Larval habitats of *Anopheles gambiae* senso lato (Diptera-Culicidae) in Anger Gute resettlement villages, western Ethiopia

Oljira Kenea¹

*Corresponding author

Email: qabanef2015@gmail.com

Mebrate Dufera¹

Email: mebratedufera@gmail.com

¹Department of Biology, College of Natural and Computational Sciences, Wollega University, Nekemte, Ethiopia.
Abstract

Background: Anger Gute is one of the national malaria surveillance Sentinel Sites in Ethiopia targeted to generate malaria data for the disease control, elimination and eradication. The objective of this work was to obtain information about the habitat characterization of An. gambiae s.l. larvae in western Ethiopian lowlands particularly in Anger Gute resettlement villages.

Methods: A longitudinal mosquito larval habitat survey was undertaken every month from January to December 2018. The study covered three rural farming villages namely Tulu Lencha, Warabo and Dalasa Makanisa. All anopheline positive larval habitats present within a 500 m radius of each village and 700 m along the major streams which were located adjacent to the villages were sampled year round to study the mosquito larval density and the habitat characteristics. Larval collections were done from various aquatic habitats in all the villages using the standard dipping method. All III and IV instar anopheline larvae collected were preserved in 70% alcohol. In Wollega University Entomology laboratory, each larva was individually mounted on a microscope slide and identified to An. gambiae s.l. by morphological criteria using identification keys for the Ethiopian mosquitoes. Characteristics of the larval habitats were determined using mean comparison of the mosquito larval density using one-way analysis of variance (ANOVA).

Results: 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%). During wet season, most of (83.3%) the larval habitats were accessed along roads. Whereas during dry season the habitats were most accessible along streams in the study localities. Significantly
higher mean densities of the mosquito larvae were obtained from aquatic habitats that had turbid standing water and in habitats near to human dwellings (<500 m).

**Conclusions:** 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.

**Keywords:** Anger Gute, *Anopheles gambiae* s.l., Larval habitat, Malaria, Road, Stream

**BACKGROUND**

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 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 emergence 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 control of the vector 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 one of the national malaria surveillance sentinel sites in Ethiopia. To the best of our knowledge, the larval habitat parameters of An. gambiae s.l. 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.

METHODS

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 located about 360 km west of Addis Ababa along the main road connecting Jimma to Bahir Dar via Nekemte. It is set in the Anger River Valley (upper Blue Nile Valley) in western
Ethiopia. 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 altitude of the area is between 1200m to 1500m above sea level and located at latitude of N9° 33’57” and longitude of E36° 37’57”. The daily mean temperature is 28°C which is a conducive 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 fails. 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.

The study design

A longitudinal mosquito larval habitat survey was undertaken every month from January to December 2018. The study covered three rural farming villages namely Tulu Lencha, Warabo and Dalasa Makanisa. These study localities were selected purposely, based on their proximity to mosquito breeding areas (streams) and malaria transmission. All anoheline positive larval habitats present within a 500 m radius of each village and 700 m along the major streams which were located adjacent to the villages were sampled year round to study the mosquito larval density and the larval 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.

**Mosquito larval sampling and processing**

Larval collections were done from various aquatic habitats in all the villages using the standard dipping method. During each survey, a habitat was first inspected for the presence of mosquito larvae visually, then 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. Sampling was done by the same individual in the morning (0900–1200 hrs) or afternoon (1400–1700 hrs) for about 30 min or less at each larval habitat. Anopheline larvae were distinguished from culicines based on their resting habits in water and the siphon. All III and IV instar anopheline larvae collected were preserved in 70% alcohol. In Wollega University Entomology laboratory, each larva was individually mounted on a microscope slide and identified to *An. gambiae s.l.* by morphological criteria using identification keys for the Ethiopian mosquitoes.

**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 intensity of light, turbidity, vegetation type, water current, distance to the nearest house, whether the habitat was natural or human made, location of the habitat. 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. 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. 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.

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

RESULTS

Habitat diversity and larval abundance
Table 1 shows spatial distribution of *An. gambiae* s.l. larvae in different habitat types in the study localities. 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%).

**Table 1: Habitat diversity and relative abundance of *An. gambiae* s.l larvae in Anger Gute Resettlement Villages from January to December 2018**

| Habitat type               | Tulu Lencha | Warabo | Dalasa Mekensa | Total   |
|----------------------------|-------------|--------|----------------|---------|
| n (%)                      | 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** |

**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.

**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, most of (83.3%) the larval habitats were accessed along foot way (roads). Whereas during dry season the habitats were most accessible along streams in the study localities (Table 2).

