Habitat characteristics and spatial distribution of *Anopheles* mosquito larvae in malaria elimination settings in Dembiya District, Northwestern Ethiopia

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Research Article

Keywords: Anopheles larvae, breeding habitat, An. arabiensis, An. pharoensis, drainage canals, Dembiya

Posted Date: May 19th, 2022

DOI: https://doi.org/10.21203/rs.3.rs-830514/v1

Abstract

The persistence and productivity of larval habitat is a major factor that regulates adult *Anopheles* mosquitoes' density and malaria transmission intensity. A study on *Anopheles* mosquitoes breeding habitat diversity, distribution, characteristics and larval density in different seasons across various habitat types is important to design effective larval control strategies. This study was aimed to investigate the *Anopheles* species composition, the productivity of larval breeding habitats, and their spatial distribution in selected localities of Dembiya District. A longitudinal study on characteristics and productivity of *Anopheles* larval breeding habitats was conducted from June 2018 to May 2019 in selected localities of Dembiya District. *Anopheles* larvae were collected using standard WHO dipper (350 ml capacity) and droppers depending on the size of the breeding habitats. Physicochemical characteristics of breeding habitats were measured and *Anopheles* mosquitoes were identified by using morphological keys and polymerase chain reaction (PCR). Logistic regression was used to assess the association of environmental factors with the presence or absence of *Anopheles* mosquitoes larvae. A total of 1,629 *Anopheles* larvae and 185 pupae were collected from both localities. These comprise *Anopheles arabiensis*, *An. pharoensis*, *An. coustani*, *An. christyi*, *An. squamosus*, *An. demeilloni*, *An. danicalicus*, and *An. cinereus*. The highest density of *Anopheles* larvae was collected from at a drying water canal (14.7 ± 3.5 larvae/dip) and the lowest larval density was recorded in rain water pools (0.2 ± 0.2 larvae/dip). The presence or absence of *Anopheles* larvae were significantly associated with physical characteristics of the breeding habitats such as turbidity (mid turbid) (AOR = 66.03; 95% CI: 2.01-2168.24, $p = 0.019$) and presence of grasses (AOR = 12.62; 95% CI: 1.29-122.78, $p = 0.029$). This study indicated that breeding sites persist and support *Anopheles* mosquito breeding activities and impact malaria control and elimination programs. Incorporating vector control strategies targeting *Anopheles* larvae as a part of malaria intervention strategies could enhance the malaria control and elimination program in the study area.

Introduction

Mosquitoes are responsible for the transmission of different types of diseases such as malaria, yellow fever, dengue, and West Nile fever (Alonso *et al*. 2011; Braack *et al*. 2018). Malaria is a life-threatening infectious disease caused by protozoan parasites of the genus *Plasmodium* that are transmitted through the bites of infected female *Anopheles* mosquitoes. Globally, an estimate of 241 million malaria cases and 627,000 deaths were reported in 2020, of which 95% of malaria cases and highest proportion of deaths were reported from a WHO Africa region (WHO, 2021). In this region 80% of all malaria deaths are recorded in children's under the age of 5. From the total malaria case across the globe 55% is recorded in sub-Sahara Africa (WHO, 2021). In Ethiopia, more than 60% of the population is at risk of malaria infection and 68% of the country's landmass is malarious (FMoH, 2014). The diverse ecology and favorable environmental conditions of the country supported the rapid development of *Anopheles* mosquitoes and *Plasmodium* parasites (Taffese *et al*. 2018).

There are more than 400 different species of *Anopheles* mosquitoes worldwide, of which around 30 are known malaria vectors (WHO, 2018). In Ethiopia, *An. arabiensis* is the primary malaria vector, whereas *An. funestus*, *An. pharoensis* and *An. nili* are secondary vectors (Gillies and Coetzee, 1987). The most important stage of the *Anopheles* mosquito life cycle such as egg-laying, larval and pupal development, and adult emergence takes place in the aquatic environment (Oyewole *et al*. 2009). Different species of *Anopheles* mosquitoes have their own preferred aquatic habitats. For instance, *An. arabiensis* prefers to breed in temporary, small, sunlit, clear, and shallow fresh-water pools (Gimnig *et al*. 2001; Edillo *et al*. 2006; Himeidan and Rayah, 2008).

The abundance and distribution of potential breeding habitats of *Anopheles* mosquitoes determine the density of adult *Anopheles* mosquitoes and transmission of malaria parasites (Carter *et al*. 2000; Rejmánková *et al*. 2013). Several environmental characteristics like climate, physical, chemical, and biological conditions of the breeding habitats have effects on the development and survival of the *Anopheles* mosquito larvae (Mereta *et al*. 2013; Roux and Robert 2019). Anthropogenic factors like agricultural expansion, construction of dams and urbanization affects the diversity and distribution of *Anopheles* mosquito breeding habitat and larval development (De Silva and Marshall, 2012). Climate change associated with these anthropogenic effects favors rapid development of *Anopheles* mosquitoes and *Plasmodium* parasite in areas with antecedently low malaria transmission (Alonso *et al*. 2011).

In Ethiopia, malaria control program mainly depends on the management of clinical malaria cases or control efforts targeting adult mosquitoes by selective indoor residual spray (IRS) and insecticide-impregnated bed nets (LLINs) (FMoH, 2014). However, these strategies are challenged by the development of drug resistant *Plasmodium* parasites and insecticide resistant vector species (Messenger *et al*. 2017; Taffese *et al*. 2018; Loha *et al*. 2019). Considering this, interests are rising to use larval source management as a part of integrated vector management in the country (Gari and Lindtjørn, 2018; Asale *et al*. 2019). Nevertheless, our knowledge about mosquitoes breeding habitat diversity, distribution and characteristics is limited and insufficient to design effective malaria intervention strategies by larval control (Rejmánková *et al*., 2013).

Therefore, the aim of this study was to assess species composition, distribution, and ecology of *Anopheles* mosquito larvae and pupae in malaria endemic localities of Dembiya District, Northwestern Ethiopia. The result of this study will provide important information to design effective vector control strategies by larval source management.

