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THE EFFECT OF ENVIRONMENTAL MODIFICATION ON MALARIA EPIDEMIOLOGY AND *ANOPHELES* BIONOMICS IN ETHIOPIA

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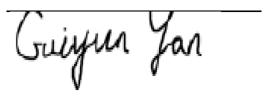
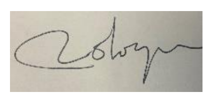
Co-promoter/s	Prof. Guiyun Yan (PhD) University of California, Irvine, USA	Signature: 	Date: January 2022
	Dr. Solomon Kibret (PhD) University of California, Irvine, USA	Signature: 	Date: January 2022
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DEDICATION

To my dad, who made education real at the place where I was born and grew up, just a midst of darkness, hard to education access. He took initiative to travel long miles on foot to meet higher official, requesting establishment of primary school and realized the enlightenment to the community from where now many educated minds serving their community in and abroad. It would be unthinkable to see this end if there was no education access in such hard-to- reach village, mainly due to my dad.

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LIST OF ACRONYMS

FMoH	Federal Minister of Health
RDT	Rapid Diagnostic Test
SPSS	Statistical Package for the Social Sciences
LSM	Larval Source Management
NMCP	National Malaria Control Program
NMCT	National Malaria Control Team
IRS	Indoor Residual Spray
DDT	Dichlorodiphenyltrichloroethane
MoH	Minister of Health
WHO	World Health Organization
EPHI	Ethiopia Public Health Institute
G6PD	Glucose-6-phosphate dehydrogenase deficiency
AIDs	Acquired Immunodeficiency Syndrome
LLINs	Long Lasting Insecticidal Nets
ITNs	Insecticide Treated Nets
CDC	Center for Disease Prevention and Control
PHCU	Primary Health Care Unit
CSP-ELISAs	Circumsporozoite Protein Enzyme-linked Immunosorbent Assays
ELISA	Enzyme-Linked Immuno-Sorbent Assay
PCR	Polymerase Chain Reaction
USA	United State of America
PHCU	Primary Health Care Unit

NERC	National Ethics Review Committee
PMI	President's Malaria Initiative
ICEMR	International Center of Excellency for Malaria Research
SP	Sulfadoxine-Pyrimethamine
ACT	Artemisinin-based Combination Therapy

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GENERAL SUMMARY

Background

Rain-fed agriculture has affected African livelihood by making people vulnerable to climate-related drought. Construction of dams and initiating irrigation schemes has therefore been widely recognized as key solutions to ensure food security and enhance economic growth in drought prone regions. In this regard, Ethiopia has embarked extensive dam constructions and irrigation expansion to promote economic development. However, such development activities may cause environmental modifications that could adversely affect the spread of vector-borne diseases such as malaria. To date, there has been limited data evaluating the impact of environmental modifications on the epidemiology of malaria and its vector bionomics.

Therefore, this study was done to evaluate impact of environment modifications on distribution and ecology of malaria vector mosquitoes at Arjo-Dedessa Sugarcane Irrigation Scheme in southwest Ethiopia. The study had three main objectives: i), it aimed to determine the existing malaria transmission dynamics in the area (Chapter 4). ii), it aimed to identify breeding habitats of malaria vector mosquitoes in the area (Chapter 5), and iii), it aimed to determine the effects of change in the agroecosystem on survivorship and development of *Anopheles gambiae* s.l, the main malaria vector in the area (Chapter 6).

Methods

Epidemiological and entomological surveys were conducted between 2017 and 2020 in Arjo-Dedessa area of Southwestern Ethiopia. Retrospective data of malaria for the period between 2008 to 2017 were obtained from health facilities near the Arjo-Dedessa Sugarcane Irrigation area. Malaria positivity rate, incidence rate, parasite species proportion, seasonality, age structure and sex distribution were analysed and discussed (Chapter 4).

Entomological surveys were also conducted that encompasses; repeated cross-sectional survey for *Anopheles* larval ecology study and life-table experiment for survivorship and development study. *Anopheles* mosquito larvae were collected seasonally from two agroecosystems, 'irrigated sugarcane plantation' ('irrigated area' hereafter) and 'non-irrigated mixed crop-covered' areas ('non-irrigated area' hereafter) during the dry (December 2017 – February 2018) and wet (June

2018 – August 2018) seasons. Mosquito habitat diversity and distribution, and larval abundance were compared between the two agroecosystems and discussed (Chapter 5).

Life-table experiments were conducted to examine the effect of environmental modification on survivorship of *An. gambiae* s.l both immatures and adults in irrigated and non-irrigated areas. The pupation rate and development time of the immatures and adult longevity and fecundity were compared between the two agroecosystems and discussed (Chapter 6).

Results

The epidemiological profile of malaria for the study area was mapped (Chapter 4). Over 10 years, 54,020 blood films were collected for malaria diagnosis in the health facilities at the area, of which 18,049 (33.4 %) were confirmed malaria cases by both microscopically and RDT**.** *Plasmodium falciparum, P. vivax*, and mixed infection (*P. falciparum* and *P. vivax*) accounted for 8,660(48%), 7,649(42.4%), and 1,740(9.6%) of the malaria cases, respectively. The study also revealed that *P. vivax* was the predominant over *P. falciparum* for four years (2010, 2014, 2015 and 2016). Malaria has been reported in all age groups, but age distribution showed that the vast majority of cases were adults above 15 years of age (73.7%). In all age groups, males were more significantly affected than females. Moreover, malaria positivity rate showed a strong seasonality. However, malaria cases were reported in all seasons across the 10 years data analyzed.

The association between environmental modification due to irrigated agriculture and *Anopheles* mosquito larval habitat diversity and distribution, and larvae occurrence is discussed in Chapter 5. In this study, 319 aquatic habitats were surveyed during the study period. Around 60% (n = 152) of the habitats were positive for *Anopheles* mosquito larvae, of which 63.8% (n = 97) and 36.2% (n = 55) were from irrigated and non-irrigated areas, respectively. The number of *Anopheles* positive habitats was two-fold higher in irrigated than non-irrigated areas. *Anopheles* larval abundance in the irrigated area was 16.6 % higher than in the non-irrigated area. Pearson's chi-square analysis showed that season, agroecosystem, and turbidity had a significant association with larval *Anopheles* occurrence.

The effect of environmental modification on mosquito survivorship and development is discussed in Chapter 6. The estimated mean survival time of female *An. gambiae* s.l in irrigated and non-irrigated area was 37.9 and 31.3 days, respectively. The estimated mean larval-to-adult development time of *An. gambiae* s.l larvae was not found to be different in both irrigated and non-irrigated areas. A survival analysis indicated that adult female *An. gambiae* s.l in the irrigated area live significantly longer than those in the non-irrigated area. Females mosquitoes showed higher longevity than males in both irrigated and non-irrigated areas.

Conclusions and recommendations

In general, malaria positivity showed a declining trend over 10 years period in the area. However, in recent years of study, it showed a slight rise, which indicates that the area needs attention to intensify the existing interventions to sustain control and enhance malaria elimination efforts.

This study found out a higher *Anopheles* mosquito breeding habitat diversity, larval occurrence and abundance in the irrigated than non-irrigated areas in both the dry and wet seasons of the year. This indicates that irrigation development activities contribute to the proliferation of suitable mosquito breeding habitats that could increase the risk of malaria transmission. Incorporating larval source management into routine malaria vector control strategies could help reduce mosquito population density and malaria transmission around irrigation schemes.

Adult *An. gambiae* s.l survivorship was found to be enhanced in irrigated area than non-irrigated area. Longer survival of adult mosquito in irrigated sugarcane plantation area may have important implications in malaria transmission. Thus, routine monitoring of entomological indices and environmental parameters in line with land use change is needed to detect any change in malaria epidemiology and device toiler-made interventions.

CHAPTER 1: GENERAL INTRODUCTION

1.1. General Overview

Malaria is a disease caused by infection with protozoan parasites of the *Plasmodium* genus. *Plasmodium falciparum, P. vivax, P. ovale, P. malariae* and *P. knowlesi* are the five species cause human malaria. From *plasmodium* species, *P. falciparum* has been the predominant one with the greatest public health impact, principally in sub-Saharan Africa. *Plasmodium vivax, P. ovale,* and *P. malariae* are also found in sub-Saharan Africa [1,2].

Regardless of remarkable progress in disease burden reduction, malaria remains a public health threat. In 2019, World Health Organization (WHO) reported an estimated 229 malaria million cases and 409, 000 deaths of malaria globally, of which 94% of cases and 94 % of deaths were in Africa [3]. According to WHO report 2020, malaria case incidence (i.e. cases per 1000 population at risk) reduced from 80 in 2000 to 58 in 2015 and 57 in 2019 globally. Between 2000 and 2015, global malaria case incidence declined by 27%, and between 2015 and 2019 it declined by less than 2%, indicating a slowing of the rate of decline since 2015 [3].

Malaria is among major public health problem in Ethiopia with a risk for the life of roughly 52% of the population [4]. *Plasmodium falciparum* and *P. vivax* are the most predominant parasite species responsible for the majority of malaria cases in the country [5,6].

Adult female *Anopheles* mosquitoes are vectors for the *Plasmodium* parasites. Globally, there are estimated more than 537 *Anopheles* species, of which 70 species are found to transmit human malaria parasites, and 41 are important vectors. In sub-Saharan Africa, there are around 140 *Anopheles* species of which approximately 20 are known to transmit malaria parasites. The most dominant and widely distributed vectors species in tropical Africa are *An. gambiae* Sensu Lato and *An. arabiensis* Patton (among *An. gambiae* complex) and *An. funestus* Giles [7].

In Ethiopia, around 46 *Anopheles* species are reported**.** *Anopheles arabiensis* is the principal vector [8]. *Anopheles funestus* group, *An. pharoensis,* and *An. nili* are considered as secondary vectors in Ethiopia [9–11]**.**

The malaria vectors have ecological preference for different ecological settings. However, because of environmental modification due to anthropogenic activities such as agricultural expansion, urbanization, population growth, deforestation, irrigated agriculture and dam construction, change in bionomics and dynamics of malaria vectors may occur [12–15]. Eventually, such change in vector bionomics might have a contribution to altering vectorial capacity of the local vector population, which in turn, become an essential factor in determining malaria transmission intensity [14,16–18].

In an effort to avert poverty, developing countries have been implementing water resource development projects like irrigated agriculture, and hydropower dams construction [14,15]. Such land-use change often leads to the creation of conducive breeding grounds for efficient malaria vectors [17,19,20]. The land-use change either singly or in combination with climatic conditions affect the biology and ecology of both parasites and their vectors, and eventually the incidence and prevalence of vector-borne infectious diseases [21,22]. Previous studies indicated that such change in land use pattern has been increased malaria transmission through proliferating vector breeding sites and changing the microclimate that governs the dynamics of vectors [14,16,23,24].

Variation in climatic conditions following environmental modifications has a profound effect on the biology and development of both malaria parasites and its vectors. Temperature and humidity are the most important climate factors that determine the success of both parasite and vectors [25,26]. The best conditions for the development of *Plasmodium* in the mosquito and transmission of the infection are when the mean temperature is within the range of 20 to 30°C [25], while the mean relative humidity is at least 60% [27].

Entomological indicators like aquatic habitat proliferation, mosquito survivorship or longevity in vector population are important determinants of malaria transmission intensity in a specific locality. These factors are highly prone to change in response to environmental modification [23]. Therefore, determining and having updated information on such variables helps device appropriate interventions in malaria elimination. Identification and management of larval habitats is the greatest tool, which has been assisting to suppress vector densities and malaria transmission [28]. Older adults that have passed through the extrinsic incubation period are potentially infective [29].

In the case of the immature stage, as shorter immature development time and less mortality, more adult population density, which increase the probability of vector-host contact [30,31].

Ethiopia, a country with more than 52 % of its population is at risk of malaria infection [32, 40], has been experiencing a massive change in land use by water resource development projects including irrigated agriculture and hydropower dam construction [23]. Arjo-Dedessa sugar development project, in southwest Ethiopia is among mega irrigation schemes with a sugarcane farm covering around 4,000 hectares with future expansion plan of 80,000 ha. Malaria is a public health problem in the study setting. However, in the area no information is available on epidemiological and entomological indicators of malaria transmission following environmental modification.

Therefore, this thesis work aimed to determine malaria transmission patterns, and to investigate the impact of environmental modification on the distribution and diversity of *Anopheles* mosquitoes breeding habitat; and development and survivorships of *An*. *gambiae* s.l at Arjo-Dedessa irrigation scheme and its vicinity, southwest Ethiopia.

1.2. Biology of Malaria Vector Mosquitoes

Understanding the ecology and behavior of the malaria vectors is relevant in monitoring their response to the existing interventions and devising tailor-made interventions [59]. There are aquatic developmental stages (egg, larva and pupa), as well as the adult stage, which is responsible for malaria transmission. The development of larval and pupal stages might be influenced by the oviposition site selection of gravid female *Anopheles* mosquitoes. Chemical cues and some physical factors direct the oviposition site selection of adult females [59,60]. Some of breeding sites of African *Anopheles* mosquitoes including shallow and sunlight-exposed temporary water bodies, permanent shaded water bodies, permanent man-made concrete structures, drainage canals and natural swamps [61]. Some species are salt-water breeders, whereas others prefer hot springs [62]. After finding the appropriate habitats, adult *Anopheles* females lay their eggs on the surface of the water. The eggs hatch to larvae, which are active feeders on decaying organic matters and microorganisms [59]. Larva subsequently molts into the second, third and fourth instar. The final instar develops into the non-feeding stage pupa, then adults emerges from pupae within a few days

(Fig. 1.2). The duration of the life cycle (usually 10-14 days in the tropics) depends on water temperature, type of larval food and species [59].

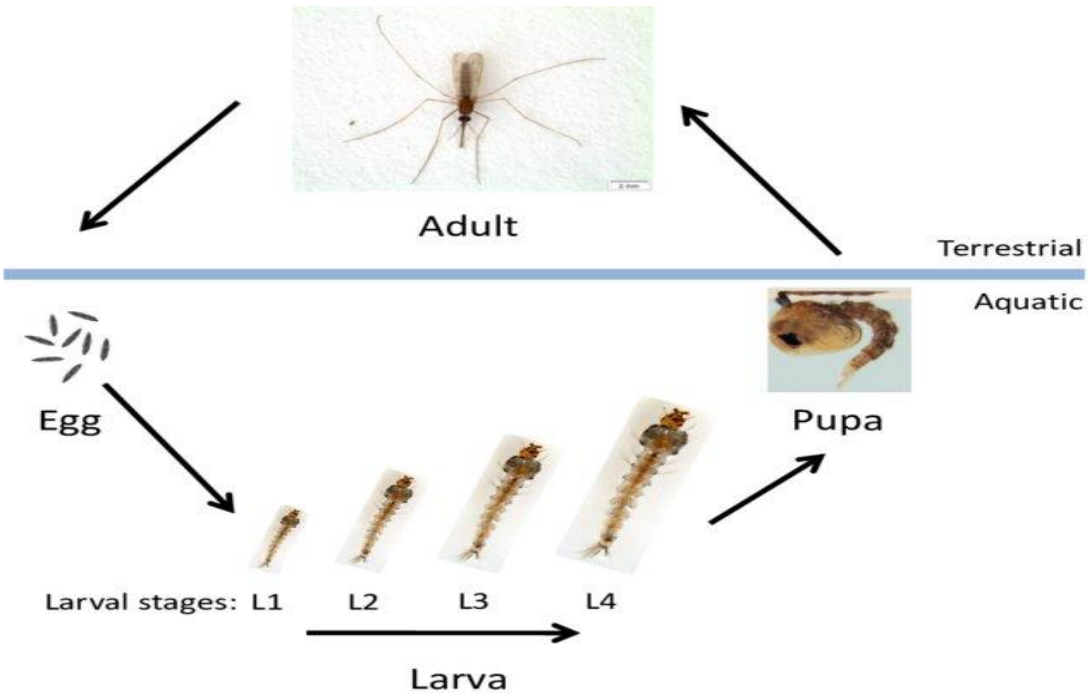

Figure 1.1: Life cycle of *Anopheles* mosquito [63]

1.3. Entomological Determinants of Malaria Transmission

The malaria transmission intensity can be measured by entomological variables, including the EIR, the longevity and feeding preferences of vectors, the susceptibility of the vectors to parasites and the length of the extrinsic incubation period of the parasites [64]. *Anopheles* mosquitoes with a higher EIR, susceptibility to parasites, longevity, and higher human-biting behaviors are potentially more important as vectors than others, and also increase the intensity of malaria transmission.

1.3.1. Vectorial capacity

Vectorial capacity is the overall ability of vector, in a given location at a specific time, to transmit a pathogen. It is measured in terms of the number of infectious bites a person receives, which is affected by vector population size, longevity, number of gonadotrophic cycles, feeding behavior and diel activity (circadian rhythm or biological clock) and others [65]. Globally, there are more than 537 *Anopheles* species, of which approximately 70 are potential vectors of malaria, and yet they are different in their competence [66]. There are intrinsic and extrinsic factors that affect the vectorial capacity of malaria vectors. There is a difference even between individuals of the same species. The factors that determine the vectorial capacity of a malaria vector species include vector longevity, strong human blood preference, susceptibility of the vector to parasite infection, and the duration of sporogonic development (the parasite development within a vector) [64].

1.3.2. Vector longevity

Age grouping of adult malaria vectors is important to help understand the epidemiology of malaria and for assessing the efficacy of vector control interventions [67]. Most anti-vector interventions, such as LLINs and IRS, are designed to shorten the lifespan of mosquitoes by killing older mosquitoes, thereby consequently reducing the burden of malaria transmission [59]. Malaria parasites undergo development in the vectors (extrinsic incubation) before transmission occurs, which comprises a significant proportion of the expected life expectancy of the vectors [59,64]. Hence, those malaria vectors that live long enough allow the parasite to complete the extrinsic incubation period, and become infectious to transmit malaria to susceptible hosts. The physiological age of female mosquitoes is determined by dissecting their ovaries and grouping them into nulliparous (young) and parous (old) using the Detinova method [68]. Nulliparous female mosquitoes have coiled tracheolar skeins, whereas the parous females have stretched tracheolar skeins. Parous mosquitoes are those that have oviposited one or more batches of eggs, and therefore, they could potentially transmit parasites because of their repeated contacts with hosts for blood meals. A female mosquito usually becomes infectious after three gonotrophic cycles. Nulliparous females have not laid their first batch of eggs, and are thus not yet infective. A more precise method of age determination is the Polovodova method, which counts the number of dilatations left after each oviposition in the ovary [69]. The number of dilatations shows the number of times a female mosquito had a blood meal and laid eggs, hence showing both age and the number of gonotrophic cycles (number of contacts with hosts). The more the number of gonotrophic cycles, the more likely the mosquito becomes infectious to susceptible hosts, but the method is difficult and laborious intensive. Another approach to mosquito longevity determination is performing a direct life-table experiment to estimate the development time and survivorship of a vector. This approach helps to determine the age structure of vector mosquito population in various environmental settings. Development and survivorship are sensitive to environmental change. In supportive environment mosquito immatures develop faster, which increase adult population and; adult live longer, which indicate the potential of multiple gonotrophic cycles, eventually intensify malaria transmission [69,70].

1.3.3. Sporogonic development cycle

The sporogonic development period is an extrinsic incubation period which is parasite development time taken in the mosquito. *Anopheles* mosquitoes take gametocytes (male and female) while feeding on infected hosts [71]. Gametocytes transform into female and male gametes inside the gut and fuse to form zygotes and then transform into the motile ookinetes. Ookinetes pass the mid-gut epithelial cells and form oocysts on the outer surface of the mosquito gut. The nuclei divide and form sporozoites in the oocysts, and yet, these stages are not infective to humans. When mature, the oocysts burst and release sporozoites, which spread throughout the haemocoel. Some enter into salivary glands and further develop to become infective to a human host.

The vector-parasite interaction is very complex having numerous factors to determine it. Temperature is well-known factor, which plays a role in parasite development inside vectors. The duration of the extrinsic cycle of the malaria parasite is shorter at a higher temperature [72]. If the extrinsic incubation period is short, the vectorial capacity may be high, even if the daily survivorship of the mosquito is relatively low [66].

1.3.4. Physiological competence of vectors to parasites

The *Plasmodium* parasite must complete the sexual stage of its life cycle (zygote to viable sporozoite in the salivary glands) in the body of female *Anopheles* mosquitoes before its transmission to humans [64,65]. The development and transmission of the malaria parasite is dependent on the competence of the vector species. Non-*Anopheles* mosquitoes may have human-vector contact and ingest malaria parasites along with human blood, but cannot support the development of the malaria parasites [73]. The absence of developmental signals, specific cellular receptors and parasite-specific resources may justify the inability of non-*Anopheles* mosquitoes to support malaria parasite development [64]. Even within *Anopheles* mosquitoes, some species are natural vectors of malaria parasites and more susceptible to human *Plasmodium* than others [74]. Consequently, only a fraction of parasites ingested complete the extrinsic incubation period in a small proportion of female *Anopheles* mosquitoes due to either the innate immune system of vectors against Plasmodia [75], or gut wall barriers during parasite development and the innate ability of a species to permit the development of the parasite [76].

1.3.5. Blood feeding behavior

The blood-feeding behavior of *Anopheles* is essential for malaria transmission due to the human-vector interactions, and those vectors that show strong anthropophagic behavior are more efficient because this behavior increases the risk of parasite transmission [77]. The genetics and physiology of the vectors (intrinsic factors) and the defensive behavior of hosts, host species, color, body heat, body mass and other (extrinsic) factors may influence the feeding patterns of vectors [78]. Nonetheless, in most conditions, blood-feeding is highly influenced by the accessibility of the hosts [77]. For instance, blood-feeding is primarily associated with reproduction, so to safeguard reproduction the mosquito may feed even on non-preferred hosts if the hosts of choice are not available [78]. *An. gambiae* has shown a tendency to feed on hosts with previous contact (last encountered hosts) than new hosts [79].

The feeding patterns of malaria vectors may also be manipulated by malaria parasites, mainly to reduce mortality and ensure efficient parasite transmission to the susceptible hosts [80]. Blood feeding frequency, persistence, and the number of probing are relatively high in infectious vectors, which in turn may increase the efficiency of parasite transmission [81]. A sporozoite-infected *An. gambia*e could be more likely to have fed on multiple hosts in one feeding cycle compared to an uninfected one [82]. A parasite-induced feeding behavioral change of infectious vectors may change the transmission pattern of malaria and the risk of infection. High feeding activities of sporozoite-infected *An*. *gambiae* might increase mortality, but it depends on the defensive behavior of hosts [83].

1.4. Malaria in Ethiopia

1.4.1. Epidemiology and burden

Malaria epidemiology in Ethiopia is diverse because of the variation in topography and ecology across the country. It has been widely reported that 68% of the Ethiopian population are at risk of malaria [33]. However, based on the current stratification, the proportion of the population at risk of malaria is about 52% with 68 (6.4%) districts having high transmission [34, 40]. The area generally is considered malarious and is characterized by strong seasonal malaria due to both *P. falciparum* and *P. vivax* [35]. *Plasmodium falciparum* has generally been presumed to be the most predominant *plasmodium* species causing malaria in Ethiopia and several surveys reported more *P. falciparum* than *P. vivax* infection [36]. However, a shift in the relative importance of *P. falciparum* and *P. vivax* has been reported, with some evidence indicating that a higher proportion of outpatient malaria cases reported since 2005 have been due to *P. vivax* [37,38]. This transition from *P. falciparum* to *P. vivax* dominance in various settings is assumed to be a result of effective malaria control interventions reducing *P. falciparum* transmission [37,38].

Malaria transmission is seasonal in most areas of Ethiopia. Transmission peaks following seasonal rains from February to March and from June to August, although with some variation in the peak and duration of malaria transmission across the country [39].

In Ethiopia, there were several malaria epidemiological strata according to altitude, temperature and rainfall. However, in 2020, the FMoH has updated malaria risk strata, based on annual parasite incidence (API) per 1,000 population (per the WHO recommendation) plus altitude and expert opinions. Accordingly, the new stratification classified risk of malaria in Ethiopia into five distinct malaria strata. The five malaria strata are high (API ≥ 50), moderate (API ≥ 10 & < 50), low (API > 5 & < 10), very low (API > 0 & ≤ 5) and malaria-free areas (API = 0) as shown in Figure 1.1. The current stratification that assumed the level of malaria burden into account ensures suitability for different strategic objectives and will guide implementation of appropriate interventions across different strata [40].

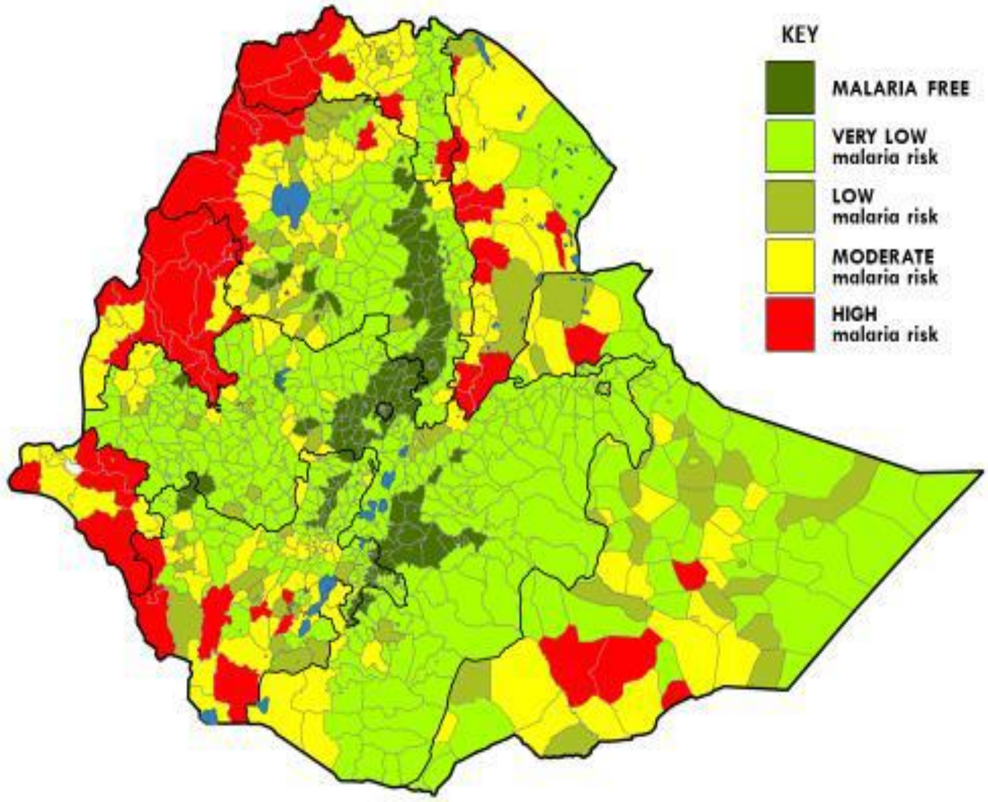

Figure 1.2: Malaria stratification map of Ethiopia, 2020 [40]

Clinical records of symptomatic *plasmodium* infections indicate that *P. falciparum* shows stronger seasonality than *P. vivax* transmission [41]. *Plasmodium vivax* infections tend to be maintained at lower densities than *P. falciparum* due to *P. vivax* parasite's host-cell preference for reticulocytes [42]. Lower density *P. vivax* infections may result in less overt morbidity and a lower probability of the affected individual seeking diagnosis and treatment than for a high-density *P. falciparum* infection. Other potential contributing factors to the less seasonal nature of *P. vivax* can take place at a lower temperature than for *P. falciparum*, generation of hypnozoites by *P. vivax* and consequent potential for relapsing infections [43], and the ability of *P. vivax* to generate gametocytes soon after initial infection and at relatively high proportions in low-density infections [42].

Malaria is generally assumed not to be present at altitudes above 2000m [44], and the FMoH of Ethiopia considers highlands over 2500m to be malaria-free [40,45]. In addition to temperature and altitude influencing the probability of mosquito survival and development of sporozoites, the local environment also, influences malaria transmission. Small-scale variation in transmission intensity has been described within communities, where increased malaria risk is associated with residence close to local water bodies, including irrigation schemes and dams [46–48].

Historically, before the scale-up in malaria intervention, Ethiopia is reported to have experienced up to 10 million cases of clinically suspected malaria a year [33]. The country was affected by a major epidemic in 1958, which had an attack rate of 30% of those at risk nationally, and a case fatality rate estimated at 5- 10 %, increasing to 20% in areas affected by food security [49]. The 1958 epidemic was attributed to an unusually extended rainy season along with uncommonly high temperatures. Subsequent major malaria epidemics in east Africa have also been attributed to unusual climatic conditions, and their severity exacerbated by nutritional crises and reduced efficacy of first-line malaria treatment [50,51], with the most recent major epidemic in Ethiopia occurring in 2003 [52,53]. In Ethiopia, epidemics have historically occurred on a cyclical basis every five to eight years, potentially a result of global climate fluctuations such as El Nino events [54], parasite resistance to first-line drug treatment or population movements [51].

1.4.2. Malaria control measures in Ethiopia

Malaria control has been performing through cases diagnosis, treatment and vector control. Expansion of microscopy services at mid-level health facilities and availability of RDTs at all health facilities, including health posts, are enabled the transition from clinical to parasitological diagnosis [33].

The current first-line treatment for *P. falciparum* is artemether-lumefantrine, an ACT, which replaced SP in 2004. In 2012, the treatment guideline was updated to improve case management of severe malaria by recommending intravenous or rectal artesunate at health posts as pre-referral treatment, as well as promoting the use of parasitological diagnosis rather than presumptive treatment of fever with antimalarial [33]. The first-line treatment for *P. vivax* remains chloroquine, but mixed infections (including RDT HRP2 and pan-*Plasmodium* lactate dehydrogenase (pan LDH) positive cases) should receive ACT. There are some indications of developing resistance of *P. vivax* to chloroquine in Ethiopia [55,56]. The treatment guidelines recommend radical cure with primaquine for patients with *P. vivax* infection residing in non-endemic areas who are being treated at health centers or hospitals, but primaquine is not recommended for use at health post level due to the risk of haemolysis and lack data on the prevalence of G6PD deficiency [57].

Vector control has been one of the pillars in malaria control effort in the country [33]. Commonly known vector control interventions are distribution of insecticide treated mosquito nets (ITNs), indoor residual spray (IRS), and larval habitat management (LSM). The history of vector control strategies were detailed in the next sections.

1.5. Malaria Vectors in Ethiopia

In Ethiopia, more than 46 species and subspecies of *Anopheles* mosquitoes were documented [8]. Few species are incriminated as primary and secondary vectors of malaria, while most species are considered as non-vectors.

