ijlpr 2022; doi 10.22376/ijpbs/lpr.2022.12.6.L140-147

International Journal of Life science and Pharma Research

ISSN 2250-0480

Research Article

Spatio-Temporal Distribution and Species Composition of Malaria Mosquitoes in Selected Districts of Benishangul Gumuz Regional State, Western Ethiopia

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Abstract: The highest malaria transmission risk as stratified by annual parasite incidence, appears largely in Benishangul Gumuz region, the lowland and midlands of the western border with Sudan and South Sudan. The effectiveness of malaria vector control strategy depends on knowledge of factors that determine vector density and distribution, affecting adult *Anopheles* vector population dynamics and malaria transmission. This study aimed to investigate the spatial-temporal distribution and species composition of *Anopheles* mosquitos in western Ethiopia. The study was conducted in three districts of Assosa Zone (Assosa, Homosha and Bambasi), Benishangul Gumuz regional state. Adult and larva of *Anopheles* mosquitoes were collected using standard techniques after the long rainy season (October 2020-December 2020) and Dry season (January to February 2021). After collection, the data were analyzed using SPSS software. A total of 2063 *Anopheles* mosquitoes (1745 larvae and 318 adults) were collected during the study period and categorized into five *Anopheles* species (*An. gambiae, An. funestus, An. coustani, An. ziemanni, and An. squamosus*). Among all species *An. gambiae* s.l. (1548, 88.7%) was the predominant species while *An. squamous* (14, 0.8%) was the least dominant. From the three districts, the highest *Anopheles* mosquito specimens were collected from Bambasi (917, 52.6% larval and 126, 39.6% adult). And out of all collection months, the highest number of larvae and adult mosquitoes was collected during October. Among larval breeding habitats, pond was highly productive for *Anopheles* mosquitoes. *Anopheles* mosquitoes prefer to breed in all breeding sites, particularly temporary and sunlit habitats. *Anopheles gambiae s.l.* predominantly occurs during all seasons in its immature and mature stages, suggesting that malaria is a health problem in the area.

Keywords: *Anopheles mosquitoes*, Assosa, Abundance, Malaria, Vector control, and Western Ethiopia

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Received On 5 April, 2022

Revised On 23 September, 2022

Accepted On 30 September, 2022

Published On 1 November, 2022

Funding This work is supported by the College of Natural and computational sciences, Assosa University (Ref Number- Asso/Uni/4/1179/13 date 21/Jul/2020".

Citation Desta Ejeta , Spatio-Temporal Distribution and Species Composition of Malaria Mosquitoes in Selected Districts of Benishangul Gumuz ,Regional State, Western Ethiopia.(2022).Int. J. Life Sci. Pharma Res.12(6), L140-147 http://dx.doi.org/10.22376/ijpbs/lpr.2022.12.6.L140-147

1. INTRODUCTION

Anopheles mosquitoes have a paramount role in the incidence and transmission of malaria pathogens. Malaria is a serious public health caused by pathogens of the genus *Plasmodium*. It is transmitted by female *Anopheles* mosquitoes[1]. There are more than forty-two species of *Anopheles* mosquitoes in Ethiopia[2]. *Anopheles arabiensis, An. funestus, An. pharoensis* and *An. nili* are the malaria vectors[3-6] while *An. arabiensis* is responsible for most malaria infections, and the other three play a minor role in transmitting the disease[7]. In Ethiopia, malaria control programs are ongoing, with various intervention strategies mainly focusing on vector contro[8-10]. In spite of noteworthy progress in control, malaria remains a severe public health challenge, and 75% of the land of Ethiopia is malarious. More than 60% of the total population of Ethiopia is at malaria risk and 54 (6.4%) districts have high transmission. According to FMOH[11], health indicator report, 69.2% of malaria cases in the country are caused by *P. falciparum*, whereas 30.2 % were confirmed to be due to *P. vivax*. In Ethiopia, malaria occurs in three-fourths of the land and is a significant health problem affecting two-thirds of the country's population. The infection is endemic in Bambasi District, Assosa Zone, Benishangul Gumuz Regional State of western Ethiopia. Currently, Ethiopia is scaling up coverage of indoor residual spraying (IRS) and us long-lasting insecticidal nets (LLINs) as *Anopheles* mosquito control strategy. And the control strategies contributed to the reduction of malaria in Ethiopia[10]. According to the malaria indicator survey 2017 of the country, areas with the highest malaria transmission risk as stratified by Annual Parasite Incidence (API) appear to be largely in the lowland and midlands of western border with Sudan and South Sudan[12]. According to the report, malaria transmission in Ethiopia is seasonal, lasting for about three months, usually from September to November, except in Benishangul Gumuz and Gambela regions, where malaria transmission exceeds six months during and after the main rainy season. The data indicated that Benishangul Gumuz region is the most malaria-affected region with 18.4% prevalence, amongst all age groups whereas the national prevalence was 1.2%. Similarly, the API level of the region is 236.6 while the national API level is 27.7. For the effectiveness of the planned malaria elimination by 2030, data related to factors that determine malaria transmission, incidence and prevalence are essential. Moreover, local-level information about malaria vector density and distribution is important to design effective vector control strategies that lead to successful malaria elimination. The objective of this study was to investigate spatiotemporal distribution and species composition of *Anopheles* mosquito in selected areas of Benishangul Gumuz regional state, western Ethiopia.

