International Journal of Tropical Insect Science (2022) 42:2325–2336
https://doi.org/10.1007/s42690-022-00755-0

ORIGINAL RESEARCH ARTICLE

Characterization of *Anopheles* mosquito larval habitats and species composition in Bambasi District, Northwestern Ethiopia

Hawi Keno[1] · Desta Ejeta[2] · Tokuma Negisho[1] · Mulugeta Wakjira[1] · Geremew Muleta[3] · Gadisa Natea[1] · Delenasaw Yewhalaw[4,5] · Eba Alemayehu Simma[1]

Received: 31 July 2021 / Accepted: 6 February 2022 / Published online: 30 March 2022

Abstract

Malaria is a widespread vector-borne disease in the tropics and subtropics causing nearly half a million deaths every year. Malaria vector control intervention mainly rely on the control of adults using Indoor residual sprayings (IRS) and long lasting insecticidal nets (LLINs). The purpose of this study was to assess the species composition of *Anopheles* mosquitoes and determine the environmental and physicochemical parameters of their breeding habitats in Bambasi district, Benshangul Gumuz regional state, northwestern Ethiopia. Three major *Anopheles* breeding habitats were identified in three Kebeles namely; drainage ditch (Keshmando), swamp (Amba 46), and stagnant water (Amba 47). *Anopheles* mosquito larvae were sampled twice a month from September 2020 to November 2020. A total of 2185 *Anopheles* mosquito larvae were collected. Of those collected larvae three *Anopheles* species (*Anopheles gambiae* s.l. *An. funestus* and *An. coustani* complex) were identified. *Anopheles gambiae* s.l was the most abundant whereas *An. funestus* and *An. coustani* were the least in all the study kebeles. Of the three kebeles, Amba 47 was found the most productive for *Anopheles* followed by Amba 46 and Keshmando. The highest mean density of larvae per dip was sampled in September in all the study sites. The three sampling sites varied in physicochemical characteristics. The findings of this study showed that dissolved oxygen (DO) was highest (7.07 ± 0.55 mg/L) in the swamps and lowest (0.32 ± 0.04 mg/L) in the drainage ditches. Conductivity across different habitats showed wide variations. There were slight variations in temperature between different habitats. Higher total dissolved solids (TDS) 12.19 ± 0.26 mg/L was recorded from the drainage ditches; whereas TDS 9.49 ± 1.62 mg/L was recorded from the swamp. Salinity in the drainage ditches and stagnant water was 5.54 ± 1.00PSU and 3.30 ± 0.97 PSU respectively. There were negative strong correlation between the larval density with temperature and EC but positive correlation between larval density with salinity. However, there was no significant correlation between *Anopheles* larval density with TDS and DO. In conclusion this study suggested that environmental and physicochemical factors could play an important role in the development of mosquito larvae. Therefore, characterizing mosquito larval habitats is important for targeted control of malaria vectors in Ethiopia.

Keywords *Anopheles* mosquitoes · Larval habitats · Physicochemical characteristic · Bambasi · Ethiopia

Abbreviations

DO	Dissolved Oxygen
EC	Electrical Conductivity
FMoH	Federal Ministry of Health
IRS	Indoor Residual Spraying
LLIN	Long-lasting Insecticidal Net
pH	Hydrogen ion concentration
PMI	President's Malaria Initiative
SPSS	Statistical package for social sciences
TDS	Total dissolved solids
WHO	World Health Organization

✉ Eba Alemayehu Simma
ebasimma@gmail.com

[1] Department of Biology, College of Natural Sciences, Jimma University, Jimma, Ethiopia

[2] Department of Biology, College of Natural and Computational Sciences, Assosa University, Assosa, Ethiopia

[3] Department of Statistics, College of Natural Sciences, Jimma University, Jimma, Ethiopia

[4] School of Medical Laboratory Sciences, Faculty of Health Sciences, Institute of Health, Jimma University, Jimma, Ethiopia

[5] Tropical and Infectious Diseases Research Center, Jimma University, Jimma, Ethiopia

Background of the study

Malaria, is transmitted to humans by the bite of adult female *Anopheles* mosquitoes (Cox 2010). In 2019, worldwide there were an estimated 229 million cases of malaria and 409 000 deaths of which 94% of the cases were reported from Africa (WHO 2020). In Ethiopia, even though there has been steady progress in the reduction of malaria cases (Deribew et al. 2017; Taffese et al. 2018; WHO 2020) over one million cases of malaria has been reported in 2019 (WHO 2020).

The genus *Anopheles* mosquito is the most studied genera among medically important insects. Of the total 465 *Anopheles* species globally described, about 70 species are known to transmit human malaria (Sinka et al. 2012). Forty one *Anopheles* mosquito species are known to transmit human *Plasmodium* parasites, of which 20 species are the dominant malaria vectors in Africa (Sinka et al. 2012). The major malaria vectors in Africa included *An. gambiae s.l., An. funestus, An. nili, An. pharoensis* and *An. moucheti* (Sinka et al. 2012, 2011).

