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### Characterization of *Anopheles gambiae senso lato* Larval Habitats in Anger Gute Resettlement Villages, Western Ethiopia

### Oljira Kenea Negasa\*, Mebrate Dufera Bekere

Department of Biology, College of Natural and Computational Sciences, Wollega University, Nekemte, Ethiopia

#### **Abstract**

The study was conducted in the Anger River Valley of Ethiopia specifically in the Anger Gute resettlement villages from January to December 2018. Larval habitat survey was undertaken in three rural farming villages namely Tulu Lencha, Warabo and Dalasa Makanisa to assess An. gambiae s.l. larval density and the habitat characteristics. Year round anopheline mosquito positive larval habitat surveys were done every month within a 500 m radius of the villages and 700 m along the major streams which were located adjacent to the villages. The mosquito larval samplings were done using the standard dipping method. All the late instar (III and IV instar) anopheline larvae sampled were preserved in 70% alcohol in the field and later identified to An. gambiae s.l. using identification keys for the Ethiopian mosquitoes in an entomology laboratory at Wollega University. Mean comparison and one-way analysis of variance (ANOVA) was used to reveal the characteristics of larval habitats and mean densities of the mosquito larvae. Results showed many diverse An. gambiae s.l. larval habitats in the villages. The major larval productive habitats were found to be rain pool puddles (35.0%) followed by river edge pools (23.5%). Most of the larval habitats were accessible along roads and streams during wet and dry season respectively. The mosquito larval abundances were significantly associated with aquatic habitats that had turbid standing water and habitats located near to human dwellings (<500 m). In conclusion, results underscore that rain pool puddles that occur along footways and roads closer to human habitation were the major An gambiae s.l. larval habitats during wet season. However, the stream edge pools that were formed along local streams were the most larval productive habitats during dry season. These findings implicate that targeting malaria mosquito larval intervention along footways and roads in wet season and along streams during dry season could result in effective malaria vector larval control in the study settings.

#### **Keywords**

Anger Gute, Anopheles gambiae s.l., Larval Habitat, Malaria, Road, Resettlement, Stream

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#### 1. Introduction

Malaria remains a major health threat in Ethiopia where only 25% of the populations live in areas that are free from the disease burden. Malaria transmission is seasonal and epidemic in Ethiopia, mainly due to altitudinal and climatic variations [1, 2]. The disease transmission peaks from September to December coinciding with the major rainy season and a minor transmission season also occurs in April—

May [3]. Anopheles arabiensis, a member of An. gambiae s. l., is the sole primary vector of malaria in the country [1]. Plasmodium falciparum and Plasmodium vivax, are the dominant malaria parasites, which account for around 60% and 40% of the overall malaria cases in the country respectively [3].

Malaria control relies on indoor residual house spraying (IRS) and long-lasting insecticidal nets (LLINs) as the key frontline life-saving malaria prevention with vector control in

<sup>\*</sup> Corresponding author

Ethiopia. Both IRS and LLINs have proven to be highly effective at reducing malaria incidence and prevalence in the country [4, 5]. Despite great progress in reducing malaria transmission, the future use of both of these interventions have been threatened by the development of insecticide resistance [6-8], difficulties in attaining adequate population coverage [9] and outdoor and early biting behavior of *An. gambiae* s.l. [10-13]. Therefore, additional methods such as larval source management that might complement IRS and LLINs are sorely needed for reducing transmission of malaria.

Larval control of malaria vectors can be an effective method to suppress vector density. Larval control of *An. gambiae* s.l. has succeeded in several parts of the world including in some parts of Ethiopia. For example, eradication of introduced *An. gambiae* s.l. from the northeast coast of Brazil and the Nile valley of Egypt [14] via anti-larval measures are good evident where source reduction was successful. A community-led larval intervention study in a dam village in Tigray, Ethiopia showed a 49% relative reduction in *An. arabiensis* abundance in the dam village [15] via community engagement source reduction activities.

However, human activities associated with settlement, agriculture, and water development projects and other environmental alterations may increase larval habitats of anopheline malaria vectors [10, 16, 17]. Rural and urban resettlement schemes in Ethiopian lowlands have generated disturbances in the natural environment, thus affecting the ecology of the species of *Anopheles* and thus the malaria transmission [12, 17, 18]. It has resulted in ecological changes due to human actions such as deforestation and establishment of new settlements in previously unsettled areas and consequently the proliferation of mosquitoes that prefer human habitation to natural settings [17].