2. MATERIALS AND METHODS

2.1 Study Area Descriptions

The study was conducted in Assosa, Homosha, and Bambasi districts of Benishangul Gumuz regional state, Western Ethiopia, from September 2020 to February 2021 (figure 1). The four largest ethnic groups reported in the Asosa Zone were the Berta (59.95%), the Amhara (23.86%), the Oromo (10.31%), and the Tigrayans (1.5%), 1.48% of the population was from Sudan; all other ethnic groups made up 2.9% of the population. The primary sources of livelihood are mixed subsistence agriculture and artisan gold mining[13].

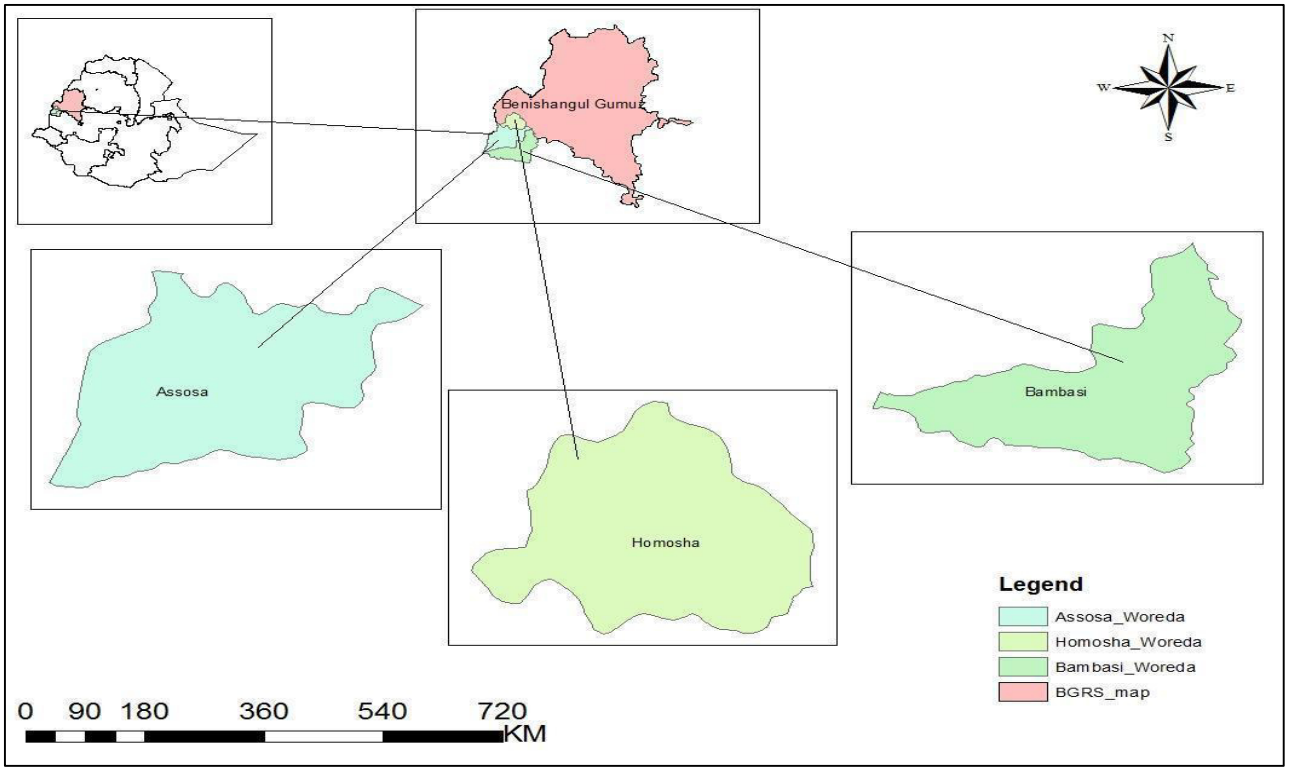

Fig 1. Map of study area

Figure 1 illustrates the three study districts selected for data collection. For the current study, data were collected from the three districts namely: Asssoa, Homosha and Bambasi. In addition, different study villages or sites were used from each study districts. The districts are located in Benishangul Gumuz regional state, western Ethiopia. The area is located 667 km to the west of Addis Ababa, the capital of Ethiopia. and is bordered by Amhara regional state in the north and northeast,

Oromia region in the south and southeast, and Gambella region in the south. It also shares an international boundary with both Sudan and South Sudan in the west.

2.2 Study Design

A cross-sectional study was conducted for larval collection, and the longitudinal entomological survey was implemented for adult Anopheles mosquito collection. The mosquito samples were collected from different villages of some selected districts of Benishangul Gumuz region. The villages were Kebele 2 and Campus area of Assosa district, Tsore and Tumet of Homosha district, and Mender 49 and Mender 46 of Bambasi district. Sample processing was conducted at Assosa University, Tropical, and Infectious Diseases Research Center. Finally, the data were analyzed by using SPSS version 16.0 software.

2.3 Entomological Survey

2.3.1 Larval Collection and Identification

Anopheles mosquito larval collection and habitat characterization were conducted in Assosa, Homosha, and Bambasi districts of Assosa zone after a long rainy season (October 2020-December 2020) and Dry season (January to February 2021). Possible breeding habitats were first inspected for the presence of mosquito larvae, and the coordinates were recorded using a GPS (Garmin Oregon 550) unit. As indicated in plate 1, standard dippers (350 ml) having a long handle (1 – 1.5 m) were used to collect water