In Ethiopia, 47 *Anopheles* mosquito species are documented (Gaffigan et al. 2018) of which *An. arabiensis, An. funestus, An. pharoensis* and *An. nili* are recognized malaria vectors. *Anopheles arabiensis* is the primary malaria vector in Ethiopia (Abose et al. 1998; Lulu et al. 1999) while, *An. funestus*, *An. pharoensis* and *An. nili* are secondary vectors occurring with varying densities, limited distribution and vector competence (White 1982; FMoH 2014). Moreover, very recently a new invasive *Anopheles* species, *An. stephensi,* has been documented in Ethiopia (Carter et al. 2018) which might complicate the malaria elimination effort of the country.

Anopheles mosquitoes undergo egg, larval, pupal and adult stages in their life cycle. The egg, larval and pupal stages are aquatic and have very small spatial dispersion. Mosquito breeding habitats are significantly higher in the rainy season than the dry season (Mahgoub et al. 2017). In the dry season, the number and size of larval habitats are generally limited and contribute to a low population of adult *Anopheles* mosquitoes transmitting malaria (Himeidan et al. 2009). Even though, *Anopheles* mosquito species exploit diverse breeding habitats that considerably vary in size, altitude, vegetation cover and topography (Minakawa et al. 2012), the majority of malaria vectors may emerge from prolific habitats, which could account only for a small proportion of the habitats. Small size breeding habitats have many advantages over larger permanent ones which increase the developmental rate or survivorship of the aquatic stage (Gu et al. 2008). Therefore, mosquito larval habitat ecology is important in determining larval densities and designing mosquito control programs (Overgaard et al. 2002; Simisek 2004).

Indoor residual spraying (IRS) and long-lasting insecticidal nets (LLINs) are the main pillars of the malaria vector control strategy in Ethiopia. In the last decade, both IRS and LLINs significantly reduced malaria incidence and prevalence in the country (Girum et al. 2019; Taffese et al. 2018; WHO 2020). However, the wide spread of insecticide resistance in the major malaria vector, *An. arabiensis* (Alemayehu et al. 2017; Messenger et al. 2017; Yewhalaw et al. 2010) could compromise the effort of malaria vector control and elimination efforts. Moreover, the plasticity in feeding, biting and resting behavior of *An. arabiensis* contributed to the existing malaria transmission in the country despite intensified malaria intervention, the scaled up of LLINs, high coverage of IRS, and improved malaria diagnosis and treatment (Degefa et al. 2015; Kenea et al. 2016; Yohannes and Boelee 2012). Therefore, besides targeting adult *Anopheles* populations it is equally important to control the immature stages of *Anopheles* mosquitoes with appropriate larval control tools. Different interventions targeting larvae of *Anopheles* mosquitoes successfully suppressed malaria vector population density and the risk of malaria infection in some African countries including Ethiopia (Castro et al. 2009; Kibret et al. 2018; Utzinger et al. 2001; Wamae et al. 2010; Mekonnen Yohannes et al. 2005).

In Ethiopia, few studies assessed the habitat of mosquito larvae and some environmental factors that affect mosquito abundance (Dejenie et al. 2011; Getachew et al. 2020; Hawaria et al. 2020; Mereta et al. 2013). However, to our knowledge, there is no study reported from Bambasi district (woreda), one of the malaria sentinel sites in Ethiopia, to characterize and assess *Anopheles* mosquito larval breeding habitats. Hence, this study aimed at characterizing *Anopheles* mosquito larval breeding habitats and determining *Anopheles* mosquito fauna in Bambasi district, Ethiopia.

Materials and methods

Description of the study area

Bambasi district is located in Assosa Zone, Benishangul-Gumuz Regional state Northwestern Ethiopia. Assosa town is the capital of the Assosa district and Benishangul-Gumuz Regional state, which is located about 600 km West of Addis Ababa. Bambasi town is 45 km East of Assosa town. It is part of the Assosa Zone, bordered by the Mao-Komo special district on the Southwest, Assosa in the Northwest, Oda Buldigilu in the Northeast and by

the Oromia Region in the Southeast. Bambasi has a longitude and latitude of 9°45′N 34°44′E with an elevation of 1668 m above sea level and is known malarious area. The district experiences mean annual rainfall and temperature values of 1381.42 mm and 28.37 °C, respectively. It is characterized by unimodal rainfall distribution with the rainy season extending from March to November while reaching a peak during the months of June to September (Mossisa et al. 2019). Therefore, the area falls within the lowland (moist Kolla) agro-ecology (Hurni et al. 2016).

The study was conducted in three Kebeles namely Keshmando, Amba 46 and Amba 47 (Fig. 1). Amba 46 and Amba 47 are characterized by having agricultural fields whereas, Keshmando is mainly a human settlement. Keshmando is located 9°36.257 ‘N and 34°41.2008 ‘E 1399 meters above sea level (masl). Amba 46 is located 9°50.88 ‘N and 34°41.316 ‘E 1446 masl. Amba 47 is located 9°52.087 ‘N and 34°41.145 ‘E 1423 masl.