Previous studies on the impact of resettlement on malaria incidence and entomological indices in Ethiopian lowlands showed higher mosquito load and malaria transmission intensity in the resettlement village compared to non-resettlement village [12]. *Anopheles arabiensis* showed 6.5-8 times more abundance in dam settlement village compared to a remote village in northern Ethiopia [15]. Likewise, mean monthly malaria incidence and anopheline larval density was significantly higher in the lowland irrigated villages as compared to non-irrigated villages in central Ethiopia [10]. The most recent study on domestic prevalence of malaria vectors found higher mean indoor density of *An. gambiae* s.l. in resettled than indigenous village in western Ethiopia [18].

However, there are remarkably few data available on the

malaria vector larval habitats in such malaria endemic settings including in Anger Gute Resettlement Villages (AGRV). Anger Gute is a national malaria surveillance sentinel site in western Ethiopia. To the best of our knowledge, characteristics of *An. gambiae* s.l. larval habitats in western lowland in general and in AGRV in particular are unknown. The present study aims to investigate larval habitats and abundance of *An. gambiae s.l.* in AGRV in western Ethiopia. Therefore knowledge acquired from the current study could help to develop an effective measure to control malaria through vector management. It also helps for involving the community who are responsible for creating and maintaining the larval habitats which may lead to a more effective and sustainable control program.

### 2. Methods

#### 2.1. The Study Area

The study was conducted in AGRVs located in Gida Ayana district (GAD) in East Wollega Zone of Oromia Regional State in Ethiopia (Figure 1). GAD is located at 110 Kms from Nekemte, the main city of East Wollega Zone. Anger Gute is one of the four sub-districts of GAD. It consists of eight resettlement villages (kebeles) all of which are hot spot malarious villages of the district. Anger Gute is set in the Anger River Valley (upper Blue Nile Valley) in western Ethiopia. It is located about 360 km west of Addis Ababa along the main road connecting Jimma to Bahir Dar via Nekemte. Anger Gute area accommodates settlement schemes within the Anger Valley with community members from different parts of the country who immigrated to the area due to food insecurity, recurrent drought and degradation of natural resources from Ethiopian high lands. The majority of the population lives in rural areas in houses made with mud or cements walls and iron roofs.

The elevation of the area ranges from 1200m to 1500m above sea level and its absolute location is N9<sup>0</sup> 33'57" and E36<sup>0</sup> 37'57" latitude and longitude respectively. The daily average temperature of the area is 28°C and is a favourable environment for mosquito population to breed. Malaria is a leading health problem in AGRVs. The major malaria transmission season is September to December following the main rain and the minor transmission is from April to May, following the spring rain falls. The most important economic activity in the area is mixed agriculture and small scale trade at the town level. Local rural residents primarily depend on farming and livestock rearing for their subsistence.

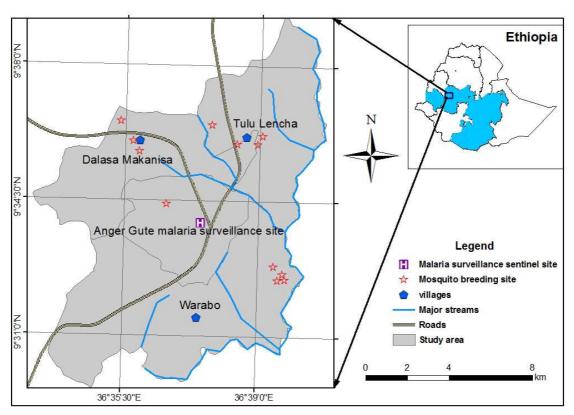


Figure 1. Map of Anger Gute Resettlement Villages, in Oromia Resgional State in Ethiopia.

#### 2.2. The Study Design

Field based longitudinal entomological studies were undertaken every month from January to December 2018 in three resettlement villages namely Tulu Lencha, Warabo and Dalasa Makanisa. The villages were selected purposely, based on a preliminary survey of their proximity to mosquito breeding areas (streams) and malaria transmission. Anopheline larval habitats were assessed within a 500 m radius of each village and 700 m along the major streams which were located adjacent to the villages to study the mosquito larval density and habitat characteristics. The major streams adjacent to the villages are namely Enjiro, Bachbach and Bishan Gonfa streams which are tributaries of the Anger River that drains into Abay (Blue Nile) River jointly with Didessa River in the upper Blue Nile Valley of Ethiopia.

# 2.3. Mosquito Larval Sampling and Processing

The standard dipping method was used for the mosquito larvae collection. Procedurally, a habitat was first inspected for the presence of anopheline larvae visually, then collection was done by dipping using a standard dipper (11.5 cm diam and 350 ml capacity), pipettes, and white plastic pans. When mosquito larvae were present, 5–10 dips was taken depending on the size of each larval habitat at intervals along the edge [16]. Sampling was done in the morning (0900–1200 hrs) or afternoon (1400–1700 hrs) for about 30 min or

less at each larval habitat by the same individual. Anopheline larvae were distinguished from culicines based on their resting habits in water and the siphon. All late instar (III and IV instar) anopheline larvae collected were preserved in 70% alcohol and later identification to *An. gambiae s.l.* was done by morphological criteria using identification keys for the Ethiopian mosquitoes at Wollega University Entomology laboratory.

