg/l, Na [0.4; 129.5] mg/l, K [0.3; 143.5] mg/l, Cl [0; 3623.9] mg/l, PO4 [0; 31.707] mg/l, NO2 [0.0074; 3.4373] mg/l and F [0, 3.566] mg/l.

The most numerous habitat type (n=22) found in the study area was tire track, followed by ponds (n=14), pooled water (n=13) and roadside ditches (n=9). The potential breeding habitats were most abundant in pastures (n=25), less were detected in agricultural area (n=19) and settlement (n=11).

Species composition

Out of 450 mosquito larvae 40 (8.9%), which were collected in 14 sites, survived to adulthood in the laboratory. These covered all habitat types and land use areas. All individuals belonged to *A. gambiae* complex. PCR analysis was successful for 12 out of 14 individuals (86%), which resulted in all individuals belonging to *A. arabiensis*. The PCR analysis was repeated for the two failed specimens that PCR amplification had failed, but again yielded no results.

Larval abundance

Binomial regression showed that probability to detect *Anopheles* mosquitoes was decreasing with increasing fluoride ion content ($p<0.05$) (Table 2). Few more parameters were close to significance. It was more likely to find anopheline larvae in habitats with harder water ($p=0.17$), which was strongly positively correlated with Ca ion content ($r=0.9$; $p<0.01$). Nitrate concentration also positively affected ($p=0.14$) the abundance of anopheline larvae. However, the binomial regression model was weak with probability of 0.022 that all slopes in the model are zero.

Table 2. Results from binomial logistic regression, showing association between physicochemical variables and the presence of anopheline larvae. Coefficients show increasing or decreasing probability to detect anopheline larvae with increasing value of the predictor, while p-value indicates the significance of each predictor.

Predictor	Coef	SE Coef	Z	p-value	Odds Ratio
Constant	-14.8858	10.0792	-1.48	0.140	
Depth (cm)	-0.13089	0.14998	-0.87	0.383	0.88
Surface	-0.03694	0.03584	-1.03	0.303	0.96
t	0.12445	0.21050	0.59	0.554	1.13
pH	1.14330	1.12460	1.02	0.309	3.14
Turbidity	0.00490	0.00660	0.74	0.457	1.00
DO	0.10128	0.12625	0.80	0.422	1.11
Hardness	0.00859	0.00629	1.37	0.172	1.01
Na	-0.00922	0.01765	-0.52	0.602	0.99
K	-0.01128	0.02162	-0.52	0.602	0.99
PO4	-0.03045	0.10271	-0.30	0.767	0.97
NO2	5.40333	3.70183	1.46	0.144	222.14
F	-1.04765	0.50637	-2.07	0.039	0.35

Larval density

The total larval density varied from 1 to 46 larvae / 10 dips. Poisson regression on all larval stages showed that conductivity (p=0.018) and dissolved oxygen (p=0.015) had a significant positive effect on the abundance of *Anopheline* larvae of all stages (Table 3), while depth, turbidity and sodium (Na) ion had an effect close to significance.

Table 3. Results from Poisson regression on the densities of different larval stages

	Estimate	Std. Error	t value	Pr(>|t|)	
Total larvae					
(Intercept)	1.939000	0.629700	3.08	0.00384	**
depth	-0.130500	0.074310	-1.756	0.08718	.
turbidity	0.003419	0.001935	1.767	0.08525	.
conductivity	0.000067	0.000027	2.47	0.01811	*
DO	0.051220	0.020130	2.545	0.01511	*
Na	-0.012100	0.006129	-1.974	0.05567	.
Young instar larvae					
(Intercept)	0.735000	0.293700	2.503	0.0164	*

conductivity	0.000078	0.000030	2.593	0.0131	*
DO	0.047230	0.019390	2.435	0.0193	*
Late instar larvae					
(Intercept)	0.089060	0.485900	0.183	0.85549	
turbidity	0.006674	0.002169	3.076	0.00377	**
conductivity	0.000089	0.000035	2.587	0.01344	*
Na	-0.015560	0.008434	-1.845	0.0725	.