Mosquito larval sampling

Mosquito larvae were sampled twice a month from September 2020 to November 2020 after the long rainy season. Different breeding habitat types such as drainage ditch, swamp and stagnant water were surveyed and searched for the presence of *Anopheles* larvae and their productivity (Fig. 2). During each survey, a habitat was first inspected for the presence of mosquito larvae and then larvae were collected using a standard dipper (350 ml). From each larval habitat, about 20 dips were taken at intervals along the edge of each larval habitat. Sampling was done by the same individual in the morning (10:00–12:00 h) or afternoon (2:00–4:00 h) for about 30 minutes. The larvae were then picked using pipette and transferred to collecting containers. From each habitat, larvae were always transferred into containers with water from the site of collections. Containers were labeled with relevant information such as date, site and number of larvae collected, and were kept in a room of the field insectary and allowed to rear in to adults. Larvae were fed with brewery yeast once per day, and exposed to sunlight twice a day for maximum 30 minutes. Pupae were collected and transferred into a beaker, placed in a cage for adult emergence.

Identification of adult *Anopheles* mosquitoes

Adult *Anopheles* mosquito samples were morphologically identified to the species level under a light microscope

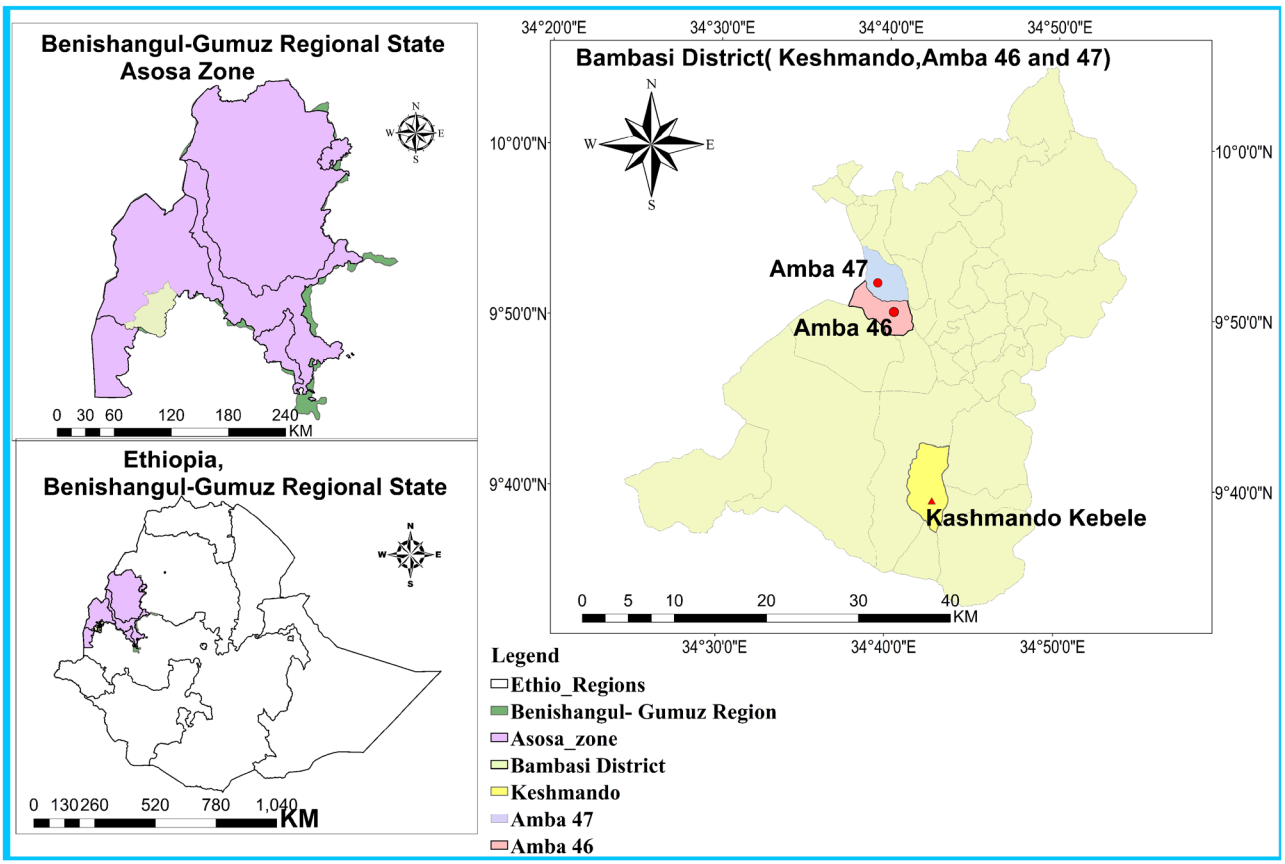

Fig. 1 Map of the study area

Fig. 2 Pictures showing *Anopheles* larval breeding sites (**A**) drainage ditch, (**B**) swamp and (**C**) stagnant water

using standard taxonomic keys (Coetzee 2020; Gillies and Coetzee 1987) in Biology laboratory, Assosa University, Assosa. Afterwards, the samples were kept individually in Eppendorf tubes over silica gel and transported to Jimma University for further identification.