# 2.4. Larval Habitat Characterization and Recording of Environmental Variables

During the survey, a habitat was first investigated for the presence of mosquito larvae visually, when the mosquito larvae were present (at least one larva), the aquatic habitat was recorded as a type of *An. gambiae s.l.* breeding habitats. Photographs of each larval habitat type were taken. Characterization of larval habitats was carried out by observing and recording the features of the different breeding sites present within the sampling locations.

The environmental variables recorded were distance to the nearest house, vegetation type, intensity of light, water current, turbidity and whether the habitat was natural or human made. Distance to the nearest house was measured with a tape when it was shorter than 100 m and by footsteps when it exceeded 100 m and recorded as near when the distances <500 m and far when >500 m to the nearest house. The type and presence of aquatic vegetation was observed

and recorded as emergent, floating, and none if no vegetation at all. Emergent plants included both aquatic and immersed terrestrial vegetation. Intensity of light was visually categorized as light and shade. Water current was determined by visual inspection and categorized as slow flowing or still. Turbidity was estimated by taking water samples in glass test tubes and holding them against a white background to categorize them as either clear or turbid [16].

#### 2.5. Data Analysis

Densities of the *An. gambiae* s.l. larvae were calculated as density per 100 dips (number of the mosquito larvae /total number of dips) x 100. Pearson's correlation coefficient was used to determine the associations between climatic variables and anopheline densities. Logistic regression analysis was used to determine relationship between larval abundance and environmental parameters of the larval habitats. Mean comparison and one-way analysis of variance (ANOVA)

were used to reveal the characteristics of larval habitats and mean densities of the mosquito larvae. Multiple regression was used to detect key environmental factors significantly associated with the mosquito larval abundance. The significance level was considered at p < 0.05. All statistical analyses were done using SPSS version 20.0.

### 3. Results

## 3.1. Larval Habitat Diversity and Abundance

Spatial distribution of *An. gambae s. l.* larvae is presented in Table 1. As it can be seen from the table, the mosquito larvae were found in many diverse habitats and were collected most abundantly from rain pool puddles (35.0%) followed by river edge pools (23.5%).

<b>Table 1.</b> Habitat diversi	ty and relative abund	lance of <i>An. gambiae</i> s	.l larvae in the stud	ly setting from.	January to December 2018.
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Habitat type	Tulu Lencha	Warabo	Dalasa Mekensa	Total	
	n (%)	n (%)	n (%)	n (%)	
Rain pool puddles	6460	9840	2940	19240 (35.0)	
Hoof prints	480	1460	1420	3360 (6.1)	
Tyre tracks	1980	2940	1430	6350 (11.6)	
Rain harvest pool	2290	-	380	2670 (4.8)	
Rice fields	1710	740	-	2450 (4.5)	
Pipe leakages	-	-	990	990 (1.8)	
Borrow pits	720	760	960	2440 (4.4)	
Stream edge pools	3200	7780	1940	12920 (23.5)	
Stream bed pools	1340	2310	830	4480 (8.2)	
Total	18180 (33.1)	25830 (47.0)	10890 (19.8)	54900	

## 3.2. Monthly Pattern of *An. gambiae* s.l. Larval Density

Larvae of *An. gambiae* s.l. occurred in the study setting every month over the study year, but with different peak periods of abundance (Figure 2). The mosquito larvae appeared to show two larval density peaks (bimodal peaks): The first small

peak larval density of the vector was observed in April before onset of rain followed by declining densities in May and June after rain started in April. However the most peak larval density of the vector was observed in August with declining density thereafter in the following months along with declining rain fall in the study setting.

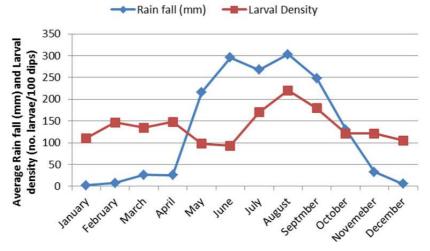


Figure 2. Average monthly precipitation and larval desnsity of An. gambiae s.l. in Anger Gute Resettlement villages in 2018.

#### 3.3. Larval Habitat Accessibility by Season

In total 348 An. gambiae s.l. larval positive habitats were frequently observed over the study period. The mosquito larval habitat locations were statistically significantly associated with season of the year. During wet season, the majority of (83.3%) the larval habitats were accessed along foot ways (roads). However, during dry season the habitats were most accessible along streams in the study setting (Table 2).