Anopheles arabiensis, member species of *An. gambiae* complex, is the major vector of *plasmodium*. *An. arabiensis* and *An. amharicus* are the two sibling species. *Anopheles arabiensis* is the most abundant and relatively anthropophagic species, and is consequently responsible for most malaria transmission [9], whereas *An. amharicus* is mainly zoophilic and has a limited distribution and therefore not involved in malaria transmission. The predominance and principal role of *An. gambiae* s.l (presumably *An. arabiensis*) in malaria transmission have been documented in the 1930s by Italian malariologists [84].

Anopheles arabiensis shows variable feeding and resting behaviors with both anthropophagic and zoophagic, and exophilic and endophilic behaviors. For instance, Tirados and colleagues have reported its anthropophagic behavior, both indoors and outdoors, in the Konso district in southern Ethiopia [85]. On the other hand, Habtewold and colleagues documented the zoophagic behavior from another locality in the same region [86]. Again a strong zoophagic behavior of *An. arabiensis* was reported in southwest Ethiopia [87]. Seventy-eight percent of *An. arabiensis* from CDC light traps had human blood meal origin in Ziway, central Ethiopia [47]. *Anopheles amharicus* has not been incriminated as a malaria vector in Ethiopia [88]. From a higher proportion of *An. amharicus* collected from cattle sheds in southwest Ethiopia only a small proportion (1.1%) of them had human blood meal origins [87,89]. A study from the Jimma area, however, showed the occurrence of *An. amharicus* in houses occupied by humans [90].

Anopheles funestus group, *An. pharoensis,* and *An. nili* are the secondary vectors of malaria identified in Ethiopia [9–11,91,92]. Several investigations in various localities of the country have shown variable results of sporozoites rates among *An. pharoensis* population [47,93–95]. Although *An. funestus* is an important vector in some parts of Africa, it is one of the secondary vectors, which has its known distribution in Gambella and around the Rift Valley Lakes in southern Ethiopia [10,91,92]. Its distribution was wide from the1930s to the 1960s, and in 1966 Rishikesh attempted to dissect the salivary glands of 339 *An. funestus* from the Zway and Awasa areas, but all were negative for sporozoites [96]. In Ethiopia, DDT spraying during the malaria eradication campaigns might probably have eliminated the population of vector species, as has been reported in other East African countries [97,98]. The other secondary vector of local importance in Ethiopia is *An. nili*. Krafsur was the first to incriminate *An. nili* from Gambella in 1970, where he reported sporozoite rates of 0.84% in 1967 and 1.57% in 1968, thereby concluding that the species was responsible for malaria transmission, mainly in the wet season [11]. This species was later shown to be rare in other parts of the country [80,91], and thus appears to be less important in malaria transmission elsewhere.

Recently, in Ethiopia, the spread of a new malaria mosquito vector, *An. stephensi*, an Asian malaria vector, has raised concern about its potential impact on malaria transmission. *An. stephensi* mosquitoes breed predominantly in urban settings, prefer water storage containers. Although transmission is a health concern in some urban settings, malaria control programs usually focus on rural settings [99]. The spread of *An. stephensi* therefore implies rethinking of existing interventions.

In many countries, the dynamics of the vectors is changing and those considered as non-vectors are becoming either potential or proven vectors of malaria [100,101]. For example, in Kenya an unidentified but highly *Plasmodium* susceptible *Anopheles* species was recently documented [102]. In Ethiopia, there is no such conclusive data on the biodiversity of malaria vectors because the role of most mosquito species are not well documented due to the limited number of entomologists to identify all species in an area [103].

The salivary glands of *An. coustani* was found to be positive for the *Plasmodium* parasite in the 1940s in Ethiopia [104]. The human biting behavior of this species was reported from the central highlands of Ethiopia [9]. In southern Ethiopia, *An. coustani* was the second dominant species [105]. After many years, *An. coustani* from Jima town was found to be positive to CSP using ELISA [106], but because of morphological misclassification and the false positivity of ELISA, there is a need to conduct further investigations using a more sensitive molecular techniques like PCR to consider it as a proven vector of malaria. Human blood was identified from *An. demeilloni* and *An. christyi* in the south-central highlands of Ethiopia [93]. *Anopheles ziemanni* was mainly biting humans outdoors in Gambella, but was negative for CSPs [94]. *Anopheles marshalli*, *An. demeilloni*, *An. squamosus*, *An. garnhami*, *An. cinereus*, *An. tenebrosus*, *An. rhodensiensis*, *An. longipalpis* and other *Anopheles* species have been documented in Ethiopia [45]. Many of them exhibit human-biting behavior, and are vectors of malaria elsewhere in Africa [107].

Thus, it is important to monitor those species that have contact with humans, since they may be involved in malaria transmission, thus complicating control and elimination efforts.

1.6. Malaria Vector Control

Since the discovery of the malaria-mosquito association, the control of malaria has relied on anti-vector control strategies [108]. The larval source management (LSM), indoor residual spray (IRS), and long-lasting insecticidal nets (LLINs) are pillars, which have been experienced to combat malaria vector and their contribution is immense for the current global malaria reduction effort [109]. In addition to LLINs and IRS, improving LSM and zoo prophylaxis are also potential candidates. In 1980, WHO recommended zoo prophylaxis as a component of vector control interventions [110]. Moreover, the integrated vector control approach, using the combination of available tools against malaria vectors, is likely the most effective.

1.6.1. Insecticide-treated nets and LLINs

Conventional ITNs were introduced in the 1970s, and both ITNs and LLINs have been widely scaled up since the 2000s for malaria vector control [109]. Since 2005, the coverage of malaria vector control interventions has tremendously increased in most malaria-endemic regions [111]. Insecticide-treated nets and LLINs are fundamental tools mainly used against indoor resting and biting malaria vectors [112]. Unlike LLINs, ITNs need frequent retreatment of insecticide, which was a challenge for the communities in malaria-endemic areas to accomplish, and it was substituted by LLINs in most places. LLINs significantly reduced malaria incidence and mortality in many malaria-endemic countries [109]. The cost-effectiveness and acceptance of LLINs make them the most important tools to control malaria vectors. The nets are designed to avoid human-vector contact, with the chemicals impregnated to repel and/or kill those mosquitoes entering houses and attempting to feed on humans under insecticide-treated nets [112]. The effectiveness of nets is guaranteed when the vectors are susceptible to insecticides [113]. The wide spread of pyrethroid insecticide resistance in the population of malaria vectors may compromise the effectiveness of nets [114]. However, those people sleeping under nets are still getting protection from the infectious bites of mosquitoes because the nets are acting as physical barriers [109].

1.6.2. Indoor residual spraying

Indoor residual spraying prevents malaria transmission by killing vectors that spread malaria parasites. Those malaria vectors that rest indoors on insecticide-sprayed wall surfaces are the most targeted species. The insecticidal activity of DDT first took place in early 1940, with DDT-based IRS bringing a radical change in malaria vector control [115]. The IRS of DDT was the cornerstone for the 1950s and 1960s malaria eradication campaign, and some countries achieved eradication, while many others reduced the geographical distribution of malaria [116]. Even after the eradication programme by WHO is phased out, IRS continued as the main stay of malaria vector control tool. It was mainly used by military personnel during World War II, and was successful in killing indoor resting malaria vectors and reducing malaria transmission [117]. DDT has a long residual effect on the wall of houses, and was either applied once or twice a year. It was introduced in many national malaria vector control programmes during the late 1940s and early 1950s [117]. Liberia was the first place used in implementing large-scale DDT house spraying in 1945 [118]. It was planned to assess the feasibility of malaria eradication in tropical Africa. The success of DDT in the 1940s and early 1950s helped to convince global communities to launch the 1955 malaria eradication programme [116]. Thus, malaria was eliminated from several countries in Europe, the Americas, Asia and Australia [119]. It also played a substantial role in shrinking the geographical distribution of malaria, mostly in Asia [116]. The least amount of success was achieved in Africa, possibly due to political conflicts, a lack of trained personnel, transportation difficulties in rainy seasons and weak health infrastructures [119]. The effectiveness of DDT against agricultural pests and household insects made prices go up (including financial constraints for DDT use in 1951), and its widespread application rapidly led to the development of vector resistance in Greece in 1949 [120].

In Ethiopia, an organized malaria control programme was first initiated at the national level in 1959, during which DDT was used in pilot projects [84]. Four pilot projects (the Upper Awash Valley, the Kobo-Chercher plain, the Dembia plain and Gambella) were established to assess the technical feasibility of malaria eradication in both highlands and lowlands. The first national malaria eradication-training center was established in Nazareth in the late 1950s. In the 1960s, a national malaria eradication service was launched based on DDT-IRS, and malaria was significantly reduced from different parts of the country [84]. However, this campaign was replaced by a malaria control programme in the 1970s aimed at reducing malaria morbidity and mortality [119].

The Division for Malaria Control (1979-1985), and the later national Malaria and Other Vector-borne Diseases Prevention and Control (1986-1993), was established under the Ministry of Health to coordinate malaria control in Ethiopia [121]. The benefit of the malaria eradication campaign was substantial in Ethiopia for those people protected by DDT, and until the 2009, only DDT was used for IRS. Malathion was only considered in areas with DDT-resistant *An.* mosquitoes [9]. In the 1980s and 1990s, the frequency of malaria epidemics and its burden was increased, as the health infrastructure collapsed due to civil war and an acute shortage of vector control personnel mainly because of retirement [103]. Starting in early 1990, the operation of IRS was decentralized to the regional and district health teams, but a lack of technical personnel at the district level became a bottleneck for the operation [84]. Both DDT and malathion were continued to be used as spray chemicals based on the status of local vector resistance. DDT use continued until 2009 and was replaced by delthametrin, which was then shortly replaced by carbamate insecticides for IRS [84], as *An. arabiensis* populations developed a resistance to pyrethroid insecticides in most parts of the country [122,123]. The extensive use of pyrethroid insecticides, both for IRS and LLINs, might shorten the efficacy of pyrethroid insecticides [114]. The resistance of *An. arabiensis* to DDT may have contributed to the rapid spreading of resistance to pyrethroid insecticides, since the two classes of insecticides have a similar mode of action [114]. The use of insecticides with a similar mode of action for IRS and LLINs was against the WHO recommendation, which encouraged using insecticides with different modes of actions to delay resistance development in public health important vectors [114].

For IRS to be effective, at least 80% of homes need to be sprayed. However, the IRS programme can face resident refusal and re-plastering, which influence the effectiveness of the operation [124]. The improper use of IRS against the guidelines on dose and application might also affect the effectiveness of IRS, thus leading to insecticide resistance development.

1.6.3. Larval source management

The use of LSM was one of the principal malaria vector control method before the investigation of the insecticidal property of DDT. It targets mosquitoes at aquatic stages to prevent the completion of the development of immature stages [125]. LSM includes habitat modification (permanently destructing breeding sites), larviciding of breeding sites (application of chemical or biological insecticides), biological control (using predators), and habitat manipulation (temporarily making the breeding sites unsuitable). It has been used by the Tennessee Valley Authority in the United States [126] and Panama during the canal construction [127]. In Brazil, *An. gambiae* (recently identified as *An. arabiensis*) [128] was successfully eliminated mainly by the well-targeted application of Paris green on breeding sites and malaria had declined with the subsequent elimination of *An. gambiae* from Brazil [129]. The same strategy of applying Paris green larviciding supplemented by pyrethrum house spraying was followed in Egypt from 1944 to 1945 to eliminate *An. gambiae* s. l [130]. Historically, Paris green and petroleum oils were the most successful and widely used chemicals for larval control. In some parts of Africa, larval control using bacterial agents has shown promising results [131].

In Ethiopia, LSM, such as the drainage of mosquito breeding sites and larviciding with Temephos (Abate), are thought to be effective in urban areas, resettlement villages, and military camps [9]. The understanding of the ecology of the vector species might determine the efficacy of larval control. For instance, *Anopheles gambiae* often breeds in small, temporary rain pools, which are numerous and difficult to locate [132]. Larval source management would be very effective if many of the mosquito breeding sites were identified and well defined [133]. Chemical or biological larviciding and habitat manipulation can play a substantial role in resistance management by killing resistant-malaria vectors in aquatic stages [134]. The malaria vectors that tend to bite and rest outdoors (less targeted by IRS and LLINs) can also be killed at the aquatic stages.

For all malaria control interventions to be effective, community involvement is a critical tool. Community engagement (CE), empowerment and mobilization is among the strategies that has been used to accelerate the achievement of malaria control. It is about carrying out targeted advocacy, communication, and social mobilization activities to promote desired positive behavior for effective implementation and proper utilization of malaria interventions. CE has been used to design public health interventions and approaches for prevention and control of malaria in a variety of countries in a range of national programs, such as promoting early testing and treatment, improving the use of LLINs, and LSM. It helps to foster ownership of anti-malaria interventions and active participation in planning and implementation of interventions. Currently, in the effort to realize malaria-free Ethiopia, the country has set enhancing CE among the key strategies. CE mechanisms at all levels of the health management system will enable a malaria service delivery that is responsive to community needs. Health extension workers and women development army are the main platforms to implement CE and mobilization. Civil society organizations and other community platforms can be considered for empower the community [40, 265].

1.7. Water Resource Development in Ethiopia

Water resource development is not a new phenomenon in the country. The Imperial government took the first initiative in water resource development in the second half of the 1950s. Large-scale water projects for agricultural purposes and power generation were constructed from the end of the 1950s, and were concentrated in the Awash valley as part of the agro-industrial enterprises that were expanding in the area at the time. They subsequently spread to the Rift Valley and the Wabe Shebelli basin. Essentially, the government's interest at the time centred almost entirely on large-scale and high technology water projects: hydro-power dams, irrigation schemes, and water supply projects for Addis Ababa and a few major towns. Since then, all large-scale schemes in the country have been constructed at the initiative of the government [266].

Until recently, the water potential of the country was not accurately known, and even today, this is still an argumentative field. There have been different estimates of the irrigation potential of the country, and the issue has not been satisfactorily resolved. The World Bank, which is 1.0 and 1.5

million ha, made one of the earliest estimations [267]. Then after, Ministry of Agriculture (MoA) estimated the total irrigable land in the country to be 2.3 million hectares [266]. On other hand, the International Fund for Agricultural Development estimated 2.8 million hectares, while the office of the National Committee for Central Planning estimated 2.7 million hectares in 1990 [268]. The then the highest estimation was made by the Indian engineering firm Water and Power Consulting Services' (WPCOS), which is 3.5 million hectares and Ethiopia Valleys Development Studies Authority (EVDSA) accepted the figure [269]. Most of these figures are derived by adding up the irrigation potential of the country's eight river basins (Abbai, Tekezzae, Baro-Akobbo, Gibe-Omo Rift valley (Lakes), Genale-Dewa, Wabe Shebelle, and Awash).

In the 1960s and 1970s, comprehensive reconnaissance and feasibility studies were carried-out on the Abbai (Blue Nile), Awash and Wabe Shebelle river basins. In 1962, a German engineering team, and in 1964, the U.S. Bureau of Reclamation undertook extensive studies of the water resource potential of the Abbai River basin, the largest basin in the country. Both reports maintained that there were high hopes for the development of irrigated agriculture in the basin. The German study, which was focused the Gilgel Abbai basin suggested that the production of oil seeds, pulses and fodder crops, using the waters of the Gilgel Abbai, would be very profitable and earn high foreign exchange [270]. The U.S. study also recommended that small-scale irrigation should be greatly encouraged but that large-scale schemes would be too costly. It argued that without a coordinated water development program in the basin there would be no prospects for agricultural development in Ethiopia. On the other hand, the Awash River basin attracted a good deal of local and international investment, and was the subject of numerous studies and surveys in the 1960s and 1970s [271]. By the beginning of the 1970s, 100,000 hectares of land was under modern irrigation in the country of which about 50 percent was located in the Awash Valley [272]. In brief, the imperial regime was keen to determine the water resource potential of the country's river basins and to invite foreign capital to invest in agro-industrial enterprises in these areas.

In the late 1980s, an Indian firm, Water and Power Consulting Service (WAPCOS), prepared a preliminary master plan for water development for the whole country [269]. Following the plan a Gilgel Gibe River hydropower project was constructed and Wabi Shebelle basin master plan was also initiated. Feasibility and reconnaissance studies of watersheds and subsidiary river valleys have been undertaken at the initiative of Water Resource Development Agency (WRDA) and

EVSDA in the 1980s. The main objective of all these ventures has been to determine the water resource development potential of the country.

In the pre-Revolution period, the main purpose of irrigation was to provide industrial crops to the growing agro-industries in the country, many of which were controlled by foreign interests, and to boost export earnings. There was a shift of emphasis in the post-Revolution period. The Derg, like its predecessor, was intense to promote large-scale water projects. Initially, irrigation was seen as part of the modernization of the country's agricultural economy. Moreover, irrigation was considered an important investment for improving rural income through increased agricultural production, and for reducing the growing pressure on the land by bringing unused land under cultivation. Later, with the recurrence and continued threat of drought, the justification for water management schemes expanded to include relieving drought and recurrent food shortages, and growing more food for the internal market to improve food security and the nutritional status of the population [271].

In conclusion knowing the national water resource development status helps health sector to predict potential associated risk and plan for mitigations accordingly. The country’s current water resource development status is not updated which hinder the understanding of it impact with respect to the public health.

1.8. Environmental Modification, Vector Bionomics and Malaria Transmission

To ensure food security and economic growth, the globe has been practicing water resource development projects such as dam construction and irrigation schemes, which trigger new interactions between the environment and humans to happen. Such development practices are leading to new epidemiological patterns of vector-borne diseases like malaria [135]. Particularly in Africa, irrigation schemes and dams have extensively proliferating to cover the growing food and energy demands of respective regions [136]. Studies showed that *Anopheles* mosquito distribution and abundance; and malaria incidence and prevalence were associated with land use patterns change such as irrigated agricultural practices [137,138], water resource developments or dam construction [14,16], and population settlements [106].

1.8.1. Malaria and irrigation schemes

Land use and land cover change like extensive irrigated agricultural practices have been the primary drivers of malaria transmission in various areas [139,140]. Although extensive irrigation projects can lead to increased agricultural production, such environmental modification can contribute to malaria transmission by both facilitate proliferation of breeding habitats for vectors of diseases and creating a microclimate that favors a vector [141,142].

Numerous studies associated irrigated agro-ecosystems with an exacerbated malaria burden with altering the vector population dynamics and the transmission patterns from seasonal to perennial or increasing the degree of endemicity [137,138,143]. In paddy fields in Burundi, a sharp increase in *P. falciparum* malaria cases was related to a higher production of *An. arabiensis* in the flooded fields [144]. Diuk-Wasser *et al.* found that irrigation schemes increased the densities of *An. gambiae s.s* in Mali [145]. In Sierra Leone, many people escaping from other endemic areas under conflict occupied an area under irrigation. The introduction of parasites plus the presence of local competent vectors conduced again to a worsened public health situation [146]. A similar study reported an exacerbated malaria burden and the change of the malaria transmission pattern from seasonal to perennial in rice-growing areas in Sudan, following increased densities of *An. arabiensis* and the immigrants from other neighboring endemic areas [147]. The study conducted in Madagascar also showed that in the sub-arid region (unstable transmission) of irrigated rice field the EIR due to *An. funestus* was 150 times higher in areas within the influence of the paddy fields when compared with farther away communities [148]. Additionally, the transmission pattern changed from seasonal to perennial, thus converted the area into a stable transmission, which was an unstable transmission area. A similar finding was reported in unstable transmission areas of the Madagascar highlands, where irrigation projects favored the proliferation of *An. funestus*, resulting in severe malaria epidemics [149]. In Ethiopia, malaria prevalence and the risk of transmission by *An. arabiensis* were found to be significantly higher in irrigated sugarcane agroecosystems compared to non-irrigated agro-ecosystems in western Ethiopia [137]. Kibret et al.(2010) reported higher *Anopheles* mosquito density in irrigated villages compared to the non-irrigated [47]. In Zimbabwe, Boelee et al. (2002) reported that the operation of irrigation scheme was augmented malaria prevalence [150].

In contrast to the negative effects of irrigated agricultural practices on vector dynamics and malaria transmission, controversial conclusions that decreased and/or had no effect on malaria prevalence in irrigated areas compared to non-irrigated were also reported. In sub-Saharan Africa, studies reported that irrigation schemes either reduced or have no impact on malaria incidence and transmission and *Anopheles* mosquito population dynamics. Ijumba & Lindsay depicted the situation where a marked increase of *Anopheles* densities due to rice fields did not result in exacerbated malaria incidence [151]. Better economic and social conditions of nearby communities, more reliable health structures, better housing conditions, and focused vector control and prevention measures, and efficient treatments were forwarded as a possible justification to the event. Mutero *et al.,* (2004) reported a lower prevalence of malaria in irrigated areas despite 30–300 times higher abundance of the local malaria vector compared to the non-irrigated area in Kenya [24]. Another study in Burkina Faso reported average malaria prevalence rates ranged from 16-58% in an irrigated village compared to 35-83% in non-irrigated villages [152]. For reduced malaria prevalence in the irrigated agroecosystem, several justifications were forwarded. First, enhanced incomes that facilitate better protective measures to be taken that reduced the contact rates between settlers and the mosquito fauna. Second, increased mosquito densities might have resulted in decreased survivorship, so the mosquitoes did not live long enough to allow the full parasite development inside them.

Cultivation of some crops also blamed to support malaria vectors development, which in turn increases vector-borne disease transmission. For instance, it was reported that maize cultivation affects *Anopheles* mosquito distribution, abundance, and malaria transmission. Maturing maize produces a copious amount of wind-borne pollen that is nutritious enough and produced over a sufficient period to support the development of at least one generation of *Anopheles* mosquitoes [51,153]. Ye-Ebiyo *et al*., (2000) reported that larvae of *An. arabiensis* readily ingest the pollen grains themselves. An aqueous extract of maize pollen markedly accelerates the rate at which larvae ingest inert particles. The feeding ability of *Anopheles* mosquito larvae on maize pollen in turbid water is enhanced by water-soluble phago-stimulatory components released from pollen grains. Accelerated larvae to the pupal development and larger adults yield were observed where maize pollen is abundant compared to little access to maize pollen [154]. Larger adult body size contributes to greater longevity and reproductive success. Additionally, maize pollination season coincides with *Anopheles* mosquito breeding time due to warmer temperatures and higher humidity.

The succession of *Anopheles* species during the different crop developmental stages has also been documented. For example, in Africa, *An. gambiae* s.s and *An. arabiensis* are more abundant during the early crop stages as they prefer to breed in sun-exposed water bodies. *An. funestus* can be found in higher abundances during later crop stages, as it presents a preference for shaded or semi-shaded breeding sites [155].

1.8.2. Malaria and dams

One of environmental modification commonly being experienced is dam construction for hydroelectric power. The era of the big dams constructed mainly for hydropower generation started with the discovery of the turbine in 1832. In sub-Saharan Africa, dams are broadly recognized as a key factor in promoting economic growth, alleviating poverty, and ensuring food security [156]. This region has the lowest per-capita water withdrawals in the world, which poses a crucial need to build dams to ensure sustainable development [157]. Thus, hundreds of large dams are under construction to accelerate economic development in the region. However, if not handled properly, such development could lead to negative public health consequences, such as malaria outbreaks.

Evidence on the effect of dams on malaria transmission in Africa is rising. The population at risk of malaria around those dams is conservatively estimated to be 15 million and it is projected to increase to 25.5 million on average by the 2050s. The number of malaria cases associated with reservoirs is also projected to reach 2.1–2.9 million in the 2050s [15]. This proliferation of large dams under construction or planned (Fig. 1.3), indicates that it is a time to look critically at various measures that need to be taken to alleviate malaria around dams [158].

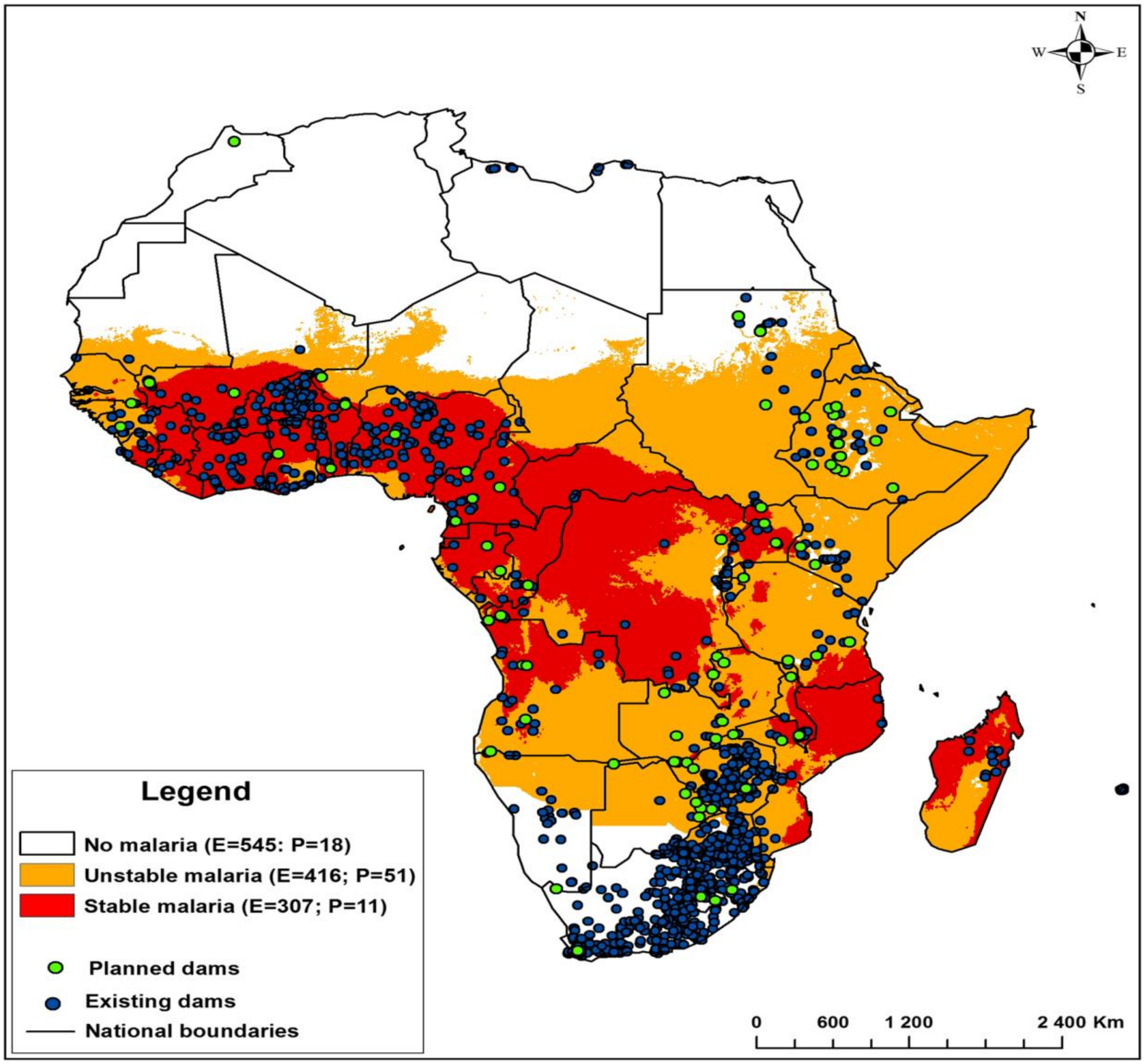

Figure 1.3: Spatial distribution of existing and planned dams in Africa with respect to the 2010 malaria stability indexing (E, existing dams number; P, planned dams numbers) [158].

The studies have shown that communities living close to reservoirs are at greater risk of contracting malaria than those living further away. For instance, in communities living close to the Koka dam of Ethiopia, malaria incidence is nearly 20-times higher than in those living more than 6 km away [159]. More several studies in sub-Saharan African countries reported impacts of dams on malaria vector distribution and abundance, and malaria transmission risk. Water resource projects which are constructed for hydroelectric power and irrigation purpose are blamed for year-round malaria transmission and increased incidence in Ethiopia [14,16,48,160]. Increased *Anopheles* densities and malaria incidence due to the operation of the dam were also reported in Senegal [161].

Man-made environmental modifications normally exert a great impact on unstable malaria transmission areas, most of the time in form of epidemics with high morbidity and mortality rates [162]. On the other hand, stable malaria transmission areas sustain the capacity to better absorb the artificial alterations, resulting in small effects on the parse very high malaria exposure [162]. Pre-and post-intervention data demonstrated that the construction and operation of large dams in Senegal, increased *Anopheles* densities but without exerting any significant influence on malaria transmission. The justification forwarded to the observed result was exacerbated mosquito densities were not accompanied by favorable conditions to allow the female mosquitoes to live long enough to become infected, permit the complete *Plasmodium* development cycle inside of the mosquito, and transmit the parasite to susceptible host [163].

The succession of species was observed around dams of the Senegal River where *An. gambiae s.s* and *An. arabiensis* were the most abundant species before the dam construction, but *An. pharoensis* became the predominant species when the project was completed, accounting for most of the new malaria infections. The Diama dam provoked an interruption of the flux of the sea salt upstream of the Senegal River, allowing the colonization of the area by *An. funestus* [163]. Since different species has its vector capacity, the ecological succession of species in such modification area has implication to malaria transmission.

1.8.3. Malaria control and water resource developments

To mitigate negative health effects, multifactorial issues should be considered before deciding to develop water resource development. In the case of dam construction, to minimize the risk of malaria, at least, a buffer zone should be put in place to avoid people living close to the dam. A study indicated that a faster water level drawdown desiccates mosquito larval habitats before the larvae complete their aquatic stage. Such manipulation of the water level by optimizing the reservoir management worked well to control malaria [164]. The filling of a reservoir will cover several isolated natural breeding sites in a determined area, although an extensive new shoreline may be used for the *Anopheles* to breed. Thus, a more extensive area that can become a potential breeding site will be defined, but this area would be easier to identify and control than the sparse natural water bodies [110].

Another strategy for mosquito population reduction is fluctuating dam water levels periodically and hence alter the flight range of local *Anopheles*. For example, during the dry season, reservoir capacity may be lowered considerably and the area under the flight range of mosquitoes may be considerably shortened, placing some communities at less risk of acquiring the disease [149]. However, at the Samuel dam in Brazil observed that, naturally reduced water levels during the dry season was exacerbated malaria incidence and *Anopheles* populations due to the exposure of new areas of land with stagnant water [165]. This indicates that such control strategies must be planned carefully. The efficiency of fluctuating reservoir water levels also depends on the correct maintenance of the banks, mainly reducing the amount of associated vegetation that may facilitate mosquitoes breeding.

In the selection of an appropriate site for water resource development projects, preliminary research on important factors is vital. The factors like 1) bionomics of local *Anopheles* species, 2) characteristics of the communities that will be placed under the influence of the project, 3) environmental variables that may govern malaria transmission and 4) the capacity of the local health system to respond efficiently to the potential risk are very critical. This knowledge would allow control programmes to forecast the negative effects on the health of that project in that specific setting and to prepare toiler-made measures to be implemented [164]. For example, different social groups such as dam workers, dislocated people or traditional tribes may present different susceptibility to health risks that need to be considered when planning an integrated control program [166,167].