samples from large breeding sites and the samples were collected twice a month[14]. The water sample was taken and filtered and the larvae were collected and preserved in a 150 ml plastic bottle. The sampling bottle was labeled with sample number, date, and area name, and transported to the entomology laboratory, Tropical and infectious diseases research center, Assosa University for counting and identification. The location, category of the breeding sites, and type of mosquito was noted in the datasheet. The whole water was collected and measured from a habitat with a small quantity of water. The larvae were counted and identified into species based on morphological characteristics using Nikon stereo zoom microscope .

Plate 1: *Anopheles* mosquito larvae collection from different sites in Benishangul Gumuz regional state

2.3.2 Adult Anopheles Mosquito Collection

Anopheles mosquito survey was conducted using by CDC light trap collection technique. CDC light traps were placed indoors near bed and outdoors with human bait at about 1m height from 6:00pm to 6:00am to collect endophagic female *Anopheles* mosquitoes (Plate 2). A CDC light trap was used for two consecutive days in four houses (one CDC/house/night) for six CDC nights totaling 24 CDC catches. The CDC light trap collections were done from October, 2020 – February, 2021 in all study sites.

Plate 2: a collection of adult Anopheles mosquitoes from different collection sites in Benishangul Gumuz region.

2.3.3 Processing and Species Identification of Adult Anopheles Mosquito

Adult *Anopheles* mosquitoes were anesthetized using chloroform and morphologically identified into species level by using taxonomic keys [14,15].

3. DATA ANALYSIS

The data were entered into an excel computer program, checked for completeness, and coded. After cleaning, the data was analyzed using SPSS version 16.0 software (SPSS Inc., Chicago, IL. 2007). Additionally, the processed data was presented using tables and graphs.

4. RESULTS

4.1 Species Composition and Abundance of Anopheles Mosquito Larvae

About 1745 immature *Anopheles* mosquitoes were collected from all areas (Bambasi, Homosha Assosaa). All the collected larvae were identified into five *Anopheles* mosquito species, namely: *An. gambiae s.l.*, *An. coustani* An. *ziemanni*, and *An. Squamous*.

Table 1: Distribution, abundance and species composition of *Anopheles* mosquito larvae in the urban and rural areas of Assosa zone, Western Ethiopia.

