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# A comprehensive analysis on abundance, distribution, and bionomics of potential malaria vectors in Anseba zone, Eritrea (2020)

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**Abstract**
In Eritrea malaria is still remains a public health concern despite the drastic decline in morbidity and mortality. With all the achievements, little has been invested in entomological studies. So the gains may be reversed considering the receptivity and vulnerability of most of the malarias areas and/or previously malarias areas. A cross-sectional entomological survey was conducted in 3 sentinel sites of Anseba zone, Eritrea during the period Aug. – Nov.2020 for a comprehensive analysis on abundance, distribution and bionomics of potential malaria vectors. Mosquito immature stages and adults were collected from different habitats using both immature and adult collection options. From the total 40 aquatic larval habitats, more than 77.5% were River edges found along the Anseba River, animal foot print, small streams, dams and swamps was among the preferred mosquito breeding habitats. Larval densities among the study sites were found to be high with approximately 20.7 larvae per dip. A total of 1746 femal mosquitoes were identified to their species level morphologically. As a result 5 species were identified *vis*. *An. gambiae*, *An. cineruse*, *An pretoriensis*, *An. pharoensis* and other 1 species not identified yet. *An gambiae* was the most dominant vector and was found to be endophlic and endophagic. The pick biting hours were found to be from 08:00pm-12:00pm, most high at 10:00pm to 12:00pm. Nearly 95% of all adult mosquitoes collected using human landing catch was between these hours.

**Keywords:** Potential malaria, abundance, distribution, and bionomics, Eritrea

## Introduction

Mosquito-borne diseases (MBDs) threaten the lives and livelihoods of millions of people worldwide [1]. According to WHO (2020), the estimated number of malaria deaths stood at 405,000 in 2018 alone. Children less than five years and pregnant mothers are the most vulnerable groups affecting by malaria; in 2018, they accounted for 67% (272,000) of all malaria deaths globally [2]. The WHO African region carries a disproportionately high share of the global malaria burden. According WHO, in 2018 the region was home to 93% of malaria cases and 94% of malaria deaths. WHO estimates that there are approx 3.2 billion people at risk of contracting malaria [2] Malaria exerts excessive continuous huge public health burden in most of developing poor countries [3, 4]. Currently, the disease burden is estimated at 45.6 million DALYs (disability-adjusted life years) [5]. Hence, it has been identified as a key contributor to weak economic growth and investment in Africa because it experiences the most intense malaria transmission in the world [6]. Likewise, malaria causes high morbidity in Eritrea especially in Gash Barka region, and it is endemic in other three zones of the country *Vis*. Northern red sea (NRS), Debub and Anseba and 70% of the estimated population resides in malaria endemic areas [7].

Malaria is caused by five species of parasite that affect humans, and all of these species belong to the genus *Plasmodium (p): P. falciparum, P. vivax, P. ovale, P.malariae* and *P. knowlesi*. Of these, *P. falciparum* and *P. vivax* are the most important. Malaria due to *P. falciparum* is the most deadly form, and it predominates in Africa [8]. *P. vivax* has a wider distribution than *P. falciparum*, because it is able to develop in the *Anopheles* mosquito vector at lower temperatures, and to survive at higher altitudes and in cooler climates.[8] Malaria is transmitted by a bite of infected female *An.* mosquitoes. Worldwide, there are approx 48 species which considered as vectors of human malaria [9].

However, *An. gambiae s.s*., the newly designated *An. coluzzi*, *An.arabiensis* and *An. funestus* are the most efficient vector mosquitoes in the world [10, 11, 12]. In Africa a comprehensive analysis on the dominant anopheles vectors of human malaria were done by Sinka *et al* (2010), revising documents/studies from 46 African countries. It was found that *An. gambiae* was the most dominant and was reported from the largest number of countries (34) and from the highest point of location [13] Sinka found that *An. funestus* and *An. Arabiensis* were the second most occurred mosquitoes followed to gambiae [13]. Similarly in Eritrea, *An. arabiensis* the member of *An. gambiae complex* is the primary malaria vector [14]. Nearly 85% of the total identified *An*. Mosquitoes, and followed by *An.d'thali* (9.4%) and *An.cinereus*. *An. d'thali* was an efficient vector dominantly collected from Anseba zone [14].

**Malaria vector species distribution in Eritrea**

Although malaria remains a major cause of morbidity in Eritrea (dramatic reduction was recorded during the past decades), little is known about the *An.* mosquito species responsible for transmission. Only 2 studies are available. The first was a study conducted in 1994 by Carrara *et al*., on vector species in western Eritrean. This study focused only on the *An. gambiae* complex and was limited in geographic range [15]. The second study was a two years study conducted in 5 malarias zones of the country, and the finding was a total of 13 species was collected or identified and of which 84.5% of the species was found to be *An*. *gambiae* and followed by *An. d'thali* (9.4%) and *An.cinereus* 3.4%) [14]. All the species and their percentage of existence are displayed on table 1.1.