Methods

Description of the study area

A longitudinal study on species composition, breeding habitat characteristics, and spatial distribution of *Anopheles* mosquito larvae and pupae was conducted from June 2018 to May 2019 in two localities (Guramba Bata and Arebiya) of Dembiya district in North Gondar Administrative Zone of Amhara Regional State, Northwestern Ethiopia (Fig. 1). The district is located at 12°39'59.99" N and 37°09'60.00" E. Kola Diba is the administrative center of the district, located 750 km north of Addis Ababa and 35 km southwest of Gondar. The district is bordered by Lake Tana in the south. Dembiya district has 45 localities with an estimated population of approximately 271,000. The majority of the population (91%) lives in rural areas, with most engaged in farming activities; the remaining 9% live in urban areas. The district has 49,528 rural households with 4.3 mean household sizes (CSA, 2007).

The elevation of Dembiya District ranges from 1500 m to 2600 m a.s.l. The agro-ecology of the District is mid-altitude (Woynadega) with a mean annual minimum temperature of 11 ^{0}C and a maximum of 32 ^{0}C, respectively. The mean annual rainfall ranges from 995 mm to 1175 mm. Land use data from the district agricultural bureau indicated that most of the land is considered as plain (87%), while mountainous, valleys, and wetland make up 5%, 4.8%, and 3.2%, respectively. Out of the total area of the District, 31% is cultivated land, 16% is none cultivable land, 5.6% forest and bush, 12.8% grazing, 8.1% is covered with water, 20.2% swamp and 4.3% is a residential area. The district receives bimodal rainfall, with the short rainy season from March to May and the main rainy season from June to September.

The major crops grown by small-holder farmers include teff (*Eragrostis tef*), maize (*Zea mays)*, barley (*Hordeum vulgare*), sorghum (*Sorghum bicolor*), and finger millet (*Eleusine coracana*). Besides, legumes and pulses such as chickpeas (*Cicer arietinum*) and cowpeas (*Vigna unguiculata*) are also grown in the district. Farmers also grow pulse and spice crops like pepper (genus *Capsicum*), niger seed (*Guizotia abyssinica*), fenugreek (*Trigonella foenum-graecum*), black cumin (*Nigella sativa*), White cumin (*Cuminum cyminum*), and rice (*Oryza sativa*) on a limited amount of farmlands.

One of the study localities, Guramba Bata (12^{0}21’57.75”N and 37^{0}20'25.31" E, altitude 1,795–1,820 m.a.s.l.), has a seasonal river “Ahya gedel” or “Nededo” which forms intermittent mosquito breeding water bodies until the end of December. Guramba Bata has one health post and one health center, 1113 households with 6008 inhabitants (2974 males and 3034 females) in 2017/18 (District Health Office report) (Fig. 1).

The second study locality, Arebiya (12^{0}20'26.59"N and 37^{0}22'16.04" E) has “Megech” river serves as a water source during a dry season and flows into Lake Tana. This locality has 1976 households and a total of 8632 inhabitants (4298 males and 4384 females) in 2017/18. Arebiya has only one health post (District Health Office report) (Fig. 1).

Collection of Anopheles **mosquitoes larvae and pupae**

The larvae and pupae of *Anopheles* mosquitoes were collected from different breeding sites such as drainage canals, artificial pit shelters, hoof prints, and rain pools (Fig. 2). The larvae collection was performed for two consecutive days per month for 12 months. Before larval sampling, habitats were inspected for the presence or absence of *Anopheles* larvae and pupae. When *Anopheles* larvae were present, repeated dips were taken using standard WHO dippers (350 ml capacity) depending on the size of each larval habitat (WHO 1975). For small habitats like hoof prints, samples taken with droppers from several sites were pooled to get appropriate larval sampling (Soleimani-Ahmadi *et al*. 2014). Sampling was performed in the morning (09:00–12:00) or in the afternoon (14:00–17:00) for about 30 minutes at each larval breeding habitat.

Species Identification

Anopheles mosquito larvae and pupae were separated from *Culicines* using a hand lens (10X) based on gross morphological characteristics (Verrone 1962; Gillies and Coetzee, 1987). The 3rd and 4th instar *Anopheles* larvae, and *Anopheles* pupae collected from each type of breeding habitat, were transferred to separately labeled mosquito breeders (BioQuip, Dimensions: 7 – 3/4" (195 mm) high x 3–5/8" (92 mm) diameter) and reared in the field laboratory based on WHO guidelines (WHO, 1975). The adult *Anopheles* that emerged from field collected larvae and pupae were used for species identification based on morphological characteristics (Verrone 1962; Gillies and

Coetzee 1987). Morphologically identified adult *An. gambiae sensu lato* was individually preserved in Eppendorf tubes with silica gel and cotton for further molecular identification.

Species identification using rDNA–polymerase chain reaction (PCR)

Preserved adult *An. gambiae s.l* specimens from field collected and reared larvae and pupae were further identified to sibling species using a ribosomal DNA polymerase chain reaction (PCR) by including the primers for *An. gambiae s.s*, *An. arabiensis*, *An. quadriannulatus* and *An. amharicus* (Scott *et al*. 1993).

Physico-chemical characteristics of larval habitats

Physical characteristics of the breeding habitats (positive or negative for *Anopheles* larvae or pupae), including habitat type, water depth, turbidity, vegetation, presence of algae, bottom substrate, habitat stability, lotic or lentic water, sunlight intensity, and distance from nearby houses were measured and recorded (Minakawa *et al*. 1999).

Chemical characteristics of the breeding habitats (positive or negative for *Anopheles* larvae or pupae) such as temperature, pH, and conductivity were measured at the field with HANNA© HI 98130 Combo pH & EC tester (Hanna Instruments Inc., Kehl am Rhein, Germany) with the probes placed 2 to 3 cm below the water surface.

Data analysis

The density of *Anopheles* larvae and pupae was expressed as the total number of *Anopheles* larvae per total number of dips taken. After checking for normality, all dependent variables were $\log_{10}(x + 1)$ transformed, and subjected to statistical analysis. Since the data were found to be normally distributed after transformation, parametric testes such as one-way analysis of variance (ANOVA) and student t-test (for independent variables with two categories) were used to analyze differences in mean larval densities among breeding habitat types and other environmental variables. When significant differences were observed in one-way ANOVA, means were separated using Tukey's HSD (Tukey's Honestly Significant Difference) test at $\alpha = 0.05$. Multiple logistic regression analysis was used to detect the best predictor environmental variables associated with the presence or absence of *Anopheles* mosquito larvae (Sattler *et al*. 2005). The percentages of species composition of *Anopheles* mosquitoes collected from each breeding habitat was calculated (number of *Anopheles* mosquitoes species * 100/ total number of species identified). Pearson correlation analysis was used to assess the relationship between larval densities and chemical characteristics such as pH, temperature, and conductivity. The data were analyzed using SPSS version 20 (Armonk, NY: IBM Corp), $p \leq 0.05$ were considered as significant.