Districts	Larvae by species					
	An. gambiae	***An. ziemanni***	***An. funestus***	***An. coustani***	***An. squamosus***	**Total**
Assosa	313 (20.22%)	5 (14.71%)	15 (18.52%)	7 (10.29%)	3 (21.43%)	343(19.7%)
Bambasi	788 (50.90%)	22 (64.71%)	47 (58.02%)	52 (76.47%)	8 (57.14%)	917(52.6%)
Homosha	447 (28.88%)	7 (20.59%)	19 (23.46%)	9 (13.24%)	3 (21.43%)	485(27.8%)
Total	**1548 (88.7%)**	**34 (1.94%)**	**81 (4.64%)**	**68 (3.9%)**	**14 (0.8%)**	**1745 (100%)**

An. gambiae s.l. was the most abundant species (1548, 88.7%) followed by An. funestus (81, 4.64%) (Table 1). However, the least common species was An. squamosus (14, 0.8%) (Table 1).

4.2 Spatial and Temporal Distribution of Anopheles Mosquito

The highest number of *An. gambiae s.l.*, the primary malaria vector in the area, was recorded from Bambasi district with a mean distribution of 157.6±196.4, and the least was from Assosa district with a mean of 62.6±91.1 (table 2).

Table 2: The mean distribution of *Anopheles* mosquito larvae in the urban and rural areas of Assosa Zone, Western Ethiopia

District	Larvae by species				
	An. coustani	***An. funestus***	***An. gambiae***	***An. ziemanni***	***An. squamosus***
Assosa (Urban)	1.4±2.2	3±4.2	62.6±91.1	1±1.4	0.6±0.9
Bambasi (Rural)	10.4±10.0	9.80±10.5	157.6±196.4	4.40±5.6	1.60±1.517
Homosha (Rural)	1.8±2.1	3.8±5.0	89.4±111.3	1.4±1.7	0.6±0.9
Overall	**4.5±7.0**	**5.5±7.3**	**103.2±136.5**	**2.3±3.6**	**0.9±1.2**

Note: the values are mean ± se

From all study months, the highest density of *Anopheles* mosquitoes was recorded during October (1,008, 57.8%) and no Anopheles mosquitoes were recorded in February (Table 3).

Table 3: Distribution, abundance, and mean of morphologically identified *Anopheles* mosquito larvae species in Assosa zone, Western Ethiopia

Months	*An. gambiae*	*An. ziemanni*	*An. funestus*	*An. coustani*	*An. Squamosus*
October	940 (60.7%) 188.0±82.3	4 (11.76%) 0.8±1.1	32 (39.5%) 6.4±4.3	27 (39.7%) 5.4±4.1	5 (35.7%) 1.0±1.0
November	409 (26.4%) 81.8±59.1	20 (58.8%) 4.0±1.0	32 (39.5%) 6.4±3.5	24 (35.3%) 4.8±4.3	6 (42.7%) 1.2±0.4
December	199 (12.7%) 39.8±28.4	9 (26.5%) 1.8±1.1	15 (18.5%) 3.0±1.9	15 (22.1%) 3.0±3.4	3 (21.4%) 0.6±0.9
January	0	1 (2.9%) 0.2±0.4	2 (2.5%) 0.4±0.9	2 (2.9%) 0.4±0.9	0
February	0	0	0	0	0
Total	**1548** **61.9±83.3**	**34** **1.3±1.7**	**81** **3.2±3.7**	**68** **2.7±3.6**	**14** **0.6±0.8**

Values are mean ±SD; (n=11)
****P<0.05 when compared with the control***

As illustrated in Table 3, the highest *Anopheles* mosquito larvae were collected and recorded after the long rainy season (October followed by November). Conversely, the lowest Anopheles larvae were recorded during the dry season.

4.3 Breeding Preference of Anopheles Mosquito

Anopheles mosquito larvae were collected and identified from different breeding sites in the study area. *Anopheles* larvae were collected from animal foot print, pond, excavator dig pit, tires, and containers. The result indicated that the most productive breeding habitats were ponds (1448; 83.21%) (Table 4) followed by plastic/metal containers (125; 7.18%), while animal footprints were detected to be least productive (30; 1.72%).