**Table 2.** Accessibility of *An. gambiae* s.l. larval habitats by season in Anger-Gute area in 2018

Habitat location	Season		– Total	
париат юсаноп	Dry	Wet	Total	
Along foot way (road)	3 (2.5)	189 (83.3)	192 (55.2)	
Along stream	118 (97.5)	30 (13.2)	148 (42.5)	
Other	0	8 (3.5)	8 (2.3)	
Total	121	227	348	

#### 3.4. Environmental Factors Associated with Larval Abundance

Table 3 depicts the characteristics of larval habitats and mean densities of An. gambiae s.l larvae. As presented in the table, higher average densities of the mosquito larvae were collected from habitats that had turbid standing water and in habitats near to human dwellings (<500 m). Higher average densities of the mosquito larvae were also significantly collected in wet season along foot ways and roads.

**Table 3.** Environmental variables of larval habitats and mean densities of *An. gambiae* s.l. larvae

CI	** * * * * * * * * * * * * * * * * * * *	An. gambiae s. l. larval density			
Characteristics	Variable	Mean ± SE	F	P	
II-liteti-i-	Natural	$149.3 \pm 7.3$	3.106	0.079	
Habitat origin	Man made	$166.1 \pm 6.0$			
Distance to the	Near	$160.9 \pm 5.1$	5.523	0.019	
nearest house	Far	$118.4 \pm 6.3$			
Season	Wet	$176.2 \pm 6.6$	30.443	0.000	
Season	Dry	$123.0 \pm 4.4$			
	Along footways	$181.0 \pm 7.5$	16.752	0.000	
Habitat access	Along stream	$131.0 \pm 4.5$			
	Other	$92.5 \pm 10.6$			
D	Temporary	$157.8 \pm 4.8$	0.021	0.885	
Permanence	Permanent	$152.0 \pm 39.2$			
Water current	Slow flowing	$127.8 \pm 13.1$	1.642	0.201	
water current	Still	$159.0 \pm 4.9$			
Intensity of	Light	$158.6 \pm 4.9$	0.931	0.335	
light	Shade	$133.3 \pm 10.9$			
	None	$160.9 \pm 5.0$	3.348	0.036	
Vegetation	Emergent	$109.0 \pm 9.5$			
	Floating	$111.6 \pm 10.6$			
T	Clear	$130.8 \pm 7.1$	5.132	0.024	
Turbidity	Turbid	$162.1 \pm 5.3$			

Four of the environmental variables were found to be significantly correlated with mean density of *An. gambiae* s.l. larvae (Table 4). Specifically the mosquito larvae were significantly correlated with distance to the nearest house,

season of the year, habitat accessibility and habitat water turbidity.

**Table 4.** Correlation between environmental variables and the mosquito larval density

Environmental variables	Density of An. gambiae s.l. larvae
Habitat origin	0.094
Distance to the nearest house	-0.125*
Season of occurrence	0.284**
Habitat access	-0.297**
Permanence	-0.008
Water current	-0.069
Intensity of shade	-0.052
Vegetation	-0.130*
Turbidity	0.121*

\*, \*\* indicates that correlation significant at 0.05 and 0.01 level respectively.

Further regression analysis detected three core environmental factors associated with the abundance of anopheline larvae (Table 5). Accordingly, the relative abundance of *An. gambiae s.l.* larvae was positively associated with habitat accessibility and season of the year where as it was negatively associated with the presence of vegetation.

**Table 5.** Multiple step-up regressions for *An. gambiae* s.l. larvae in relation to habitat characteristics

Character	R <sup>2</sup>	Coefficient	SE	Standard coefficient	t	p
(Constant)		145.7	35.5		4.105	0.000
Access	0.088	-26.9	11.5	-0.164	-2.333	0.020
Season	0.101	32.7	13.0	0.175	2.505	0.013
Vegetation	0.113	-24.7	11.6	-0.110	-2.126	0.03

### 4. Discussion

## 4.1. Habitat Diversity and Larval Abundance

This study has identified nine An. gambiae s.l. larval habitats in the study setting namely: rain pool puddles, stream edge pools, tyre tracks, stream bed pools, hoof prints, rain harvesting pools, rice fields, borrow pits and pipe leakage pools, of which the former two habitats were the most predominant An. gambiae s.l. larvae productive habitats. The occurrence, sustenance and dimensions of all the larval habitats except stream edge and stream bed pools are dependent on rain water in the study area. However the stream edge and stream bed pools rely on water from three main local streams specifically Enjiro, Bachbach and Bishan Gonfa that flow adjacent to the study villages. These habitat types were previously reported from elsewhere in the country [16, 19-23] and from other parts of Africa as well [24-26]. However, to our knowledge, the habitat types were first report from the study area, specifically from the Anger Valley which is part of the Blue Nile Valley of Ethiopia where the Anger Gute National Malaria Surveillance Sentinel Site is

located. Most of the previous reports of *An. gambiae* s.l. larval habitats came from the Ethiopian Rift Valley [16, 19, 20, 27], Northern Ethiopian River Valleys [15, 22, 28], Ghibe-Omo River Valley [23, 29] and the Baro-Akobo River Valley in south western Ethiopia [21, 30]. Therefore these malaria mosquito productive habitats are first report from the upper Blue Nile Valley of Ethiopia.