Regression was further applied to early and late instar larval densities separately. Poisson regression showed conductivity and dissolved oxygen to have a significantly positive effect on the density of young instar larvae.

Density of late instar larvae were significantly increasing with increasing turbidity ($p<0.01$) and conductivity ($p<0.05$), while Na ion content negative effect ($p=0.0725$) on larval density.

The scatterplots in figures 8, 9 and 10 show association between larval densities and physicochemical variables that had a significant effect.

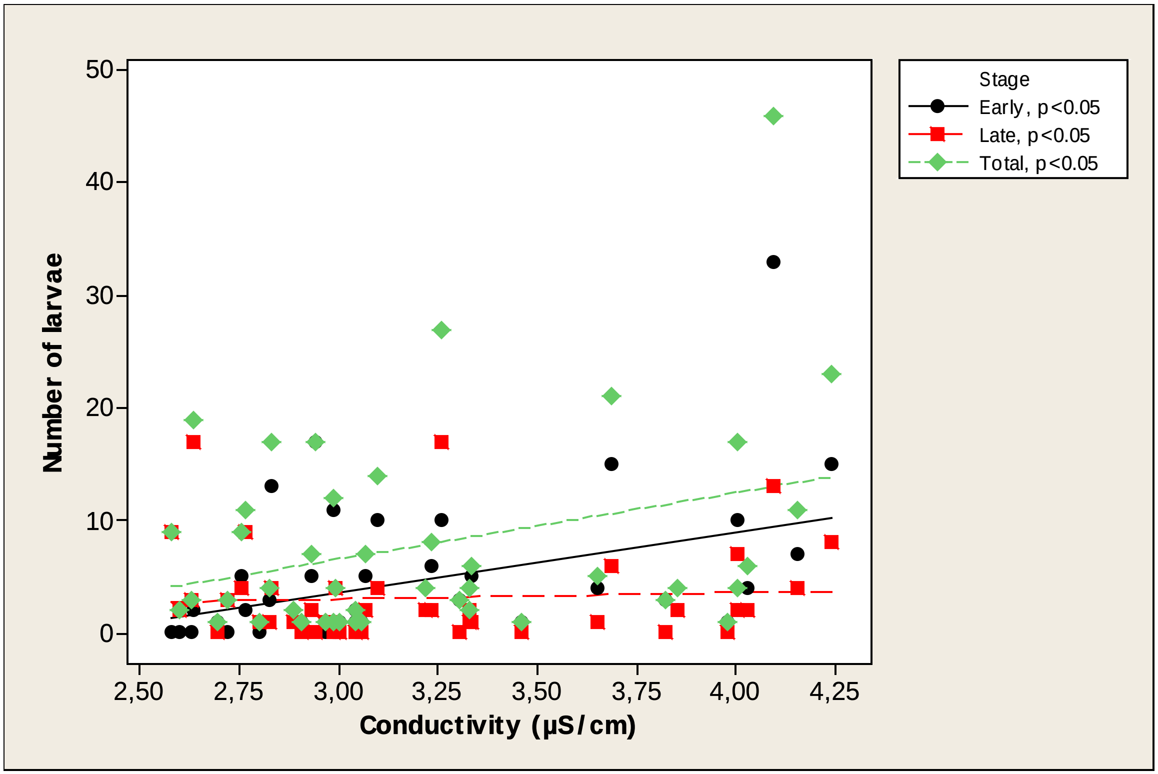

Figure 8. Relationship between larval density and conductivity. Conductivity is shown on a logarithmic scale.