Results

Breeding habitat types and abundance of Anopheles **larvae and pupae**

During the one-year study period, a total of 108 potential larval habitats (60.2% from Arebiya and 39.8% from Guramba Bata) were assessed for the presence of *Anopheles* larvae. From the total sampled potential habitats only 41 were positive for *Anopheles* larvae and pupae (Table 1). From which, the predominantly encountered larval habitats were rain pools (17.6% (n = 19)), and river pool (17.6% (n = 19)). More than 80% of the larval breeding habitats were recorded during the long rainy season. Whereas during the dry season, the distribution of *Anopheles* larvae and pupae were restricted to water pools at riversides, pits dug for plastering a house, and temporary habitats around the hand pump water well.

The highest mean densities of *Anopheles* larvae were recorded from breeding sites in drainage canals (14.7 ± 3.5 larvae/dip), followed by abandoned burrow pits dug for plastering houses (8.8 ± 3.1 larvae/dip), swamps (3.8 ± 1.2 larvae/dip), and hoof prints (3 ± 1.2 larvae/dip). In addition, *Anopheles* larvae were also recorded from puddles (2.7 ± 2.7 larvae/dip), water pools at riversides (2.0 ± 0.9 larvae/dip), and tire tracks (0.4 ± 0.4 larvae/dip) (Table 1). The difference in mean densities of *Anopheles* larvae and pupae among habitats was statistically significant ($F_{8, 99}$ = 9.85, $p = 0.000$) and ($F_{8, 99}$ = 3.46, $p = 0.001$) respectively (Table 1).

Table 1
Distribution of *Anopheles* mosquito larvae and pupae in different breeding habitats in the two localities of Dembiya District, Northwestern Ethiopia.

Habitat type	No. of breeding sites surveyed (%)	No. of dips	No. of larvae collected	Mean (larvae/dips) ± se	No. of pupae collected	Mean (pupae/dips) ± se
Riversides	19 (17.6)	99	279	2.0 ± 0.9^{ab}	48	0.3 ± 0.1^{ab}
Burrow pits	14 (12.96)	70	421	8.8 ± 3.1^{bc}	49	0.99 ± 0.4^{ab}
Drainage canals	12 (11)	48	566	14.7 ± 3.5^{c}	59	1.6 ± 0.6^{b}
Tire tracks	8 (7.4)	24	9	0.4 ± 0.4^{a}	3	0.1 ± 0.1^{a}
Hoof prints	7 (6.5)	19	63	3.0 ± 1.2^{ab}	5	0.2 ± 0.2^{ab}
Swamps	14 (12.96)	56	205	3.8 ± 1.2^{ab}	9	0.2 ± 0.1^{ab}
Rain pools	19 (17.6)	69	30	0.2 ± 0.2^{a}	0	0.0 ± 0.0^{a}
Puddles	7 (6.5)	33	56	2.7 ± 2.7^{a}	12	0.6 ± 0.6^{ab}
Streams	8 (7.4)	30	0	0.0 ± 0.0^{a}	0	0.0 ± 0.0^{a}
Total	108(100%)	448	1629		185	

The mean larval and pupal densities with different letter designations in a column are significantly different with Tukey's HSD post hoc analysis at α = 0.05.

Species composition and monthly distribution of Anopheles **larvae and pupae**

The species composition of *Anopheles* mosquitoes identified from the two study sites during the study period is presented in Table 2. A total of 1,629 *Anopheles* larvae and 185 pupae were collected from the two localities. From the total collected immature stages of *Anopheles* mosquitoes, 52.3% (852) larvae and 65.9 (122) pupae were from Arebiya and 47.7% (777) larvae and 34.1% (63) pupae were from Guramba Bata. The difference in mean *Anopheles* larval and pupal density between the two study sites were not statistically significant (t (106) = -0.454, $p = 0.651$) and (t (106) = 0.70, $p = 0.485$) respectively.

From the total collected *Anopheles* larvae and pupae, 835 females and 788 males have successfully emerged into adults. The rest 191 larvae and pupae were not able to emerge to adult. Therefore, only 835 female *Anopheles* mosquitoes were subjected to species identification based on morphological features. All *An. gambiae s.l* samples used for species identification using PCR were found to be *An. arabiensis*. Eight species of *Anopheles* such as *An. arabiensis*, *An. pharoensis*, *An. coustani*, *An. christyi*, *An. squamosus*, *An. demeilloni*, *An. danicalicus*, and *An. cinereus* were identified (Table 2). From the total identified *Anopheles* species, *An. arabiensis* (59.2%) was dominant followed by *An. pharoensis* (35.3%) and *An. coustani* (2.99%). The least common species were *An. dancalicus* and *An. cinereus* (Table 2).

The monthly distribution of *Anopheles* larvae and pupae showed that the highest density of *Anopheles* larvae in Arebiya was collected in June, September, October, and May (Fig. 3a). In the meantime, less density of *Anopheles* larvae was recorded during July and August, which is corresponding to pick monthly rainfall, which creates unstable larval breeding habitat due to over flooding. Similarly, in Guramba Bata high larval density was recorded starting from June, August, September, and October (Fig. 3b). However, the density of *Anopheles* larval density sharply declines in both study areas during a dry season (January, February, and March) (Fig. 3a **& b**).

Table 2
Species composition and abundance of *Anopheles* mosquitoes collected from the two localities of Dembiya District, Northwestern Ethiopia (Values in parenthesis are percentages).