To tackle the negative health impact, several strategies of environmental management, biological and chemical control methods, and population education approaches may be considered in settings with land-use change. Impregnated bed-nets have been proven a very useful tool for reducing human-vector contact rate, thus reducing considerably malaria transmission in many settings [168]. In areas under the influence of dams in Senegal and Ethiopia, a reduction of malaria prevalence was observed in communities provided with impregnated bed-nets [169]. Indoor residual spray is the common alternative methods to alleviate the malaria burden. At the Uttaranchal dam in India, a better socioeconomic status, knowledge of the risk factors for malaria by nearby communities, and sound vector control measures such as IRS, eliminated the disease transmission among risk communities [149].

It is important to bear in mind that pressure due to control measures may affect the behavior of malaria vectors. This scenario was observed at the Tucurui dam in Brazil, where the local malaria vector, *An. darlingi* population shifted to a more exophilic behavior due to control measures such as IRS and impregnated curtains, coupled with a change in human behavior [170]. Another challenge in control intervention is insecticide resistance of malaria vectors due to the extensive use of pesticides in agriculture and insecticides in public health. This was confirmed by recording higher resistance levels in mosquito populations close to the paddy fields (space correlation), and higher resistance level during the spraying periods of rice fields (time correlation) [171]. To manage insecticide resistance problems in such development area, environmental management and biological agents for malaria control have been underlined [172]. It is important to understand the bionomics of all potential malaria vectors of a specific area when applying environmental management strategies, as a specific intervention may deter a species to colonize the site but may benefit the adaptation of another local species with a different vector capacity [173].

Environmental manipulation consists of actions that produce temporary conditions that are unfavorable for mosquito proliferation in water development projects. For instance, potential breeding sites reduction may be achieved by lining irrigation canals to avoid the formation of standing water bodies, with proper edge maintenance and vegetation removal [136]. This was observed between well-planned and unplanned rice-fields in the irrigation scheme of Mwea in Kenya where larval densities were higher in the unplanned rice-growing villages as their canals and flooded fields were not properly drained, providing more opportunities for *Anopheles* to breed [174]. Intermittent irrigation is a typical and efficient method to control mosquito populations in paddy fields, consisting of repetitive dry stages of the crops, not allowing the water to be on the cultures for the larval development time of the vector [175].

A warranting strategy to reduce negative health impacts is establishing water development projects far enough from the communities, so that they are out of the flight range of the local *Anopheles* species [169].

1.9. Statement of the Problem and Rationale of the Study

Developing countries, particularly sub-Saharan Africa, have been experiencing environmental modification due to water resource development such as irrigated agricultural practice, dam construction, wetland cultivation and the like [23,143,176]. The primary aim of such land use and land cover change is to ensure food security in line with increasing population growth. However, such environmental modifications are blamed for facilitating unintended public health problems like malaria. Previous studies indicated aforementioned environmental modifications have been increased malaria transmission risks by creating favorable conditions for vectors and parasites [14,160]. Irrigated agroecosystem contributed to increased malaria incidence and prevalence. Following irrigation, the density of mosquitoes usually increases often leading to a rise in malaria transmission [20,137,177]. Increased *Anopheles* densities and malaria incidence due to the operation of dams for hydropower and irrigation schemes were also reported by several studies [14,15,161,178].

On another side, studies reported that irrigation schemes either reduced or has no impact on malaria incidence and transmission and *Anopheles* mosquito population dynamics. Ijumba & Lindsay depicted the situation where a marked increase of *Anopheles* densities due to rice fields did not result in exacerbated malaria incidence [179]. Also in Kenya, Mutero *et al.,* (2004) reported a lower prevalence of malaria in irrigated areas despite 30–300 times higher abundance of the local malaria vector compared to non-irrigated area [24]. In Tanzania, 2.6 times lower EIR and low malaria transmission was reported in irrigated villages compared to control villages [151]. In Burkina Faso, average malaria prevalence rates ranged from 16-58% in an irrigated village compared to 35-83% in non-irrigated villages [152]. The study in Mali revealed a two-fold reduction of annual malaria incidence after the implementation of the irrigation scheme [145,180,181].

Ethiopia, a country with more than 52 % of its population is at risk of malaria infection [32, 40], has been experiencing a massive change in land use by water resource development projects including irrigated agriculture and hydropower dam construction [176]. The studies related to the impact of water resource development on malaria transmission and its vector bionomics have been conducted in some settings and they indicated that such environmental modifications have been contributing to year-round malaria transmission and increased incidence in the country [4,137]. Despite rapped expansion of irrigation agriculture and dam construction, the study related to the impacts of environmental modification on malaria transmission and its vector dynamics is limited both in number and in areas covered. Therefore, to realize the country's malaria elimination goal, adequate and updated information on malaria epidemiology and its vector bionomics in the area with environmental modifications is vital.

Arjo-Dedessa sugar development project at southwest Ethiopia, is one of the mega irrigation schemes with a sugarcane farm covering around 4,000 hectares with the future expansion plan of 80,000 ha, which supplies a state-owned sugar factory. The area is historically known to be a wildlife sanctuary, which was a forest before. Long ago, the government, partly, settled residents evacuating from other part of the country to establish their lives through subsistence farming. Historically, malaria is a public health problem at the area. However, to the best of my knowledge, there are no studies on how the development activities have been influencing malaria transmission and its vector bionomics at the area. This gap urges to conduct study to assess malaria transmission dynamics (Chapter 4), malaria vector's breeding site distribution and diversity (Chapter 5), and survivorship and development of local malaria vectors (Chapter 6). A better understanding of the disease transmission dynamics in a setting with rapid environmental change helps monitor and predict future epidemics and devise tailor-made intervention strategies. Identifying major breeding sites and their distribution, and determining survivorship and development in response to land-use change are also important to plan supplemental vector control tools to support the existing interventions.

1.10. Conceptual Framework

Understanding the complex relationship between land-use change and malaria transmission is crucial if we are to provide practical evidence to guide interventions aimed at improving the health and quality of life among people living in the area undertaking environmental modification due to water resource development projects.

Based on the literature, the conceptual framework proposed describing the relationship between environmental modification and malaria transmission intensity. Figure 1.3 illustrates how environmental modification due to irrigated agriculture and malaria transmission are linked to each other.

In principle, proximity to irrigation scheme implies proximity to new bodies of standing water that can serve as *Anopheles* larval breeding sites. The reality of this general expectation largely depends on the ecology of the local vectors. In particular, it requires that the new bodies of standing water having suitable chemical, physical and biological characteristics like temperature, relative humidity, light intensity, surrounding vegetation, turbidity, predators, etc., compatible with the larval habitats for local vector species. Consequently, the creation of new breeding sites might have an effect on the development of vector species, increased density, enhanced survival rates, and fecundity among them in terms of their role in local transmission. The outcome is illustrated on the right as increases or decreases in malaria transmission. The central boxes reflect the entomological factors that influence the level of vector competence. The boxes to the top represent anthropogenic factors related to the physical environment thought to influence mosquito bionomics. The surrounding environment's climatic conditions are influenced by anthropogenic factors, which in turn influence entomological factors, as indicated to the left of the illustration diagram (Fig. 1.3).

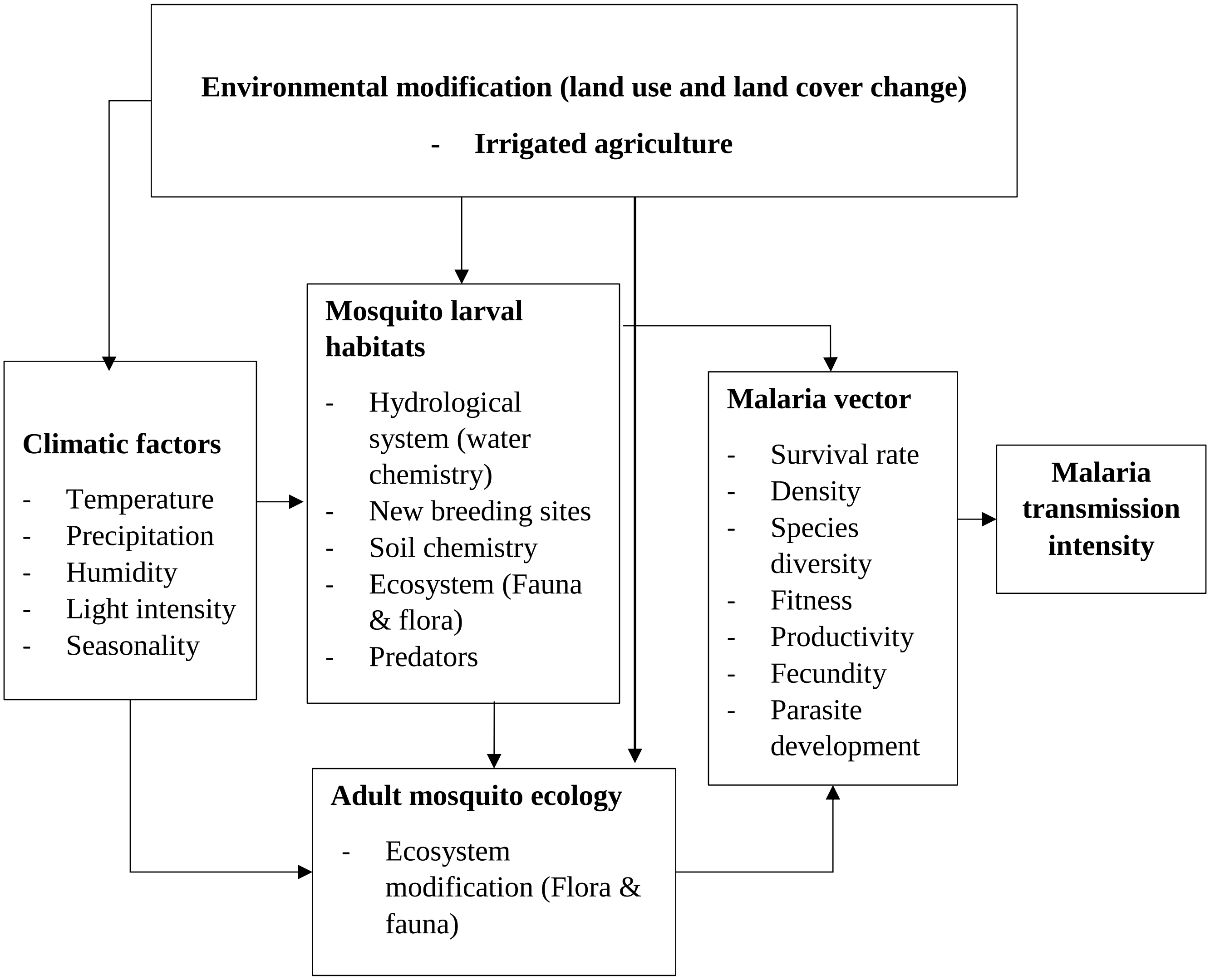

Figure 1.4: Conceptual framework

1.11. Significance of the Study

Ethiopia has been practicing massive environmental modification due to the expansion of water resource development. In the meantime striving to eliminate malaria, the primary health problem in the country. Environmental modification like irrigated agriculture has a linkage with malaria transmission. This study is aimed at determining the impact of environmental modification on malaria transmission through assessing the bionomics of local malaria vectors. Therefore, the information generated from this study will have various implications. First, it informs the country's policy makers in developing evidence-based and specific malaria interventions while enhancing irrigated agricultural projects. The information helps to revise the existing policy in line with the dynamic environment. This information can be adopted to other settings with a similar practice. Second, in Ethiopia, the study addressing the effect of irrigated sugarcane plantation on the ecology of malaria vectors are very limited and no study encountered addressing malaria vector survivorship. Thus, this study is the first of its kind in the area and the information generated will serve as a base to build-up further researcher work. Third, this study will also help the community in Arjo-Dedessa sugar development and its vicinity. To ensure the health of the community the sugar development factory's administration is structured to have a health department, which aimed to deliver both preventive and curative service to the factory and surrounding communities. Thus, the result of this study will help the factory health department as a road map in the malaria control effort at the area. Fourthly, the findings will help national health system mainly malaria control program in approaching local-specific intervention's strategies in malaria elimination effort.

CHAPTER 2: RESEARCH OBJECTIVES

2.1. General Objective

The main objective of the study is to investigate the impact of environmental modification due to extensive irrigated agricultural practice on the bionomics of malaria vectors and malaria transmission in southwest Ethiopia.

2.2. Specific Objectives

1. To determine malaria transmissions pattern around Arjo-Dedessa irrigation scheme, southwest Ethiopia.
2. To determine effects of environmental modification on the diversity and positivity of *Anopheles* mosquitoes' aquatic habitats in Arjo-Dedessa irrigation scheme, southwest Ethiopia.
3. To determine *Anopheles gambiae* s.l survivorship, development and fecundity in Arjo-Dedessa irrigation scheme, southwest Ethiopia.

CHAPTER 3: GENERAL METHODS AND MATERIALS

3.1. Description of Study Area

The study was conducted at Arjo-Dedessa sugar development site and its vicinity, Oromia Regional State, southwest Ethiopia (Fig. 3.1). The site is situated between three districts, Jimma-Arjo district, Dabo-Hana and Buno-Bedele district. Arjo-Dedessa irrigation development site is one of the largest projects in the country.

Extensive irrigated agriculture represents the most important environmental change in the area. The irrigation development areas were covered with a massive irrigated sugarcane plantation ('irrigated area' hereafter); whereas the surrounding areas were covered with other, non-irrigated mixed field crops common in the area ('non-irrigated area' hereafter). Historically, the area is known to be a wildlife sanctuary called 'Dedessa wildlife sanctuary', known by its large forest. In 2006, a large-scale sugarcane plantation farm that feeds state-owned sugar development factory was established. Currently, the farm covers more than 4,000 hectares of land, with an expansion plan of 80,000 ha in the next ten years. The irrigation scheme pumps water from Dedessa River, one of the major tributaries of the Blue Nile River basin. In addition to the Dedessa River, seasonal streams and springs are abundant in the area. The altitude of the area ranges from 1,350 m above sea level with mean annual rainfall of 1,477 millimeters.

The area is known to be malaria-endemic area. While LLIN and IRS were routine practiced, larval habitat management through community involvement was rarely applied except during epidemic years. However, in the area no study has been encountered addressing local malaria disease transmission patterns and its vector bionomics.

Local communities depend on subsistence farming. They mainly practice smallholder non-irrigated farming that involves both field crops cultivation and livestock rearing. Maize, peanut, sorghum, rice, wheat, coffee and fruit trees such as mango are some of the common crops at the area.

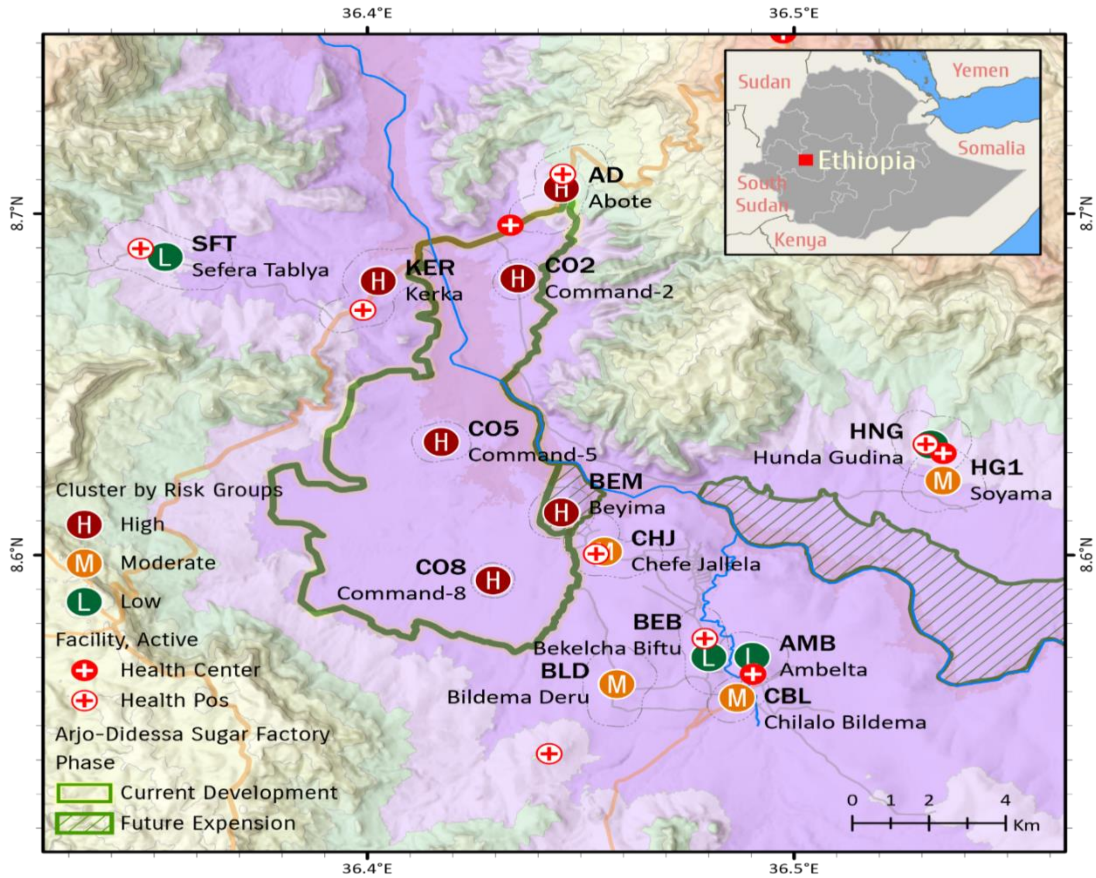

Figure 3.1: Map of the study area

3.2. Malaria Epidemiological Study

3.2.1. Study design, period, and data collection

In the study, a health facility-based retrospective study design was conducted for the malaria transmission pattern study. It was accomplished by reviewing the malaria morbidity records from registers of health facilities. Monthly malaria confirmed case data from 2008 to 2017 was extracted from 11 health facilities at Arjo-Dedessa sugar development site and its vicinity (Chapter 4).

3.3. *Anopheles* Mosquito Larval Ecology Study

3.3.1. Study design and period

In this study, a repeated cross-sectional design study was deployed. *Anopheles* mosquito larvae were surveyed from two agroecosystems, 'irrigated' and 'non-irrigated' areas during the dry (December 2017–February 2018) and wet (June 2018–August 2018) seasons. Mosquito habitat diversity, larval occurrence, and abundance were compared between the irrigated and non-irrigated areas (Chapter 5).

3.3.2. Larval survey

Prior to sampling, the study site was classified as irrigated area and non-irrigated area. Then, the areas are further classified into clusters. Mosquito larvae were sampled following the WHO standard larval survey procedure using a standard dipper (350 ml, Bio Quip Products, Inc. California, USA) [182]. All *Anopheles* larvae samples were transported to the field insectary and reared to adult stage for morphological identification using taxonomic keys [183].

To characterize the aquatic habitat, environmental variables including habitat type, crop type, turbidity, exposure to sunlight, distance to the nearby house, vegetation, substrate types, land use and land cover were assessed during the survey [184,185]. Geographic coordinate readings of each surveyed aquatic habitat were recorded using Geographic Positioning System (GPS). A detailed description of the larval survey is provided in chapter 5.

3.4. *Anopheles Gambiae* s.l Survivorship Study

3.4.1. Study design and period

A life-table experiment was conducted to examine the effect of environmental modification on the survivorship of *An. gambiae* s.l both immatures and adults in two different settings, irrigated and non-irrigated areas, from August to October 2019.

3.4.2. *Anopheles gambiae* s.l immatures survivorship experiment

A blood-engorged *An. gambiae* s.l were collected from indoor of the houses and animal shelter at study areas using mouth aspirator. Mosquitoes were kept in paper cages at field insectary with an oviposition substrate to facilitate egg-laying. Collected eggs were let to hatch and newly hatched first instar larvae were used for the experiment. The development and survival of larvae was followed under washbasins (mimic natural habitat) placed in sugarcane plantation and other field crop covered area. A detailed description of the experiment set-up and data record is provided in chapter 6.

3.4.3. *Anopheles gambiae* s.l adult survivorship experiment

In this study, *An. gambiae* s.l adults emerged from the larval survivorship experiments were used. Twenty-five female and 25 male mosquitoes within 24 hours post-emergence were transferred into the paper cage (21.5 cm x 9 cm). Then, the cages with mosquitoes were placed in irrigated area and non-irrigated area. Mosquito cages were suspended from the roof structures that were designed for the experiment purpose at 2 m above the ground and the mosquitoes were followed until the last mosquito get die. A detailed description of the experiment set-up and data record is provided in chapter 6.

3.4.4. Microclimate data collection

During experiment, microclimate data were recorded using HOBO data loggers (Onset Computer Corp., MX2202, Bourne, MA and Onset Computer Corp., MX2301, Bourne, MA).

3.5. Data Analysis

For each objective, based on the nature of data, specific data analysis was performed (chapter 4, chapter 5 and chapter 6). To satisfy the assumptions of individual statistical analyses, in each statistical test, the basic assumptions have been checked before running the analysis. Test of significance was done assuming α at 0.05 and a p-value less than 0.05 was considered significant. All analyses were done using IMB SPSS statistical software version 25 (SPSS Inc, Chicago, IL, USA), R version 3.5.2 and Microsoft Excel (Version 2016, Microsoft Corporation, Washington, U.S). Spatial data aggregation, analysis, and visualization were produced with ArcGIS Pro 2.5 [186].

3.6. Dissemination of findings

Results of the study were published in peer-reviewed journals for scientific communities. As an end-user, the finding was communicated to Arjo-Didessa Sugar Factory community. Again, the parts of this finding was presented on the national research workshop held at Sokoru, September 2021, Ethiopia. The dissertation booklet will be availed in the library of Jimma University. Moreover, the communication of the findings will be continued whenever & wherever opportunities found to do so.

3.7. Ethical Consideration

The proposal was reviewed and approved by the Institutional Review Board (IRB), Institute of Health, Jimma University. Letters of permission were also obtained from the Arjo-Dedessa factory administration office, Buno-Bedele and East-Wollega Zonal Health Departments. Verbal consent was sought from the heads of all health facilities before the data collection.

CHAPTER 4: EPIDEMIOLOGICAL PROFILE OF MALARIA AT ARJO-DEDESSA SUGARCANE PLANTATION SCHEME AND ITS VICINITY (Adopted from Hawaria et al 2019)

Hawaria *et al. Malar J* (2019) 18:145
https://doi.org/10.1186/s12936-019-2777-z

Malaria Journal

RESEARCH Open Access

Ten years malaria trend at Arjo-Didessa sugar development site and its vicinity, Southwest Ethiopia: a retrospective study

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Abstract

Background: The trend analysis of malaria data from health facilities is useful for understanding the dynamics of malaria epidemiology and inform for future malaria control planning. This study was conducted to determine the malaria trend at Arjo-Dedessa sugar development site and its vicinity, southwest Ethiopia, from 2008 to 2017.

Methods: Monthly malaria confirmed case data from 2008 to 2017 was extracted from 11 health facilities based on laboratory registers at Arjo sugar development site and its vicinity, southwest Ethiopia. Both positivity rate and malaria incidence rate were calculated. Changes in malaria parasite species and seasonality were analyzed; age structure and sex distribution were compared between different study periods. Trend in malaria incidence and climatic impact were analyzed and past LLIN and IRS campaigns were used as dynamics modifier.

Results: Over 10 years, 54,020 blood film were collected for malaria diagnosis in the health facilities at the area, of which 18,049(33.4%) were confirmed malaria cases by both microscopically and RDT**.** *Plasmodium falciparum*, *P. vivax*, and mixed infection (*P. falciparum* and *P. vivax*) accounted for 48, 42.4, and 9.6% of the malaria cases, respectively. The study also revealed that *P. vivax* was the predominant over *P. falciparum* for four years (2010, 2014, 2015 and 2016). There was a remarkable reduction of overall malaria infection during the 10 years.

Malaria has been reported in all age groups, but age distribution showed that the vast majority of cases were adults age 15 years and above 13,305 (73.7%). In all age groups, males were more significantly affected than females ($\chi 2 = 133.0$, d.f. = 2, $p < 0.0001$). Moreover, examination of malaria positivity rate showed a strong seasonality ($\chi 2 = 777.55$, d.f. = 11, $p < 0.0001$). However, malaria cases were reported in all seasons across 10 years in the study area.

Conclusion: In general, malaria positivity showed a declining trend over 10 years period in the area. However, current prevalence shows it is public health burden and needs attention for further intensification of interventions. In the study area, both *P. falciparum* and *P. vivax* co-exist and *P. vivax* is more prevalent than *P. falciparum* in almost half of the years. Therefore, malaria interventions should be strengthened in the study area.

4.1. Background

Malaria remains a major public health burden globally in general, and in sub-Saharan Africa in particular, including Ethiopia. In 2018 alone, World Health Organization (WHO) reported 228 million cases and 405, 000 deaths of malaria globally, of which 93% of cases and 94 % of deaths were in Africa [187]. Currently, there is a global initiative to eliminate malaria and consequently, a remarkable result in malaria control has been achieved. In Ethiopia, the fight against malaria has shown notable progress in controlling the disease over the last two decades in Ethiopia. Following this, Ethiopia has also set a goal to eliminate the disease by 2030 [58,188,189]. The interventions which have been contributing to such significant decline include; the distribution of long-lasting insecticide-treated nets (LLIN), indoor residual spraying (IRS); and introduction of prompt and effective treatment with Artemisinin-based Combination Therapy (ACT) to treat uncomplicated *P. falciparum* malaria and environmental management [190–192].

In Ethiopia, two parasite species, *P. falciparum* and *P. vivax* are predominant parasite species accounting 60% and 40% of malaria cases, respectively [33,193,194]. The malaria transmission in Ethiopia is seasonal with unstable transmission patterns in most areas, however, it was year-around in some lowland areas. The peak malaria transmission in Ethiopia occurs in general from September to December and March to May [36,194,195]. The unstable transmission patterns make the country prone to cyclic epidemics occurring every five to eight years [58]. However, information is scarce on malaria transmission pattern in some endemic areas of Ethiopia, which is vital for evidence-based intervention by the local health system.

Climatic change, which is an attribute to environmental modification, determine the dynamics of malaria by limiting the survival of malaria vectors and the rate of *Plasmodium* development in the vector mosquitoes [196,197]. Thus, human environmental modifications such as extensive irrigated agricultural practices, dam construction, and the like in particular area can have an impact on the trend of malaria transmission [14,198]. In Ethiopia, there are rapid ecological changes following the development activities [199]. Arjo-Dedessa sugar development site is one of the largest development projects in the country. There is environmental modification due to huge irrigated sugarcane plantation farm. Irrigation of sugarcane experience in its vicinity has been created a large area of malaria vector breeding habitats which may have a significant impact on malaria transmission. However, malaria transmission pattern has not been yet described around the irrigation scheme.

This study aimed at determining the malaria trend and transmission pattern for the past 10 years at the health facilities as a proxy measure for the trend of malaria at Arjo-Dedessa sugarcane development site and its vicinity, which may contribute to the evidence-based decision for malaria control strategies.

4.2. Methods and Materials

4.2.1. Study setting

The study was conducted at Arjo-Dedessa sugar development site (Arjo-Dedessa sugar factory) and its vicinity. The study setting description has been given in chapter 3.

Eleven health facilities nearby the Arjo-Dedessa irrigation scheme (Health Posts, Health Centers and a Hospital) were included in this study. The health facilities included in the study are: Arjo Health Center, Arjo Primary Hospital, Abote-Dedessa Health Post, Arjo Sugar factory Clinic, Kolo-Sirri Health Center, Kolo-Sirri Health Post, Alberta Health Post, Bildima-Deru Health Post, Karkaha Health Post, Sefera-Tabiya Health Post and Bakalcha-Biftu Health Post (Fig. 4.1).

4.2.2. Study design and period

A health facility-based retrospective study was conducted by reviewing the malaria morbidity records from registers of health facilities at Arjo-Dedessa irrigation scheme and its vicinity. Monthly malaria confirmed case data from 2008 to 2017 was extracted from 11 health facilities based on the laboratory register. The study included all malaria records of those individuals who were diagnosed using a Microscope or RDT. The timeline consideration was based on data availability and quality in the facilities. All available malaria morbidity registration books were collected from the selected health facilities. Health facilities included in the study were selected based on their proximity to the irrigation scheme. All records of patients who presented at the health facilities and were treated as malaria patients were included in the study.

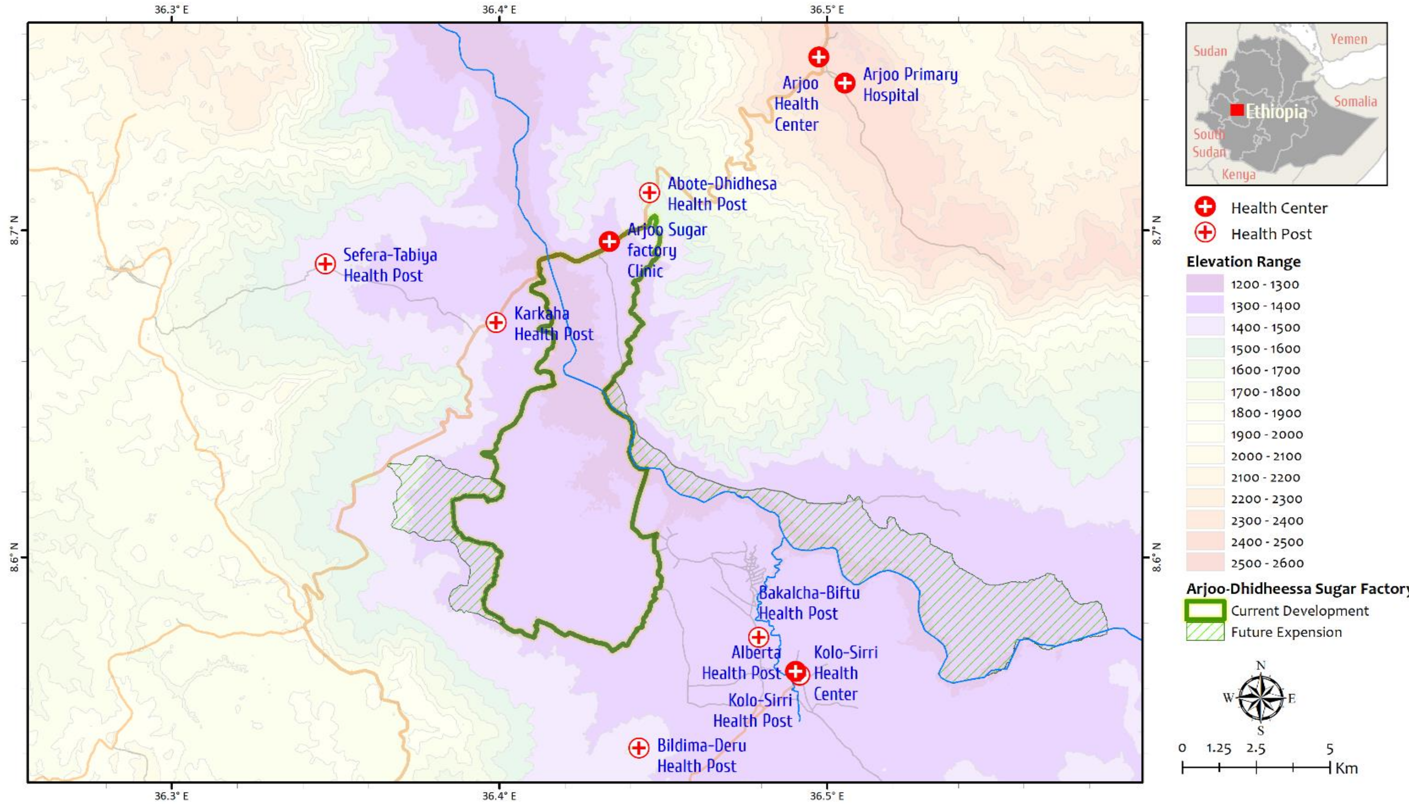

Figure 4 1: Map of the study site and health facilities.