The results also show that the mosquito larvae were obtained most plentifully from rain pool puddles followed by stream edge pools. In line with this study, *An. gambiae s.l.* larvae were found to be the most abundant in rain pool habitats during the rainy season and in stream and river edge pools during the dry season in Ethiopia [19, 20, 22, 23, 27] and elsewhere in Africa [24].

## 4.2. Monthly Pattern of *An. gambiae* s.l. Larval Density

Anopheles gambiae s.l. larvae appeared to show two larval density peaks over the study year: one low peak in April and the other highest peak in August with declining density thereafter in the following months. These results could be explained by the influence of stream water in dry season and rain water during wet season on the larval habitats. The first peak density of the mosquito larvae that was observed in April might be due to large pockets of water pools along streams that proliferate larval mosquito before onset of rain. Whereas the highest mosquito larval density peak that was observed in August was due to the impact of rain fall. The study area receives unimodal rain that occurs from April to September and the densities of the mosquito vector are mainly driven by the seasonal precipitation. This finding corroborates with the work of Abose et al. [1] who observed peak larval density of the malaria vector in August and a sharp declining of the mosquito larval density in the following months.

# 4.3. Productive Larval Habitat Location and Accessibility by Season

Results show that during the wet season, *An. gambiae* s.l. larvae productive habitats were predominantly found along footways and roads. However during the dry season, the most productive habitats were concentrated along streams in the study setting. These could be attributed to larval breeding site preference of the mosquito vector and the impact of habitat water desiccations by season. Previous reports indicate that *An. gambiae* s.l. breeds in sunlit rain water pools that were free of vegetation during the wet season [31, 32] and in residual pools along drainage systems in dry season [16, 19, 22, 23]. The mosquito larvae preferred vegetation free rain pools that were located along footways and roads than elsewhere in the study setting due to the influence of fast

growing standing vegetation and associated fauna that might act as larval predators [32, 33] during rainy season. In tropical savannah, like the present study localities, most of the ground is covered by fast growing vegetation during the rainy season and as a result mosquito breeding sites are colonized by flora and fauna successions and these factors will limit sunlit breeding mosquitoes such as *An. gambiae* s.l. to vegetation free areas particularly along footways and roads.

However, in dry season, mosquito breeding sites are limited to drainage areas such as streams, rivers and lakes due to lack of rain water and the impact of desiccation. But after onset of rain, the streams will increase and most larval habitats located along streams will be submerged by overflow of the stream water and succession of flora and fauna in the drainage areas [23, 32]. This will act as natural control of the mosquitoes and warrant further investigation of the impact of stream flooding and flora and fauna succession on malaria mosquito population dynamics in the study setting and elsewhere in the country. These results imply that deforestation of dry lands, wetlands and streamline and riverine forests may proliferate the malaria mosquito breeding and need special concern and action in the study settings and elsewhere in the country. On the other hand, afforestation and wetland conservation may reduce the malaria mosquito breeding and malaria transmission in Ethiopia and warrant further study. This calls for soil, vegetation and wetland conservations in the study area and elsewhere in the country for the larval control of the malaria vector via source reduction and environmental protection measures. The impact of ecofriendly methods of malaria vector control such as afforestation and wetland conservation on the mosquito population dynamics warrant further study in the study area and elsewhere in Ethiopia.

## 4.4. Environmental Factors Associated with Larval Abundance

Significantly higher mean densities of *An. gambiae s.l* larvae were obtained from aquatic habitats that had turbid water. This agrees with the most recent report from Ethiopia [23] and also consistent with several reports from other parts of Africa [24, 34, 35] which showed that *An. gambiae s.l.* usually prefers turbid rain pool water. Turbidity was associated with eroded soils that accumulated after rains or when the habitats with muddy substrates became disturbed by animals drinking water [23]. Previous reports by Miller *et al* [25] also revealed that *An. gambiae s.l.* larvae are amphibious and are capable of terrestrial displacement in drying muddy aquatic habitats whereby they can reach in standing water pools. The mosquito larvae have developed adaptive selection to live and grow in turbid habitat water

following fertilizer application [36].