Species	Arebiya	Guramba Bata	Total
An. arabiensis	297	197	494 (59.2)
An. phareonsis	113	182	295 (35.3)
An. christyi	6	2	8 (1)
An. squamosus	3	0	3 (0.4)
An. coustani	9	16	25 (2.99)
An. demeilloni	2	4	6 (0.7)
An. dancalicus	0	1	1 (0.1)
An. cinereus	0	3	3 (0.4)
Total	430 (51.5)	405 (48.5)	835 (100)

Species specific monthly distribution of Anopheles **mosquitoes**

The species-specific monthly distribution of the dominant *Anopheles* mosquitoes is indicated in Fig. 4a **& b**. The highest density of *An. arabiensis* in Arebiya was recorded during June (16.5%), September (9.1%), and May (21.6%) (Fig. 4a). Similarly, in Guramba Bata the highest density of *An. arabiensis* was recorded during June (12.8%), July (10.4%), and September (12.3%), but its number sharply declined after the end of the long rainy season (Fig. 4b). The number of *An. pharoensis* reached its peak around the end of the main rainy season in the two study areas (Fig. 4a **& b**).

Anopheles **species specific breeding habitat types**

The associations of species-specific *Anopheles* larvae in different breeding habitat types are presented in Table 3. *An. arabiensis* larvae were collected from drainage canals, burrow pits, water pools at riversides, tire tracks, hoof prints, and puddles (Table 3). A high number of *An. arabiensis* was collected near the edge of a small temporary and permanent habitat with still and mid turbid water, grass, and had full sunlight access. *An. pharoensis* was collected from a wide range of permanent habitats such as water pools at riversides, swamps, and grassy burrow pits (Table 3). The breeding habitats of *An. pharoensis* were relatively turbid, full of vegetation, and had partial sunlight access. *An. coustani* was collected from permanent habitats such as riverside water pools, burrow pits, and swamps (Table 3). The most common breeding sites of this species were usually permanent habitats with relatively turbid water, vegetation, and partial sunlight access.

Table 3
Species-specific spatial distribution of *Anopheles* mosquitoes in the two localities of Dembiya District, Northwestern Ethiopia (Values in parentheses are the percentages).

	***Anopheles* mosquito larval breeding habitat types**								
Species	Riverside	Burrow pit	Drainage canal	Tire track	Hoof print	Swamp	Rain pool	Puddles	Total
An. arabiensis	78 (15.8)	148 (29.9)	174 (35.2)	5 (1)	26 (5.3)	26 (5.3)	11 (2.2)	26 (5.3)	494 (59.2)
An. pharoensis	83 (28.1)	69 (23.4)	64 (21.7)	-	-	79 (26.8)	-	-	295 (35.3)
An. coustani	9 (36)	5 (20)	4 (16)	-	-	7 (28)	-	-	25 (2.9)
An. christyi	1 (12.5)	5 (62.5)	1 (12.5)	-	-	1 (12.5)	-	-	8 (0.96)
An. demeilloni	-	2 (33.3)	4 (66.7)	-	-	-	-	-	6 (0.7)
An. squamosus	-	2 (66.7)	-	-	-	1 (33.3)	-	-	3 (0.4)
An. cinereus	3 (100)	-	-	-	-	-	-	-	3 (0.4)
An. dancalicus	-	-	1 (100)	-	-	-	-	-	1 (0.1)
Total	174 (20.8)	231 (27.7)	248 (29.7)	5 (0.6)	26 (3.1)	114 (13.7)	11 (1.3)	26 (3.1)	835 (100)

Association of Anopheles larval densities with physical characteristics of larval breeding habitat types

The associations of mean larval densities with physical variables of the breeding habitat types are presented in Table 4. The densities of *Anopheles* larvae were significantly associated with shallow depth (≤ 0.5 m), medium turbidity, availability of grasses, muddy bottom substrates, distance to the nearest house (≤ 100 m), and presence of algae ($p \leq 0.05$) (Table 4).

Table 4
Association of physical characteristics of breeding habitats with *Anopheles* mosquito larval density, Dembiya District, Northwestern Ethiopia.

Physical factors	Variables	Densities of *Anopheles* larvae		
		Mean ± se	*p*	F
Average depth	≤ 0.5 m	5.2 ± 1.01	0.040	2.144
	≥ 0.5 m	1.8 ± 0.57		
Turbidity	Low	0.63 ± 0.45	0.000	19.899
	Med	6.96 ± 1.24		
	High	0.55 ± 0.37		
Vegetation	No vegetation	0.69 ± 0.43	0.000	-4.89
	Grass	7.44 ± 1.29		
Bottom substrate	Muddy	6.74 ± 1.08	0.000	8.231
	Clay	0.44 ± 0.34		
	Sandy	3.21 ± 3.21		
Water current	Stagnant	4.69 ± 0.96	0.180	-1.35
	Flowing	2.34 ± 0.92		
Light intensity	Full sunlight	4.28 ± 0.81	0.687	0.404
	Shaded	3.57 ± 1.84		
Distance	≤ 100m	7.04 ± 1.22	0.000	7.76
	100 -200m	0.53 ± 0.53		
	201-300m	0.00 ± 0.00		
	301–400m	0.17 ± 0.17		
Algae	Present	10.16 ± 2.16	0.000	5.66
	Absent	1.74 ± 0.40		
Water persistency	Temporary	2.6 ± 0.70	0.99	1.66
	Permanent	5.15 ± 1.18		

A bivariate analysis showed that average depth (COR = 2.54; 95% CI: 0.98–6.6, $p \leq 0.05$) turbidity (COR = 28.4; 95% CI: 3.61-224.27, $p \leq 0.05$), vegetation (COR = 17.82; 95% CI: 5.62–56.54, $p \leq 0.05$), distance to the nearest house (COR = 32.7; 95% CI: 4.15-257.27, $p \leq 0.05$) and presence of algae (COR = 4.92; 95% CI: 1.99–12.11, $p \leq 0.05$) were significantly associated with the presence or absence of *Anopheles* larvae (Table 5).

Table 5
A bivariate and multivariate analysis of association of physical factors of breeding habitats with presence or absence of *Anopheles* mosquito larvae, Dembia District, Northwestern Ethiopia.