4.2.3. Malaria morbidity data collection

In Ethiopia, malaria cases are treated both clinically and using both microscope and RDT as per the national malaria diagnosis and treatment guideline [195]. Both the presumptive and confirmed cases are recorded on registration books at PHCU and reported to the next higher level of a health management system. The study included all malaria records of those individuals who were diagnosed using Microscope or RDT over the past 10 years. The timeline consideration was based on data availability and quality in the facilities.

All available malaria morbidity registration books were collected from the health facilities. All records of patients who presented at the health facilities and treated as malaria patients were included in the study. The data was extracted and entered into Microsoft Excel Worksheet. The parameters recorded included health facility's name, residence, date of examination, result, sex, age, and parasite species. The cases with incomplete records of important variables such as, date, age, sex, and examination result were excluded from the study. Trained laboratory technicians collected data.

LLIN campaign data. Since there were no records of LLIN coverage in the study area for the past 10 years, national LLIN campaign data, i.e., the total number of LLIN distributed nationwide, was used as a reference for malaria control interventions. LLIN campaign data was obtained from the Federal Ministry of Health.

4.2.4. Meteorological data collection

Meteorological records were obtained from Arjo meteorological station. Variables recorded included average monthly maximum, minimum and mean temperature, monthly cumulative precipitation and relative humidity.

4.2.5. Data analysis

Malaria infection positivity rate was calculated as the number of confirmed cases over the total examined at all study health facilities. The Incidence rate was calculated as cases per 1,000 people per year based on the current catchment population and 2007 Ethiopia census assuming a constant increase in catchment population during the study period. Age was grouped as <5 years, 5–14 years, and ≥15 years. Age and sex distributions were compared between 2008–2014 and 2015–2017 using $\chi 2$-test. Seasonality was determined by monthly positivity rate of total infections and by species. Species composition was calculated annually.

Trends, climatic and intervention effects was analyzed using the following model:

$$C_{t+1} = \alpha + \gamma t + \beta_1 C_t + \beta_2 LLIN_t + f(CLIM) + \varepsilon_t$$

$$f(CLIM) = \beta_3 T_{max} + \beta_4 T_{min} + \beta_5 T_{mean} + \beta_6 P_{recip} + \beta_7 H_{umid}$$

Where α is a constant, γ measures the trend, parameters of β measures carry-on effect (autocorrelation), LLIN effect and climatic effects, including maximum, minimum and mean temperature, precipitation and relative humidity, and ε_t is a random error term. Parameters were estimated using maximum likelihood estimation (MLE) and the best model was selected by the Akaike information criterion (AIC). To determine whether there was a significant decline in incidence since 2015, analysis was carried out firstly by using data from 2008–2014 and then using all data.

4.3. Results

4.3.1. General characteristics and malaria parasite species

Over 10 years, 54,020 suspected malaria cases were diagnosed in the health facilities at the study area, of which 18,049(33.4 % positivity rate) were confirmed malaria cases by both microscopically and RDT. There were three peak years, i.e., 2009, 2010 and 2013 (Fig. 4.2). *Plasmodium falciparum*, *P. vivax*, and mixed infection accounted for 8,660(48.0%), 7,649(42.4%), and 1,740(9.6%) of malaria cases, respectively. Although overall slight predominance of *P. falciparum* over *P. vivax* was observed, *P. vivax* was dominant over *P. falciparum* for four years, i.e., 2010, 2014, 2015 and 2016 (Fig. 4.2 & 4.3). Following the malaria peak in 2013, there was a remarkable decline in malaria cases due to the decline of *P. falciparum* (average of 1146(51.6%) between 2008 and 2014 to 213(33.2%) between 2015 and 2017) (Fig. 4.2 & 4.3). The proportion of mixed infections was down from average of 231(9.7%) between 2008 and 2014 to 41(6.2%) between 2015 and 2017 (Fig. 4.2 & 4.3).

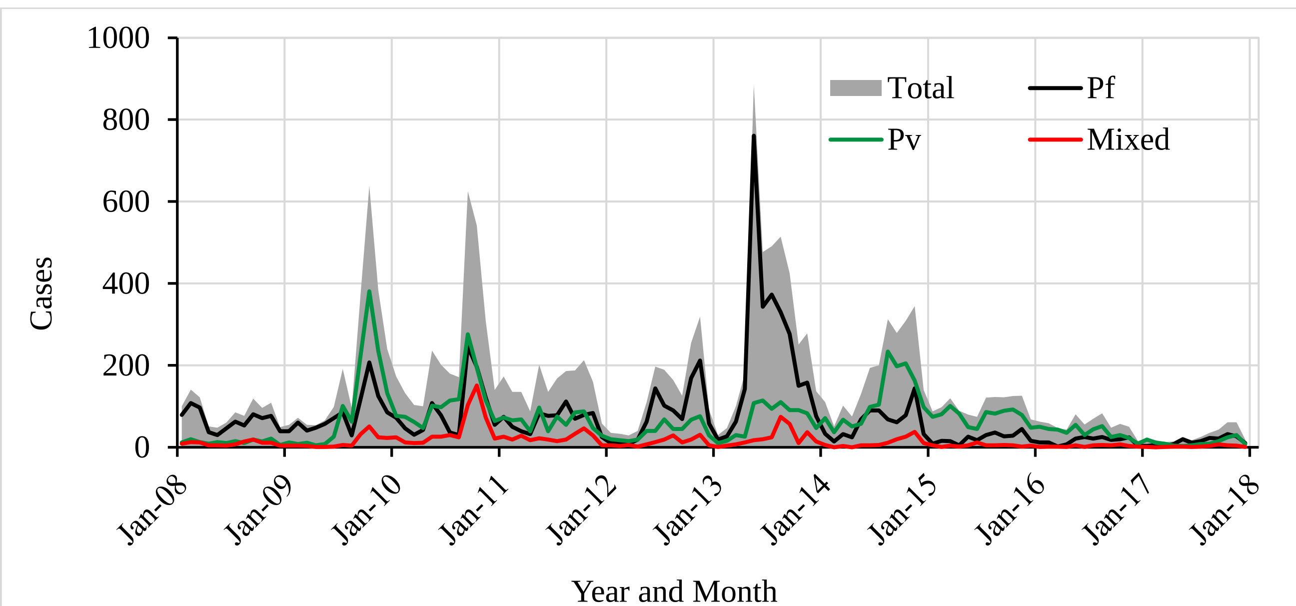

Figure 4.2: Annual trend of malaria cases at Arjo-Dedessa sugar development site and its vicinity, southwest Ethiopia (2008 – 2017).

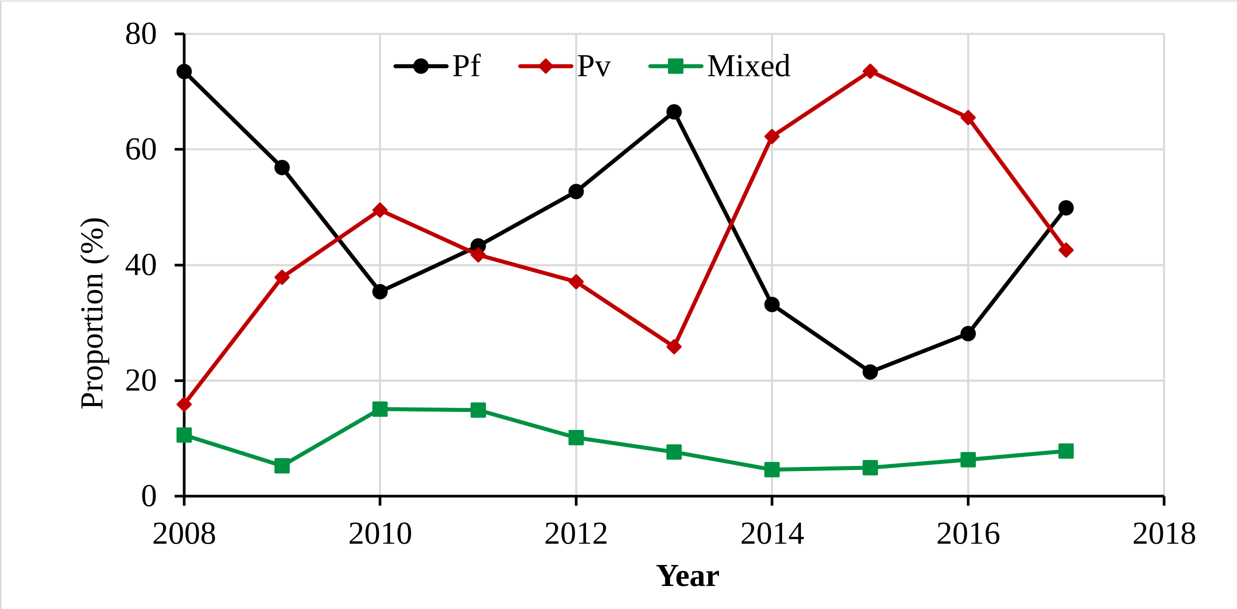

Figure 4.3: Proportion of *Plasmodium* species at Arjo-Dedessa sugar development site and its vicinity, southwest Ethiopia (2008 – 2017).

4.3.2. Age and sex distribution and their change over time

Of the total patients examined, 31,954(59.2 %) were males and 22,066(40.8%) were females. Of the total malaria cases confirmed, 11,644(64.5%) were males and 6,405(35.5%) females. Males were increasingly dominant in malaria cases, between 2008 and 2014 males accounted for 63.8% (10,082/15,792) of all cases compare to 69.2% (1,562/2,257) between 2015 and 2017 ($\chi 2 = 68.26$, d.f. = 1, $p < 0.0001$) (Fig. 4.4).

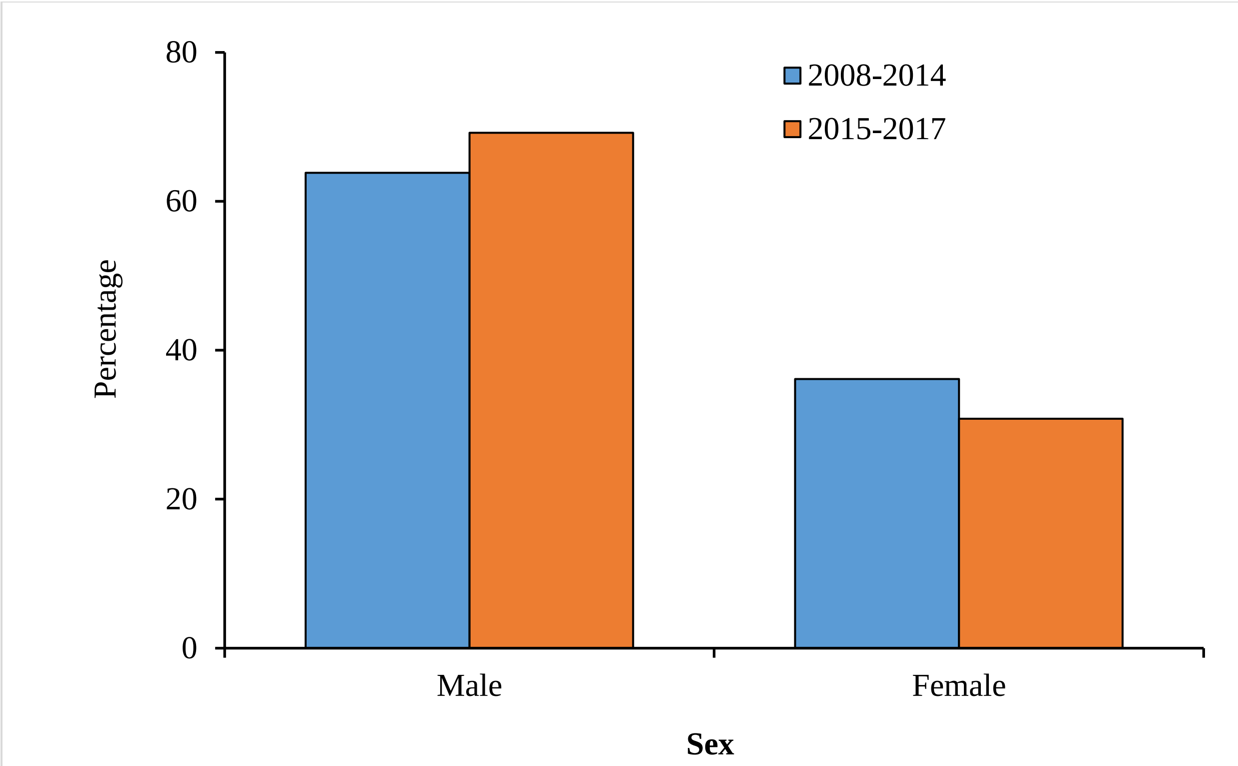

Figure 4.4: Distribution of malaria cases in different year periods across the sex at Arjo-Dedessa sugar development site and its vicinity, southwest Ethiopia (2008 – 2017).

Age distribution showed that vast majority of cases were adults age 15 years and above 13,305(73.7%) (Fig. 6). Proportion of adult cases increased from 72.1% (11,390/15,792) between 2008 and 2014 to 84.2% (1,915/2,257) between 2015 and 2017 ($\chi 2 = 284.15$, d.f. = 1, $p < 0.0001$) (Fig. 4.5).

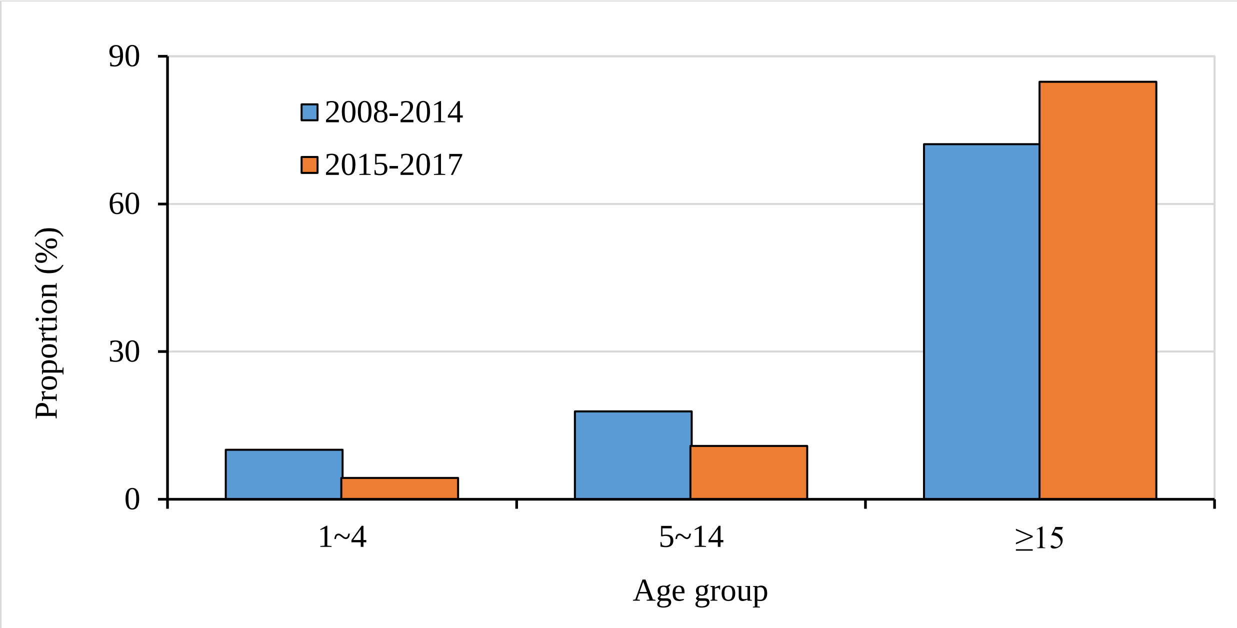

Figure 4.5: Distribution of malaria cases in different year periods across the age at Arjo-Dedessa sugar development site and its vicinity, southwest Ethiopia (2008 – 2017).

Cross-examination revealed that, in all age groups, males were more affected than females, and the difference was significant ($\chi2 = 133.0$, d.f. = 2, $p < 0.0001$) (Fig. 4.6).

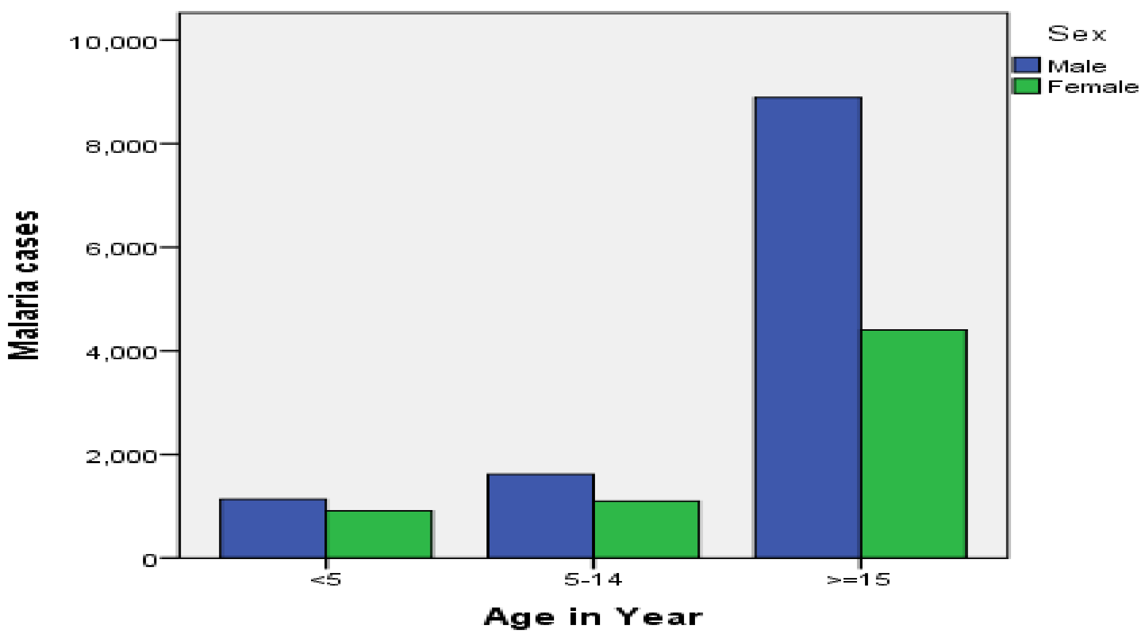

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Figure 4.6: Malaria cases by sex and age group at Arjo-Dedessa sugar development site and its vicinity, southwest Ethiopia (2008-2017).

Cross comparison also found that *P. falciparum* was the predominant parasite in children below 15 years, however, *P. vivax* and mixed were more pronounced in adults ($\chi 2 = 171.2$, d.f. = 2, $p < 0.0001$) (Fig. 4.7).

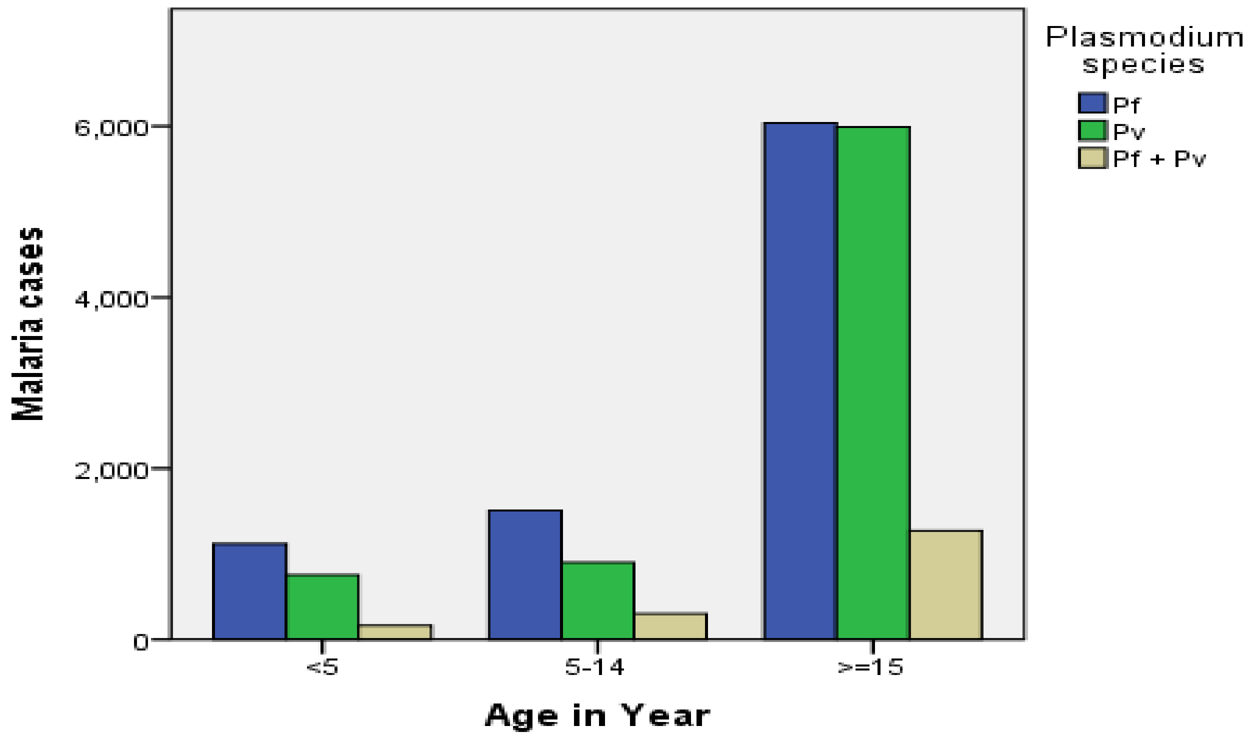

Figure 4.7: Distribution of *plasmodium* species by age group at Arjo-Dedessa sugar development site and its vicinity, southwest Ethiopia (2008 - 2017).

4.3.3. Seasonal variations in malaria positivity rate

Examination of malaria positivity rate showed a strong seasonality ($\chi 2 = 777.55$, d.f. = 11, $p < 0.0001$). The peak season started in May and ended in November with the highest confirmed cases between September and November after the long-rainy season (Fig. 4.8). However, there was a significant difference in peak seasons between *falciparum* and *vivax* parasites ($\chi 2 = 563.52$, d.f. = 1, $p < 0.0001$) (Fig. 4.9). *Plasmodium falciparum* peaked in May and dominated from May to July, *P. vivax* peaked in September, and the two species showed a similar proportion from August to December (Fig. 4.9).

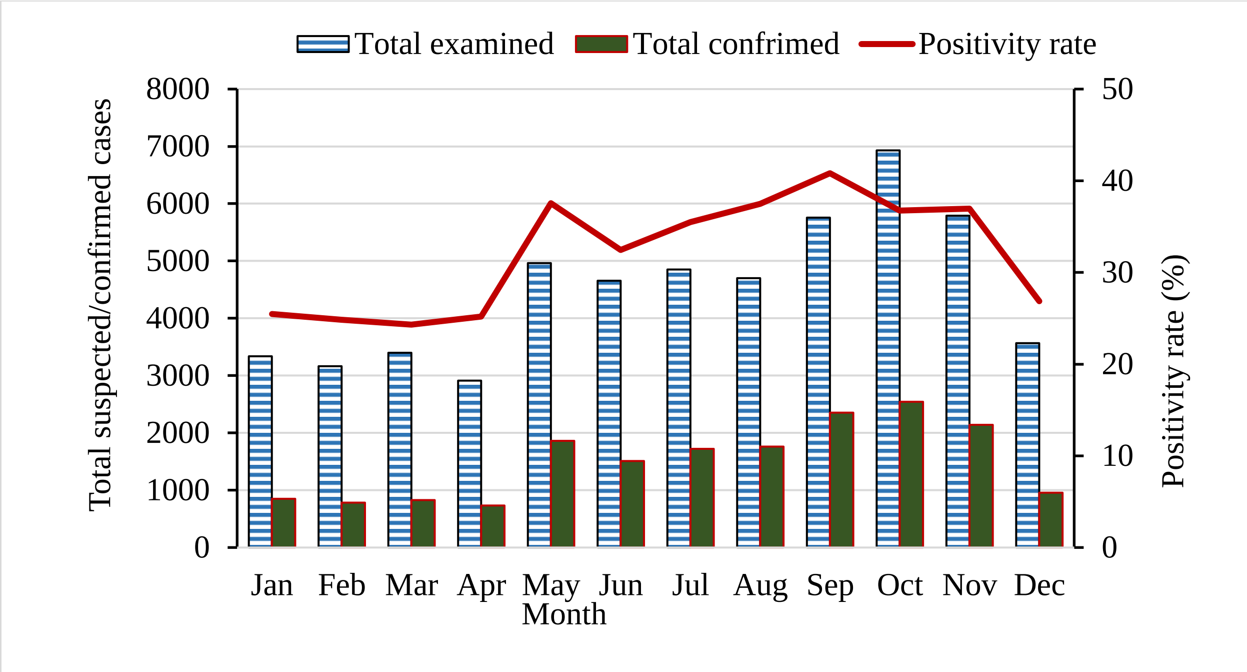

Figure 4.8: Seasonal dynamics in total malaria positivity rate at Arjo-Dedessa sugar development site and its vicinity, southwest Ethiopia (2008 – 2017).

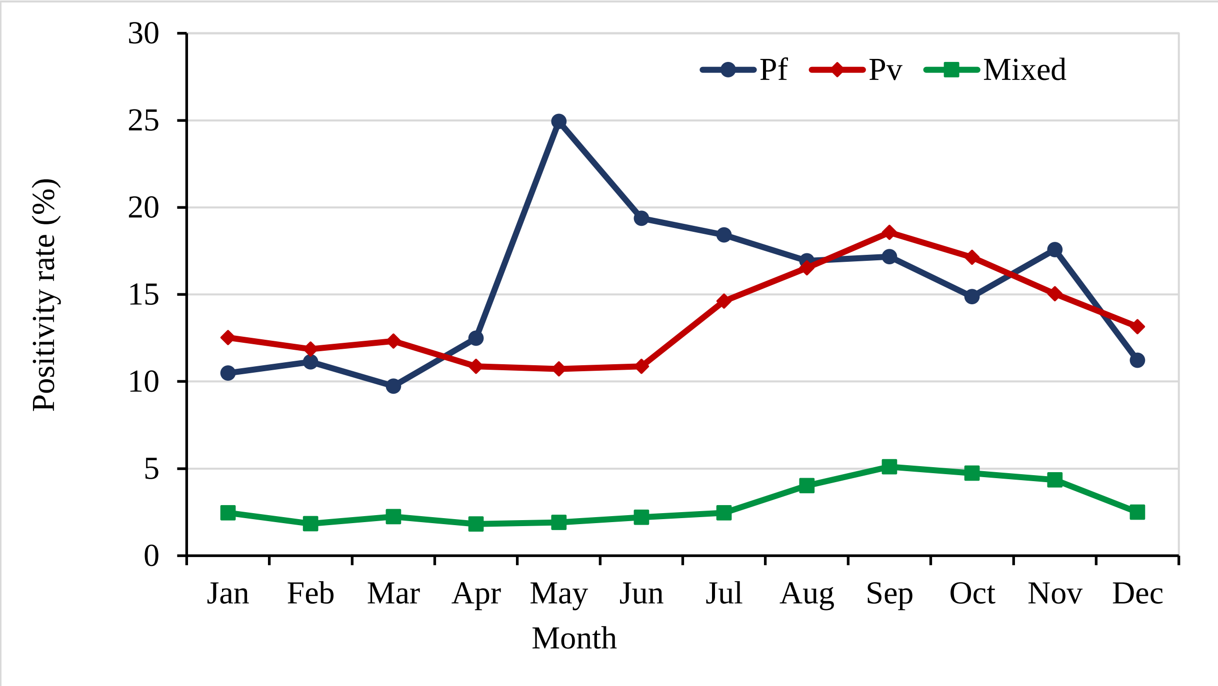

Figure 4.9: Seasonal dynamics in total malaria positivity rate and parasite species at Arjo-Dedessa sugar development site and its vicinity, southwest Ethiopia (2008 – 2017).

4.3.4. Trend in incidence rate and climatic effect

Total malaria cases. Table 1 showed the results of modeling analysis for the total malaria cases ($R^2 = 0.552$, adjusted $R^2 = 0.536$, $F_{6,113} = 34.806$, $P < 0.001$). In addition to the significant one-month lagged carry-on (infections carried from last month) effect, two-month lagged precipitation had significant positive impact on total cases, an increasing trend before 2013 and a decreasing trend (represented by time) after 2013 was observed (Table 1).

***Plasmodium falciparum* cases.** Table 1 showed the results of modeling analysis for *P. falciparum* cases ($R^2 = 0.524$, adjusted $R^2 = 0.498$, $F_{6,111} = 20.345$, $P < 0.001$). For *P. falciparum* cases, in addition to significant one-month lagged carry-on effect and two-month lagged precipitation effect, one-month lagged minimum temperature had a positive impact on cases. A similar temporal trend was revealed with modelling analysis, i.e., an increasing trend before 2013 and a decreasing trend (represented by time) after 2013 (Table 1).

***Plasmodium vivax* cases.** Table 1 showed the results of modeling analysis for *P. vivax* cases ($R^2 = 0.674$, adjusted $R^2 = 0.659$, $F_{6,111} = 40.285$, $P < 0.001$). For *P. vivax* cases, again one-month lagged carry-on effect, two-month lagged precipitation effect and similar temporal trends were significant factors (Table 4.1).

Table 4.1: Modeling analysis of the trend in clinical malaria incidence and climatic effect.