Table 4: Total number and mean of *Anopheles* larvae by habitat type in Assosa zone, Western Ethiopia

Species	Breeding habitat types					
	Pond	**Excavator dig**	**Container**	**Old tyres**	**Animal foot**	**Total**
An. gambiae	1303 (90%) (118.5±94.2)	64 (78%) 64.00±0	113 (90 %) 113.00±0	45(81.8%) 45.00±0	23 (76.6%) 23.00±0	1548 (89%) 61.9±83.3
An. ziemanni	23 (1.6%) 2.2±1.9	3 (2.4%) 3.00±0	3 (2.4%) 3.00±0	2(3.6%) 2.00±0	2 (6.7%) 23.00±0	34 (2%) 1.4±1.7
An. funestus	61 (4.2%) 5.7±3.8	4 (4.9%) 4.00±0	6 (4.8%) 6.00±0	5(9.1%) 5.00±0	3 (10%) 3.00±0	81 (4.7%) 3.2±3.7
An. coustani	51 (3.5%) 4.8±3.9	9 (11%) 9.00±0	2 (1.6%) 2.00±0	2(3.6%) 2.00±0	2 (6.7%) 2.00±0	68 (3.9%) 2.7±3.6
An. squamosus	10 (0.7%) 0.9±0.8	2 (2.4%) 2.00±0	1 (0.8%) 1.00±0	1(1.8%) 1.00±0	0 (0%) 0±0	14 (0.8%) 0.6±0.8
Total	**1448 (83.2)**	**82 (4.7)**	**125 (7.2)**	**55(3.2)**	**30 (1.7)**	**1740**

The result revealed that almost all larvae were collected from stagnant and sunlit habitats in all study areas. Many larvae were collected from the habitat that was near to house (less than 100m) (1437), and natural habitats (430) were found to be productive breeding sites compared to artificial ones. Most of the larvae were collected from habitats that are covered by vegetables. (1060).

4.4 Species Composition and Spatial Distribution of Female Anopheles Mosquito

A total of 318 of adult *Anopheles* mosquitoes were collected and identified to five of *Anopheles* mosquito species namely; *An. gambiae s.l., An. funestus*, *An. coustani, An. ziemanni* and *An. squamous* (Table 5).

Table 5: Distribution and abundance of morphologically identified adult *Anopheles* species in Assosa zone, Western Ethiopia

District	Adult by species					
	An. gambiae	***An. ziemanni***	***An. funestus***	***An. coustani***	***An. squamosus***	**Total**
Assosa	1 (33.3)	8 (44.4)	29 (23.8)	46 (28.7)	-	84(26.4)
Bambasi	9(6.7)	1 (6.7)	52 (42.6)	59 (36.9)	3 (100)	126(39.6)
Homosha	5 (60.0)	9 (50.0)	41 (33.6)	55 (34.4)	-	118 (37.1)
Total	**15 (4.7)**	**18(5.7)**	**122 (38.4)**	**160 (50.31)**	**3(0.94)**	**318(100)**

The numbers in the parenthesis are percentages.

This study shows that *An. coustani* is the most abundant female *Anopheles* mosquito species in Assosa zone with an overall mean of 2.7 followed by *An. funestus* (2.0). Similarly, the primary malaria vector, *An. gambiae s.l.* was majorly reported from Bambasi district (39.6%). The highest percentage was recorded from Bambasi (126, 39.6%) district, followed by Homosha (118, 37.1%) districts, whereas the lowest mean was recorded from Assosa district (84, 26.4%) (Table 6).

Table 6: The mean distribution of female *Anopheles* mosquito in the study area

District	Adult by species				
	An. coustani	*An. funestus*	*An. Gambiae*	*An. ziemanni*	*An. squamosus*
Assosa	2.3±1.6	1.5±1.4	0.3±0.6	0.4±0.8	-
Bambasi	3.0±2.7	2.6±3.3	0.1±0.2	0.1±0.2	0.25±0.4
Homosha	2.8±2.2	2.1±1.6	0.5±0.8	0.5±0.8	-
Overall	**2.7±2.2**	**2.0±2.3**	**0.3±0.6**	**0.3±0.7**	**0.1±0.2**

4.5 Seasonal Distribution of Anopheles Mosquito

Out of the total *Anopheles* mosquitoes collected during the study period 31.8% were recorded in October, whereas the lowest density was recorded in February (9, 2.83%).