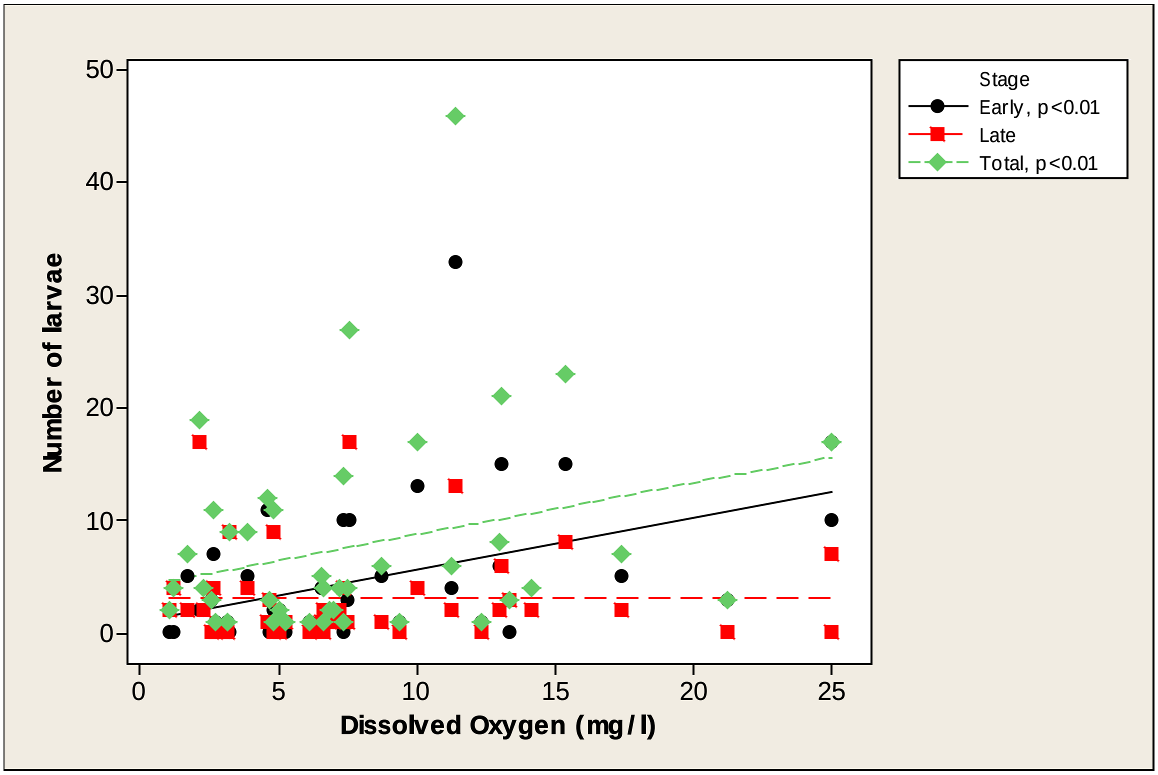

Figure 9. Relationship between larval density and the amount of dissolved oxygen.