On the other hand, this finding seems to contradict previous reports by Kenea, Balkew and Gebre-Michael [16] and Dejenie, Yohannes and Assmelash [28] who found that the preference of An. gambiae s.l. is to clear water than turbid water. This could be attributed to larval survey season and the larval habitat substrate types. During the rainy season, most larval habitat water is turbid by surface rain water runoff and flood unlike dry season larval habitat where there is no more soil erosion, flood and rain water runoff. Larval habitat substrate type also affects water turbidity. Previous reports show that An. gambiae s.l. breeds in clear water in sandy and rocky habitat substrate types such as sand pools [16, 22] and rock pools [26] as compared to muddy rain pool puddles and hoof prints [23]. This suggests that the mosquito vector flexibly breeds in turbid and clear water pools in the study setting and elsewhere based on breeding season and larval habitat substrate types.

The mosquito larvae were also most abundantly observed in habitats nearby human houses (<500 m). This finding corroborates several previous studies [16, 20, 22, 23] that support the mosquito breeds in habitats closer to human habitation as compared to those habitats far from houses. The mosquito prefers to lay eggs in habitats closer to human habitation to conserve energy lost flying long distances in search of egg laying sites and blood meal sources [37] and this anthropophilly behavior of *An. gambiae s.l.* will contribute for vectoring malaria transmission.

Results also identified three core environmental factors significantly associated with the relative abundance of *An. gambiae s.l.* larvae namely season of the year, vegetation and habitat location that were found to be key predictors of *An. gambiae s.l.* larval occurrence and abundance. The positive association between *An. gambiae s.l.* larval abundance and season of the year would be expected because the larval habitats and the mosquito populations expand during the wet season particularly from September to December coinciding with the major rainy season and from April to May during a minor rain [2]. Larval productivity depends on rainfall and subsequent changes in water table and river levels [23, 38].

The presence of vegetation was negatively associated with abundance of *An. gambiae s.l.* larvae. The negative effect of vegetation cover on the mosquito larval abundance is consistent with prior observations in Ethiopia [16, 31, 32, 34] and elsewhere in Africa [34, 37]. The works of Munga et al. [34] and Mwangangi et al. [37] evident that deforestation and cultivation of wet lands created conditions favourable for *An. gambiae s.l.* larval production.

Habitat location either along road or along stream was found to be key predicator for occurrence and abundance of the mosquito larvae. The breeding of *An. gambiae s.l.* along streams, rivers and lakes have been established in the country [1, 16, 19, 22, 23, 27]. However, to our knowledge, significant association of the mosquito larvae with footways and roads has not been reported so far. This observation underscores the importance of the malaria vector larval habitats along roads during wet season and along major drainages such as streams, rivers and lakes during dry season. This calls for policy makers and malaria control agents to target along footways and roads in wet season and along the drainages in dry season for the malaria vector larval control efforts.

In the end, as a limitation of the study, *An. gambiae s.l.* molecular identification in to sibling species was not done. The study targeted *An. gambiae s.l.* larvae productive habitats. Therefore, detailed investigation on the biotic, physical and chemical factors in both productive and non-productive *An. gambiae s.l.* larval habitats is needed for more evidence based application of appropriate larval control measures in the study settings.

### 5. Conclusion

The malaria mosquito An gambiae s.l. breeds most abundantly in rain pool puddles along footways and roads closer to human habitation during wet season and in stream edges along local streams in dry season in Anger Gute Resettlement villages. These findings suggest that targeting malaria mosquito larval intervention along footways and roads in wet season and on stream edges along local streams in the dry season could result in effective larval control of the malaria vector in the study setting. Conversely, significant relative abundance of the malaria mosquito larvae along roads in wet season and their total decline from along local streams in the same season implicates the impact of the streamline vegetation and forests on the mosquito larvae in the resettlement village setting. This calls for soil and wetland conservations in the study area and elsewhere in the country for the larval control of the malaria vector via source reduction and environmental protection measures. The impact of eco-friendly methods of malaria vector control such as afforestation and wetland conservation on the mosquito population dynamics warrant further study in the study area and elsewhere in Ethiopia.

### **Abbreviations**

ANOVA: analysis of variance; AGRV: Anger Gute Resettlement Villages; IRS: indoor residual spraying; GAD: Gida Ayana District; LLINs: long-lasting insecticidal nets

### **Authors' Contributions**

OK and MD conceived and designed the study. All authors were involved in proposal writing and participated in the field coordination and data collections. OK analyzed the data and drafted the manuscript. All authors revised and approved the final version of the manuscript.