Larval habitat characterization

Environmental and physicochemical characteristics of each larval habitat were observed, measured and recorded during the larval collection. The environmental variables included intensity of light, being natural or artificial, water current, vegetation covering, the presence of algae, substrate type, turbidity and permanence of the habitat were recorded during the study. The physicochemical parameters such as salinity, dissolved oxygen (DO), electrical conductivity (EC), total dissolved solid (TDS), and water temperature were measured on site. The measurements were done two times following the larval collection period using a multi-probe meter (Palintest micro 800; Tyne and Wear, UK, NE11 0NS). The multi- probe meter was calibrated for each parameter following the standard procedure of user manual for the Palintest micro 800.

Each habitat was visually inspected for the presence or absence of algae. The proportion of vegetation cover estimated visually and expressed as the presence or absence of vegetation. Turbidity was measured by putting water sample in a clear glass container and placing it against a white background and recorded as either slightly turbid or turbid (Minakawa et al. 1999; Shililu et al. 2003). The substrate type was categorized as mud, stone if the pool was lined with stones that were large in size (rocks generally larger than 10 cm in diameter) and gravel when the stones were small in size but larger than sand. The proportion of the water surface exposed to sunlight was estimated visually by assessing the proportion of the water surface shadowed at midday (Sattler et al. 2005). Light intensity was visually categorized as sunlit (the habitat that received full sunlight throughout the day) or shaded. Water current was categorized as still or flow. Habitats were categorized as artificial or natural.

Data analysis

Anopheles mosquito species composition, spatial distribution and mean larval density data were analyzed using SPSS version 20. Percentile scores were used to compare abundance of *Anopheles* larvae among habitat types and distribution of their species within the habitats. Variations in larval mean density of the collected larvae among habitat types and environmental factors (parameters) of the larval habitats were analyzed using ANOVA. Mean larval density in all breeding habitats and study kebeles was calculated as the number of *Anopheles* mosquito larvae (early or late) per dip divided by the number of dips taken from each larval habitat (Sattler et al. 2005). Correlation analysis was employed to analyze the association between temperature, dissolved oxygen, total dissolved solids and conductivity to the larval density of *Anopheles* and P values < 0.05 considered significant during the analysis.

Results

Species composition of *Anopheles* mosquitoes

A total of 2185 *Anopheles* mosquitoes larvae were collected from three aquatic habitats during the study period. Of the total, 595, 765 and 823 larvae were collected from Keshmando, Amba 46 and Amba 47 sites respectively. The total number of mosquito larvae collected from Amba 47 (Fig. 3) was higher than *Anopheles* mosquito collected from the other two sites ($P < 0.05$).

A total of 1786 adults emerged from larval collections and morphologically identified as *Anopheles gambiae s.l.,*

An. funestus and An. coustani. An. gambiae s.l. was greater in number than the other two species (Table 1). *Anopheles gambiae s.l.* was more abundant in all study sites and breeding habitats. The highest density of *An. gambiae* s.l was collected throughout the study period at Amba 47 as compared to Keshmando and Amba 46. Overall, the number of adult *An. funestus* and *An. coustani* that emerged from the larval collections were low compared to *An. gambiae* s.l. In Keshmando, the abundance of *An. gambiae* s.l. was found to be 99.80% whereas *An. coustani* was 0.2%. At Amba 46 and Amba 47 the density of *An. gambiae* s.l. was 99.5% and 98.85% while *An. funestus* was 0.5% and 1.15% respectively (Table 1).

Table 1 Species composition of adult *Anopheles* mosquitoes reared from larvae, Bambasi Woreda, western Ethiopia, from September 2020 to November 2020

Sites	*An. gambiae s.l*	*An. funestus*	*An. coustani*	Total
Keshmando	492	0	1	493
Amba 46	592	3	0	595
Amba 47	690	8	0	698
Total	1774	11	1	1786

Anopheles larval productivity

Three habitat types (drainage ditch, swamp and stagnant water) were identified as breeding sites in the study area. *Anopheles* mosquito larvae were collected and identified from different breeding sites as shown in Fig. 4. There was variation in larval productivity across breeding habitats. The most productive habitat for *Anopheles* mosquito was a stagnant water from Amba 47 with 21.24 larvae per dip followed by a swamp from Amba 46 and drainage ditch from Keshmando with a mean larval density of 19.75 and 14.83 larvae per dip, respectively (Table 2).

The highest number of *Anopheles* mosquito larvae was recorded in September whereas the lowest was recorded in November in all the study sites (Table 3).

Abundance and distribution of *Anopheles* mosquitoes in breeding sites

Table 2 depicts the abundance and spatial distribution of *Anopheles* species in different breeding habitats during the study period. *An. gambiae* s.l. adult reared from larvae were obtained most abundantly from stagnant water (690) and swamps (592). Higher number of the total *An. funestus* adults reared were obtained from stagnant water. *Anopheles coustani* was the only species sampled from the drainage ditch and generally absent from the other two habitat types. The abundance of larvae per number of dips of sampling from the different habitat types revealed that *An. gambiae* s.l. were the most abundant species in stagnant water ($p < 0.05$).