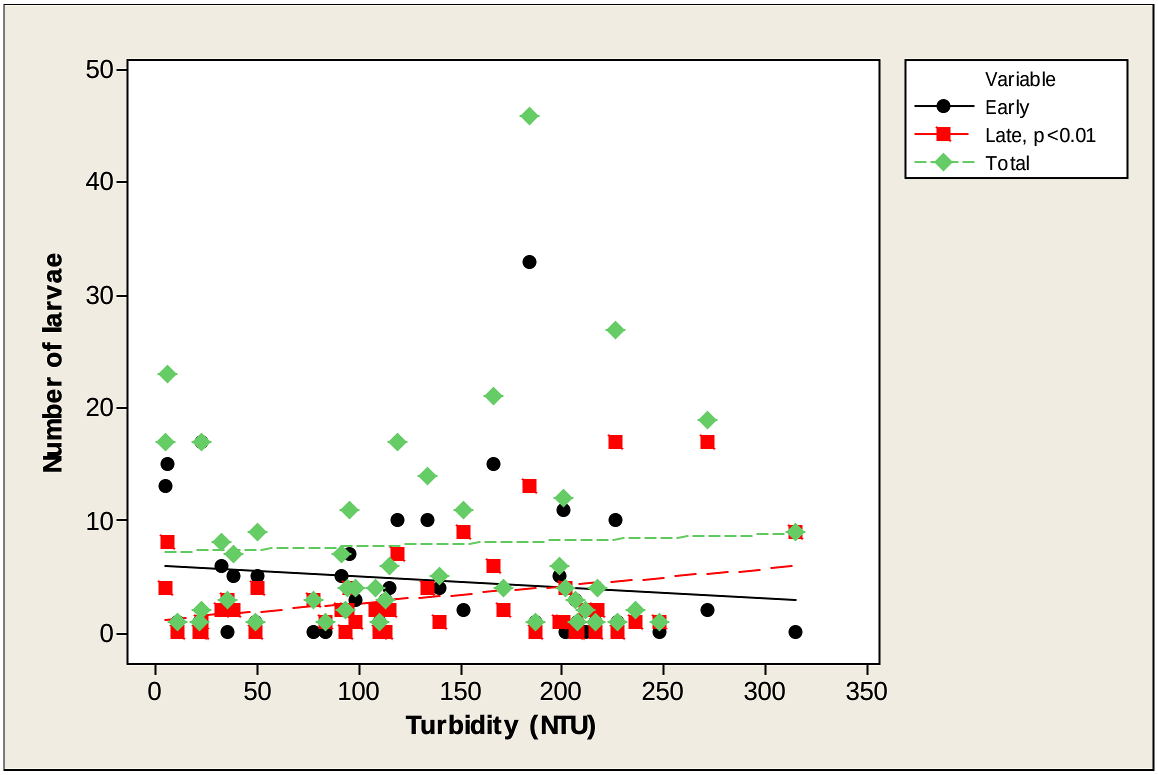

Figure 10. Relationship between larval density and turbidity.

Larval densities were further compared between the categories of habitat type, land use and shading using non-parametric Kruskal-Wallis test, while the larval densities in habitats with and without aquatic vegetation were compared using Mann-Whitney U-test. Habitat type did not have any significant effect on the density of the larvae (Table 4). There was a significant difference among land use types in the density of early instar larvae.

Table 4. Kruskal-Wallis* and Mann-Whitney** test results, comparing median larval densities between categories that describe different habitat characteristics.

Category	n	Median		
		Early	Late	Total
Habitat type*				
Pond	10	5.0	2.0	6.5
Pooled water	11	4.0	2.0	6.0
Roadside ditch	5	3.0	1.0	4.0
Tire track	18	2.0	1.50	3.5
p-value		0.15	0.741	0.502
Land use*				
Crop	19	2.0	1.0	4.0
Pastures	25	4.0	2.0	5.5
Settlement	11	0.0	2.0	4.0
p-value		0.008	0.305	0.409
Shading*				
Exposed	34	2.5	2.0	4.5
Partly shaded	7	2.0	1.0	3.0
Shaded	3	0.0	2.0	3.0
p-value		0.559	0.607	0.663
Aq. Vegetation**				
No	32	2.0	2.0	4.0
Yes	12	5.5	1.5	8.0
p-value		0.013	0.927	0.144
Algae**				
No	39	2.0	2.0	4.0
Yes	5	2.0	2.0	4.0
p-value		0.671	0.506	0.941

The density of early instar larvae was significantly affected by land cover type (p=0.008). The median was lowest in the settlement, while highest in pastures. The median of late instar larvae did not differ significantly between the land cover areas (Figure 11).