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### References

- [1] Abose T, Ye-Ebiyo Y, Olana D, Alamirew D, Beyene Y, Regassa L, et al.: Re-Orientation and definition of the role of malaria vector control in Ethiopia; the epidemiology and control of malaria with special emphasis to the distribution, behavior and susceptibility to insecticides of anopheline vectors and chloroquine resistance in Ziway, Central Ethiopia and other areas. Addis Ababa; 1998.
- [2] Abeku TA, Oortmarssen GJ, Borsboom G, de Vlas SJ, Habbema JDF. Spatial and temporal variations of malaria epidemic risk in Ethiopia: factors involved and implications. *Acta Tropica* 2003, 87: 331-340.
- [3] Deribew A, Dejene T, Kebede B, Tessema GA, Melaku YA, Misganaw A, et al.: Incidence, prevalence and mortality rates of malaria in Ethiopia from 1990 to 2015: analysis of the global burden of diseases 2015. *Malaria Journal* 2017, 16: 271.
- [4] Otten M, Aregawi M, Were W, Karema C, Medin A, Bekele W, et al. Initial evidence of reduction of malaria cases and deaths in Rwanda and Ethiopia due to rapid scale-up of malaria prevention and treatment. *Malaria Journal* 2009, 8: 14.
- [5] Alemu A, Muluye D, Mihret M, Adugna M, Gebeyaw M. Ten year trend analysis of malaria prevalence in Kola Diba, North Gondar, Northwest Ethiopia. *Parasite & Vectors* 2012, 5: 173.
- [6] Balkew M, Getachew A, Chibsa S, Olana D, Reithinger R, Brogdon W. Insecticide resistance: a challenge to malaria vector control in Ethiopia. *Malaria Journal* 2012, 11: 139.
- [7] Massebo F, Balkew M, Gebre-Michael T, Lindtjorn B. Blood meal origins and insecticide susceptibility of Anopheles arabiensis from Chano in South-West Ethiopia. *Parasite & Vectors* 2013, 6: 44.
- [8] Gari T, Kenea O, Loha E, Deressa W, Hailu A, Balkew M, et al. Malaria incidence and entomological findings in an area targeted for a cluster randomized controlled trial to prevent malaria in Ethiopia: results from a pilot study. *Malaria Journal* 2016, 15: 145.

- [9] Solomon T, Loha E, Deressa W, Gari T, Overgaard HJ, Lindtjørn B. Low use of long-lasting insecticidal nets for malaria prevention in south-central Ethiopia: A communitybased cohort study. PLoS ONE 2019, 14.
- [10] Kibret S, Petros B, Boelee E. Tekie H. Entomological studies on the impact of a small-scale irrigation scheme on malaria transmission around Ziway, Ethiopia. Ethiop. *J Dev Res* 2010, 32: 418-438.
- [11] Yohannes M, Boelee E. Early biting rhythm in the afrotropical vector of malaria, Anopheles arabiensis, and challenges for its control in Ethiopia. *Med Vet Entomol* 2011, 26: 103-105.
- [12] Degefa T, Zeynudin A, Godesso A, Haile Michael Y, Eba K, Zemene, E, et al. Malaria incidence and assessment of entomological indices among resettled communities in Ethiopia: a longitudinal study. *Malaria Journal* 2015, 14: 24.
- [13] Kenea O, Balkew M, Tekie H, Gebre-Michael T, Deressa W, Loha E, et al. Human-biting activities of Anopheles species in south-central Ethiopia.. Parasite & Vectors 2016, 9: 527.
- [14] Killeen GF, Fillinger U, Kiche I, Gouagna LC, Knols BG. Eradication of Anopheles gambiae from Brazil: lessons for malaria control in Africa? *Lancet Infect Dis* 2002, 2: 618–627.
- [15] Yohannes M, Haile M, Ghebreyesus TA, Witten KH, Getachew A, Byass P, et al. Can source reduction of mosquito larval habitat reduce malaria transmission in Tigray, Ethiopia? *Trop Med Inter Health* 2005, 10: 1274-1285.
- [16] Kenea O, Balkew M, Gebre-Michael T. Environmental factors associated with larval habitats of anopheline mosquitoes (Diptera: Culicidae) in irrigated and major drainage areas in the middle course of the Rift Valley, central Ethiopia. *J Vec B Dis* 2011, 48: 85-92.
- [17] Deressa W, Ali A, Berhane Y. Review of the interplay between population dynamic and malaria transmission in Ethiopia. *Ethiop J Health Dev* 2006, 20: 137-144.
- [18] Kenea O, Tekie H. Domestic Prevalence of Malaria Vectors and Self-reported Malaria Episode with respect to Ownership and Utilization of Long-lasting insecticidal nets in selected Resettlement and Indigenous Villages in Sasiga District, Western Ethiopia. *Journal of Biology, Agriculture and Healthcare* 2016, 6.
- [19] Animut A, Gebre-Michael T, Balkew M, Lindtjørn B. Abundance and dynamics of anopheline larvae in a highland malarious area of south-central Ethiopia. *Parasite & Vectors* 2012, 5: 117.
- [20] Gone T, Balkew M, Gebre-Michael T. Comparative entomological study on ecology and behaviour of Anopheles mosquitoes in highland and lowland localities of Derashe District, southern Ethiopia. *Parasite & Vectors* 2014, 7: 483.
- [21] Wondwosen B, Birgersson G, Seyoum E, Tekie H, Torto B, Fillinger U, et al. Rice volatiles lure gravid malaria mosquitoes, Anopheles arabiensis. Scientific Reports 2016, 6.
- [22] Kindu M, Aklilu E, Balkew M, Gebre-Michael T. Study on the species composition and ecology of anophelines in Addis Zemen, South Gondar, Ethiopia. *Parasite & Vectors* 2018, 11: 215.
- [23] Getachew D, Balkew M, Tekie H. Anopheles larval species composition and characterization of breeding habitats in two localities in the Ghibe River Basin, southwestern Ethiopia. Malaria Journal 2020, 19: 65.