Distribution of *Anopheles* larvae density during the study period

During the study period, September was the most productive month for *Anopheles* mosquito in all breeding sites. In September, the highest number of larvae per dip was sampled from Amba 46 followed by Amba 47. In October and November the trend was changed and Amba 47 was found to be more productive followed by Amba 46. The lowest larval density was sampled in November in all the study sites (Table 3).

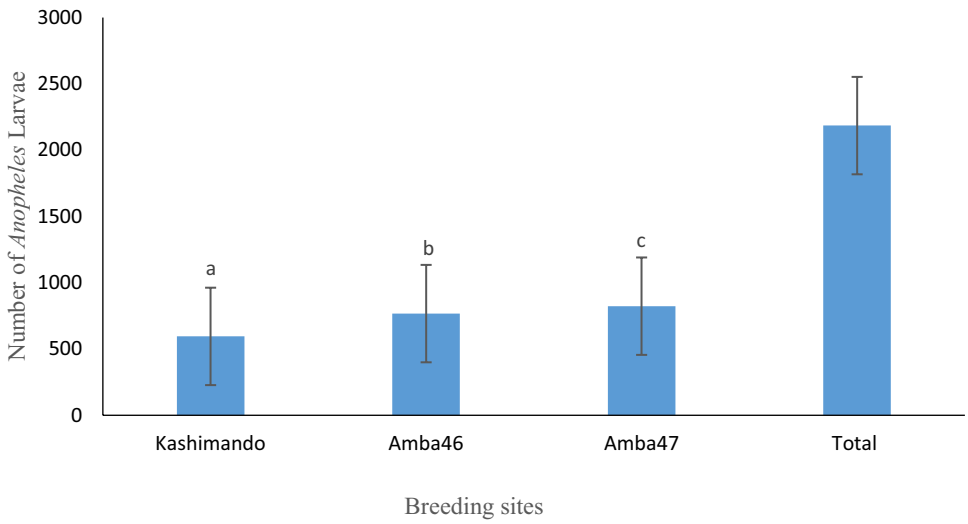

Fig. 3 Abundance of *Anopheles* larvae collected from different localities, Bambasi Woreda, Northwestern Ethiopia, from September 2020 to November 2020; [a,b,c],Mean values with different letters are significantly different ($P < 0.05$)

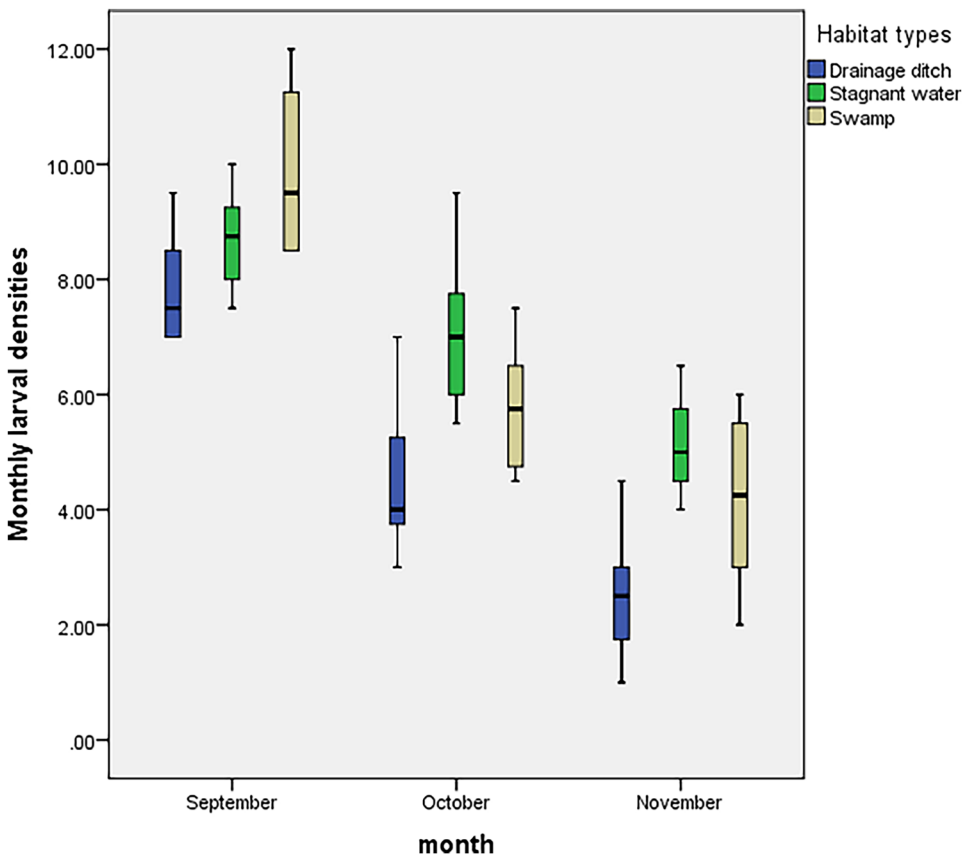

Fig. 4 Mean monthly larval densities of *Anopheles* larvae of the three habitats, Bambasi Woreda, Northwestern Ethiopia, from September 2020 to November 2020

Habitat characteristics and distribution of *Anopheles* larvae

Habitat characteristics and abundance of *Anopheles* species in each breeding site are depicted in Table 4. *Anopheles gambiae* s.l. was greater in larval density in turbid and natural habitats than that of slightly turbid and artificial habitats. Similarly, its density was higher in sunlit habitats and mud than in shaded and mudy habitats with little gravel. This species preferred temporary, still and turbid water with full sunlight and mud substrates. *Anopheles funestus* were greater in sunlit turbid water. Similarly, *An. funestus* obtained from temporary and natural habitats with mudy and with emergent vegetation and algae. *Anopheles coustani* were recorded from temporary habitats with turbid, water, shaded, mudy with little gravel and in artificial habitats. *Anopheles coustani* was absent from habitats with muddy substrate types.