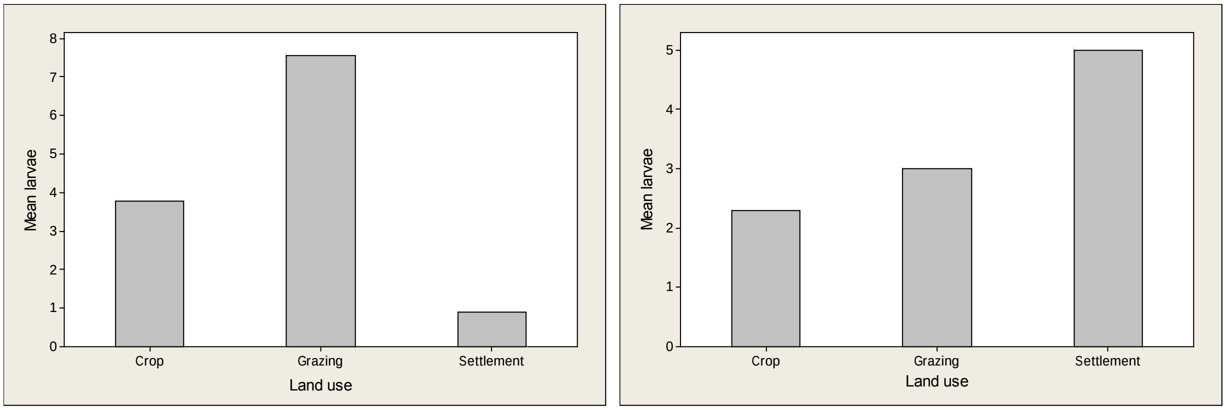

Figure 11. Mean densities of early (left) and late (right) instar larvae in different land use areas.

Shading and presence of algae in the habitat had no significant effect on larval densities. Presence of aquatic vegetation had a positive effect on density of early instars (p=0.013), but not on late instars.

HABITAT HETEROGENEITY

All physicochemical parameters of the habitats were compared between land use areas and habitat types using non-parametric Kruskal-Wallis test. The results are summarized in Table 5 and Table 6.

Table 5. Kruskal-Wallis tests applied to compare medians of water physicochemical parameters (WPP) in sites within different land cover categories**.**

WPP	Crop	Grazing	Settlement	p
n	19	25	11	
depth [cm]	6	7.583	5.5	**0.049**
t [˚C]	30.2	29.4	27.5	0.079
surface area [m^2]	2	20	1.5	**0.000**
pH	8.8	9.42	8.25	**0.000**
turbidity [NTU]	134	98.15	151.2	**0.026**
conductivity [µS/cm]	1137	2920	629	**0.001**
salinity [ppm]	0.47	1.38	0.3	**0.001**
DO [mg/l]	4.82	10.79	3.24	**0.000**
chlorophyll A [µg/l]	487.4	788.4	346.3	**0.019**
TDS [mg/l]	432	625	284	0.059
hardness	400	290	350	**0.014**
Mg [mg/l]	24.32	12.16	17.02	0.180
Ca [mg/l]	120.24	80.16	100.2	0.079
Na [mg/l]	15	12.5	5.5	**0.041**
K [mg/l]	15.5	10	12.5	0.197
Cl [mg/l]	24.9	34.7	18.1	**0.010**
PO_4 [mg/l]	30.7	28.4	22.4	0.387
NO_2 [mg/l]	30.1	26.1	28.6	0.708
F [mg/l]	26.5	31.8	22.1	0.218

More than half of the measured parameters differed significantly between different land use categories. These include depth, surface area, pH, turbidity, conductivity, salinity, dissolved oxygen, chlorophyll A, hardness and the amount of Na and Cl ions.

The depth differed significantly between the land use categories, with the highest median in pastures. Surface area in the pastures was about ten times higher compared to the other land cover categories. Pastures also had the highest pH value. Water was most turbid in the settlement, followed by crop land and pastures. The three correlating parameters: conductivity, salinity and Cl ion content had the highest values in the pastures, followed by agricultural fields and settlement. The same was true for dissolved oxygen and Chlorophyll A. The water was hardest in the crop land and softest in the grazing area. Ca and Mg ion content follow the same pattern, but without significant differences.