Factors	**Variables**	**OR (95% CI)**		***p*-value**
		COR	AOR	
Average depth	≤ 0.5	2.5 (0.98–6.60)*	0.4 (0.04–4.32)	0.450
	≥ 0.5	1	1	
Turbidity	Low	3.6 (0.35–37.36)	22.5 (0.66-774.51)	0.084
	Med	28.4 (3.61-224.27)*	66 (2.01-2168.24)	0.019
	High	1	1	
Vegetation	No vegetation	1	1	
	Grass	17.8 (5.62–56.54) *	12.6 (1.29-122.78)	0.029
Bottom substrate	Muddy	21.7 (2.64-177.76)	8.6 (0.24-308.82)	0.238
	Clay	0.00 (0.00)	0.00 (0.00)	0.997
	Sandy	1	1	
Water current	Stagnant	0.36 (0.12–1.05)	0.1 (0.01–1.29)	0.077
	Flowing	1	1	
Light intensity	Full sunlight	2.1 (0.76–5.79)	2.21 (0.25–19.92)	0.479
	Shaded	1	1	
Distance	≤ 100m	32.7 (4.15-257.27)*	27.99 (0.65-1215.17)	0.083
	100 -200m	1.39 (0.08–23.71)	0.99 (0.01-132.14)	1.00
	201-300m	0.00	0.00	
	301–400m	1		
Algae	Present	4.92 (1.99–12.11)*	6.94 (0.78–62.09)	0.083
	Absent	1		
Water persistency	Temporary	1.15 (0.51–2.61)	1.51 (0.23–9.99)	0.67
	Permanent	1		
Surface debris	Low	1.81 (0.54–6.08)	7.90 (0.53-117.17)	0.133
	Medium	1.11 (0.34–3.68)	0.723 (0.07–7.89)	0.791
	High	1	1	
* indicates statistically significant values at $p = 0.05$				

The final model for the parameters associated with presence or absence of *Anopheles* larvae showed that turbidity (AOR = 66.03; 95% CI: 2.01-2168.24, $p = 0.019$) and vegetation cover (AOR = 12.62; 95% CI: 1.29-122.78, $p = 0.029$) were key physical factors which determine the densities of *Anopheles* larvae (Table 5).

Correlation of chemical characteristics of larval habitat with Anopheles **density**

The total density of *Anopheles* larvae was positively correlated with temperature (r = 0.331 and $p = 0.013$) and pH (r = 0.697 and $p = 0.00$). However, the density *Anopheles* larvae was negatively correlated with conductivity (r= -0.321, $p = 0.016$) (Table 6).

Table 6
Correlation of chemical characteristics of breeding habitat with *Anopheles* mosquito larval density in Dembiya District, Northwestern Ethiopia.

Chemical factors	Larval density
Temperature (°C)	0.331*
pH	0.697**
Conductivity (µS/m)	-0.321*
**. Correlation is significant at the 0.01 level	
*. Correlation is significant at the 0.05 level	

Discussion

This study showed that the spatiotemporal distribution and species composition of *Anopheles* mosquito larval density were greatly affected by the physicochemical characteristics of the breeding habitat and the rainfall pattern of the localities. Describing larval habitat characteristics in terms of environmental attributes and identifying relationships between breeding habitat and larval density is important to develop novel methods of vector control by targeting the aquatic stage of *Anopheles* mosquitoes in areas with high vector intervention strategies.

During this study rain pools, river pools, burrow pit, swamp, drainage canal, tire track, hoof print, puddle, and stream were the encountered larval breeding habitats, of which rain pools and river pools were dominantly observed breeding habitats during the rainy season. Rain during the rainy season produces many rain pools and river edges, which are potential sites for larval development. In concurrent with this study, *Anopheles* mosquitoes prefer to breed at the edges of rivers and streams, in temporary rain pools, ponds, dams, drainage ditches, burrow pits, rice fields, swamp margins, roadside puddles, and in tree holes close to human dwellings (Shililu *et al*. 2003; Yohannes *et al*. 2005; Omlin *et al*. 2007). In addition, similar habitat types were recorded in previous studies elsewhere in Ethiopia (Mereta *et al*. 2013) and Kenya (Imbahale *et al*. 2011).

The distribution of *Anopheles* larvae during the dry season was limited to water pools at riversides, pits dug for plastering a house, drainage canals, and water pools around hand pump water well. This is because during the dry season the water will be confined to temporary habitats such as riversides, burrow pits, drainage canals, and swamps which are important for the reproduction of *Anopheles* mosquitoes. Similarly, studies showed that the number and size of *Anopheles* breeding habitat are reduced during the dry season (Animut and Negash 2018). This restricted distribution of *Anopheles* mosquito breeding habitat during the dry season makes them more vulnerable for larval management. Larval management during a dry season is less costly than management during a wet season in areas where the dry season is associated with limited breeding habitats (WHO, 2013). Dry season larval management will hamper the exponential reproduction rate of *Anopheles* larvae at the end of the long rainy season hence it will limit malaria transmission (Animut and Negash 2018). This approach could be effective to reduce malaria transmission associated with insecticide resistant malaria vectors and outdoor host seeking mosquitoes, as it manages the immature stage (egg, larvae, and pupae) confined in a small aquatic environment (Killeen *et al*. 2002).

The result of this study indicated that the density of *Anopheles* larvae varies among habitat types, where a significantly highest density was recorded from water pools at drainage canal and the grassy edge of burrow pits. This larval density variation in the different habitats could be explained by the spatiotemporal differences in food resource and predation pressure in different habitats and the complex interaction between physicochemical factors such as water turbidity, depth, temperature, salinity, and dissolved oxygen (Mala and Irungu 2011; Kipyab *et al*. 2015; Roux and Robert 2019).

Anopheles mosquito species such as *An. arabiensis*, *An. pharoensis*, *An. coustani*, *An. christyi*, *An. squamosus*, *An. demeilloni*, *An. danicalicus*, and *An. cinereus* were identified from the two study areas. This result is in line with finding from the west Gojam zone (Animut and Negash 2018), south-central Ethiopia (Animut *et al*. 2012), and Central Ethiopia (Kenea *et al*. 2011). The highest density of *Anopheles* larvae was recorded during the rainy seasons. The reason is that rainfall and humidity strongly affect the availability of larval habitat, *Anopheles* species, and distribution (Imbahale *et al*. 2011).