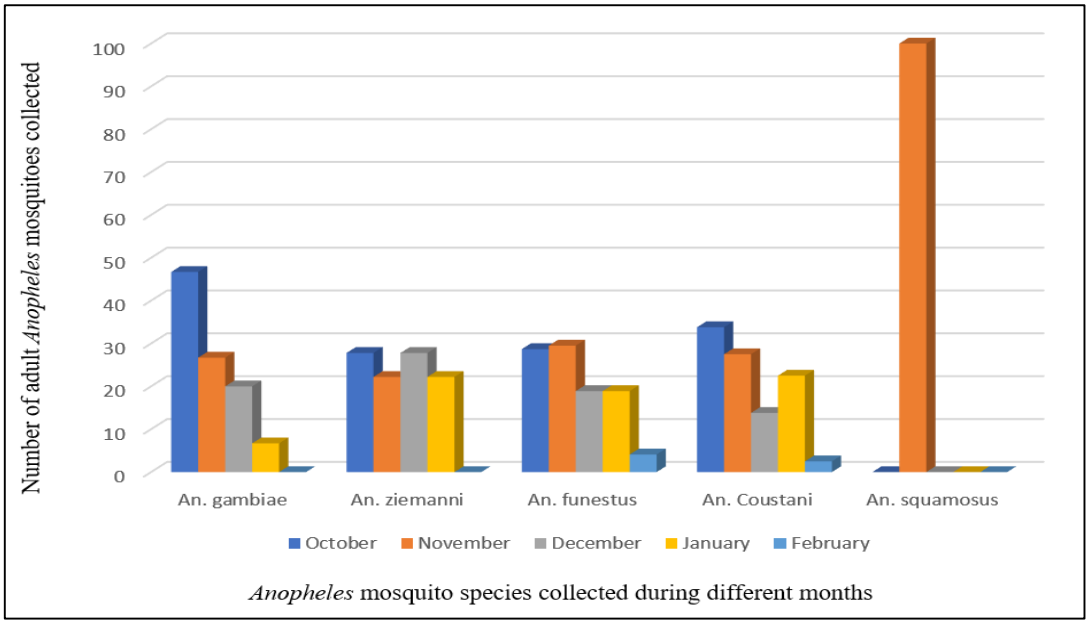

Fig 2: Distribution and abundance of morphologically identified adult Anopheles mosquito species in Assosa zone, Western Ethiopia

As illustrated in table 7, November and December were months with high numbers of *Anopheles mosquitoes*. *An. coustani* and *An. funestus* were identified from October to February, while *An. gambiae s.l.* and *An. ziemanni* were identified from October to January (Figure 2 and Table 7). *An. squamosus* was only identified from samples collected during November (Figure 2 and Table 7).

Table 7: The mean distribution of *Anopheles* mosquito adult in the study area from October 2020 to February 2021

Months	*An. coustani*	*An. funestus*	*An. gambiae*	*An. ziemanni*	*An. squamosus*
October	1.8±0.8	1.9±1.2	0.3±0.6	0.4±0.9	0
November	3.7±2.4	3.0±4.0	0.3±0.9	0.3±0.7	0.3±0.5
December	4.5±1.9	3.0±1.8	0.6±0.8	0.4±0.8	0
January	3.00±1.7	1.9±1.6	0.1±0.3	0.3±0.8	0
February	0.3±0.9	0.4±0.9	0	0	0
Overall	**2.7±2.2**	**2.0±2.3**	**0.3±0.6**	**0.3±0.7**	**0.1±0.2**

Different Anopheles mosquitoes were collected from the study sites different study months. As illustrated in Table 7, the primary malaria vector, An. gambiae s.l. was collected from October to January.

5. DISCUSSION

In the present study, *Anopheles mosquitoes* were identified across the three districts of Assosa zone, western Ethiopia. Five species of the genus *Anopheles* mosquitoes (*An. gambiae s.l., An. funestus, An. coustani, An. ziemanni* and *An. squamosus*) were collected and identified from both immature and adult stages during the study period. *An. gambiae s.l.* were found to be the dominant *Anopheles* species (1548, 88.7%) in larvae collection. This result is similar to research done in Ghibe River. The existence of the deadly malaria vector, *An. gambiae s.l.* in most breeding habitats was in line with other previous studies in Ethiopia[16-19]. Note that *An. gambiae s.l.* was not further identified to the sibling species label. However, the previous reports from nearby study areas identified that all *An. gambiae s.l.* were identified as *An. arabiensis.* Though it was highest in its immature stage, *An. gambiae s.l.* (*An. arabiensis*) was found to be low in its adult collection. This could be due to its exophagic nature. Even though it is reproductively high with predominant larvae, its zoophilic or animal host