Parasite species	Term	Estimate	t-value	Prob>|t|
Total cases	Total cases from last month	0.543	7.42	<0.0001
	Precipitation (2-month lagged)	0.196	3.58	0.0005
	Time (month since Jan 2008)	-0.478	-1.90	0.0599
	(Time-61.5)*(Time-61.5)	-0.025	-2.80	0.0060
P. falciparum	*P. falciparum* cases from last month	0.530	6.73	<.0001
	Mixed cases two months ago	-0.683	-2.01	0.0470
	Precipitation (2-month lagged)	0.132	3.25	0.0015
	Minimum temperature (1-month lagged)	14.461	2.53	0.0128
	Time (month since Jan 2008)	-0.494	-2.54	0.0124
	(Time-61.5)*(Time-61.5)	-0.016	-2.43	0.0169
P. vivax	*P. vivax* cases from last month	0.836	9.50	<.0001

Mixed cases from last month	-0.855	-2.95	0.0038
Precipitation (2-month lagged)	0.101	3.98	0.0001
Time (month since Jan 2008)	-0.248	-2.15	0.0335
(Time-61.5)*(Time-61.5)	-0.009	-2.40	0.0182

The models predicted well of the three major peaks in 2009, 2010 and 2013 (Fig. 4.10).

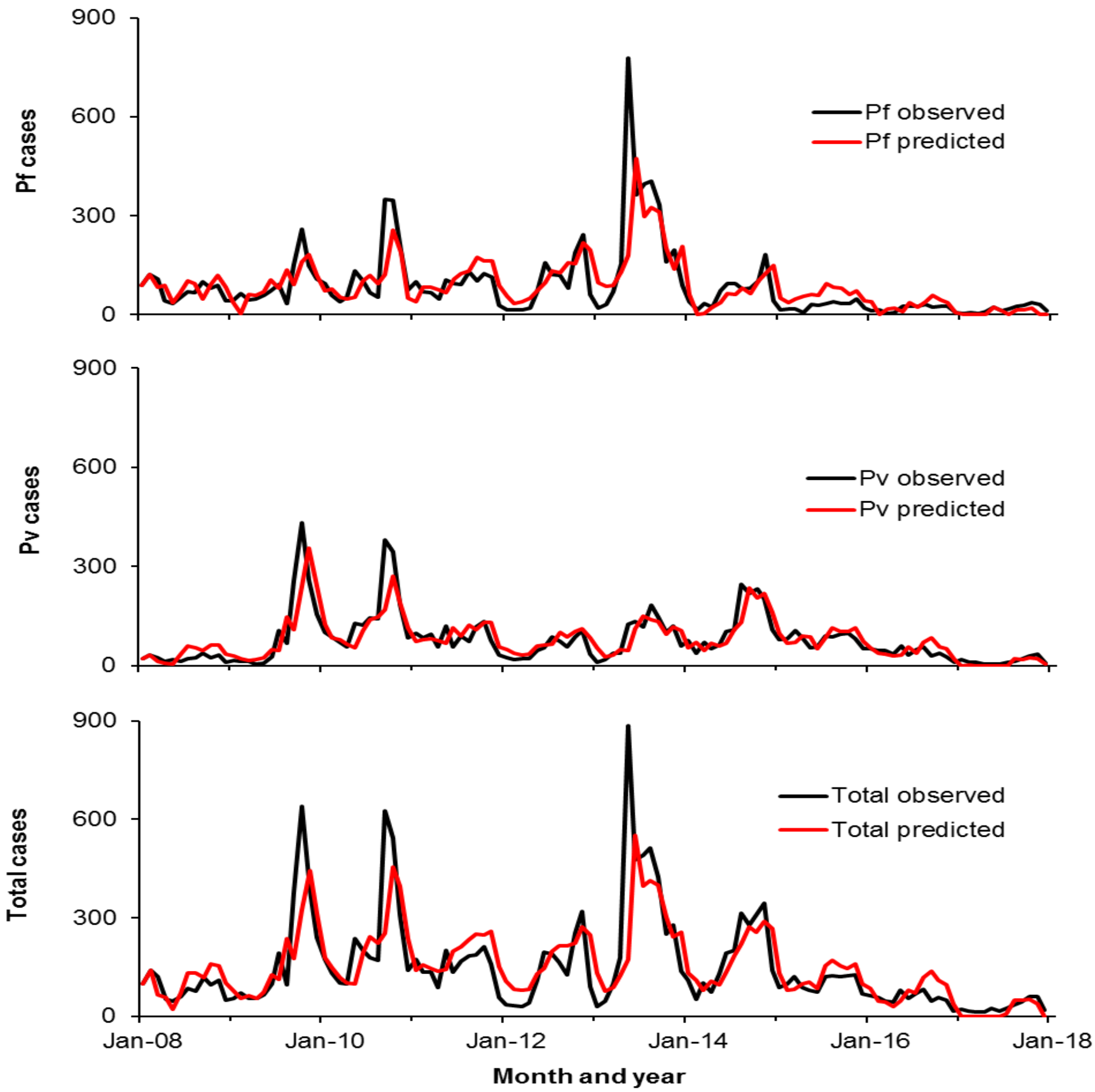

Figure 4.10: Models prediction.

4.4. Discussion and Conclusion

The results of this study indicated that malaria was a major public health burden in the area. Over the 10 years, a 33.4% annual mean positivity rate of malaria was reported. This was lower than the findings of studies conducted at Kola Diba health center and Adi Arkay, northwest Ethiopia [190,200]. The observed differences might be due to a difference in micro-epidemiological settings. The variation in malaria diagnosis techniques and the skills of the laboratory personnel in detecting and identifying malaria parasites might also be one of the reasons for the discrepancies. Likewise, the implementation of malaria prevention and control activities might differ from one area to another which indicates that the interventions in this area might have been stronger.

This study also revealed that malaria cases due to *P. falciparum*, *P. vivax,* and their mixed infection accounted for 48, 42.4 and 9.6% of the cases, respectively. This is not consistent with the national malaria parasite distribution pattern in Ethiopia [58], that *P. falciparum* and *P. vivax* accounting for 60 and 40% of the cases, respectively. The national figure estimation of malaria parasites indicates the average distribution in the country as a whole, while this study is limited to a small malaria-endemic setting in a country that could have resulted in the variation of the species prevalence. Similarly, the results of the present study is not consistent with the reports from other parts of the country, which revealed malaria cases due to *P. falciparum*, *P. vivax* and their mixed infection accounted for 68.9, 28.8, and 2.3% of the cases, respectively [200]. Another similar study also reported *P. falciparum* and *P. vivax* accounted for 75 and 25% of malaria morbidity, respectively [190]. This study also shows a trend in malaria parasite species shift in which *P. vivax* has become predominant over *P. falciparum* in the years 2010, 2014, 2015, and 2016. Such a trend in malaria parasite prevalence shift had also been reported by another similar study [201]. It has been reported that *P. falciparum* is a more prevalent and fatal malaria in Ethiopia. Because of this the intervention activities of malaria mainly focus on *falciparum* malaria , which might be a plausible reason for the observed trend shift [42,202]. Another possible reason might be climate variability due to environmental modification at the area and *P. vivax* might have developed resistance for the currently available drug, chloroquine [201,203].

The present study also revealed a higher positivity rate of malaria among males (64.5%) than females (35.5%). This result is in agreement with previous local studies [190,200,204,205], which had indicated that higher malaria prevalence in males than females. Age distribution showed that the majority of cases were adults age 15 years and above followed by the age group of 5 – 14 years. Such results, had been reported by other studies [190,200], and similar studies had also reported more susceptibility in the age range of 5-14 years [205,206]. The possible justification for malaria affected males might be because, the life of the community depends on farming and most of the time males in the reproductive age group are engaged in farming activities during which they are more likely exposed to the infective mosquito bites. The proportion of adult cases increased from 72.1% between 2008 and 2014 to 84.2% between 2015 and 2017, following the decline of the average malaria positivity rate from 38.2% to 17.8% between 2008 – 2014 and 2015 – 2017, respectively. It has been reported that at high transmission intensities, children acquire immunity rapidly and so are not susceptible to disease when they get older. On the other hand, at low transmission settings there is less disease among younger children, and consequently, older children acquire less immunity and remain susceptible [207–209]. Thus, the observed malaria burden among the adult age group might be due to dropping transmission at the area. Other potential explanations for increasing malaria cases among older children and adult could be preferential ITN use for younger children. Recent studies support the view that adults are also emerging as a population that deserves monitoring on the basis that they are reported to be at increased risk of malaria probably due to the declining antimalarial immunity that follows decreased exposure to parasites which could be a new challenge for elimination [208,210]. Therefore, as control interventions could induce change in malaria epidemiology like sex and age structure, the control strategies needed to be timely updated to contemporary epidemiological context to be able to respond to over-time transmission dynamics [211].

Over the study period, there was a declining trend of total malaria cases. This overall decline of total malaria could be attributed to the decline in *P. falciparum, P. vivax,* and mixed infection. The possible reason for this decline of malaria might be the increased attention to scaled-up malaria control interventions by the national malaria control program (NMCP) of Ethiopia since 2004. Ethiopia launched multiple interventions for malaria prevention and control throughout the country including the study area [191,193,212]. The national malaria elimination strategic plan currently recommends key intervention methods including prompt diagnosis using rapid diagnostic tests (RDT), artemisinin combination therapy (ACT) as the first-line drug to treat uncomplicated *P.*

falciparum malaria, and targeting the vector using indoor residual spraying (IRS), long-lasting insecticide-treated nets (LLINs) and environmental management [36,212]. Moreover, in the past decades, malaria control and prevention activities were intensified in the study area as it has been for other parts of the country. Community awareness creation about malaria prevention and control methods, increased accessibility of LLINs and high coverage IRS were the major interventions employed by NMCP [212]. The sum total of these efforts might have resulted in observed decline of malaria positivity rate in the study area.

In general, despite a fluctuating trend, the result showed a successive decline in malaria prevalence starting from 2013 to 2016. However, in recent years it showed a slight rise, which indicates that, the area needs attention to intensify the existing interventions to enhance malaria elimination efforts.

The peak season of total positivity rate started in May and ended in November with the highest confirmed cases between September and November after the long-rainy season. This finding is consistent with reports from other parts of Ethiopia [201,205,213], which had shown the highest peak of malaria in September, October and November. In Ethiopia, the peak malaria transmission occurs between September to December following the June to September long rains [58,194]. Some localities also experience persistent malaria, because the environmental and climatological situations permit the continuous availability of vector breeding sites [191]. Despite significant seasonality, in the study area, malaria cases were reported year-round over 10 years. In the study area, there is a sugar development site with mega irrigated sugarcane plantation, which could increase the proliferation of mosquitoes breeding habitats. Such extensive irrigation activities can modify the environment in a favor of malaria transmission [16,47,192,203]. In this study, the environmental modification effect on malaria vector bionomics and its implication to malaria transmission intensity is addressed in the next chapters.

The study indicates 10 years of malaria cases at the health facilities as a proxy measure in the malaria transmission trend. The analysis was done using secondary data and sometimes such data might have a problem, which could be the limitation of the study. Thus, the finding should be interpreted judiciously.

CHAPTER 5: IMPACT OF ENVIRONMENTAL MODIFICATION ON *ANOPHELES* MOSQUITO AQUATIC ECOLOGY (Adopted from Hawaria et al 2020)

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Infectious Diseases of Poverty

RESEARCH ARTICLE Open Access

Effects of environmental modification on the diversity and positivity of anopheline mosquito aquatic habitats at Arjo-Dedessa irrigation development site, Southwest Ethiopia

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Abstract

Background: Irrigated agriculture is key to increase agricultural productivity and ensure food security in Africa. However, unintended negative public health impacts (e.g. malaria) of such environmental modification have been a challenge. This study assessed the diversity and distribution of breeding habitats of malaria vector mosquitoes around Arjo-Dedessa irrigation development site in Southwest Ethiopia.

Methods: *Anopheles* mosquito larvae were surveyed from two agroecosystems, 'irrigated' and 'non-irrigated' areas during the dry (December 2017–February 2018) and wet (June 2018–August 2018) seasons. Mosquito habitat diversity and larval abundance were compared between the irrigated and non-irrigated areas. The association between *Anopheles* mosquito larvae occurrence and environmental parameters was analysed using Pearson chi-square. Multiple logistic regression analysis was used to determine primary parameters that influence the occurrence of *Anopheles* larvae.

Results: Overall, 319 aquatic habitats were surveyed during the study period. Around 60% (*n* = 152) of the habitats were positive for *Anopheles* mosquito larvae, of which 63.8% (*n* = 97) and 36.2% (*n* = 55) were from irrigated and non-irrigated areas, respectively. The number of *Anopheles* positive habitats was two-fold higher in irrigated than non-irrigated areas. *Anopheles* larval abundance in the irrigated area was 16.6% higher than the non-irrigated area. Pearson's chi-square analysis showed that season ($\chi2 = 63.122$, d.f. = 1, $p < 0.001$), agroecosystem (being irrigated or non-irrigated) ($\chi2 = 6.448$, d.f. = 1, $p = 0.011$), and turbidity ($\chi2 = 7.296$, d.f. = 2, $p = 0.025$) had a significant association with larval *Anopheles* occurrence.

Conclusions: The study showed a higher *Anopheles* mosquito breeding habitat diversity, larval occurrence and abundance in the irrigated areas than non-irrigated in both dry and wet seasons. This indicates that irrigation development activities contribute to the proliferation of suitable mosquito breeding habitats that could increase the risk of malaria transmission. Incorporating larval source management into routine malaria vector control strategies could help reduce mosquito population density and malaria transmission around irrigation schemes.

5.1. Background

Irrigation schemes are key to increase agricultural productivity, ensure food security, promote economic growth and alleviating poverty in the developing world [214]. However, past experience shows that inadequate consideration of the impact of environmental modification on the distribution of vector-borne diseases could lead to public health challenges [215]. Malaria is one of the major public health challenges that occur around irrigation schemes in Africa [216,217].

The distribution of malaria is mainly governed by the spatial and temporal distribution of malaria vectors in different ecological settings. Environmental modifications such as the construction of irrigation schemes could alter the existing ecological setting and favor the breeding of mosquitoes by providing additional aquatic habitats [23]. Such environmental changes may also lead to the change in mosquito vector diversity, distribution, abundance and proliferation. Studies are thus required to understand the dynamics of mosquito breeding habitats that can be created due to environmental modifications. Identifying the source of mosquitoes helps decision-makers to implement tailor-made mosquito vector interventions.

In Ethiopia, malaria is among leading public health problem and 68% of the population lives in malarious areas [4]. Although more than 42 species of *Anopheles* mosquitoes have been documented, *An. arabiensis* is the most widely distributed primary vector of malaria in the country. The major malaria vector control strategies encompass use of long-lasting insecticidal nets (LLIN), indoor residual spraying (IRS); and artemisinin-based combination therapy treatment [192].

In recent years, Ethiopia has seen extensive irrigation development aimed to improve its crop production and promote economic growth [176]. The impact of such large-scale water resources development schemes on malaria risk, however, has been poorly studied. As the country is striving to eliminate malaria from endemic areas by 2030 [193], it is important to identify risk factors associated with malaria in different settings. Understanding malaria vector mosquitoes larval ecology, diversity and distribution is therefore crucial to devise intervention measures [218–220].

This study aims to assess the impact of large-scale irrigation on the malaria vector mosquitoes larval breeding and abundance. It evaluates how irrigated areas affect availability of positive larval habitats as compared to non-irrigated areas. Furthermore, the study describes the major breeding habitats of *Anopheles* mosquitoes in the area.

5.2. Methods and Materials

5.2.1. Study setting

The study was conducted at Arjo-Dedessa irrigation development scheme and its vicinity, in southwest Ethiopia. The detail description of the study setting has been given in chapter 3.

5.2.2. Study design and period

A repeated cross-sectional study design was applied to assess the effect of irrigation activities on *Anopheles* mosquitos' larval habitat diversity and distribution. The study was conducted during the dry (December 2017–February 2018) and wet (June 2018–August 2018) seasons. Larval abundance in two different agroecosystems, irrigated and non-irrigated areas, were compared.

5.2.3. Sampling site selection

The study site was first classified into 'irrigated' and 'non-irrigated' areas. Irrigated areas were considered as 'risk' areas for malaria and constituted irrigated sugarcane farms and their surroundings within 1 km radius. Non-irrigated areas were those with low risk of malaria located outside the irrigation farms between 2 and 5 km from the irrigation schemes. These areas were further classified into clusters (a village with 100–150 households) and twelve clusters (six from irrigated and six from non-irrigated area) were selected and surveyed for aquatic mosquito larval breeding habitats.

5.2.4. Larval survey

All accessible potential mosquito breeding habitats (i.e. any water-containing structure) were surveyed for mosquito larvae. The larval surveys were conducted thoroughly within the estimated 1 km radius distance from the center of each cluster. Mosquito larvae were sampled following the WHO standard larval survey procedure using a standard dipper (350 ml, Bio Quip Products, Inc. California, USA) [182]. For larger breeding habitats, presence and absence of the larvae were determined after 20 dips. For habitats that were too small, dipping was done using pipettes. Water sampled by dipper was poured into a white sorting tray and checked for mosquito larvae. Larvae were identified morphologically and sorted by genus as *Anopheles* and *Culex*. *Anopheles* larvae were further sorted and the corresponding counts were recorded. All *Anopheles* larvae samples were poured into a plastic container and transported to the field insectary to rear them to adult stage for morphological identification using taxonomic keys [183]. All culicine larvae were discarded after counting at the sampling sites.

Plate 5.1: Field mosquito larval survey at Arjo-Dedessa, Ethiopia.

5.2.5. Rearing and identification of *Anopheles* mosquito's species

All *Anopheles* larvae samples were reared to adult mosquitoes following the methods provided by the Malaria Research and Reference Reagents Resource [63]. To maintain the same aquatic environment, the larvae were allowed to grow in the water that was collected from the field. The combination of '*Cerfami*' and '*Bravo instant yeast'* was provided as an additional food source for the larvae. Pupae were collected daily and left in a paper-cup until adults emerged. After emergence, male and female *Anopheles* were sorted, counted and recorded. All adult *Anopheles* mosquitoes were examined under a dissecting microscope and morphologically identified to species complex using the identification key of Gillies and Coetzee [183].

5.2.6. Habitat characterization

During the survey, environmental variables related to larval habitats were assessed. The variables recorded include habitat type, crop type, turbidity, exposure to sunlight, distance to the nearby house, vegetation, substrate types, land use and land cover. The distance to the nearest house was measured either using a tape meter when it was shorter than 100 m or visually when over 100 m. Habitats exposure to sunlight was visually determined as shaded, partially shaded, or sunlit. Substrate type was classified as muddy or sandy. The presence or absence of vegetation was determined visually. Vegetation type was categorized as emergent, submerse, floating, shed, or mixed. Land-use type was also grouped into the cultivated land/crop, grassland/pasture, wetland/swamp, road, and shrub land. Turbidity was classified as clear, turbid, and more turbid [184,185].

All the data were recorded offline using the Android-based tablet PC with ODK Collect application [221] including the coordinates of each surveyed aquatic habitat with the built-in Geographic Positioning System (GPS) sensor.

5.2.7. Data analysis

Anopheles larvae occurrence was defined as the presence or absence of the larvae. The density of *Anopheles* larvae was estimated as the number of larvae per dip for each habitat type. Larval abundance was calculated as the number of larvae collected in each type of habitat. Pearson chi-square analysis was applied to assess the association between *Anopheles* mosquito larvae occurrence and environmental parameters linked to larval habitats. Multiple logistic regression analysis was used to determine primary parameters that influence the occurrence of *Anopheles* larvae. Test of significance was done assuming α at 0.05 and a p-value less than 0.05 was considered significant. All analyses were done using Microsoft Excel (Version 2016, Microsoft

Corporation, Washington, U.S) and SPSS statistical software version 25 (SPSS Inc, Chicago, IL, USA). Spatial data aggregation, analysis, and visualization were produced with ArcGIS Pro 2.5 [186].

5.3. Results

5.3.1. Mosquito larval habitat types and positivity

Overall, 319 mosquito habitats were surveyed, of which 180 (56.4%) were from irrigated area, and the remaining 139 (43.7%) were from non-irrigated area (Table 5.1). Habitat types included swamps/marshy (*n* = 83; 26.0%), rain pool (*n* = 75; 23.5%), stream shoreline (*n* = 31; 9.7%), spring seepage (*n* = 24; 7.5%), tire trucks/road puddle (*n* = 21; 6.6%), animal foot print (*n* = 21; 6.6%), irrigation canal 14 (4.4%), hippo trench 13 (4.1%), man-made pool 8(2.5%), farm ditch 5 (1.6%), drainage ditch 5 (1.6%), pit 5 (1.6%), rice puddle 5 (1.6%) and other 5 (1.6%).

Among the surveyed larval habitats, 80.6% (*n* = 257) were positive for mosquito larvae (either *Anopheles* and/or culicine) and *Anopheles* mosquito larvae were found in 59.1% (*n* = 152) habitats (Table 5.1). The majority of *Anopheles* mosquito breeding habitats were from the irrigated area (63.8%; *n* = 97) while the remaining 36.2% (*n* = 55) were from the non-irrigated area.

A total of 17 different types of mosquito breeding habitats was encountered in the irrigated area, of which 14 (83%) were positive for *Anopheles* larvae. In the non-irrigated area, seven of the 13 (58.3%) surveyed mosquito breeding habitats were positive for *Anopheles* larvae (Table 5.1). The association between the occurrence of *Anopheles* mosquito larvae and type of agroecosystem was statistically significant ($\chi 2 = 6.448$, d.f. = 1, p = 0.011).

Table 5.1: Potential mosquito breeding habitats and their positivity in irrigated and non-irrigated areas, in and around Arjo-Dedessa sugar development site, southwest Ethiopia (2017–2018).

Sites	Habitat type	Number of habitat surveyed	Positive for *Anopheles* n (%)	Positive for *Anopheles* & culicine n (%)	Positive for *Anopheles* alone n (%)	Positive for culicine alone n (%)
	Rain pool	46	26 (56.5)	21 (45.3)	5 (11.2)	15 (32.6)
Irrigated area	Swamp	23	13 (56.5)	10 (43.5)	3 (13.0)	6 (26.1)
	Stream shoreline	19	11 (58.0)	10 (52.6)	1 (5.3)	7 (36.8)
	Tire track/road puddle	19	14 (73.7)	10 (52.6)	4 (21.1)	3 (15.8)
	Spring seepage	16	7 (43.8)	4 (25.0)	3 (18.8)	5 (31.3)
	Hippo trench	13	4 (30.8)	3 (23.1)	1 (7.7)	8 (61.5)
	Animal foot print	10	7 (70.0)	5 (50.0)	2 (20.0)	1 (10.0)
	Earth bottom irrigation canals	14	5 (35.7)	5 (35.7)	-	8 (57.2)
	Drainage ditch	4	1 (25.0)	1 (25.0)	-	2 (50.0)
	Man-made pools	7	6 (85.7)	5 (71.4)	1 (14.3)	1 (14.3)
	Pit	3	-	-	-	3 (100.0)
	Farm ditch	2	2 (100.0)	1 (50.0)	1 (50.0)	-
	Water container	1	-	-	-	1 (100.0)
	Rice puddle	1	1 (100.0)	1 (100.0)	-	-
	Swamp	60	32 (52.5)	29 (47.5)	3 (4.9)	19 (31.1)

	Rain-pool	29	8 (27.6)	6 (20.7)	2 (6.9)	9 (31.0)
	Stream shoreline	12	7 (58.3)	6 (50.0)	1 (8.3)	2 (16.7)
Non-irrigated area	Animal foot print	11	4 (36.4)	3 (27.3)	1 (9.1)	1 (9.1)
	Spring seepage	8	1 (12.5)	-	1 (12.5)	4 (50.0)
	Man-made pools	5	3 (60.0)	2 (40.0)	1 (20.0)	2 (40.0)
	Farm ditch	3	-	-		2 (66.7)
	Pit	2	-	-	-	1 (50.0)
	Rock pool	1	-	-	-	1 (100.0)
	Drainage ditch	1	0	0	-	1 (100.0)
	Tire track/road puddle	2	-	-	-	-
	Rice puddle	4	-	-	-	-

-: *Not applicable*

5.3.2. *Anopheles* larval density

Mean mosquito larval density varied significantly across different types of breeding habitats in both irrigated ($F = 2.610$, d.f. = 13, $p = 0.004$) and non-irrigated ($F = 2.800$, d.f. = 6, $p = 0.02$) areas during the study period. In the irrigated area, hoof prints had the highest mean larval density (3.7 larvae/dip) followed by hippo trenches (1.0 larvae/dip) and man-made pool (1.0 larvae/dip). Similarly, the highest mean larval density in the non-irrigated area was observed in hoof prints (1.7 larvae/dip) followed by rain pools (0.7 larvae/dip) and stream shoreline (0.7 larvae /dip).

There was no significant difference in mean larval density between irrigated and non-irrigated areas. Likewise, the mean larval density between dry and wet seasons was not significant ($p > 0.05$). However, the overall larval abundance in the irrigated area was higher by 16.6% when compared to the non-irrigated area.

5.3.3. Characteristics of *Anopheles* breeding habitats

The majority (70–71%) of *Anopheles* breeding habitats were located within 500 m from nearby houses in the irrigated and non-irrigated areas. About half of the mosquito breeding habitats had vegetation cover, mainly emerging vegetation. The majority of habitats were found to be turbid in both irrigated (75.3%) and non-irrigated (61.8%) areas. Most of the *Anopheles* mosquito breeding habitats were fully exposed to sunlight. Concerning land-use types, in irrigated area, 46.4% and 32.9% of habitats were cultivated/cropland and grassland/pasture, whereas in non-irrigated area, 43.6% and 40.0% were wetland/swamp and grassland/pasture, respectively (Table 5.2).

Table 5.2: Physical characteristics of the *Anopheles* larvae breeding habitats from the irrigated and non-irrigated area, in and around Arjo-Dedessa sugar development site, southwest Ethiopia (2017–2018).

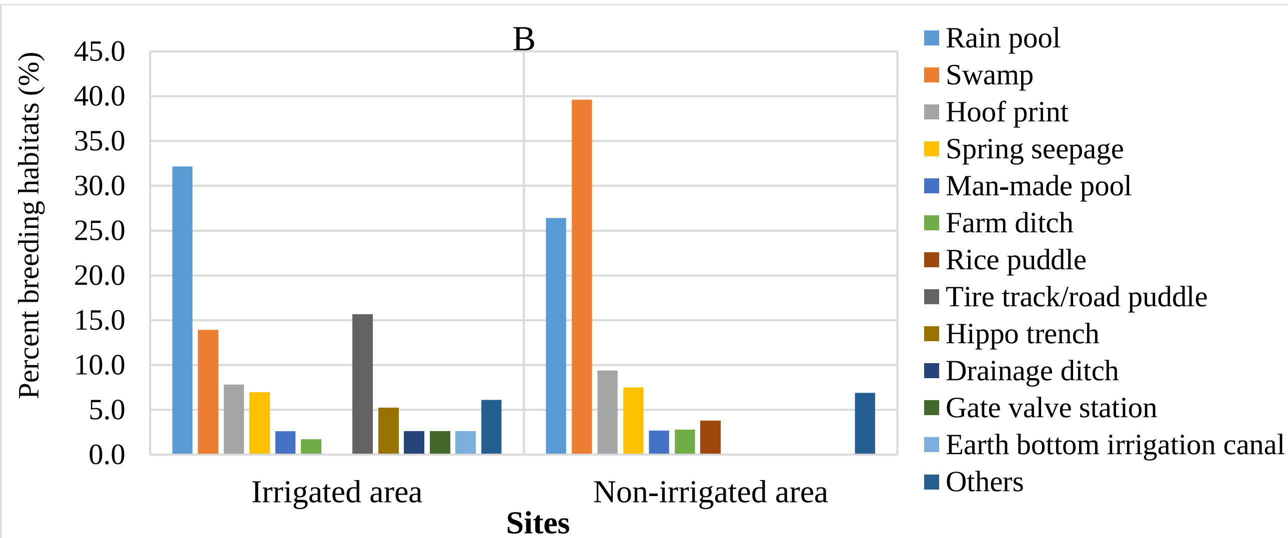
Physical characteristics		Sites		Total
		Non-irrigated area n (%)	Irrigated area n (%)	n (%)
Substrate	Muddy	54 (98.2)	97 (100.0)	151 (99.3)
	Sandy	1 (1.8)	-	1 (0.7)
Vegetation presence	No	21 (38.2)	46 (47.4)	67 (41.1)
	Yes	34 (61.8)	51 (52.6)	85 (55.9)
Vegetation type (*n* = 85)	Emergent	25 (73.5)	31 (60.7)	56 (65.8)
	Submersed	9 (26.5)	8 (15.7)	17 (20.0)
	Floating	-	2 (3.9)	2 (2.3)
	Shaded	-	5 (9.8)	5 (5.8)
	Mixed	-	5 (9.8)	5 (5.8)
Turbidity	Clear	21 (38.2)	23 (23.7)	44 (28.9)
	Turbid	19 (34.6)	47 (48.5)	66 (43.5)
	More turbid	15 (27.2)	26 (26.8)	41 (26.9
Exposure to sun	Shady	-	1 (1.0)	1 (0.7)
	Partially shady	2 (3.6)	9 (9.3)	11 (7.2)
	Sunlit	53 (96.4)	87 (89.7)	140 (92.1)
Seasonality	Permanent	5 (9.1)	24 (24.7)	29 (19.1)
	Temporal	50 (89.9)	73 (75.3)	122 (80.3)
Land use type	Shrub land	2 (3.6)	8 (8.2)	10 (6.6)
	Grassland/pasture	22 (40.0)	32 (32.9)	54 (35.5)
	Wetland/swamp	24 (43.6)	8 (8.2)	32 (21.1)
	Cultivated land/cropland	6 (10.9)	45 (46.4)	51 (33.5)
	Road	1 (1.8)	4 (4.1)	5 (3.3)
Distance from nearby house	Less than 100 m	2 (3.6)	5 (5.2)	7 (4.6)
	Between 100 m & 200 m	3 (5.5)	15 (15.5)	18 (11.8)
	Between 200 m & 500 m	33 (60.0)	47 (48.2)	80 (52.6)
	No house with in 500m	17 (30.9)	30 (31.1)	47 (30.9)

-: *Not applicable*

5.3.4. Seasonal *Anopheles* larval habitat diversity

During the dry season, stream shorelines, rain pools, swamp/marsh, spring seepages, hippo trenches (Plate 5.2) and Earth bottom irrigation canals were the most frequently encountered mosquito breeding habitats in the irrigated area. In the non-irrigated area, swamps/marshes and stream shorelines were the most common larval habitats during the dry season (Fig. 5.1A).

During the wet season, rain pools, tire tracks/road puddles and swamps/marshes, were the predominant mosquito breeding habitats in the irrigated area; while swamps and rain pools the most commonly encountered larval habitats in the non-irrigated area (Fig. 5.1B).