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

| Habitat location          | Season   | Total   |
|---------------------------|----------|---------|
|                           | Dry      | Wet     |         |
| 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     |

**Environmental factors associated with larval abundance**
The characteristics of larval habitats and mean densities of An. gambiae s.l larvae are shown in Table 5. Significantly higher mean densities of the mosquito larvae were obtained from aquatic habitats that had turbid standing water and in habitats near to human dwellings (<500 m). Higher mean densities of the mosquito larvae were also significantly collected in wet season along foot ways and roads.
Table 3: Characteristics of larval habitats and mean densities of *An. gambiae* s.l. larvae

| Characteristics     | Variable       | An. gambiae s. l. larval density |
|---------------------|----------------|----------------------------------|
|                     |                | Mean ± SE | F   | P    |
| Habitat origin      | Natural        | 149.3 ± 7.3 | 3.106 | 0.079 |
|                     | Man made       | 166.1 ± 6.0 |      |      |
| Distance to the nearest house | Near | 160.9 ± 5.1 | 5.523 | 0.019 |
|                     | Far            | 118.4 ± 6.3 |      |      |
| Season              | Wet            | 176.2 ± 6.6 | 30.443 | 0.000 |
|                     | Dry            | 123.0 ± 4.4 |      |      |
| Habitat access      | Along footways | 181.0 ± 7.5 | 16.752 | 0.000 |
|                     | Along stream   | 131.0 ± 4.5 |      |      |
|                     | Other          | 92.5 ± 10.6 |      |      |
| Permanence          | Temporary      | 157.8 ± 4.8 | 0.021 | 0.885 |
|                     | Permanent      | 152.0 ± 39.2 |      |      |
| Water current       | Slow flowing   | 127.8 ± 13.1 | 1.642 | 0.201 |
|                     | Still          | 159.0 ± 4.9 |      |      |
| Intensity of light  | Light          | 158.6 ± 4.9 | 0.931 | 0.335 |
|                     | Shade          | 133.3 ± 10.9 |      |      |
| Vegetation          | None           | 160.9 ± 5.0 | 3.348 | 0.036 |
|                     | Emergent       | 109.0 ± 9.5 |      |      |
|                     | Floating       | 111.6 ± 10.6 |      |      |
| Turbidity           | Clear          | 130.8 ± 7.1 | 5.132 | 0.024 |
|                     | Turbid         | 162.1 ± 5.3 |      |      |
Correlation analysis of each environmental variable with abundance of *An. gambiae* s.l. larvae revealed four variables to be significantly correlated with density of the mosquito larvae (Table 4). *Anopheles gambiae* s.l. larvae were significantly correlated with distance to the nearest house, season of the year, habitat accessibility and habitat water turbidity.

### Table 4: Correlation coefficients between environmental variables and densities of *An. gambiae* larvae

| 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*                               |

*Correlation significant at 0.05 level; **Correlation significant at 0.01 level.

Further regression analysis detected key environmental variables 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^2  | 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 |

**DISCUSSION**

**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 breeding sites. The availability, persistence 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 collected most abundantly 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].

**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.
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. Whereas during the dry season, the most productive habitats were concentrated along streams in the study localities. 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.

**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 pointed out 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]. Miller *et al* [25] also demonstrated 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. 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 [16, 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 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 near to human dwellings (<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 the mosquitoes will contribute for vectoring malaria transmission.

Results also identified three key environmental variables 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 [34, 37]
evident that deforestation and cultivation of natural swamps created conditions favorable for An. gambiae s.l. breeding.

Habitat location either along road or along stream was found to be key predictor 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 footways and roads during wet season and along streams, rivers and lakes during dry months. This calls for policy makers and malaria control agents to target the mosquito larval habitats along footways and roads in wet season and along nearby streams, rivers and lakes in dry season for malaria control, elimination and eradication 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.

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 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.

**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.

**Acknowledgments**

Special thanks are extended to Mr. Tekalign Gebre for mapping the study area and Mr. Tekilu Marga and Mr. Abera Oljira for coordinating and participating in field mosquito larvae collection and processing. The authors are deeply grateful to the office of vice president for research community engagement and technology transfer, Wollega University for financial support and proving field facilities.

**Competing interests**

The authors have declared that they have no competing interests.
Availability of data and materials

The data sets generated and/or analysed during the current study are available from the corresponding author on reasonable request.

Consent for publication

Not applicable.

Ethical approval

Ethical approval was obtained from the Research Ethics Review Committee (RERC) of Wollega University (Ref: WU,RD, 214,2011).

Consent statement from the human volunteer

Not applicable.

Funding

This study was financially supported by the office of vice president for research community engagement and technology transfer, Wollega University (Project number: WUR&TTF-003). The funder has no role in the design of the study, data collection, analysis, interpretation and in writing the manuscript.

REFERENCE

1. Abose T, Ye-Ebiyo Y, Olana D, Alamirew D, Beyene Y, Regassa L, et al.: Re-Orientaion 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 community-based 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 afro-tropical 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. Qualitative 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. Ovposition 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.

**Figure legends**

Figure 1: Geographical location of Anger Gute Resettlement Villages, Ethiopia
Figure 2: Mean Monthly rain fall and larval density of *An. gambiae* s.l. in Anger Gute Resettlement villages in 2018.