Table 6. Kruskal-Wallis tests applied to compare medians of water physicochemical parameters (WPP) in sites within different habitat types**.**

WPP	Pond	Pooled water	Roadside ditch	Tire track	p
n	14	13	6	22	
depth [cm]	8.833	5.75	8.292	5.833	**0.009**
t [˚C]	30.5	29.6	27	29.7	0.101
surface area [m^2]	19.813	3.5	38.75	2	**0.000**
pH	9.515	8.59	8.68	8.8	**0.000**
turbidity [NTU]	101.07	133.7	24.85	178.9	**0.002**
conductivity [µS/cm]	7150	980	839.5	1053	**0.000**
salinity [ppm]	3.66	0.46	0.38	0.44	**0.000**
DO [mg/l]	13.02	6.97	8.01	4.81	**0.000**
chlorophyll A [µg/l]	898.1	385.5	360.9	488.9	**0.005**
TDS [mg/l]	1730	257	380.5	422.5	0.288
hardness	260	330	295	360	0.170
Mg [mg/l]	12.16	19.46	14.59	19.46	0.671
Ca [mg/l]	68.14	80.16	88.18	100.2	0.258
Na [mg/l]	9.325	13.5	13	9.5	0.703
K [mg/l]	11.25	12	6.5	15.5	0.115
Cl [mg/l]	259.92	34.99	12.5	27.49	**0.005**
PO_4 [mg/l]	3.304	2.18	1.357	1.904	0.650
NO_2 [mg/l]	0.3315	0.3388	0.3118	0.3388	0.851
F [mg/l]	0.6257	0.48	0.5143	0.4543	0.278

Habitat types differed significantly in nine recorded physicochemical parameters. Ponds and roadside ditches were deeper than pooled water habitats and tire tracks. Ponds and roadside ditches had the highest surface area, followed by pooled water and tire tracks. Ponds had the highest pH, conductivity, salinity and chloride content. Tire tracks were the most turbid habitats, followed by pooled water, ponds and roadside ditches. Ponds had much higher DO and chlorophyll A content compared to other three habitat types. The habitats did not differ significantly in other parameters.

DISCUSSION

The study shows that landscape and its elements were important for productivity and distribution of mosquito breeding sites in the area of village Chano, where *Anopheles arabiensis* was the only collected anopheline species. The larvae were present in all land cover categories within habitats covering a wide range of physicochemical characteristics. However, female *Anopheles* showed preference for oviposition in more stable breeding sites located closer to the shore of Lake Abaya where the aquatic vegetation was present. We observed that the early *Anopheles arabiensis* larval stages were more abundant in the pastures, followed by agricultural land and settlement, while the density of late instar larvae did not differ significantly between the three different land cover categories. Densities of the early instar *A. arabiensis* larvae were positively associated with conductivity, concentration of dissolved oxygen and the presence of aquatic vegetation. These features were linked to larger and more stable habitats, clustered by the lake shore. In contrary, the density of late larval instars was increasing with increasing turbidity. Higher turbidity levels were recorded in habitats that were small and shallow.

A number of studies has shown that *A. arabiensis* mosquitoes prefer shallow temporary habitats with bare soil and no aquatic vegetation (Fillinger et al., 2009; Gimnig et al., 2001; Huang et al., 2006; Kenea et al., 2011; Munga, Minakawa, Zhou, Mushinzimana, et al., 2006; Ndenga et al., 2011; Sattler et al., 2005; Wanji et al., 2009). Such temporary pools are more favorable for *Anopheles* mosquito survival, as they lack aquatic predators, which are the main regulators of larval populations (Ndenga et al., 2011; Wanji et al., 2009). There is also less interspecific competition for food sources with other aquatic insect and mosquito species, which are better adapted to shaded habitats. Despite their preference for temporary and small breeding sites, there is evidence that Anophelines are capable of ovipositing in grassy aquatic habitats when typical puddles with bare soil are unavailable (Huang et al., 2006). This supports the idea that due to dry climatic conditions and intense evaporation of small habitats during the sampling period, females were laying more eggs in less favorable habitats that were permanent and contained aquatic vegetation. In this way gravid females could offset the lack of small habitats by laying eggs in larger and more stable water bodies to make sure their progenies would have enough time to develop. This leads to observation that the gravid female mosquitoes oviposited more intensively at the breeding sites reported as less favorable, but the survival rate was lower at such sites as smaller proportion of larvae reached late stages.