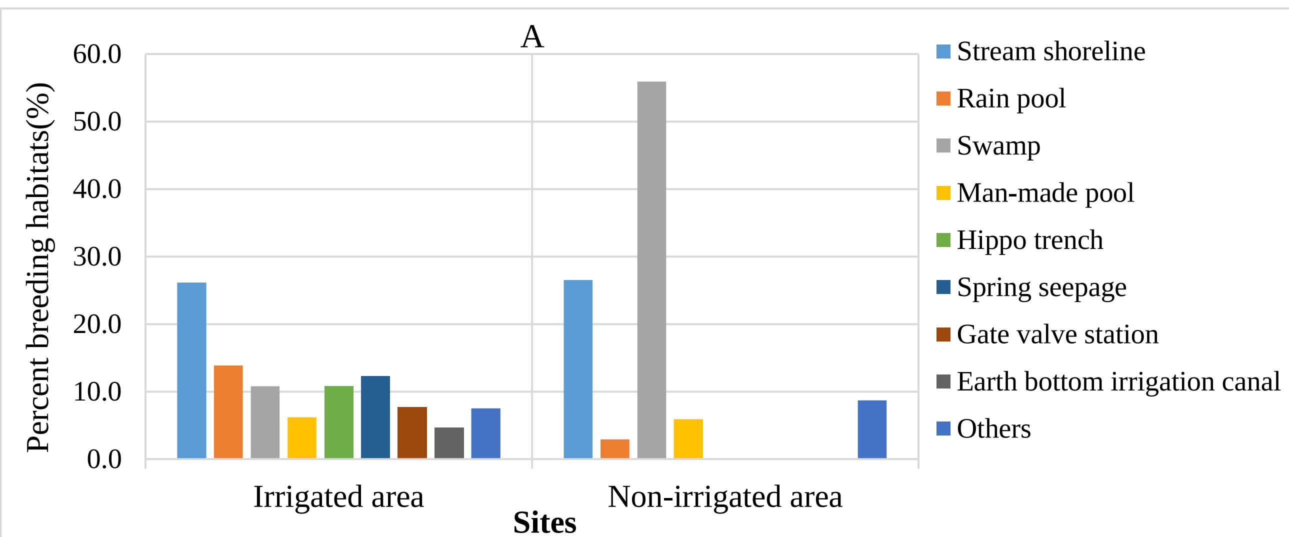


Figure 5.1: Proportion of habitat diversity in dry (A) and wet (B) seasons in the irrigated and non-irrigated areas around Arjo-Dedessa development site, Southwest Ethiopia (2017–2018).

Plate 5.2: *Anopheles* mosquito breeding habitats (A) hippo-trenches (B) rain-pool (C) shoreline (D) man-made pool (E) swamp/marsh (F) earth bottom irrigation cannel, Arjo-Dedessa sugar developmental site and its environs, Southwest Ethiopia (2017–2018).

The association between *Anopheles* larval occurrence and seasons was statistically significant in both irrigated ($\chi2 = 7.284$, d.f. = 1, $p = 0.007$) and non-irrigated area ($\chi2 = 11.429$, d.f. = 1, $p = 0.001$). A higher number of *Anopheles* larval positive habitat was recorded in the wet season than dry season

(Fig. 5.2). Generally, more diverse mosquito breeding habitats were observed in the irrigated area than the non-irrigated area during the study period.

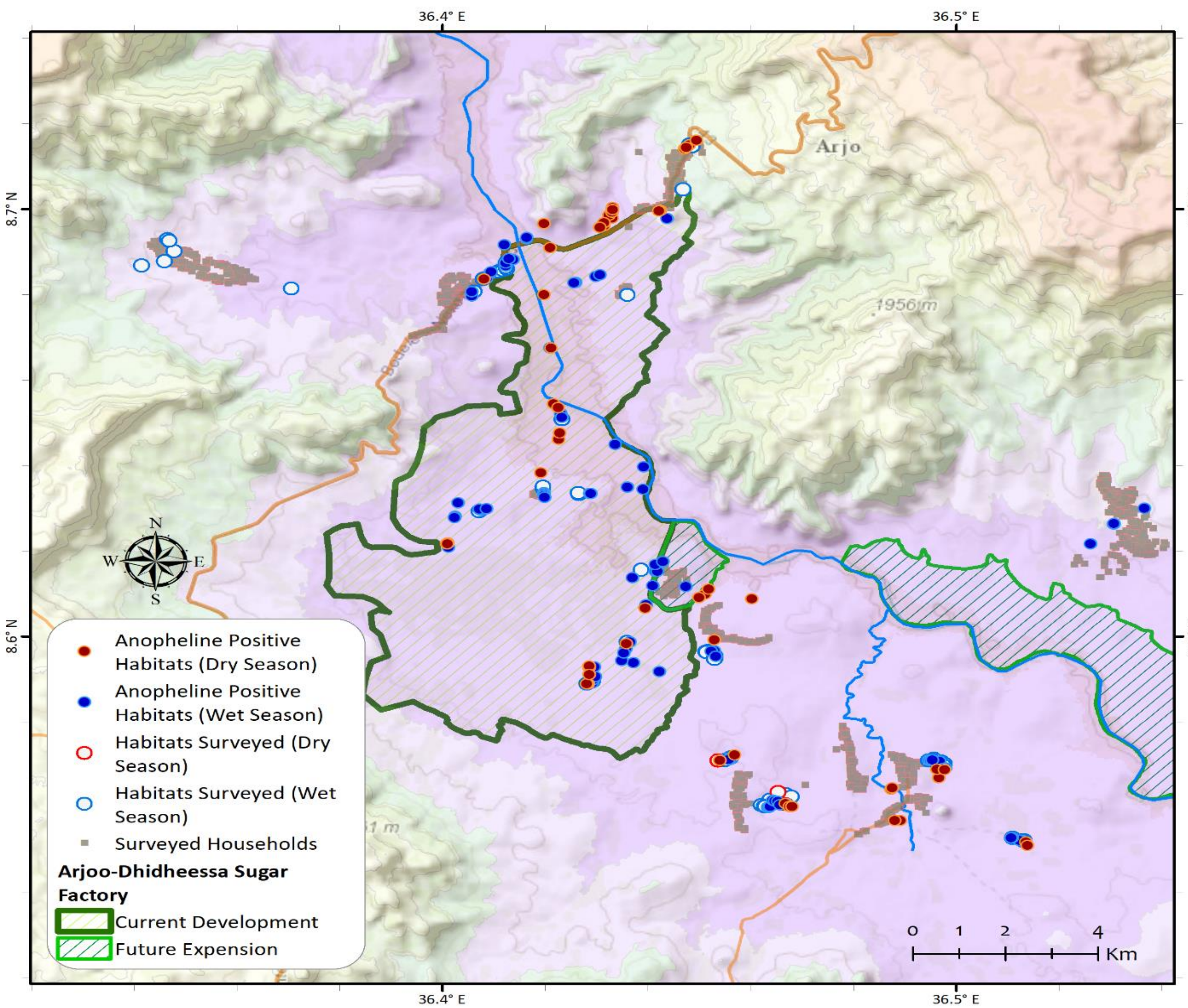


Figure 5.2: Distribution of breeding habitats positive for *Anopheles* mosquito larvae in wet and dry seasons around Arjo-Dedessa development site, Southwest Ethiopia (2017–2018).

5.3.5. *Anopheles* larvae abundance

A total of 1523 *Anopheles* larvae (1195 early, 348 late instars) and 5287 culicine were collected during the study period (Fig. 4). Out of the total *Anopheles* larvae collected, 58.3% (n = 888) and 41.7% (n = 635) were from the irrigated and non-irrigated areas, respectively. In the irrigated area, rain pools, tire trucks/road puddles, stream shorelines, and swamps were the major sources of *Anopheles* larvae, all together accounting for 65.4% of the total larval collection. In the non-irrigated area, swamps were the most productive habitats followed by rain pool and stream shoreline, together accounting for 88.6%

of the total larval samples (Fig. 5.3). Overall, *Anopheles* larval abundance was generally higher in the irrigated than non-irrigated areas both during the dry and wet seasons.

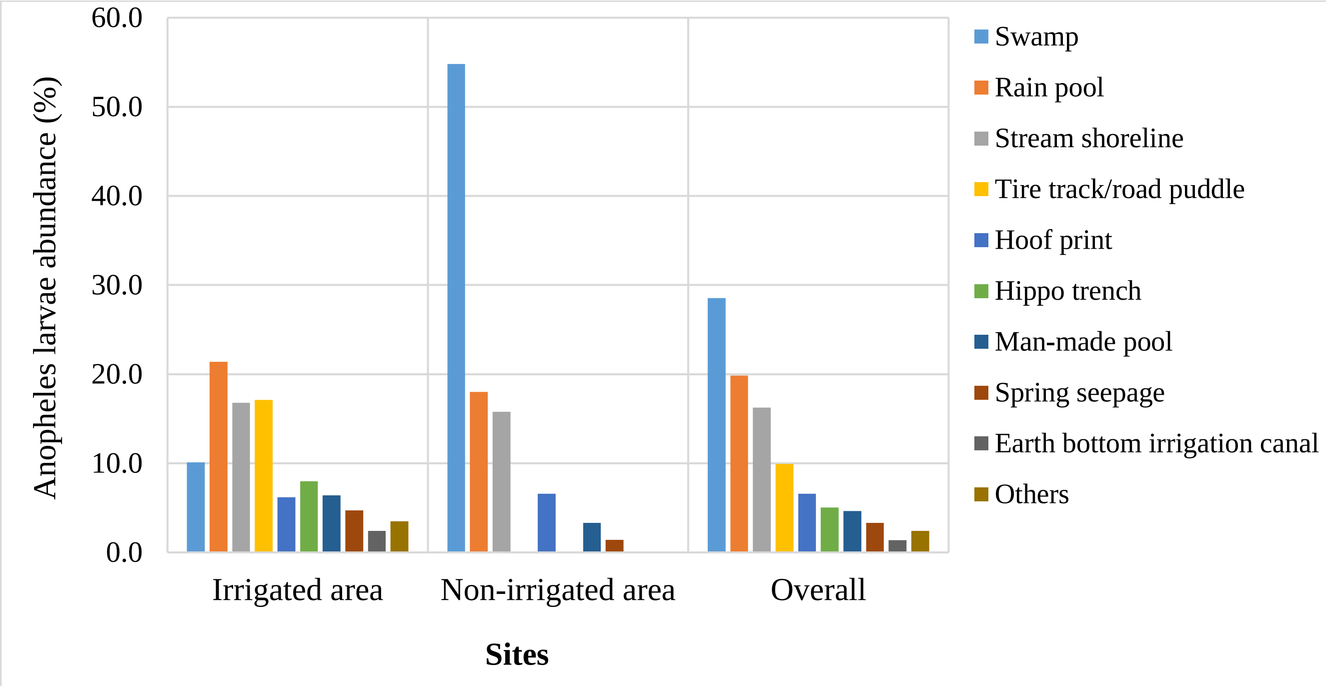


Figure 5.3: *Anopheles* mosquito larval abundance in irrigated and non-irrigated areas, in and around Arjo-Dedessa sugar development site, Southwest Ethiopia (2017 – 2018).

*Others includes: used tire, rock pool, water container, natural pond, and pit

In the irrigated area, *Anopheles* larval samples were mainly collected from stream shorelines and hippo trenches during the dry season and from rain pools and tire tracks/road puddles during the wet season. In the non-irrigated area, swamps were major sources of *Anopheles* larvae both during the wet and dry seasons. Overall, a higher abundance of *Anopheles* larvae was noted in the irrigated than non-irrigated areas during the study period (Fig. 5.4A&B).

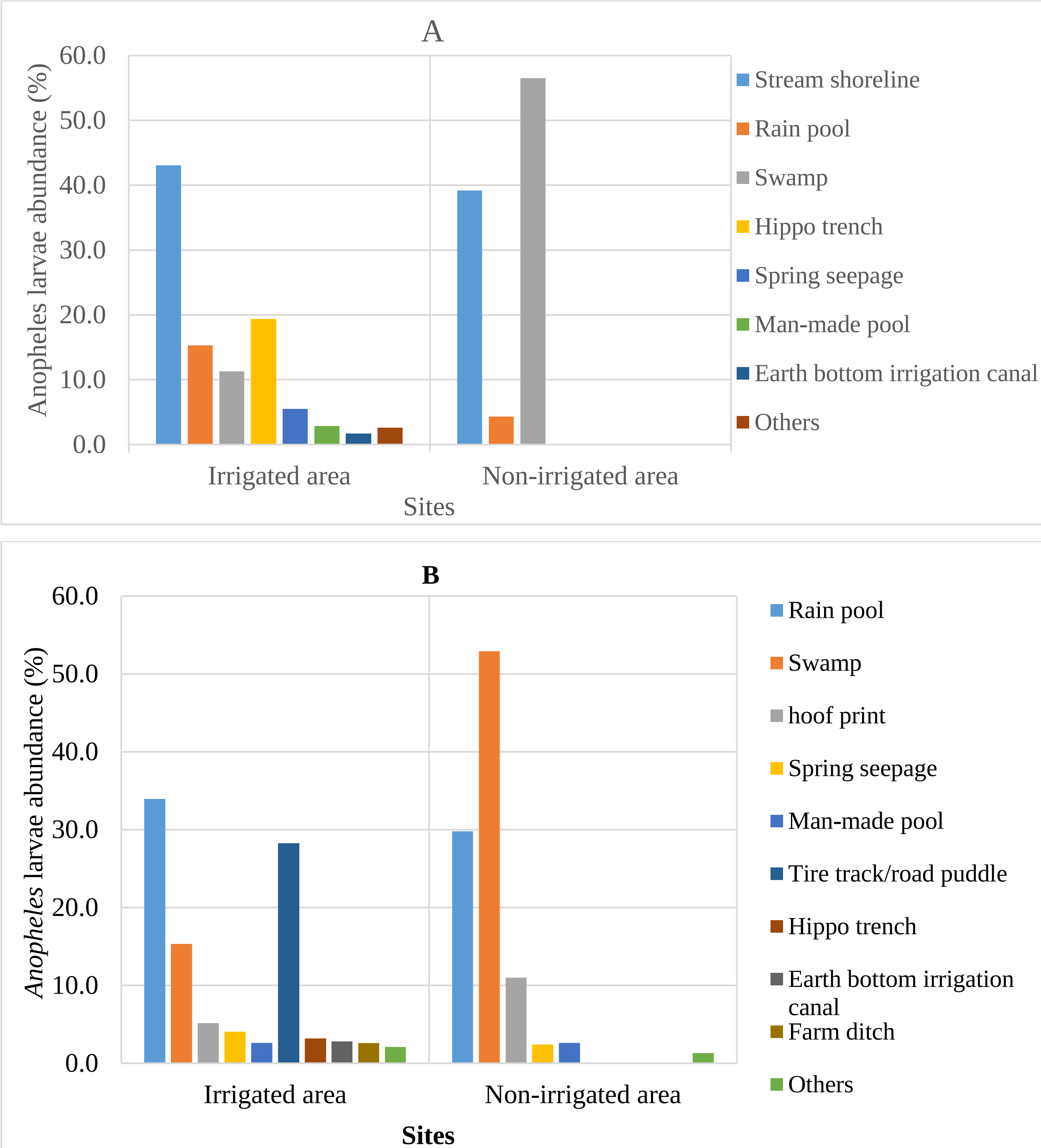


Figure 5.4: *Anopheles* mosquito larval abundance in irrigated and non-irrigated areas during dry (A) and wet seasons (B), in and around Arjo-Dedessa sugar development site, Southwest Ethiopia (2017–2018).

5.3.6. Association between environmental parameters and *Anopheles* mosquito's larvae occurrence

Results of Pearson's chi-square analysis showed a significant association between *Anopheles* larvae occurrence and environmental parameters, season ($\chi 2$ = 63.122, d.f. = 1, p < 0.001), agroecosystem (being irrigated or non-irrigated) ($\chi 2$ = 6.448, d.f. = 1, p = 0.011), and turbidity ($\chi 2$ = 7.296, d.f. = 2, p = 0.025). Multiple logistic regressions indicated that agroecosystem type was the primary predictor for *Anopheles* mosquitoes larval occurrence (OR = 1.844, 95% CI: 1.153–2.949, p = 0.011) (Table 5.3).

Table 5.3: Logistic regression analysis for *Anopheles* larvae occurrence, around Arjo-Dedessa sugar development site, southwest Ethiopia (2017 – 2018).

Variable	B	S.E.	Wald	d.f.	Sig.	OR (95%CI)
Site	0.612	0.240	6.528	1	0.011	1.844 (1.153 - 2.949)
Habitat turbidity	-0.165	0.106	2.438	1	0.118	0.848 (0.689 - 1.043)
Season	-0.322	0.257	1.571	1	0.210	0.725 (0.438 - 1.199)
Constant	0.748	0.516	2.102	1	0.147	

5.3.7. *Anopheles* mosquito species composition

About half (*n* = 755; 49.6%) of the *Anopheles* larval collections reared were emerged to adults, of which 349 were females and 406 were males (Fig. 6). The majority (73%) of them were from the irrigated area. Overall, four *Anopheles* species (*Anopheles gambiae* s.l *An. coustani*, *An. pharoensis,* and *An. squamosus*) were recorded. In the irrigated area, *An. gambiae* s.l was the predominant species (84.8%) followed by *An. coustani* (10.0%), whereas in the non-irrigated setting, *An. coustani* (54.8%) was the most common species followed by *An. gambiae* s.l (39.8%) (Fig. 5.5).

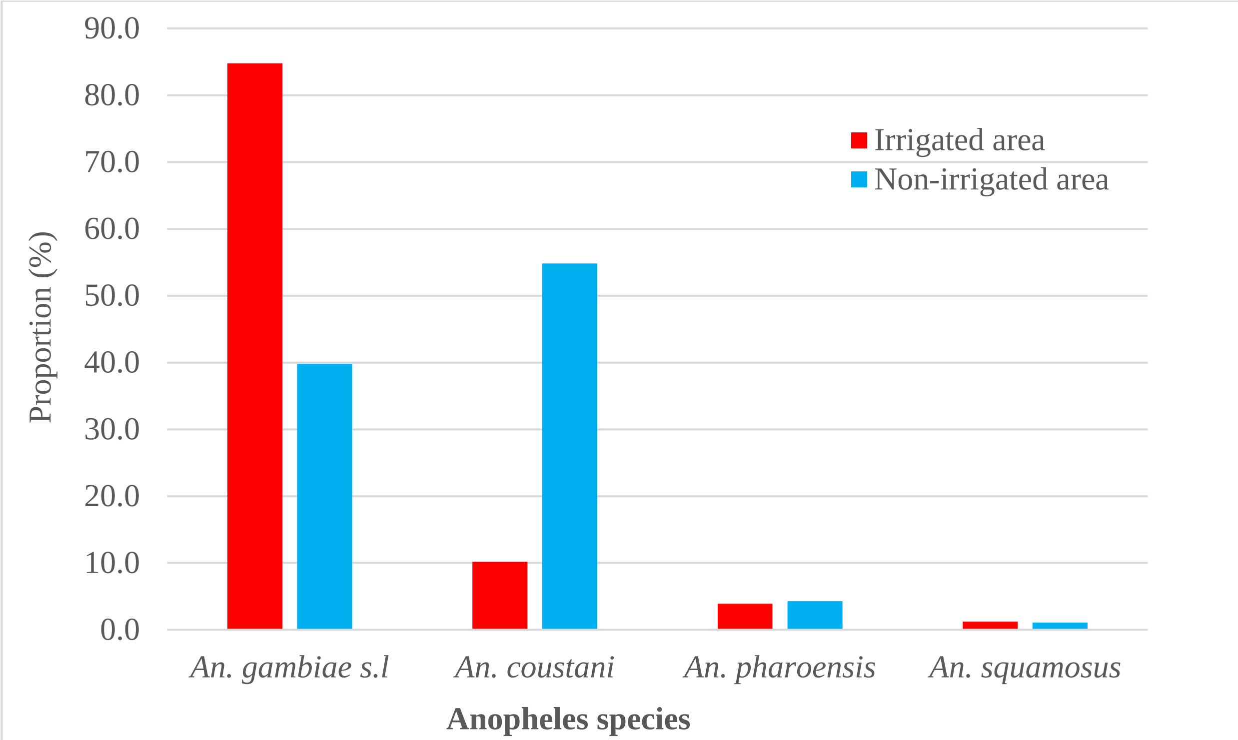


Figure 5.5: Composition of adult female *Anopheles* mosquito species in irrigated and non-irrigated areas, in and around Arjo-Dedessa sugar developmental site, Southwest Ethiopia (2017–2018).

5.4. Discussion and Conclusion

The study revealed that the *Anopheles* mosquito breeding habitats were diverse in the irrigated areas. The diversity of mosquito breeding habitats in the irrigated area was two-fold higher than in the non-irrigated area, indicating that the irrigation development contributed to the proliferation of malaria mosquito breeding habitats. Improper ground excavation, frequent vehicles and machineries movements during planting and harvesting, lack of maintenance, and poor environmental management contributed to the formation of numerous mosquito breeding habitats in the irrigation project area as noted elsewhere in Africa [23,137,199]. Similarly, several studies elsewhere in Africa have suggested that changes in land use have influenced malaria vector larval habitat availability and distribution [20,222]. The findings from the present study are also in agreement with previous studies in central Ethiopia where a higher larval and adult abundance of the malaria vectors was recorded in the irrigated than non-irrigated villages [47]. A study conducted in western Ethiopia reported that higher malaria prevalence and transmission risk increased due to high vector abundance in the irrigated sugarcane agroecosystem than non-irrigated agroecosystem [137]. Generally, an increase in mosquito breeding habitats results an increase in vector density and eventually leading to increased malaria transmission [223,224].

Most of the mosquito breeding habitats identified in this study were previously reported elsewhere [220,225,226]. However, the nature and formation of some of the habitats made them specific and unique to the study area and thus can be a target for intervention. For instance, the mosquito habitat like hippo-trench was specific to the irrigated area. Hippo-trenches were deep excavation, around 2 m, canal structures designed to prevent the hippos from entering into the sugarcane farm. The trenches were situated at the periphery of the farm and designed to collect water from surrounding River, or streams or springs (Fig. 5.3). During the rainy season, the trenches remained filled with water but became shallow and conducive for mosquito breeding during the dry season. A study conducted in Kenya suggested that habitat size is an important determinant of habitat stability and mosquito occurrence [227]. Identifying vector-breeding habitats is important to target them for larval management.

In the irrigated area, rain pools, tire tracks/road puddles, and swamps were found to be the major breeding habitats for *Anopheles* mosquitoes during the wet season, while stream shoreline and hippo-trenches provided larval breeding grounds during the dry season. On the other hand, in the non-irrigated area, swamps and rain pools were the major larval breeding habitats during the wet season, while swamps and stream shorelines were common breeding grounds during the dry season. This showed that targeting these habitats through larval management could help significantly reduce the vector mosquito population abundance and eventually reduces malaria transmission intensity in the area. In Africa, larval source management has been shown to be very effective in areas where mosquito breeding habitats are distinct and accessible [228]. Studies showed that when larval management is integrated with LLINs and IRS, a great improvement would be seen in malaria control efforts than IRS and LLINs alone [131,229]. The present study indicated the availability of distinct mosquito breeding habitats during the dry and wet seasons, indicating the potential use of larval source management to reduce the mosquito population.

The difference in *Anopheles* larval occurrence between the irrigated and non-irrigated areas could partly be due to the differences in the microclimate in the two agroecosystems. About two-third of *Anopheles* positive breeding habitats were found to be turbid. A study conducted in Ethiopia reported that *An. arabiensis*, the major malaria vector in the country, lays more eggs in the turbid water proximity to pollen-shedding maize farms than clear water [230]. The possible explanation for the preference of turbid water over clear might be due to differences in soil nutrients that influence the enrichment of bacteria that serve as a food source of larvae, and possibly as oviposition attractants [231].

This study had several limitations. The study did not include data of microclimate variation between the two agroecosystems. The variation in microclimate during the study period may have an influence on mosquito larval habitat productivity.

In conclusion, *Anopheles* mosquito breeding habitat diversity, positivity and abundance were found to be higher in the irrigated than non-irrigated areas during the dry and wet seasons. The findings of this study suggest that irrigation development activities amplify the proliferation of aquatic breeding habitats for malaria vector mosquitoes that may lead to a higher risk of malaria transmission. Identifying major malaria vector breeding habitats helps devise tailor-made interventions such as larval source management to reduce the risk of malaria around irrigation schemes.

Moreover, the next chapter addresses the effect of irrigated agricultural practice on the development, survivorship and fecundity of local malaria vectors.

CHAPTER 6: IMPACT OF ENVIRONMENTAL MODIFICATION ON MOSQUITO SURVIVORSHIP (Adopted from Hawaria et al 2020)

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Parasites & Vectors

RESEARCH Open Access

Survivorship of *Anopheles gambiae* sensu lato in irrigated sugarcane plantation scheme in Ethiopia

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Abstract

Background: To ensure food security, the sub-Saharan Africa has shown massive water resource development in recent years. However, such environmental modifications affect the survivorship and development of mosquitoes that are vectors of diseases. This study was aimed at determining the effects of irrigated agricultural practices on the development and survivorship *Anopheles gambiae* s.l in southwest Ethiopia.

Methods: Life-table experiment was conducted to determine the effect of environmental modification on survivorship of both immatures and adults *An. gambiae* s.l in irrigated sugarcane plantation and non-irrigated field crop areas. The pupation rate and development time of the immatures and adult longevity and fecundity were compared between the two settings.

Results: The estimated mean survival time of female *An. gambiae* s.l in the irrigated and non-irrigated areas was 37.9 and 31.3 days, respectively. A survival analysis showed that adult females of *An. gambiae* s.l placed in the irrigated area lived significantly longer than those at the non-irrigated area ($\chi 2 = 18.3$, d.f. = 1, $p < 0.001$) and *An. gambiae* s.l females lived significantly longer than males in both areas ($p < 0.001$).

Conclusions: Adult *An. gambiae* s.l survivorship was found to be enhanced in irrigated sugarcane plantation area compared to non-irrigated field crop area. Longer survival of adult mosquito in irrigated sugarcane plantation area could have important implications in malaria transmission.

6.1. Background

Mosquito survivorship is an important factor that determine vectorial capacity, and malaria transmission potential [232]. For example, *Anopheles* mosquito needs to survive beyond the extrinsic incubation period of the *Plasmodium* parasites to be able to transmit malaria; the longer it lives the higher the number of bites it causes [72]. Malaria vector's immatures survivorship and enhanced larval-to-pupal development rate increase adult population density, which in turn affect vectorial capacity of mosquito populations in a particular setting [3, 4].

Mosquito survivorship and development may be affected by environmental factors. Temperature (both water and ambient), relative humidity, rainfall, nutrient availability are key environmental factors governing the dynamics of malaria vectors including development and survival [233–235]. These factors can strongly be influenced by variation in land use and land cover change such as vegetation cover, landscape, distance to water body [23,236]. Zhong *et al* [18] and Wang *et al* [237] reported enhanced survivorship and development of both adult and larvae of *An. sinensis* and *An. minimus*, major malaria vector in China with higher ambient temperature due to land use and cover change. Fine-scale variation in microclimate across different landscapes is blamed for shapes variation in mosquito population dynamics [238].

To avert poverty, developing countries have been implementing water resource development projects like hydropower dams and agricultural development irrigation schemes [14,23,176]. Previous studies indicated that such change in land use and land cover has been increased malaria transmission through proliferating vector breeding sites and changing the microclimate that governs the dynamics of the vectors [12,14,16,23,47,199,218,239].

Ethiopia, a country with more than 75% of the total area is malarious [32], has been experiencing a massive change in land use and land cover by water resource development projects including irrigation schemes, and hydroelectric power projects [143]. Arjo-Dedessa sugar development project site is among mega irrigation schemes with a sugarcane plantation covering around 4,000 ha. with a future expansion plan of 80,000 ha, which supplies a state-owned sugar factory [240]. The area is historically known to be a wildlife sanctuary. Long ago, the government, partly, settled residents evacuating from other parts of the country to establish their lives through subsistence farming. The area is endemic to malaria [240]. *Anopheles* larval ecology study demonstrated higher malaria vector breeding habitat diversity, larval occurrence, and abundance at irrigated sugarcane plantation areas (chapter 5).

However, how this massive environmental modification has been influencing the development and survivorship of major malaria vectors in the area is not yet understood. Understanding malaria vector bionomics in line with environmental modification helps to model malaria transmission for better evidence-based interventions, which will have a profound effect in realizing the country's malaria elimination goal by 2030 [241]. We hypothesized that land use and land cover changes, especially massive irrigated agriculture alter the development and survivorship of malaria vectors in the areas.

Therefore, the objective of this study was to determine the effects of irrigated agricultural land development on the development and survivorship of *An. gambiae* s.l. The knowledge of vector response to environmental modification will give a better understanding of malaria transmission dynamics, which is useful for predicting the impact of environmental modification on malaria transmission intensity and set forward tailored interventions.

6.2. Methods and Materials

6.2.1. Study setting

The study was conducted in Arjo-Dedessa irrigation development scheme, southwest Ethiopia. The detail description has been provided in chapter 3.

6.2.2. Study design and period

Life-table experiments were conducted to determine the development and survivorship of *An. gambiae* s.l from August to October 2019.

6.2.3. Experiment site selection

The experiments were done in two different agroecosystem settings: areas covered with irrigated sugarcane plantation area ('irrigated area' hereafter) and areas covered with other field crops common in the area ('non-irrigated area' hereafter).

6.2.4. *Anopheles gambiae* sensu lato larval survivorship experiment

Adult mosquito collection and larvae hatching

Blood-engorged *An. gambiae* s.l were collected indoor and animal shelter from study area using mouth aspirator. All collected mosquitoes were kept in paper cages at field insectary. An oviposition substrate consisting of a Petri dish lined with a filter paper disk on wet cotton wool put in each cage for egg-laying. Collected eggs were let to hatch and newly hatched first instar larvae were used for the experiment.

Experiment

Plastic washbasin (34 cm x 14 cm) was used to imitate natural larval breeding habitat. The washbasins were exposed to the outdoor environment for a week prior to the experiment for acclimation. Then, two liters of rainwater and 1 kg of soil from the same area were added to each washbasin and left for a day. The washbasins were kept at each selected site of the two different areas, (irrigated area and other non-irrigated areas). Fifty newly emerged first instar *An. gambiae* s.l larvae were transferred into each washbasin with eight replicates for each site. The water level in the washbasins was checked daily and maintained by adding water if needed. To prevent other insects from invading the washbasins or other mosquitoes from laying eggs, the washbasins were placed inside an insect-proof 61 × 61 × 61 cm^3 BugDorm tent (BioQuip, Rancho Dominguez, (BD2120), CA, USA) (Plate 6.1). All sides of the BugDorm tent were made of clear polyester netting materials, so that sunlight was not blocked. The homogeneity of washbasins had the advantage over the natural habitats, which were highly variable in habitat size, larval food conditions (e.g. organic matters), vegetation coverage, light shade, competitors, and predators. Each day the number of surviving larvae and their developmental stage, and mortality was recorded. Pupae were counted and removed daily. Removed pupae were collected in the waterproof paper cup for adult emergence.