The drainage ditches located close to the houses were turbid. They contain a lot of debris from vegetation and plants. They were formed by road construction authority. They were temporary because dried up during the dry season. Some of the habitats were with partial sunlight as there were large plants at the edge of habitats whereas some of the habitats were completely exposed to sunlight. Others were muddy with little gravel, still water and man-made habitats.

Table 2 Density of *Anopheles* species in different habitat types, Bambasi Woreda, Nothwestern Ethiopia

Anopheles spp.	Drainage ditch	Swamp	Stagnant water	Total
An. gambiae s.l	492	592	690	1774
An. funestus	0	3	8	11
An. coustani	1	0	0	1
Total	493	595	698	1786

Table 3 Comparison of mean larval density of *Anopheles* by area and month, Bambasi Woreda, Northwestern Ethiopia

Study sites	Month		
	September	October	November
Keshmando	7.78^{Ac}	4.50^{Bc}	2.55^{Cc}
Amba 46	9.85^{Aa}	5.65^{Bb}	4.25^{Cb}
Amba 47	8.7^{Ab}	7.37^{Ba}	5.17^{Ca}

*Means followed by different superscripts (lowercase) in the same column are significantly different at ($p < 0.05$)

*Mean followed by different superscripts (uppercase) across the raw are significantly different at ($p < 0.05$)

Table 4 Habitat characteristics and abundance of *Anopheles* species in the three breeding sites, Bambasi Woreda, Northwestern Ethiopia, from September 2020 to November 2020

Habitat characteristics	Variables	No. of habitats	Number of *Anopheles* species		
			An. gambiae s.l	*An. funestus*	*An. coustani*
Turbidity	Turbid	2	1182	8	1
	Slightly turbid	1	592	3	0
Permanence	Temporary	3	1774	11	1
	Permanent	0	0	0	0
Light intensity	Full light	2	1282	11	0
	Partial light	1	492	0	1
Vegetation & Algae	Absent	0	0	0	0
	Present	3	1774	11	1
Water current	Still	3	1774	11	1
	Flow	0	0	0	0
Substrate type	Mud	2	1282	11	0
	Mud with little gravel	1	492	0	1
Origin of habitat	Natural	2	1282	11	0
	Artificial	1	492	0	1

The swamp and stagnant water were located in farmlands (natural and still habitats). The swamp was slightly turbid. The stagnant water was turbid because it contains mud and other organic debris. There was a lot of vegetation and algal bloom. Breeding sites were temporary because they dried during the dry season. Temporary and natural habitats with sunlit, still and turbid water were the most productive breeding sites for *An. gambiae* s.l. (Table 4).

Physicochemical characteristics of the habitats

The three sampling sites varied in physicochemical characteristics. The findings showed that dissolved oxygen (DO) was highest (7.07 ± 0.55 mg/L) in swamp and lowest (0.32 ± 0.04 mg/L) in drainage ditch. Conductivity across different habitats showed wide variations, 18.46 ± 0.05µS/cm for drainage ditch and (14.13 ± 1.98µS/cm) for swamp. There were slight variations in temperature between different habitats. TDS in drainage ditch was higher (12.19 ± 0.26 mg/L); whereas it was lowest (9.49 ± 1.62 mg/L) in swamp. Salinity in drainage ditches and stagnant water was 5.54 ± 1.00PSU and 3.30 ± 0.97 PSU respectively (Table 5).

A negative strong correlation exists between the larval densities with temperature and EC while moderate correlation with Salinity. However, there was no significant correlation for Anopheles larval density with TDS and DO (Table 6).

Discussion

Of the forty seven species and subspecies of *Anopheles* mosquitoes in Ethiopia (Gaffigan et al. 2018), three of them (*An. gambiae* s.l.., *An. funestus* and *An. coustani*) were documented in the present study. The most abundant species was *An. gambiae* s.l. while, very few *An. funestus* and *An. coustani* were recorded. Unlike our findings large numbers of *An. coustani* was observed from adult collection in Bambasi (PMI 2020). The variation might be due to differences in collection, sampling period and also these species rarely feature in larval surveys of *Anopheles* species. The current study revealed differences in the abundance and distribution of *Anopheles* breeding habitats in the different sampling sites.