An. arabiensis was the dominant species identified in the study area, similar to report from southwest Ethiopia (Getachew *et al*. 2020), Addis Zemen, South Gondar, Ethiopia (Kindu *et al*. 2018), South-central Ethiopia (Animut *et al*. 2012), and Western Kenya (Kweka *et al*. 2012). The highest density of *An. arabiensis* was recorded in June and September when the rainy season starts and retreats. Similar observations have shown that populations of *An. arabiensis* usually increase as the rains withdraw (Getachew *et al*. 2020). The result of this study indicated that *An. arabiensis* was dominantly distributed in small temporary habitats with still and less turbid water and grasses and full sunlight access. The reason for this could be the presence of less larval predation pressure in small temporary habitats than permanent habitats and temporary habitats with full sunlight access provide warmer water, which resulted in a high algal density (source of food for larvae) and rapid development of larvae to pupae (Gimnig *et al*. 2002). In concurrent with this study, *An. arabiensis* was reported to breed in small, temporary habitats with algae such as footprints, rain pools, puddles, tire tracks, and garden wells (Mattah *et al*. 2017). These species were also identified from temporary habitats such as swamps, irrigation canals, sand pools, canal leakage pools, water harvesting pools, and brick-making pits in central Ethiopia (Kenea *et al*. 2011).

Anopheles pharoensis was dominantly distributed in aquatic habitats with turbid, full of vegetation, and partial sunlight access. Different reports also supported this observation, claiming that the density of *An. pharoensis* was higher in aquatic habitats with floating vegetation and with shady conditions (Teklu *et al*. 2010).

Physical characteristics of the *Anopheles* mosquito breeding habitat such as low turbidity and the presence of grass were the limiting factors that determine the presence or absence of *Anopheles* mosquito larvae in this study. In addition, the density of *Anopheles* mosquito larvae was positively correlated with temperature and pH and negatively correlated with water conductivity. Conductivity measures the amount of inorganic matter and ions in water, therefore as turbidity of the water increases due to flooding conductivity also increases proportionally, which in turn affects the development of the larval population (Edillo *et al*. 2006). Low water turbidity and full sunlight access increase the water temperature and hence leads to a rapid larval development (Paaijmans *et al*. 2010). This result coincides with previous works conducted in the highlands of Ethiopia (Dejenie *et al*. 2011), Kenya (Minakawa *et al*. 1999), and Tanzania (Emidi *et al*. 2017).

Conclusions

Anopheles mosquitoes such as *An. arabiensis*, *An. pharoensis*, *An. coustani*, *An. christyi*, *An. squamosus*, *An. demeilloni*, *An. danicalicus*, and *An. cinereus* were identified during this study, of which *An. arabiensis* was the dominant species. Breeding habitats such as drainage canals and burrow pits served as a potential reproduction site of *Anopheles* mosquitoes in the study area. The distribution of *Anopheles* mosquito larvae is greatly affected by physicochemical characteristics of the breeding habitats such as water turbidity, vegetation cover, pH, temperature, conductivity, and season. A malaria control and elimination program in the study area should incorporate larval management strategies by using larvicides and source reduction as a part of IVM (community-based habitat modification during a dry season when the number of breeding habitat is limited). Further study on identification of the natural predators of *Anopheles* mosquitoes larvae/pupae in the study area is recommended.

Abbreviations

CL
Confidence Limit
DF
Degree of freedom
COD
Crud odd ratio
AOD
Adjusted odd ratio
SE
Standard error
PCR
Polymerase chain reaction

Declarations

Ethics approval

Not applicable

Consent to participate

Not applicable

Consent for publication

Not applicable

Availability of data and materials

The data sets supporting the conclusions of this article are provided in the manuscript.

Conflicts of interest/Competing interests

The authors declare that there is no conflict of interest

Funding

This study was financed by Addis Ababa University.

Authors' contributions

MT, HT, YW and SD designed the study. HT, YW and SD supervised and MT conducted the field work, rearing and identification experiments. MT conducted the statistical analyses. MT developed first draft, HT, YW and SD revised the manuscript. All authors read and approved the final manuscript.

Acknowledgements

The authors want to acknowledge Woldia University for providing a study leave and Addis Ababa University for providing financial support from Thematic Research Fund. We also express our appreciation to Aklilu lemma institute of pathobiology, Addis Ababa University, staff members for their technical assistance.