Plate 6.1: Insect-proof BugDorm tent with washbasins inside.

6.2.5. *Anopheles gambiae* sensu lato adult survivorship experiment

In this experiment, *An. gambiae* s.l adults emerged from the larval survivorship experiments were used. Twenty-five female and 25 male adult mosquitoes within 24 hours post-emergence were transferred into the paper cage (21.5 cm x 9 cm). The cages were covered with nylon mesh to prevent mosquito escape. Then, the cages were placed in irrigated sugarcane plantation and non-irrigated field crops covered area in five replicates for each site. Mosquito cages were hanged from the roof structures of small temporary shelters (2m high) constructed for the experiment purpose for rain protection (Plate 6.2). To prevent ants from reaching and scavenging on mosquitoes, grease was applied to the suspension twines. Mosquitoes were provided with 10% sucrose solution and blood meal every day for 20 minutes from the human arm (DH). An oviposition substrate consisting of a Petri-dish lined with filter paper disk on wet cotton wool was placed for egg-laying. An oviposition substrate in each cage was examined daily for the presence of eggs and the number of eggs laid was examined under a dissecting microscope, counted, and recorded. The cages were examined daily for the numbers of surviving and dead mosquitoes. Then, the dead mosquitoes were recorded and removed from the cage daily.

Plate 6.2: Roof structure from which cages with adult *An. gambiae* s.l mosquitoes suspended.

6.2.6. Microclimate data collection

For the larval survivorship experiment, HOBO data loggers (Onset Computer Corp., MX2202, Bourne, MA) were placed in each washbasin, 1 cm below the water surface, and then hourly water temperature and light intensity were recorded for the entire experiment duration. For the adult survivorship experiment, HOBO data logger (Onset Computer Corp., MX2301, Bourne, MA) was kept nearby the experiment setup at 2 m above ground and then the hourly ambient temperature, relative humidity, and light intensity were recorded during the entire duration of the experiment.

6.2.7. Data analysis

The pupation rate of *An. gambiae* s.l larvae was calculated as the proportion of first instar larvae that developed into pupae. Mean larval-to-pupal development time was calculated. Kaplan-Meier survival analysis was performed to determine the variation in daily survivorship of mosquitoes placed in two different land use and land cover areas. A log-rank test was used to determine the significance of difference between the two survival curves. Daily average, minimum, and maximum temperature, relative humidity, and light intensity were calculated from the hourly record data to determine the effect of different land use and cover on the microclimate of local niches where mosquitoes were tested for survivorship. Independent sample t-test was performed to compare pupation rate, development time and microclimate differences across irrigated and non-irrigated areas. The analyses was performed using IMB SPSS Statistics 25, R 3.5.2, and Microsoft Excel 2016.

6.3. Results

Around 300 blood-engorged, *An. gambiae* s.l were collected from indoor and outdoor (cow shelter) using aspirators. Eight hundred first instar larvae hatched from the field-collected mosquitoes were introduced to the experiments in both irrigated and non-irrigated areas, 400 each.

6.3.1. Survivorship and development time of *An. gambiae* sensu lato larvae

The proportion of larvae that completed development from first instar larvae to pupae in irrigated area and non-irrigated area was 79.4 % (95%CI: 0.66 – 0.93) and 84.5% (95%CI: 0.77 – 0.92), respectively. Statistical analysis showed that the difference in pupation rate was not significant between irrigated and non-irrigated areas (T-test = 2.22, p = 0.208) (Fig. 6.1). The mean larval-to-adult development time of *An. gambiae* s.l larvae in irrigated area and the non-irrigated area was 12.5 and 12 days, respectively. Similarly, the median larvae-to-pupae development time in irrigated was 12.5(95%CI: 10.2 – 14.8) days and at non-irrigated area 12(95%CI: 9.7 – 14.2) days. Kaplan-Meier survival analysis showed no significant difference in larval survivorship between the two areas ($\chi 2$ = 2.62, p = 0.106) (Fig. 6.2).

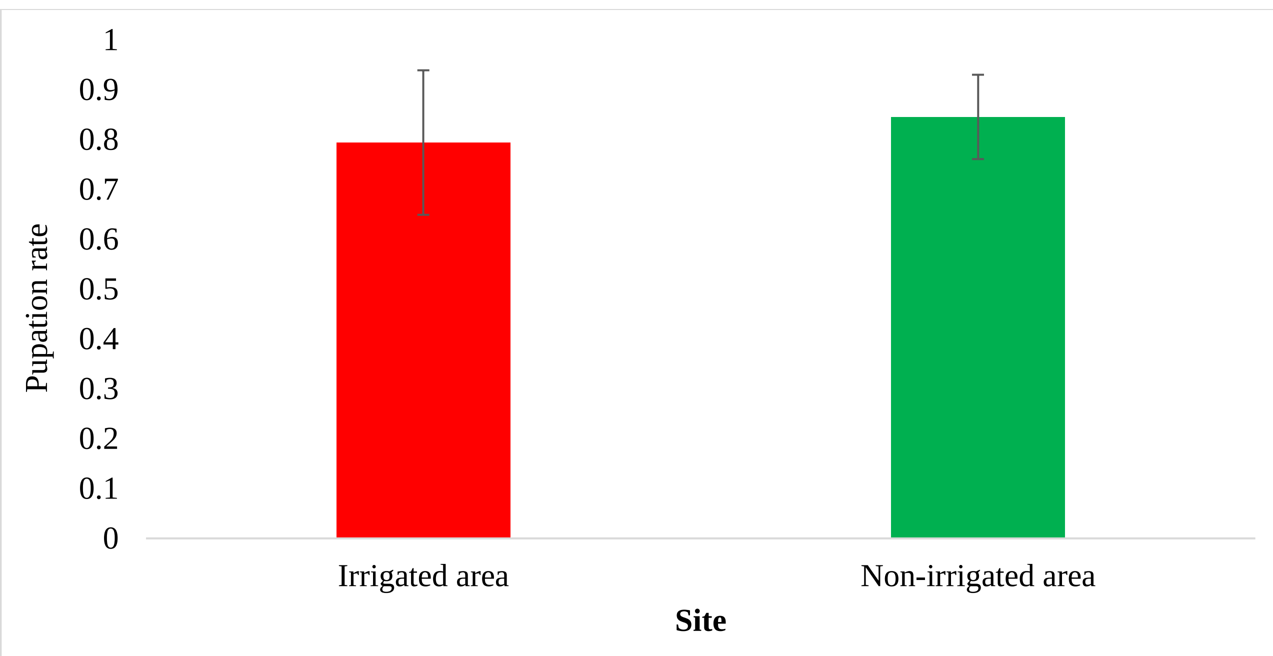

Figure 6.1: Pupation rate of *An. gambiae* s.l larvae in irrigated and non-irrigated areas, Southwest Ethiopia, 2019.

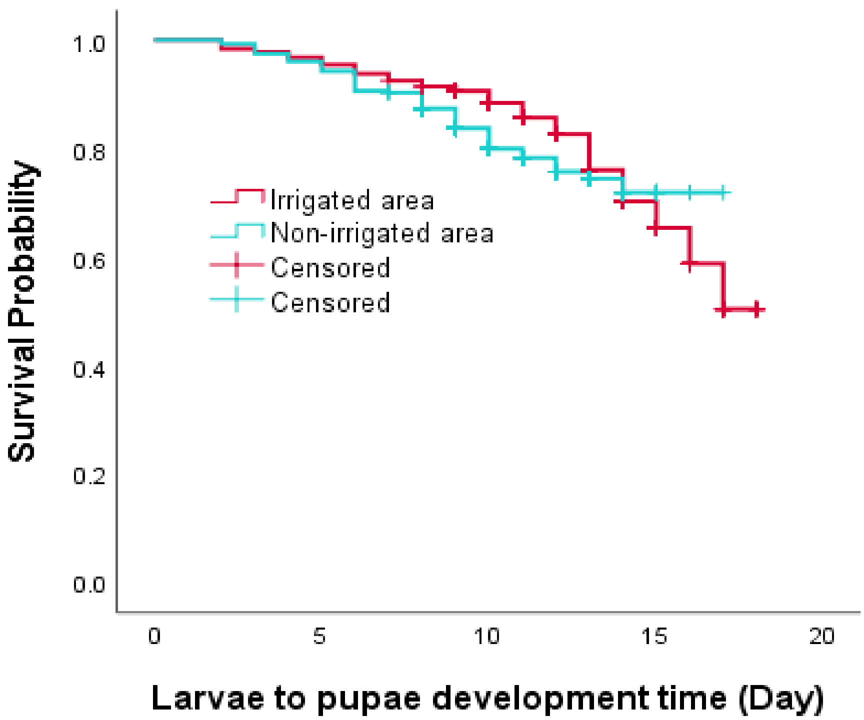

Figure 6.2: Survivorship of *An. gambiae* s.l larvae in irrigated and non-irrigated areas, Southwest Ethiopia, 2019.

6.3.2. Adult *An. gambiae* sensu lato survivorship and fecundity

Survival analysis showed that female *An. gambiae* s.l placed at the irrigated area were survived significantly longer than those in the non-irrigated area ($\chi2 = 18.3$, d.f. = 1, $p < 0.001$) (Fig. 6.3). The estimated mean survival time of female *An. gambiae* s.l in the irrigated and non-irrigated area was 37.9 and 31.3 days, respectively. Again, female mosquitoes showed a higher median survival period (41.0 days) in irrigated than non-irrigated area (31.0 days). A similar result was found that male *An. gambiae* s.l survived longer in irrigated area than non-irrigated area ($\chi2 = 23.1$, d.f. = 1, $p < 0.001$) with the mean survival time of 31.8 and 24.2 days, respectively (Fig. 6.3). The median survival period for male mosquitoes was 33.0 days in irrigated area and 24.0 days in non-irrigated area (Table 6.1). Male *An. gambiae* s.l survival was decreased compared to females at both irrigated ($\chi2 = 14.9$, $p < 0.001$) and non-irrigated areas ($\chi2 = 20.9$, $p < 0.001$).

Table 6.1: Means and medians of survival time for adult *An. gambiae* s.l in irrigated and non-irrigated areas, Southwest Ethiopia, 2019.

	Female *An. gambiae* s.l		**Male *An. gambiae* s.l**	
Site	Mean with 95%CI	Median with 95%CI	Mean with 95%CI	Median with 95%CI
Irrigated area	37.9 (34.8 – 41.5)	41.0 (35.9 – 46.1)	31.8 (28.9 – 34.7)	33.0 (28.3 – 37.7)
Non-irrigated area	31.3 (28.5 – 34.1)	31.0 (27.9 – 34.1)	24.2 (21.8 – 26.6)	24.0 (20.3 – 27.6)

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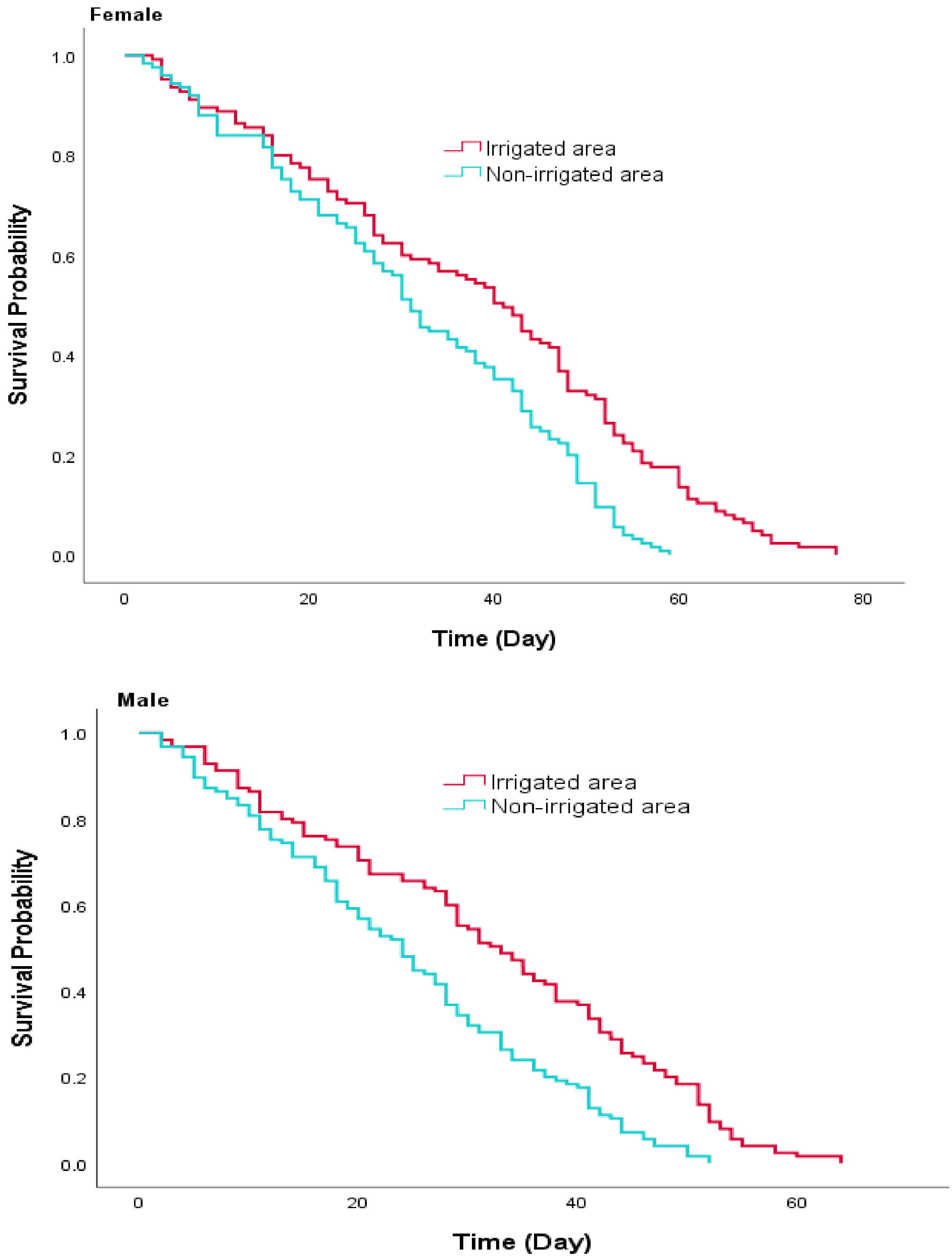

Figure 6.3: Survivorship of adult *An. gambiae* s.l in irrigated and non-irrigated area, Southwest Ethiopia, 2019.

Of 7,737 eggs laid by the female mosquitoes throughout the experiment period, 5,125 (66.2%) were from the mosquitoes placed in irrigated area and 2,612 (33.8%) were from mosquitoes in the non-irrigated area. The study showed that the fecundity of mosquitoes was 96.2% higher in the irrigated area (80 eggs/day) than in the non-irrigated area (average 33 eggs/day). The mean number of eggs laid was (41±S.E. 11.63 eggs/mosquito) and (21±5.61 eggs/mosquito) in irrigated area and non-irrigated area, respectively. Statistical analysis showed that the difference in fecundity was significant between irrigated and non-irrigated area (T-test = 2.83, P = 0.002).

6.3.3. Aquatic habitat microclimate during larval survivorship experiment

An independent sample t-test analysis on microclimate difference between two study settings indicated that mean hourly water temperature (°C) in washbasins placed at field crop area was by 1.1°C higher than washbasins in sugarcane plantation area (T-test = -2.85, p = 0.004). Similarly, mean light intensity (lum/ft2) in washbasins placed at non-irrigated area (Mean = 497.4±982.2) was significantly higher than in washbasins at irrigated area (Mean = 372.7± 664.8), (T-test = -2.47, p = 0.014) (Fig. 6.4 & Table 6.2). Mean maximum and minimum temperature and light intensity were also significantly higher in washbasins at non-irrigated area compared to irrigated area (Fig. 6.4).

Table 6 2: Mean hourly temperature and light intensity in washbasins in irrigated and non-irrigated areas, Southwest Ethiopia, 2019.

Microclimate	**Irrigated area (M±S.E.)**	**Non-irrigated area (M±S.E.)**	**t**	**d.f.**	**p**
Mean temperature (°C)	23.3±5.7	24.4±6.3	-2.85	1068	0.004
Mean maximum temperature (°C)	24.4±6.5	25.4±7.2	-2.53	1068	0.012
Mean minimum temperature (°C)	22.5±5.1	23.4±5.4	-2.83	1068	0.005
Mean light intensity (lum/ft^2)	372.7± 664.8	497.4±982.2	-2.47	1068	0.014
Mean maximum light intensity (lum/ft^2)	713.0±1311.7	931.0±1698.0	-2.28	1068	0.018
Mean minimum light intensity (lum/ft^2)	174.4±311.9	229.1±495.5	-2.21	1068	0.027

M±SE, Mean ± Standard Error

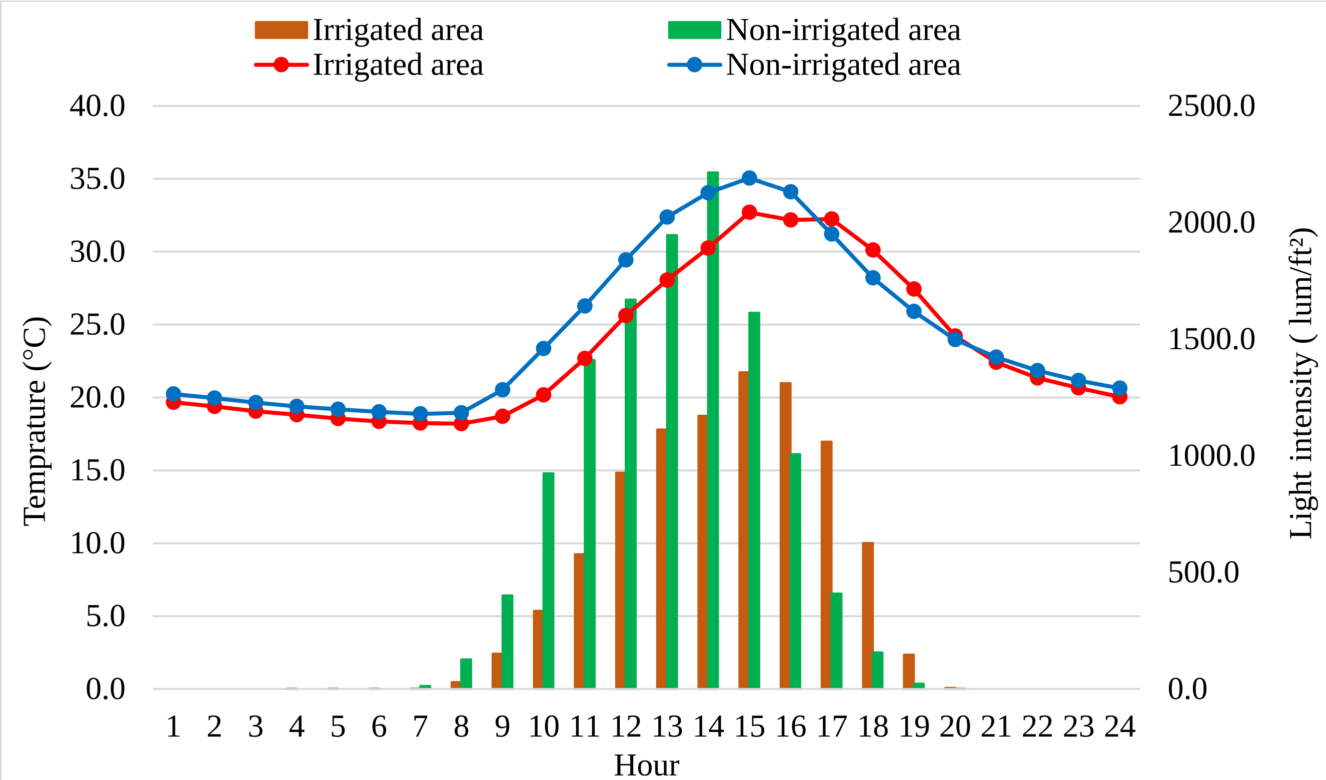

Figure 6.4: Mean hourly temperature and light intensity 24-hour daily cycle in washbasins in irrigated and non-irrigated areas, Southwest Ethiopia, 2019.

6.3.4. Ambient microclimate during adult survivorship experiment

An independent sample t-test showed that there was no difference in ambient hourly average, maximum and minimum temperature and relative humidity between irrigated area and non-irrigated area. However, there was a significant difference in light intensity between the two sites ($p = 0.001$) (Fig. 6.5 & Table 6.3).

Table 6 3: Hourly microclimate condition of mosquito niches in irrigated and non-irrigated areas, Southwest Ethiopia, 2019.

Microclimate	Irrigated area (M±S.E.)	Non-irrigated area (M±S.E.)	t	d.f.	p
Mean temperature (°C)	21.56±4.80	21.60±4.81	-0.26	3176	0.790
Mean maximum temperature (°C)	22.22±5.09	22.24±5.10	-0.09	3176	0.927
Mean minimum temperature (°C)	20.90±4.56	20.92±4.56	-0.12	3176	0.904
Mean relative humidity (%)	82.65±15.73	82.30±14.58	-0.63	3176	0.522
Mean maximum relative humidity (%)	86.11±13.77	86.55±11.80	-0.92	3176	0.339
Mean minimum relative humidity (%)	78.95±17.78	78.12±17.23	1.31	3176	0.187
Mean light intensity (lum/ft^2)	324.3±517.5	709.0±1242.3	-11.7	2952	0.001
Mean maximum light intensity (lum/ft^2)	571.7±982.5	1106.8±1834	-10.3	2952	0.001
Mean minimum light intensity (lum/ft^2)	180.1±267.5	366.6±663.4	-10.7	2952	0.001

M±SE, Mean ± Standard Error

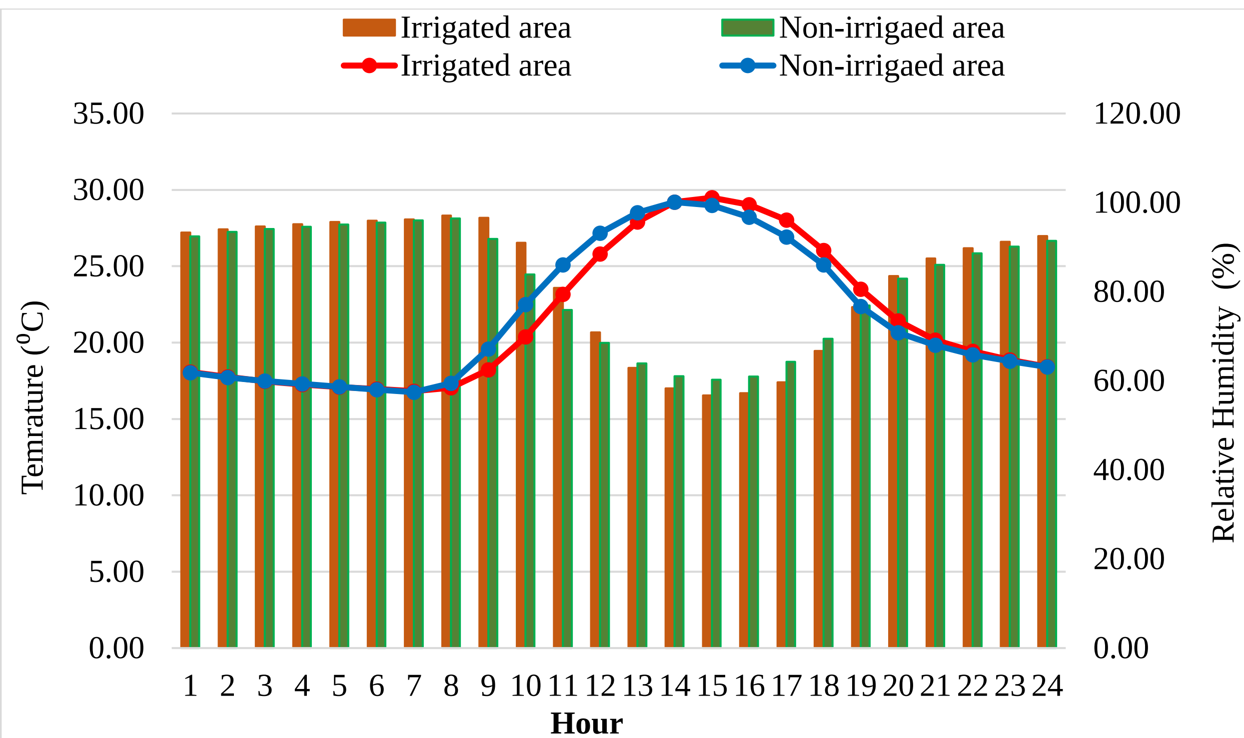

Figure 6.5: Mean hourly ambient temperature and relative humidity 24-hour daily cycle in irrigated and non-irrigated areas, Southwest Ethiopia, 2019.

6.4. Discussion and Conclusion

In this study, the effects of environmental modification on the development, survivorship, and fecundity of malaria vector mosquitoes was investigated. It is hypothesized that irrigated area enhances development, survivorship and fecundity compared to non-irrigated area due to better microclimate and nutrients following environmental modification. However, the study showed no significant difference in the development and survivorship of *An. gambiae* s.l immatures between the two areas.

Variation in vegetation cover may affect the radiation flux and energy balance of the land surface and thus may modify the microclimate [242]. By the time experiment was conducted, the sugarcane plantation was at its maturity stage, which is dense and leafy, which could partly limit direct sunlight from reaching the washbasins, whereas the surrounding crops field were relatively less dense. The mean hourly water temperature in the non-irrigated area increased by 1.1°C compared to irrigated area and higher light intensity in non-irrigated compared to irrigated area. This could partly explain the observed 5.1% more pupation rate in the non-irrigated area compared to irrigated area. Studies reported elsewhere indicated that an increased temperature due to land use and cover change increased larval survival rate [17,19,237,243–245]. Tuno et al. [244] reported that the survivorship of *An. gambiae* larvae was reduced from 56% in habitats fully exposed to sunlight to 1.5% in habitats with forest canopy coverage in western Kenya. Wang et al. [237] also reported pupation rate of *An. minimus*, malaria vector in China, to be 52.5%, 12.5% and 3.8% in the deforested, banana plantation and forested areas, respectively, which is far lower than present findings, 79.4% and 84.5% at irrigated and non-irrigated areas, respectively.

Nutrient availability may affect the survival, pupation rate and developmental time. The potential food source of *Anopheles* larvae may include but not limited to bacteria, fungi, debris and organic matter. The abundance and structure of microbes such as algae and photosynthetic cyanobacteria in aquatic habitats may have changed in response to land use and cover [246,247]. Organic matters and debris in the soil at different settings may not be the same, which could possibly vary with change in surrounding land use and land cover. Kebede et al. [248] reported that maize pollen provides nutrition for larval Anopheline mosquitoes showing that the incidence of malaria was about 10 times higher in high maize-cultivation areas. In the case of this study, the debris of sugarcane plantations and other field crops might not be the same but the result showed both areas are supporting mosquito development, which needs further investigation of a soils' biological and chemical composition in relation to mosquito immatures development.

The higher pupation rate and longer survivorship of *An. gambiae* s.l immatures generally could increase vectorial capacity to enhance malaria transmission. Based on this finding alone, we cannot conclude that the irrigated area is encountering less or equal malaria risk compared to surrounding environs. In the irrigated area, significantly more diverse breeding sites and larvae abundance has been observed compared to its surrounding (Chapter 5). Thus, more diversified breeding sites with a 79.4% pupation rate could certainly overweigh the malaria burden over surrounding environs with less habitat diversity and relatively the same pupation rate.

Adult *An. gambiae* s.l placed in the irrigated area survived longer than those in the non-irrigated area. Adult female mosquitoes survived longer than males at both settings. The findings of mosquito longevity was in line with previous studies elsewhere. For instance, Okech et al. [249] reported mean survival of 33 days for *An. gambiae* s.l mosquitoes in western Kenya, which is 6 days shorter than this finding. Gary and Forster [250], found that *An. gambiae* s.l mosquitoes had a median survival time of 29 days under insectary conditions, but in this study, the median survival time for female *An. gambiae* s.l was 41 and 31 days at irrigated area and non-irrigated area, respectively. The longer survival of mosquito in the irrigated area indicates that *An. gambiae* s.l is well adapted to the environmental conditions. Enhanced survival of malaria vector is among the determinants of increased mosquito vectorial capacity [251]. A long life of an adult female mosquito increases her opportunities to encounter an infected human host, and the extrinsic incubation period of malaria parasites so that they can reach the salivary glands after an infective blood-meal, and be transmitted in later blood-meals to uninfected hosts [70,232,252]. This has an implication for malaria transmission at the locality.

The experiment set-up at both study settings made the same and human blood and sugar were provided in the same fashion. Thus, the only difference was the environment where the experiments were situated, being irrigated area and non-irrigated area. There was no significant difference in mean, maximum, and minimum hourly ambient temperature; and relative humidity between the two environments. Previous studies indicated that *An. arabiensis*, a primary vector in Ethiopia, generally prefers areas with low humidity and high temperature [253]. A similar study also demonstrated that reduced humidity and increased temperatures following deforestation create a more suitable environment for adult *An. arabiensis* to survive longer [243]. Therefore, in the study setting the determinants involved in supporting better survival of adult *An. gambiae* s.l at irrigated area warrants further investigation.

The average daily fecundity of *An. gambiae* s.l mosquitoes in the irrigated area was 96.2% higher compared to non-irrigated area. Increased survival together with enhanced fecundity of malaria vector in irrigated sugarcane plantation area suggests that the longevity and biotic potential of *An. gambiae* s.l in the area is very high, favoring increased population density and thus the species could contribute greatly to malaria transmission. Better survival and fecundity in the irrigation area in this study are in agreement with the study conducted in Ethiopia at the laboratory level demonstrating that gravid *An. arabiensis* females attracted to sugarcane pollen volatiles [254].

This study has several limitations. The experiment was done at a one-time point of the maturity stage irrigated sugarcane plantation. The microclimate conditions in irrigated area during the seedling/germinating stage, tillering stage, grand growth stage and maturity stage [255] could not be the same, which in turn influenced the mosquito survivorship. The information on chemical and nutrient composition of the soil used as a substrate was not included in the study. Moreover, the experiments were conducted under controlled conditions for all potential biological factors that may influence mosquito survival, such as predators and competitors, which might lead to overestimated survival time than actual.