- [24] Shililu J, Ghebremeskel T, Seulu F, Mengistu S, Fekadu I, Zerom M, et al. Larval habitat diversity and ecology of anopheline larvae in Eritrea. J Med Entomol 2003, 40: 921– 929.
- [25] Miller JR, Huang J, Vulule J, Walker ED. Life on the edge; African malaria mosquito (*Anopheles gambiae* s.l.) larvae are amphibious.. *Naturwissenschaften* 2007, 94: 195–199.
- [26] Hamza AM, Rayah EE. AQualitative Evidence of the Breeding Sites of *Anopheles arabiensis* Patton (Diptera: Culicidae) in and Around Kassala Town, Eastern Sudan. *International Journal of Insect Science* 2016, 8: 65-70.
- [27] Tesfaye S, Belyhun Y, Teklu T, Mengesha T, Petros B. Malaria prevalence pattern observed in the highland fringe of Butajira, Southern Ethiopia: A longitudinal study from parasitological and entomological survey. *Malaria Journal* 2011, 10: 153.
- [28] Dejenie T, Yohannes M, Assmelash T. Characterization of Mosquito Breeding Sites In And In The Vicinity Of Tigray Microdams. *Ethiopian Journal of Health Science* 2011, 21: 58-68.
- [29] Jaleta KT, Hill SR, Seyoum E, Balkew M, Gebre-Michael T, Ignell R, et al. Agro-ecosystems impact malaria prevalence: large-scale irrigation drives vector population in western Ethiopia. *Malaria Journal* 2013, 12: 350.
- [30] Krafsur E. The bionomics and relative prevalence of Anopheles species with respect to the transmission of Plasmodium to man in western Ethiopia. *J Med Entomol* 1977, 25: 180–194.
- [31] Ye-Ebiyo Y, Pollack RJ, Spielman A. Enhanced Development in Nature of Larval Anopheles Arabiensis Mosquitoes Feeding on Maize Pollen. Am J Trop Med Hyg 2000, 63.

- [32] Kiszewski AE, Teffera Z, Wondafrash M, Ravesi M, Pollack RJ. Ecological succession and its impact on malaria vectors and their predators in borrow pits in western Ethiopia *Journal* of Vector Ecology 2013, 39: 414-423.
- [33] Mereta ST, Yewhalaw D, Boets P, Ahmed A, Duchateau L, Speybroeck N, et al. Physico-chemical and biological characterization of anopheline mosquito larval habitats (Diptera: Culicidae): implications for malaria control. *Parasite & Vectors* 2013, 6: 320.
- [34] Munga S, Noboru M, Guofa Z, Okeyo-Owuor J, Andrew K, Guiyun Y. Oviposition Site Preference and Egg Hatchability of Anopheles gambiae: Effects of Land Cover Types. *Journal* of Medical Entomology 2004, 42: 993-997.
- [35] Walker K, Lynch M. Contributions of Anopheles larval control to malaria suppression in tropical Africa: review of achievements and potential. *Med Vet Entomol* 2007, 21: 2-21.
- [36] Kibuthu TW, Njenga SM, Mbugua AK, Muturi EJ. Agricultural chemicals: life changer for mosquito vectors in agricultural landscapes? *Parasite & Vectors* 2016, 9: 500.
- [37] Mwangangi JM, Mbogo CM, Muturi EJ, Nzovu JG, Kabiru EW, Githure JI, et al. Influence of biological and physicochemical characteristics of larval habitats on the body size of Anopheles gambiae mosquitoes along the Kenyan coast. *Journal of Vector Borne Diseases* 2007, 44: 122-127.
- [38] Hardy AJ, Gamarra JGP, Cross DE, Macklin MG, Smith MW, Kihonda J, et al. Habitat hydrology and geomorphology control the distribution of malaria vector larvae in rural Africa. *PPLoS ONE* 2013.