Anopheles larvae breed in various types of habitats, varying from large permanent to small temporary water bodies (Service 2008). Three larval habitat types were identified

Table 5 The physicochemical parameters of larval habitat, Bambasi Woreda, Northwestern Ethiopia, from September 2020 to November 2020 (Mean ± SD)

Area	Temp (°C)	TDS (mg/L)	DO (mg/L)	EC (µS/cm)	Salinity (PSU)
Drainage ditch	29.45 ± 0.93	12.19 ± 0.26	0.32 ± 0.04	18.46 ± 0.05	5.54 ± 1.00
Swamp	29.08 ± 1.36	9.49 ± 1.62	7.07 ± 0.55	14.13 ± 1.98	1.22 ± 1.05
Stagnant water	29.35 ± 0.98	6.05 ± 1.45	3.27 ± 0.12	12.73 ± 0.75	3.30 ± 0.97

TDS total dissolved salt, *DO* dissolved oxygen, *EC* electric conductivity

Table 6 Correlation coefficient between larval density and physicochemical parameters of larval habitat, Bambasi Woreda, Northwestern Ethiopia, from September 2020 to November 2020

	Variable	1	2	3	4	5	6
1	*Anopheles mosquito larval density*	1					
2	Temp (°C)	-0.742**	1				
3	TDS (mg/L)	-0.194	-0.028	1			
4	DO (mg/L)	0.357	-0.083	-0.297	1		
5	EC (µS/cm)	-0.578**	0.466*	0.561**	-0.617**	1	
6	Salinity (PSU)	-0.395*	0.109	0.359	-0.889**	0.771**	1

as breeding sites in this study, namely swamp, drainage ditch and stagnant water. All habitats were the most common breeding sites in the area. Hawaria and his co-workers also reported similar habitat types from Arjo-Didessa, Ethiopia (Hawaria et al. 2020). In this study, different *Anopheles* species were collected from various habitat types but, the most abundant species was *An. gambiae* s.l. Stagnant water was the most prolific habitat for *Anopheles* larvae.

This study revealed that *Anopheles gambiae* s.l. was the most predominant in stagnant water and least abundant in the drainage ditches. *Anopheles coustani* were collected only from the drainage ditch. On the other hand *An. funestus* had its highest number in stagnant water and the lowest were collected from the swamp. In contrast *An. funestus* group mainly prefers swamps (Dida et al. 2018). Habitat type also influences the abundance of *An. funestus*. *Anopheles funestus* mainly prefer to breed in permanent water bodies; this could be the reason for very rare occurence in swamps, drainage ditch and stagnant water. The major reason for this was, *An. funestus* larvae are associated with larger, semi-permanent water bodies with emergent vegetation and algal bloom (Gimmg et al. 2001). This study was conducted following the long rainy season but, for *An. funestus* dry season was favorable than the wet season. Thus, the findings of this study agrees with (Umar 2014), which reported that *An. gambiae* s.l. was responsible for malaria transmission during the wet season while *An. funestus* has been confirmed to be responsible for the transmission of malaria during the dry season.

The physical characteristics of the breeding sites documented during the study were; turbidity, presence and absence of vegetation and algae, habitat permanence, water condition, the origin of habitat and exposure to sunlight. The larvae occurred in a wide range of habitats, but most species prefer turbid water. *Anopheles gambiae* s.l. abundance was associated with turbid water. Similarly, the previous findings reported by Munga et al., 2005 suggested that *An. gambiae* s.l. exploit turbid water for oviposition. This indicates that during the rainy season, *An. gambiae* s.l. prefers turbid water. The other observation contradicts the present findings by Shililu et al. (2003) who recorded higher anopheline larval densities from clear aquatic habitats. Also, *An. gambiae* s.l. was documented from a slightly turbid habitat (Teklu et al. 2010). *Anopheles gambiae* s.l. was collected from a slightly turbid habitats with emergent vegetation and open sunlit conditions. Few *An. funestus* was collected, where the habitats were turbid. This indicated that *An. funestus* prefers clear water to turbid water. A similar finding was reported from Tanzania that *An. funestus* prefer clear water (Nambunga et al. 2020).

Highest *An. gambiae* s.l. was collected in the study area. The three habitats identified in this study were temporary and they dried at the end of the study period. This finding agrees with the previous report that *An. gambiae* s.l. prefers temporary breeding sites (Kenea et al. 2011).

Moreover, it was observed that the characteristic substrate type for anopheline larva habitat in the study area was mud as high anopheline larvae were observed to occur in larval habitats than other substrate types of mud with little gravel. Soil provides nutrients for the enrichment of bacteria that serve as food sources for larvae and possibly oviposition attractants. This observation is in agreement with previous reports by Minakawa et al., 1999 who described that anopheline larvae generally do not prefer habitats such as water tanks without soil substrates.

Vegetation was also an important predictor for *Anopheles* larvae presence and abundance. A similar finding was reported for *An. gambiae* s.l. larvae by Mwangangi et al., 2007. The presence of vegetation could help the larvae to hide from their predators. Algae was an important factor in the abundance of *An. gambiae* s.l. larvae in swamp, drainage ditch and stagnant water body; that algae favored the abundance of *An. gambiae* s.l. as it was the main source of its food. The presence of algae with anopheline larval occurrence or abundance is also in agreement with the finding of Gimnig and his coworkers who reported that algal food is a key for larval abundance (Gimnig et al. 2002).