Another important aspect to take into account is the survival of the adult mosquitoes in dry conditions, which is strongly dependent on relative humidity (Gullan & Cranston, 2005). Rainfall is considered to play crucial role in mosquito population dynamics as it provides the medium for the immature stages, as well as increases the relative humidity and hence the longevity of the adult mosquito (Lindsay & Martens, 1998; Protopopoff et al., 2009). In the lack of precipitation, mosquitoes have to find strategies to avoid desiccation. Abogaye-Antwi and

Tripet (2010) found that access to water and food availability influences the survival of adult mosquitoes, as the use of water and body lipid reserves affect desiccation resistance. This could lead to clustering of the adults around areas with a permanent source of water, even though such areas would not provide favorable breeding sites. Here we can draw a conclusion that the spatial distribution of the breeding sites of *Anopheles* mosquitoes depends on local climatic conditions. The surroundings of Lake Abaya could support the survival of these mosquitoes in dry conditions, while the inhabited area could be more favorable during the rainy season, as the proximity to the blood meal is an important predictor of *Anopheles* larval distribution and densities (Kenea et al., 2011; Le Menach et al., 2005; N. Minakawa et al., 1999; Wanji et al., 2009).

The larvae of *A. arabiensis* were found in habitats with salinity up to 9.73 ppm. Though they are reported to be strictly limited to sites of low salinity (Jawara et al., 2008), they have been occasionally been found in brackish habitats (Sinka et al., 2010). However, no upper limit of salinity has been reported for this species. We found a positive association between the density of mosquito larvae and conductivity, which is an indicator of the salinity. This agrees with Gouagna et al. (2012) and Olayemi et al. (2010) who also found that the density of larvae correlated positively with conductivity. In contrast, Fillinger et al. (2009) reported that pools, which were characterized by low conductivity, were the most productive breeding sites. In our study, most of the breeding sites with the highest salinity values were located near the lake, which were more favored for oviposition due to geographical location as discussed above, rather than the salinity itself. This indicates how well *Anopheles* mosquito larvae are adapted to fluctuating environmental conditions.

We found higher densities of late anopheline stages with increasing turbidity. Some surveys found higher *A. arabiensis* mosquito densities in clear water (Robert, Awono-Ambene, & Thioulouse, 1998; Sattler et al., 2005; J. Shililu, Ghebremeskel, Seulu, et al., 2003), while other reported higher densities in turbid waters (Awolola, Oduola, Obansa, Chukwurar, & Unyimadu, 2007; Fillinger et al., 2009; Mala & Irungu, 2011; Sinka et al., 2010). Ye-Ebiyo et al. (2003) observed better larval development in relatively clear water. They suggest that increased densities in turbid water may be a result of continued oviposition at the end of the seasonal rains, as the habitats evaporate and become more turbid and scarce. They add, that inert particles in the water medium may negatively affect young instar larvae by preventing them from feeding effectively and reducing the chance of survival. On the other hand, if turbidity is caused by suspended organic material, such as maize pollen, it may provide additional food source for the development of immature *Anopheles* stages. However, this was not the case in our survey, as maize was not flowering when sampling took place.

Beside these factors, overall larval density was increasing with increasing levels of dissolved oxygen. DO might be important for cuticular respiration, allowing mosquito to cover their demand of oxygen for survival. This could be beneficial not only for longer feeding on greater variation of food source in the water column, but also avoiding predators. Secondly, dissolved oxygen was strongly correlated with chlorophyll A content. Chlorophyll A reflects the amount of chlorophyll in living algal and cyanobacterial cells in water, which act as an important food source for *Anopheles* mosquito larvae (Gimnig et al., 2001; Mala & Irungu, 2011; J. Shililu, Ghebremeskel, Seulu, et al., 2003; Tuno et al., 2005).