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Figures

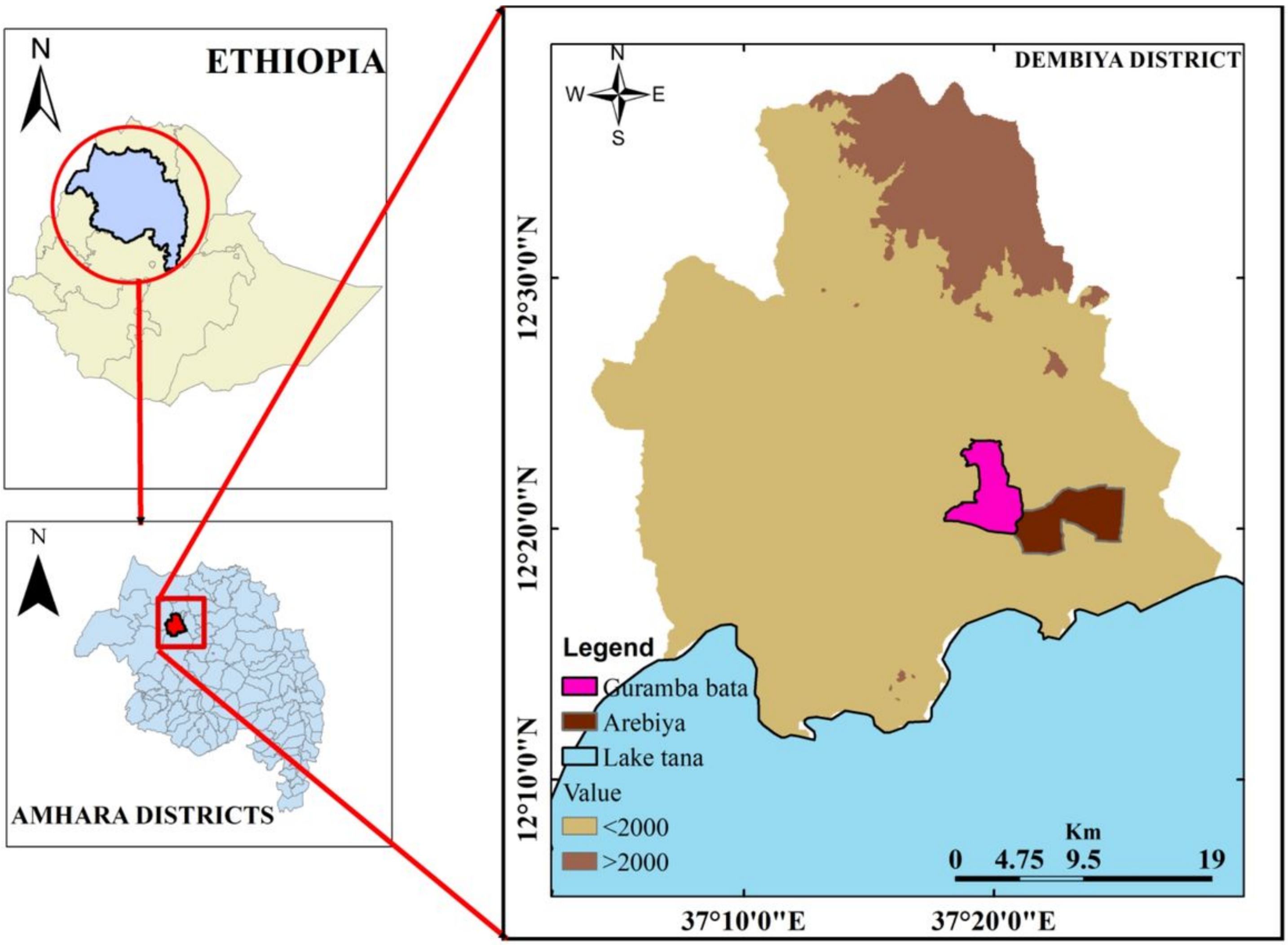


Figure 1

Map of the study area (Tarekegn et al.*, 2021)*

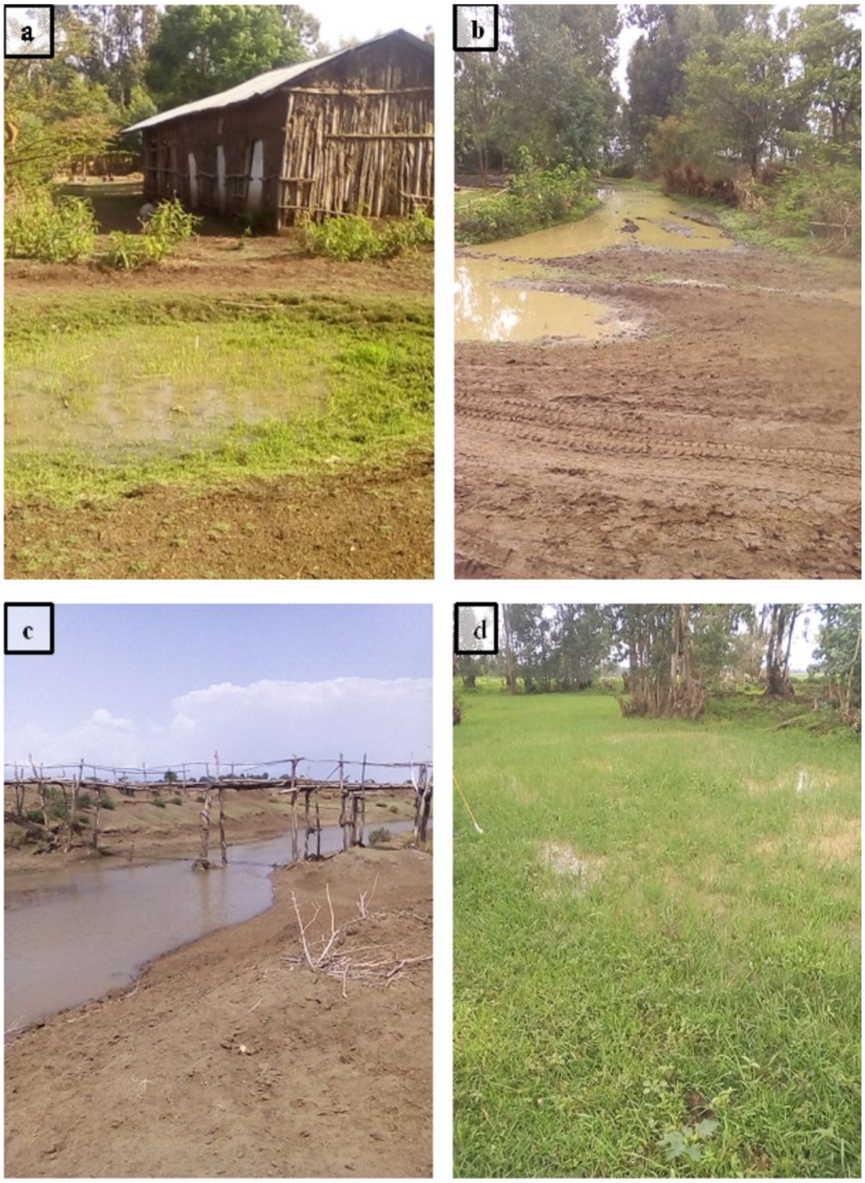
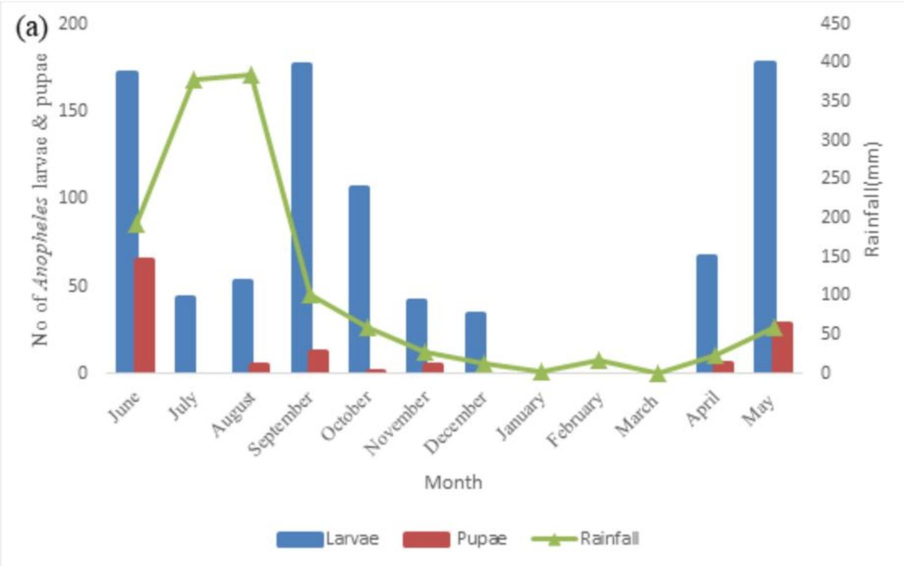