In conclusion, irrigated sugarcane plantation significantly enhances the survivorship and fecundity of adult *An. gambiae* s.l, the major malaria vector in Ethiopia. The study results on survivorship parameters of malaria vector mosquitoes under a variety of environmental conditions are helpful to model the impact of environmental modification on vector population dynamics and help devise tailor-made vector control strategies. Moreover, longer survivorship of adult mosquitoes in sugarcane plantation area calls for larval management to reduce the vector population and subsequent malaria transmission.

CHAPTER 7: GENERAL DISCUSSION, CONCLUSIONS AND RECOMMENDATIONS

7.1. General Discussion

To alleviate poverty, many developing countries in sub-Saharan Africa have been experiencing rapid land use and land cover change. Water resource development projects including irrigated agriculture and dam construction are the common environmental modification that are blamed to increase malaria transmission risk [23]. To measure the impact of such development projects on vector-borne disease transmission, the baseline information on disease epidemiology and vector entomological profile need to be understood to plan tailor-made interventions. By doing so, any change in disease epidemiology and vector bionomics as a consequence of environmental modification can be detected and controlled before causing a devastating impact on public health.

This study generated epidemiological and entomological information on malaria transmission around irrigated agricultural areas in southwestern Ethiopia. The study examined the malaria transmission dynamics, malaria vectors aquatic ecology; and their development, survivorship, and fecundity.

The ten-year malaria transmission pattern demonstrated in this study (Chapter 4) indicated that malaria was a major public health burden in the area. Malaria cases due to *P. falciparum*, *P. vivax* and their mixed infection accounted for 48, 42.4 and 9.6% of the cases, respectively, which is not consistent with the national malaria parasite distribution pattern and some other studies in the country that showed the dominance of *P. falciparum* over *P. vivax* [58,200,213]. In four years of study, the *P. vivax* has become predominant over *P. falciparum*. The national estimation indicates the average distribution in the country as a whole, while this study is limited to a small malaria-endemic setting, which could be a possible reason for the inconsistence of parasite proportion. Intervention focus on *P. falciparum* and climate variability due to environmental modification at the area might be a reason for observed trend shift [42,202].

The results indicate a higher positivity rate of malaria among males than females and the majority of cases were adults age 15 year and above. Similar findings were reported in previous studies [190,200,204,205]. The livelihood of the studied community is depending on farming and most of the time males in the reproductive age group are engaged in farming activities at night time during which they are more likely exposed to infective mosquito bites, which could be a possible reason for more malaria in the adult male population. Another probable reason for more malaria burden among the older age group might be due to dropping transmission at the area. Recent studies supported a view that adults are emerging as a population at increased risk of malaria probably due to the declining antimalarial immunity that follows decreased exposure to parasites which could be a new challenge for elimination [208,210]. As control interventions could induce change in malaria epidemiology like sex and age structure, the control strategies needed to be timely updated to contemporary epidemiological context to be able to respond to over-time transmission dynamics [211]. It has also been claimed that at high transmission settings, children acquire immunity and so are not susceptible to disease when they get older. On the other hand, at low transmission, like this study setting, there is less disease among younger children, and consequently, older children acquire less immunity and remain susceptible [207–209]. Other potential explanations for increasing malaria cases among adult could be a preferential LLIN use for younger children.

This study also showed a successive declining pattern in malaria prevalence in the Arjo area. However, in recent years it showed a slight rise, which calls revisit the existing interventions to enhance malaria elimination efforts while increasing irrigated agricultural development. The increased proliferation of mosquitoes breeding habitats and enhanced adult mosquito longevity and fecundity at irrigated sugarcane plantation area observed in this study might be contributing to observed prevalence rising [16,41,47,203]. Thus, evidence-based monitoring of any change in diseases epidemiology needs to be there.

Secondary data from health facilities was used just to describe the existing malaria epidemiology, despite its drawback that sometimes might have accuracy problems. Since no other report of malaria epidemiology at the area, the information generated by this study will serve as baseline information to monitor potential future malaria dynamics as the change in land use get intensified in the future.

In the epidemiological study, ten-year data was used for analysis. In Ethiopia, it is assumed that malaria epidemics usually happen between five to eight-year. Using ten-year data in the study helps understand the long time dynamic in disease epidemiology at the area. All health facilities in and around Arjo-Dedessa irrigation scheme were included in the study which reduces the sampling bias of the study.

The primary goal of this study was to evaluate the impact of environmental modification on malaria transmission risk through investigating entomological indicators. Chapter 5 presented *Anopheles* mosquitoes' aquatic ecology with respect to different agroecosystems. The study demonstrated that the diversity of *Anopheles* mosquito breeding habitats in the irrigated area was two-fold higher than the non-irrigated area, indicating that the irrigation development has been contributed to the proliferation of malaria mosquito breeding habitats. In line with breeding habitat diversity enhanced larvae occurrence, and abundance was observed in irrigated sugarcane area. This finding is in consistence with the findings from studies elsewhere in Africa that suggested changes in land use have influenced malaria vector larval habitat availability and distribution [20,47,222].

The environmental modification due to irrigated sugarcane farming process is attributed to an increase in vector breeding sites proliferation, larval abundance, and occurrence compared to the non-irrigated area. Increased breeding habitats obviously will end in increased malaria vector density and eventually increased malaria transmission intensity [223,224]. A study conducted in western Ethiopia reported higher malaria prevalence and increased transmission risk due to high vector abundance in the irrigated sugarcane agroecosystem than non-irrigated agroecosystem [137].

Identifying vector-breeding habitats is important to target them for larval management. Most of the mosquito breeding habitats identified in this study were previously reported elsewhere [220,225,226]. However, the nature and formation of some habitats made them specific and unique to the study area and thus can be a target for intervention. In Africa, larval source management is very effective in areas where mosquito breeding habitats are distinct and accessible [228]. Studies showed that when larval management is integrated with LLINs and IRS, a great improvement would be seen in malaria control efforts than IRS and LLINs alone [131,229]. Lack of proper environmental management while implementing water resource development projects has been noted in Africa [23,137,199].

A higher *Anopheles* mosquito species composition was recorded in the irrigated area compared to non-irrigated area. This implies that the environmental conditions in the irrigated area have been favoring diversity of mosquito species, which in turn may have a vector control implication. The variation in environmental conditions may have an influence on mosquito larval habitat productivity, larval occurrence and abundance. However, the specific environmental parameters inducing such variation have not been addressed, which is the limitation of the study.

In this study, *Anopheles* aquatic ecology survey was done both in dry and wet seasons which helps understand the seasonal distribution and diversity of *Anopheles* aquatic habitats to target them in larval habitat management. A comparative approach of irrigated sugarcane plantation and non-irrigated area could helps measure the impacts of environmental modification on *Anopheles* breeding habitat diversity, larval occurrence and abundance.

Malaria vectors development and survivorship are other entomological indices of malaria transmission risk, which are sensitive to environmental modification. Chapter 6 presented the evaluation of the effects of environmental modification on the development and survivorship of malaria vector mosquito population. In line with the hypothesis, adult *An. gambiae* s.l mosquitoes placed in the sugarcane plantation area lived longer compared to the non-irrigated crop field area. The mosquito longevity result of this study is longer compared to previous studies elsewhere. Okech et al. [249] reported mean survival of 33 days for *An. gambiae* s.l mosquitoes in western Kenya, which is 6 days shorter than our finding. Similarly, Gary and Forster [250] found that *An. gambiae* s.l mosquitoes had a median survival time of 29 days under insectary conditions, which is 12 days shorter compared to this study. The longer survival of mosquito at the irrigated area indicates *An. gambiae* s.l is well adapted to the environmental conditions.

Measurement of female mosquito vector survival is an important biological determinant of malaria transmission intensity [256,257]. A long-lived adult female mosquito increases its opportunities to encounter an infected human host and successfully live through the extrinsic incubation period of malaria parasites so that the parasite can reach salivary glands after an infective blood-meal and become infectious [70,232,251,252]. Malaria parasites require more than 10 days of incubation inside female mosquito vectors (extrinsic incubation period) before they become infectious [25,71,258,259]. While there is uncertainty about mosquito survival in the field, crude estimates suggest the median lifespan of African malaria vectors is 7–10 days [260]. Thus, only relatively old mosquitoes can transmit the parasite [261]. As a result, even minor reductions in mosquito survival can have exponential impacts on parasite transmission [258]. Accordingly, accurate estimation of both mosquito abundance and longevity is essential for the assessment of the impact of various vector control measures.

The generational life-table experiment was done to study the development and survivorship of *An. gambiae* s.l. Examination of ovarian trancheoles structure is one of the methods that has been widely used to determine the physiological age of malaria vectors [262–264]. It is assumed that after each egg-laying, dilatation is observed at the region of follicular tube where the egg originated from. In theory, the number of dilatations in the ovarioles must equal the number of egg-laying in female mosquitoes, which is an indicator of physiological age. This method gives an indirect estimate of mosquito physiological age. In this study generational life-table experiment was used which gives direct estimate of age of mosquitoes.

During the survivorship study, the microclimate data of the area where the experiments were set was collected and no significant difference in hourly ambient temperature and relative humidity were observed between the two settings. However, previous studies demonstrated that decreased humidity and increased temperatures following deforestation create a more suitable environment for adult *An. arabiensis* to survive longer [243,253]. Therefore, for a better understanding of exactly which environmental parameters are contributing to the observed survival difference between irrigated areas and non-irrigated area further investigations are warranted.

In this study, average daily fecundity was also found to be enhanced in irrigated area than non-irrigated area. Thus, the result suggests augmented reproductive and growth performance of *An*. *gambiae* s.l in the irrigated area and thus contribute to the species success for malaria transmission. The findings complement the study conducted in Ethiopia at the laboratory level demonstrating gravid *An. arabiensis* females attracted to sugarcane pollen volatiles [254].

The study showed no significant difference in survivorship of *An. gambiae* s.l immatures between the two settings. During the immature survivorship experiment, the mean hourly water temperature in the non-irrigated area increased by 1.1°C compared to irrigated area. This result partly could explain the observed 5.1% more pupation rate in the non-irrigated area compared to the irrigated area. Vegetation cover disparity may affect the radiation flux and energy balance of the land surface and thus may modify the microclimate [242]. By the time of experiment, sugarcane plantation was at the maturity stage, which is dense and leafy that partly could limit direct sunlight reach to washbasins, whereas the surrounding field crop area was relatively less dense. In consistence with this study, increased larval survival rate with increased temperature due to land use and cover change was reported from studies elsewhere. For instance, Tuno et al. [244] demonstrated that the survivorship of *An. gambiae* larvae was reduced from 56% in habitats fully exposed to sunlight to 1.5% in habitats with forest canopy coverage in Western Kenya. Wang et al. [237] also reported an enhanced pupation rate in the deforested area compared to a banana plantation and forested areas for *An. minimus*, a malaria vector in China.

Microclimate conditions are not the only determinant to mosquito immatures development and survivorship. Nutrient availability, predators and competitors also have an incredible role. In present study, the experiments were performed in a confined environment where predators and competitors are controlled but the nutrient availability may affect the survival, pupation rate and development time.

The soil from the respective site was used as a substrate. Organic matters and debris in the soil at different settings might not be the same, which could possibly vary with changes in surrounding land use and land cover change. The debris of sugarcane plantation and other field crops might not be the same that needs further investigation of a soil's biological and chemical composition in relation to mosquito immatures development. Kebede et al., reported that maize pollen provides nutrition for larval *Anopheles* mosquitoes and 10 times higher malaria incidence in massive maize cultivation areas [248]. In this study area, among the field crops, maize was a dominant one. Consequently, in addition to microclimate difference, debris and organic matters in substrate could have augmented increased pupation rate and shorten development time in non-irrigated field crop areas.

In general, having significantly more diverse *Anopheles* mosquito breeding sites and larvae abundance with 79.4% pupation rate and longer adult *An. gambiae* s.l survival positions irrigated sugarcane plantation area at higher risk to encounter malaria compared to its environs.

7.2. Limitation and strength of the Study

Limitation of the study

The study was conducted using different study design, different data sources. However, these studies are not immune from methodological limitations:

- Epidemiological study was done using secondary data records from health facilities that might have a problem. Cases in the community that were not visited health facilities seeking treatment are not included in the analysis. If all cases in the community were included, the observed trend might have been changed significantly. The inaccuracy and missing of data are other limitations of the retrospective study.
- During larval habitat survey, many small stagnant water bodies might be overlooked, which limits a clear description of habitat diversity and distribution. This is one of the common challenges in entomological study, particularly aquatic ecology. In this study, very few physical parameters were assessed to characterize larval habitat. There are many more physical, chemical and biological parameters, which can determine the occurrence and abundance of larvae. Incorporating the biological and chemical characteristics of larval habitats would give a more clear image of different land use pattern's impact on malaria vector aquatic ecology. Microclimate data is the potential determinant of larval occurrence and habitat productivity. However, it was also not included in the

analysis of larval ecology study. Integrating microclimate data in to analysis would help understand more the effect of environmental modification in the occurrence and abundance of *Anopheles* larvae.

- Generational life-table experiments were done in the wet season. The response of the mosquito population to different seasons could not be the same. Further study of including various seasons is needed to have a complete understanding of the impacts of environmental change on the development and survivorship of malaria vectors in the area. In addition, sugarcane should pass through four stages of growth before harvesting. This study was done during the maturity stage. The surrounding microclimate could not be the same throughout all growth stage, which in turns influence development and survivorship of malaria vectors. It would be better if the data were collected in different seasons and through all development stages of sugarcane plantations. The information on chemistry and nutrient's composition of a soil used as a substrate was also not investigated. Moreover, the experiments were conducted in a controlled manner of all possible biological factors that could influence mosquito survival rate like predators and competitors, which might lead to overestimated survival rate than actual.
- One of the limitations of my Ph.D. research was that the study did not include adult mosquito behavior and their malaria transmission potential with respect to water resource development practice.

Strength of the study

- In epidemiological study, ten-year data was used for analysis. In Ethiopia, it is assumed that malaria epidemics usually happen between five to eight year. Using ten-year data in the study helps understand long time dynamic in disease epidemiology at the area. All health facilities in and around Arjo-Dedessa irrigation scheme were included in the study which reduces the sampling bias of the study.

- Entomological study was conducted by using both repeated cross-sectional and experimental study design. In *Anopheles* aquatic ecology study, the survey was done both in dry and wet seasons which helps understand seasonal distribution and diversity of *Anopheles* aquatic habitats to target them in larval habitat management. Comparative approach of irrigated sugarcane plantation and non-irrigated area could helps measure the impacts of environmental modification on *Anopheles* breeding habitat diversity, larval occurrence and abundance.

- The generational life-table experiment was done to study the development and survivorship of *An. gambiae* s.l. Examination of ovarian trancheoles structure is one of the methods that has been widely used to determine physiological age of malaria vectors [259–261]. It is assumed that after each egg-laying, dilatation is observed at the region of follicular tube where the egg originated from. In theory, the number of dilatations in the ovarioles must equal to the number of egg-laying in female mosquitoes, which is an indicator of physiological age. This method gives indirect estimate of mosquito physiological age. In this study generational life-table experiment was used which gives direct estimate of mosquitoes age.

7.3. Conclusions

- Despite a declining trend of the overall positivity rate, malaria remains public health burden in the area, which needs attention for further intensified interventions in line with the irrigated agriculture expansion. The data demonstrated that in four study years, *P.vivax* showed a predominance over *P. falciparum*, which is the peculiar result, indicating a shift in malaria parasite trend. The age group of 15 years and above was more affected and males were more affected than females. Although there was significant seasonal variation, malaria cases were reported year-round in the area. Therefore, malaria interventions should be strengthened to sustain control and move towards elimination in such water resource development project areas.

- *Anopheles* mosquito breeding habitat diversity, positivity, and abundance were found to be higher in the irrigated area than non-irrigated areas during both dry and wet seasons. The results suggest that irrigated agricultural development activities augment the proliferation of aquatic breeding habitats for malaria vector mosquitoes that may lead to a higher risk of malaria transmission to the surrounding community. Identifying major malaria vector breeding habitats helps devise tailor-made interventions such as larval source management to reduce the risk of malaria around irrigation schemes.

- The study results demonstrated that irrigated sugarcane plantation significantly enhances the survivorship of adult *An. gambiae* s.l and fecundity. The findings on development, survivorship and fecundity of malaria vector in different agroecosystems is helpful to model impact of environmental modification on vector population dynamics and support to develop tailor-made vector control strategies. Since vector survivorship is a critical factor in the mosquito–malaria cycle, generational life-table study is expected to be a useful tool in malaria early warning systems for the purpose of forecast, early detection of epidemics, and intensity of disease prevalence in endemic areas.

- Overall, having significantly and highly diverse *Anopheles* mosquito breeding sites and larvae abundance with 79.4% pupation rate and longer adult *An. gambiae* s.l survival with enhanced fecundity put irrigated sugarcane plantation area at higher risk to encounter malaria. This calls for the need for larval management to reduce the vector population and consequently malaria transmission.

7.4. Recommendations

Operational

a) Malaria interventions should be strengthened to sustain control and move towards elimination in such water resource development project corridors.
b) Introduction of sound monitoring and surveillance systems proximal to such irrigation scheme area would facilitate the systematic evaluation of the overtime impact of environmental modification on the bionomic of malaria vector and eventually malaria transmission. This would greatly improve our understanding of the role of an irrigation scheme in either promoting or reducing malaria transmission.
c) Routine larval source management should be integrated with other vector control strategies to help reduce the malaria burden in the area.
d) Targeting major *Anopheles* breeding habitats might enable more efficient use of available resources to control malaria through larval source management.

For policy

a) Area-specific vectors monitoring and surveillance systems could be designed in line with environmental modification.
b) Supplementary interventions could be implemented in the area nearest to the irrigation scheme.

c) Local vector survivorship and fecundity profile could be considered to maximize the impact of interventions.

Future research prospective

a) The information generated by this study will serve as baseline information to monitor potential future malaria dynamics as the change in land use get intensified in the future. Future studies should document the change in malaria transmission as the irrigation structure become fully operational at full capacity.
b) The impact of larval source management, in combination with LLINs and IRS, needs to be evaluated at irrigation scheme.
c) *Anopheles* mosquito development, survivorship, and fecundity need to be investigated in different seasons and all development stages of sugarcane plantation. In addition, for a better understanding of exactly which environmental parameters are contributing to observed survival and fecundity variance between irrigated and non-irrigated areas further investigations are needed.
d) The relationship between environmental modification and biological and chemical properties of mosquito aquatic habitats needs to be studied explicitly.
e) The biological and chemical composition of the soil at irrigated sugarcane plantation area in relation to mosquito immatures development needs to be studied.
f) The pupation rate and productivity of field *Anopheles* breeding habitats in relation to land-use and cover pattern needs to be studied
g) The stability of *Anopheles* mosquito breeding habitats in relation to land use pattern needs to be examined.

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ANNEX

Annex I: CV of Ph.D. candidate

Personal Information	
	Dawit Hawaria Yirgalem, 184, Ethiopia +251916044383 hawaria.dawit@gmail.com Sex: male | Date of birth 10/01/1988 | Nationality : Ethiopian
Work Experience	
Oct.2013 – Present	Ast. Professor, Yirgalem Hospital Medical College, Yirgalem. In addition to teaching I have also worked in varies responsibilities and duties: 1. Currently, Yirgalem Hospital Medical College Vice provost for Research and Community Service 2. Have been worked on the position of Yirgalem Hospital Medical College Vice Provost for Development & Administrative affairs 3. Coordinator Public Health department and Community Based Education
Feb. 2013 – Sept.2013	Chief Environmental Health expert, Surveillance coordinator, Arbegona District, SNNPR, Ethiopia
Nov. 2009 – Dec. 2011	Environmental health expert, Disease Prevention and Health Promotion officer, Arbegona District, SNNPR, Ethiopia.
Education Background	
Since 2016 – Now	Ph.D. fellow in Tropical and Infectious Diseases, Jimma University, Ethiopia
2011 – 2013	Master's degree of Environmental Health, Jimma University, Ethiopia
2007-2009	Bachelor degree of Environmental Health, Wolaita Sodo University, Ethiopia

Personal Skills	
Organizational /managerial skills	- Vice provost, Coordinator of social and population health department and community based education, Yirgalem Medical College, Ethiopia. - Coordinator of district public health surveillance activities, Arbegona District
Computer skills	Microsoft office
Other skills (software)	I have also an experience of using statistical tools: SPSS, ArcGIS, EndNote, Bioinformatics tools.

Publications

1. **Hawaria D**, Kibret S, Demissew A *et al,* (2021). Survivorship of *Anopheles gambiae* sensu lato in irrigated sugarcane plantation scheme in Ethiopia. *BMC Parasites and Vectors*; doi: 10.1186/s40249-019-0620-y
2. **Hawaria D,** Demissew A, Kibret S *et al,* (2020). Effects of environmental modification on the diversity and positivity of anopheline mosquito aquatic habitats at Arjo-Dedessa irrigation development site, Southwest Ethiopia. *BMC Infect Dis of Poverty*. 9:9. doi: 10.1186/s40249-019-0620-y
3. **Hawaria D,** Getachew H, Zhou G *et al,* (2019). Ten years malaria trend at Arjo-Didessa sugar development site and its vicinity, Southwest Ethiopia: a retrospective study. *BMC Malar J.* 18:145 https://doi.org/10.1186/s12936-019- 2777-z
4. **Hawaria D**, Santiago RD and Yewhalaw D (2016). Efficient attractants and simple odor-baited sticky trap for surveillance of Anopheles Arabiensis Patton mosquito in Ethiopia. *J infect Dev Ctries;* 10(1):082-089.doi:10.3855/jidc.6841
5. Jiang AL, Lee MC, Zhou G, Zhong D**, Hawaria D**, *et al*, (2021). Predicting distribution of malaria vector larval habitats in Ethiopia by integrating distributed hydrologic modeling with remotely sensed data. Scientific Reports; 11:10150 DOI: 10.1038/s41598-021-89576-8
6. Dabaro D, Birhanu Z, Negash A, **Hawaria D,** Yewhalaw D. (2021) Effects of rainfall, temperature and topography on malaria incidence in elimination targeted district of Ethiopia. BMC *Malar J DOI: 10.1186/s12936-021-03641-1*
7. Demissew A, **Hawaria D**, Kibret S *et al,* (2020). Impact of sugarcane irrigation on malaria vector Anopheles mosquito fauna, abundance and seasonality in Arjo-Didessa, Ethiopia. *BMC Malaria J DOI:10.21203/rs.3.rs-37850/v1*
8. Dona A, Abera M, Alemu T and **Hawaria D** (2018): Timely initiation of postpartumcontraceptive utilization and associatedfactors among women of child bearing agein Aroressa District, Southern Ethiopia: a community based cross-sectional study. *BMC Public health J*; 18:1100

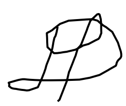
Innovation and Services to the Community at Large

As a great achievement, I had developed a new tool for malaria vector (*anophiline* mosquito) surveillance in Ethiopia. The work has been presented to scientific community through publishing it in international journal and to the general community. Through this work, I have participated in electronic media program (Ethiopia Broadcast Service_ETV) to elucidate the importance of developed tool in malaria prevention effort to the public and how they can use it, which is my the remarkable contribution to the community.

Certificates	1. Certificate of Human Subject Research for Biomedical Investigators under requirements set by University of California, Irvine 2. Certificate of short course completion on Pandemic Preparedness with One Health Approach at University of Heidelberg, Germany 3. Certificate of Merit from Yirgalem Medical College for legislation development 4. Certificate of recognition for presentation of scientific paper at the 29^{th} annual conference of EPHA 5. Certificate of completion of module based comprehensive systematic review training (Module_1) 6. Certificate of completion of module based comprehensive systematic review training (Module_2) 7. Certificate of completion of e-resource and data management prepared by DAAD-ITOCA

Scientific Paper Presentation	1. Attended 28th Ethiopia Public Health Association Scientific Conference held in Harar, February 19-22, 2017. 2. Offered oral presentation on The 8th Malaria Research Symposium held in Addis Ababa, February 17-18, 2017. Presentation topic: "Efficient attractants and simple odor-baited sticky trap for surveillance of *Anopheles* arabiensis Patton mosquito in Ethiopia" 3. Have also recently presented a poster on The 29th Ethiopia Public Health Association Scientific Conference held in Addis Ababa, February 26-28, 2018. Presentation topic: "Spatio-temporal Analysis of Malaria Occurrence in Aleta_Cuko District, Southern Ethiopia: A retrospective study"

Memberships	I am active member of: 1. Ethiopia Public Health Association, Since 2011 2. Ethiopian Environmental Health Professional Association, Since 2017 3. Malaria Research Network, Since 2016 4. Ethiopian Tropical and Infectious Disease Society, Since 2017
References	1. Delenasaw Yewhalaw (PhD), Head, TIDRC, Jimma university Email: delenasawye@yahoo.com 2. Solomon Kibret (Visiting professor), Scientific Director, California Department of Health West Valley Mosquito and Vector Control District, California, USA Email: s.kibret@gmail.com 3. Guiyun Yan (Professor), University of California, Irvine Email: guiyuny@uci.edu 4. Admasu Arsicha (MPH), Provost, Yirgalem Hospital Medical College - Email: admasuarsicha@yahoo.com



Dawit Hawaria

Annex II: Dissertation declaration Form

Letter for Declaration

I, the under signed, declared that this is my original work, has never been presented in this or any other university, and that all the resources and materials used for the thesis have been fully acknowledged.

Name: Dawit Hawaria Logita

Signature: Date: August 2021

This dissertation has been submitted for examination with my approval as **promoter.**

Delenasaw Yewhalaw, Ph.D.
Professor of Medical Entomology
Jimma University, Tropical and Infectious Diseases Research Center (TIDRC)
Jimma, Ethiopia
Phone: (+251)917804352
Email: delenasawye@yahoo.com

Signature: Date: August 2021

Annex III: Mosquito Larval Habitat Survey tool

1. **ID of the site: ____________________**

2. **Collection site**

   (1) Region: _________________ (2) District: ________________ (3) Cluster: _________________

3. **Geographic coordinates:**

   (1) Latitude: _________________ (2) Longitude: ________________(3) Elevation: _________

4. **Habitat Type:**

   (1) Drainage ditch,
   (2) River edge/Reservoir shoreline,
   (3) Swamp/Marshes,
   (4) Puddle (water area < 50 m^2)
   (5) Animal footprint,
   (6) Tire track/Road paddle,
   (7) Natural pond (water area > 50 m^2)
   (8) Man-made pond,
   (9) Natural pond/Rain pool,
   (10) Rock pool,
   (11) Water container,
   (12) Irrigation (concrete lining) Canal/structure,
   (13) Irrigation (earth bottom) Canal/structure,
   (14) Brick pit,
   (15) River/ stream side
   (16) Hippo Trench
   (17) Others____________________

5. **Substrate**: (1) Clay/muddy, (2) Sandy (3) Gravel

6. **Presence of vegetation:** (1) Emergent, (2) Submerse, (3) Floating, (4) Shed

7. **Habitat vegetation coverage in percentage (%): ________________________________**

8. **Turbidity:** (1) Clear (2) Low (3) Medium (4) High

9. **Temperature: ____________**

10. **pH: _________________**

11. **Electrical conductivity: ______________**

12. **Dissolved Oxygen:___________________**

13. Exposure to sunlight: (1) Shaded, (2) Partially Shaded, (3) Sunlit

14. Seasonality: (1) Temporal (2) Sem-Permanet (3) Permanent

15. Land use Type (surrounding environment):

(1) Forest
(2) Shrubland
(3) Grassland/Pasture
(4) Wetland/Swamp
(5) Cultivated land/Cropland
(6) Urban and built-up
(7) Road
(8) Barren/Bare Rock
(9) Water
(99) Others________________

16. Irrigation Method at the area:

(1) Surface (Canal) Irrigation,
(2) Pipe/Sprinkler Irrigation,
(3) Drip Irrigation,
(4) Stream Irrigation,
(5) Other Irrigation________________

17. Crop Type at the area (choice one or more) :

(1) Sugarcane
(2) Teff
(3) Wheat
(4) Barley
(5) Corn/maize
(6) Vegetable
(7) Coffee
(8) Potato
(9) Bean
(10) Rice
(11) Sorghum

18. Distance to nearby House:

(1) Less than 100 meters,
(2) 100 ~ 200 meters,
(3) 200 ~ 500 meters,
(4) No visual houses within 500 meters

19. Habitat Measure:

(1) Length (m): __________(2) Width (m): __________ (3) Depth (m): _________

20. Larvae present: (1) Yes (2) No

If present, record the following information:

Number of dips	**Mosquito species**					
	Anopheles			**Culicine**		
	Larvae instar 1/2	Larvae instar 3/4	Pupae	Larvae instar 1/2	Larvae instar 3/4	Pupae

Date of survey: __________________ Hour of collection: _______________

Name of the collector: ______________________________ Signature: ________________

Annex IV: Tools used to extract malaria cases from morbidity register at health facility

Case ID	Date of examination		Age	Sex	District	Kebele	Health facilities	Result	*Parasite* species	Test method
	Year	Month		*(M/F)*				*(P/N)*	(*Pf /Pv/Mixed*)	*(Microscopy/RDT)*
1.										
2.										
3.										
4.										
5.										
6.										
7.										
8.										
9.										
10.										
11.										
12.										
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14.										
15.										
16.										
17.										
18.										
19.										
20.										

21.										

Annex V: Immature *An. gambiae* s.l life-table experiment daily record format

Day	R1						R2						R3					
	Alive				**Dead**	**pupae**	**Alive**				**Dead**	**pupae**	**Alive**				**Dead**	**pupae**
	L1	L2	L3	L4			L1	L2	L3	L4			L1	L2	L3	L4		
1																		
2																		
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19																		

Annex VI: Adult *An. gambiae* s.l life-table experiment daily record format

Day	R1		R2		R3		R4		R5	
	Dead		**Dead**		**Dead**		**Dead**		**Dead**	
	Female	**Male**	**Female**	**Male**	**Female**	**Male**	**Female**	**Male**	**Female**	**Male**
1										
2										
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Annex VII: Ethical clearance certificate

JIMMA UNIVERSITY
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We are in the Community

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Ref. No IHRPGD/3243/18
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Date 06/06/2018

Institutional Review Board (IRB)
Institute of Health
Jimma University
Tel: +251471120945
E-mail: zeleke.mekonnen@ju.edu.et

To: Mr. Dawit Hawaria

Subject: Ethical approval of research protocol

The IRB of institute of health has reviewed your research project entitled:

"Impacts of the environmental modification on the ecology of *Anopheles* Mosquito and malaria transmission intensity, Southwestern Ethiopia"

This is to notify that this research protocol as presented to the IRB meets the ethical and scientific standards outlined in national and international guidelines. Hence, we are pleased to inform you that your protocol is ethically cleared.

We strongly recommended that any significant deviation from the methodological details indicated in the approved protocol must be communicated to the IRB before they are implemented.

With regards!

Zeleke Mekonnen (PhD)
Associate Professor, Health Research and Postgraduate Director

Institute of Health, Jimma University

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