The present study showed that anopheline larvae were collected from still waters. Similar findings were reported from Eritrea, which showed that still water is the

main larval habitats for anopheline mosquitoes (Shililu et al. 2003). The main reason for the high abundance of anopheline larvae in still water could be the favorable situation in which larvae can stay close to the surface with their spiracle open to the air for breathing. Moreover, high water current and flooding are detrimental to *Anopheles* larval survival as a result of the physical harm to the larvae and reduction in their oxygen tension (Okogun 2005).

Anopheline larval abundance was highly associated with natural habitats compared to artificial habitats. This indicates that environmental variables of larval breeding habitats regulate the abundance of mosquitoes (Kenawy et al. 2013; Godwin R.A. Okogun et al. 2003; Paaijmans et al. 2008).

The findings of the present study indicated that various physicochemical parameters in mosquito breeding sites at various levels had some influence on mosquito vector oviposition, survival and spatial distribution. High salinity was were recorded in drainage ditch, due to higher turbid breeding sites. Large number of *An. gambiae* s.l. were collected in stagnant water which had low salinity compared to drainage ditch habitats. This indicates that high salinity influences the oviposition of mosquito larvae. Low salinity was observed in swamps, which *An. gambiae* s.l. and *An. funestus* larvae can survive, this suggests that *An. gambiae* s.l. larvae can survive in water with either high or and low salinity.

High EC and TDS were recorded in the drainage ditch that might be due to high turbidity and high salinity in the area. *Anopheles* larvae abundance was lower in water with high levels of salinity and conductivity. A similar study reported that high water conductivity due to high salinity and other dissolved ions have a negative impact on the primary production of mosquito larvae (Closs et al. 2003). This findings are inconsistent with a study conducted from Nigeria that conductivity and TDS appeared to have no influence on *Anopheles* larval density (Imam and Deeni 2015). Also, this finding disagrees with *Anopheles* larvae abundance in water with high salinity and conductivity (Emidi et al. 2017).

Temperature is one of the most important water quality parameters. It affects water chemistry and the functions of aquatic organisms. It influences the amount of oxygen that can be dissolved in water, the rate of photosynthesis by algae and other aquatic plants and the metabolic rates of organisms. In this study slightly varied temperatures were recorded ranging from 29.08 ºC – 29.45 ºC. It was reported from a study conducted in Ghana that a temperature range between 30 ºC – 36.2 ºC found the most suitable conditions for the development of the *Anopheles* larvae (Opoku and Ansa-Asare 2009). Previous studies reported that moderate temperatures were necessary for the optimum growth of *Anopheles* larvae; they further described that high temperatures usually accelerated their growth (Minakawa et al. 1999). Additionally, other studies observed that warm water also allowed more microorganisms to grow, which is food sources for mosquito larvae (Sunahara et al. 2002).

Anopheles mosquito larvae were abundantly collected from stagnant water which has a tolerable DO (4 mg/L or less) required by most mosquito species. This finding is in agreement with the study reported by Mereta et al. 2013. High DO record in swamp site might be due to the abundance of vegetation which increased photosynthetic activity. Low dissolved oxygen, in drainage ditch habitats, might have been caused by organic pollution resulting from high input of solid and liquid waste from households as the site is in close proximity to human habitation.

Conclusion and recommendation

Among the three *Anopheles* species, *Anopheles gambiae* s.l. was the most abundant species in three of the larval habitat sites (drainage ditch, swamp and stagnant water) identified in the study area. The density of *Anopheles gambiae* s.l. was high in temporary, turbid, muddy, still water, with sunlight and some vegetations and algal bloom. Each habitat in Bambasi had different physicochemical characteristics that were key determinants of the presence of *Anopheles* larvae. The abundance of the three identified mosquito species larvae was found to vary according to different environmental and physicochemical factors which determined the quality of water of the breeding habitats. Thus, combinations of these factors contributed to the differential abundance of the larval mosquitoes observed at the sampling sites. Therefore, it is highly important to clearly identify the breeding sites of *Anopheles* mosquito and the contributing factors for their productivity so as to implement larval control strategies together with the current IRS and LLINs malaria vector control interventions. Future work should focus on longitudinal sampling including other breeding sites such as hoof cattle prints, artificial containers and ponds to properly identify productive mosquito larval habitats and implement integrated malaria vector management.

Acknowledgements The authors are grateful to the department of Biology, Jimma University for the financial support of the study. We are also grateful to the Department of Biology, Assosa University for the logistic support.

Authors' contributions HK, DE, DY and EA conceived and designed the study. HK performed the field and laboratory experiments and drafted the manuscript. DY and EA supervised the experiments. GM did statistical analysis. TN, MW, GN and DY critically reviewed the manuscript. All authors read and approved the final manuscript.

Funding The study was financially supported by Department of Biology, Jimma University, Ethiopia.

Availability of data and material The datasets supporting the conclusions of this article are included within the article.

Code availability Not applicable.

Declarations

Ethics approval Not applicable.

Consent to participate Not applicable.

Consent for publication Not applicable.

Conflicts of interest The authors declare that they have no competing interests.

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