Figure 2

Anopheles *mosquito breeding habitats types. (**a**). Artificial pit, (**b**).Drainage canal, (**c**). River, (**d**). Swamp*



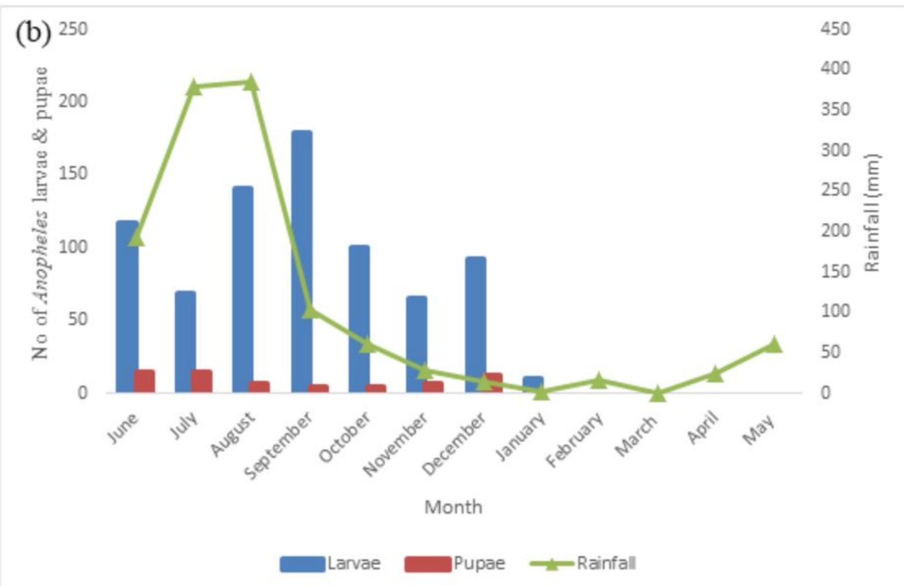


Figure 3

Monthly distributions of *Anopheles* mosquito larvae and pupae in Arebiya (**a**) Guramba Bata (**b**) study sites from June 2018-May 2019.

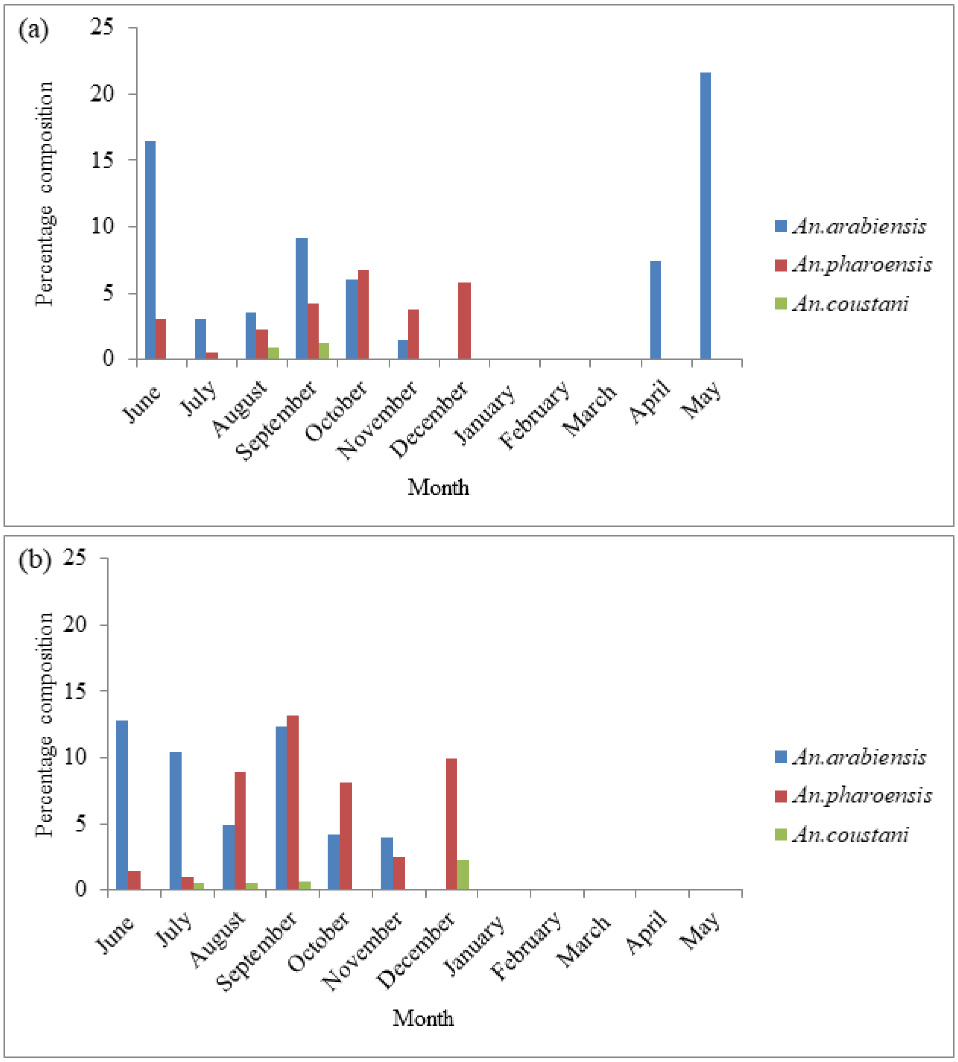


Figure 4

Monthly distribution of the dominant *Anopheles* mosquito species in Arebiya (**a**) and Guramba Bata (**b**) study sites from June 2018-May 2019.