A. arabiensis was the only mosquito species present in mosquito positive habitats during the sampling period. Massebo et al. (2013) was collecting adult mosquitoes in the same Chano village. He regarded *A. arabiensis* as predominant species, comprising 75% of all *Anopheles* species caught in the area. Ribeiro et al. (1996) and Taye et al. (2006) also reported *A. gambiae* s.l., namely *An. arabiensis* as the principal vector species in another malarious village Sille, situated approximately 30 km South-West from our study area near lake Chamo. Other species reported by these authors include *A. coustani, A. pharoensis, A. funestus, A. nili, A. marshallii, A. garnhami* and *A. demeilloni,* which differ in ecology and morphology (Gilles & Coetzee, 1987). *A. arabiensis* could be the only anopheline species found due to the fact that the sampling took place in the end of the rainy season as the weather was getting hot and arid. Adult mosquitoes of the species *A. arabiensis* perform better under conditions of low relative humidity, compared to adults of other *Anopheles* species, and are better able to exploit drier areas and seasons (Kirby & Lindsay, 2009; C.J.M. Koenraadt, Githeko, & Takken, 2004; J. I. Shililu, Maier, Seitz, & Orago, 1998). Larvae are also reported to be better competitors in higher temperatures compared to other Anopheline larvae (Coetzee, Craig, & le Sueur, 2000; Sogoba et al., 2007). On the other hand, Massebo et al. (2013), Ribeiro et al. (1996) and Taye et al. (2006) were collecting indoor and outdoor resting adult mosquitoes around human dwellings all year round, which could have resulted in bigger sample sizes and higher probability to detect more species during the season when climatic conditions are more favorable for dispersal of the adults as well as the breeding habitats are more numerous. Furthermore, we may not have sampled the habitats where other anopheline species would breed. For example, *Anopheles funestus* larvae are associated with larger, semi-permanent bodies of water containing aquatic vegetation and algae (Gimnig et al., 2001).

We also do not exclude the possibility that there were more species in our samples, as only 8,9% of mosquitoes survived to adulthood, which were later identified to species level. However, our results agree with surveys from other localities in Ethiopia, which report *A. arabiensis* as the dominant vector species in the country with relative abundance ranging from 32% to 87% (Kenea et al., 2011; PMI, 2013; Tirados et al., 2006; Yohannes et al., 2005). *Anopheles arabiensis* has also been reported as predominant species in the neighboring Eritrea (Shililu et al., 2003b). Meanwhile Gimnig et al. (2001) report *A. gambiae s.s.* and *A. arabiensis* as the dominant *Anopheles* species in Kenya. They both belong to *A. gambiae s.l.* complex, but the former is not found in Ethiopia (Hunt, Coetzee, & Fettene, 2000).

CONCLUSIONS

1. *Anopheles arabiensis* was the dominant vector species in the Chano village, located in the Southern Nations, Nationalities and Peoples Region.
2. Many physicochemical characteristics of the breeding habitats varied throughout the landscape depending on the land cover or habitat type. The habitats located in pastures were characterized as the most saline, had highest pH values and highest DO and chlorophyll A content. Ponds and roadside ditches were significantly larger in surface area and deeper, while tire tracks and puddles were shallow and had highest turbidity.
3. We did not detect significant association between presence of the *Anopheles* mosquito larvae and abiotic or landscape characteristics.
4. Early instar larvae of *A. arabiensis* were more numerous in habitats that were more stable, but the survival in such habitats was lower. Water quality may be not the main factor influencing site selection for oviposition when the climatic conditions are not favorable for the survival of the adult and immature stages. Therefore, in order to understand the dynamics of mosquito larval populations, they should be studied throughout the year as seasonality plays an important role in the temporal and spatial variation of *Anopheles* mosquitoes in the area. This knowledge could be applied in vector control, aiming mosquito populations when they are mostly vulnerable.

Taking into account that our survey was carried out at the end of the rainy season, it would be interesting to investigate the densities and distribution of *Anopheles* mosquitoes during the rainy seasons, when the rainfall is intense providing a number of breeding habitats and more favorable climatic conditions for the survival of the adult mosquitoes. Additional mosquito sampling techniques, such as sampling adult mosquito with carbon dioxide traps or light traps, could be carried out to provide more information on the distribution of adult mosquitoes as well as the species composition, as we might have overlooked or not accessed some of the potential breeding sites of other anopheline species. This could also provide information on relationship between the distribution of adults and larval populations.

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