preference may have led it to animals outside the human habitation[20]. Furthermore, in the current study, the collection was conducted exclusively inside the house during the *An. arabiensis* are known to prefer outside feeding (exophagic). The mature and immature stage mosquitos were collected from Bambasi and Homosha which have rural settings, and the least was from the Assosa district (urban setting). This finding agrees with the report of Medrano *et al.*[21]. This might be because of potential breeding sites, water resource development and other anthropogenic factors. In this study, larval distribution of Anopheles mosquito was associated with breeding habitat nature and types. Larvae were collected from various breeding habitats, including pond, excavator dig pit, containers, old tires, and animal footprint. Most of *Anopheles* mosquito larvae were collected from ponds and roadside ditched. This result is in agreement with a report from Ghibe, Southwestern Ethiopia[16]. Similarly, it was previously reported that *An. arabiensis,* which is the only vector sibling species of *An. gambiae s.l.* in Ethiopia usually breeds in temporary pools of rainwater and is better adapted to sunlit water pockets[22]. Whereas, animal foot prints were found to be least productive (30; 1.72%) for *Anopheles* breeding. *Anopheles* larvae were abundant in habitats with vegetation cover, sunlit, habitat nearer to living house. *Anopheles gambiae* density was associated with habitats with smaller perimeters, sunlit, and high vegetation cover. This study is similar with studies conducted in the Lake Victoria basin, western Kenya[23,24]. This might be due to the presence of high nutrients and minerals in ponds for *Anopheles* mosquito to feed. In contrast to this study, it was observed that habitats that had low vegetation cover and lack of emergent plant has more *Anopheles* larvae than the habitat without vegetation in a study conducted in Ghibe River Basin, southwestern Ethiopia[16]. This difference could be due to differences in species, study location, and period. Anopheles mosquitos were collected in a five-month sampling period October, 2020 to February, 2021. An abundance and distribution of *Anopheles* mosquito larvae are associated with breeding seasons, and the study found that the mosquitoes breed in all seasons and are highest in October. Anopheles distribution in the study setting occurs throughout five months, but it has a seasonal pattern with an incidence peaking during October/November. This research data suggest that larval counts and density of Anopheles mosquitoes increase after long rainy months. This finding was in line with other studies reported from different parts of the country[16,19], which said high larval abundance during and after the rainy season (June-November) in Ethiopia. This is also in agreement with research conducted in Bure district, Northwestern Ethiopia[23] and research on the impact of dams on mosquito abundance in Ethiopia[4,7]. This could be due to the creation of small ponds, water pockets that form suitable breeding habitats, and conducive meteorological factors (ambient temperature, relative humidity, and rainfall). Lamidi[27], and Donovan *et al.*[28], revealed that larval counts and density of Anopheles mosquitoes are known to be high during rainy seasons and decline during dry seasons. This is obviously because of the loss of some habitats and the decline in mosquito populations during the dry season.

6. CONCLUSION

Small, temporary, and sunlit habitats are the most productive breeding sites for mosquitoes in the study sites. *Anopheles gambiae s.l.*is a highly distributed species in western Ethiopia. Even though Anopheles mosquito larvae were predominant in and after the wet season, larvae were recorded during all seasons, suggesting that malaria transmission is a serious health problem in the area. They are attributed to its feeding behavior for effective malaria control in both immature and mature stages of *An. arabiensis* should be targeted during the planning of malaria vector control strategies.

7. AVAILABILITY OF DATA AND MATERIALS

The datasets used and/or analyzed during the current study are available from the corresponding author upon reasonable request.

8. FUNDING ACKNOWLEDGEMENT

We acknowledge the resources and financial support for the study were provided by the College of Natural and computational sciences, Assosa University (Ref Number-Asso/Uni/4/1179/13 date 21/Jul/2020. Furthermore, the generous support for carrying out the study at Tropical and Infectious Diseases Research Center, Assosa University is also acknowledged.

9. ACKNOWLEDGEMENTS

We acknowledge the community of study districts (Assosa, Homosha and Bambasi).

10. CONFLICT OF INTEREST

Conflict of interest declared none.

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