**Table 1:** Species composition and relative abundance of adult anopheles mosquitoes collected between October 1999 and April 2001 in Eritrea

| Species | Location | | | | | |
|---|---|---|---|---|---|---|
| | Gash Barka | Anseba | Debub | Maekel | NRS | Total |
| *An. gambaies.l* | 1,294 | 606 | 147 | 8 | 69 | 2,124 |
| *An. d.thali* | 0 | 216 | 2 | 0 | 17 | 235 |
| *An. cinereus* | 0 | 12 | 24 | 48 | 0 | 84 |
| *An.squamosus* | 0 | 0 | 0 | 20 | 0 | 20 |
| *An. rhondesiensis* | 0 | 19 | 1 | 0 | 0 | 20 |
| *An.rupicolus* | 0 | 11 | 0 | 0 | 1 | 12 |
| *An. harperi* | 1 | 0 | 0 | 0 | 0 | 1 |
| *An. demeilloni* | 0 | 3 | 1 | 0 | 0 | 4 |
| *An. garnhami* | 0 | 3 | 0 | 0 | 0 | 3 |
| *An. funestus* | 0 | 0 | 2 | 0 | 0 | 2 |
| *An. chrysti* | 0 | 0 | 0 | 3 | 0 | 3 |
| *An. wellcomi* | 0 | 0 | 1 | 0 | 3 | 4 |
| *An. pharoensis* | 0 | 0 | 0 | 1 | 0 | 1 |
| Total | 1,295 | 870 | 178 | 80 | 90 | 2,513 |

**Materials and methods**

**Study Design**

This study was carried out as a cross-sectional entomological surveys during the period (Aug. –Nov.2020)

**Study Area**

The study was carried out in Anseba zone, Eritrea. Anseba is located in the $15^0$ 31'- $17^0$ 32' north altitudes & $36^0$ 53'-$38^0$ 54' east longitudes. The zone is bordered by NRS from the north and north eastern, Gash Barka region from south and south west and by central region from south and south eastern. It has a total of 9 subzones, 92 'administrative areas' and 388 villages. According to zonal malaria control program (ZMCP), annual report (2018), Anseba zone has a total population of 394,419 evenly distributed throughout the zone (7074km$^2$). The climate prevailing in the zone is tropical. The temperatures range in the area is between 19 - 40 ºC, with the altitude of 744-2404 meters above the sea level (masl). Anseba zone has humidity level between 35-77%.

One of the major rivers of the country named Anseba river flows through more than half of the nine sub zones, such as Aditekelezan, Elabered, Keren, Hamelmalo and Habero sub zones. And there are plenty number of dams, wells and streams.

**Study populations**

Larva, pupa and adult of *An.* mosquitoes.

**Sampling Strategy and Sample Size**

To achieve the study objectives, the surveillance was implemented in the previously selected sentinel sites, *Vis.* Elabered, Hagaz and Filfle. From these 3 sentinel sites two villages/sites were randomly selected from each and the surveillance was conducted on the planned interval for consecutive months. Larval instars well collected from different breeding habitats in the selected locations. Ten dips, using the scoping methods were taken from each positive site for larval collection and, hence, density /10 dips of anopheline larvae in each habitat recorded (WHO, 1992). In addition, more samples of larvae were taken to the insectary and rear to adult for species identification. Likewise, adult mosquitoes were collected from houses using PSC (WHO, 1992), light trap from in and outdoor and from possible resting places by mouth aspirator/procopac (electrical aspirator) and the collected mosquitoes were preserved on silica gel in Eppendorff tubes for species identification.

**Data collection**

**Larval Collection**

Anopheles mosquito larvae were monthly collected from all available breeding sites for a period of 04 months (Aug. - Nov. 2020). Sampling was conducted by using standard larvae collection procedures. The collected larvae were transported to the insectaries and reared in trays and fed on fish powder (larval food). The emerged pupae were timely collected from the trays using pipettes and were placed in plastic cups containing water and placed inside adult cages. Emerged adults were fed 10% solution of sugar and were killed by using drops of chloroform in a piece of cotton and identified under dissecting microscope by morphological characters.

**Adult Collection**

Adult mosquitoes were collected using Pyrethrum spray sheet collections (PSC), light trap and human landing catch (HLC) collections.

**PSC/Knockdown (Kd) Method**

The PSC/Kd method was done early in the morning between 06:00-08:00 am (WHO, 1992). The entire floor and furniture of each selected rooms were covered using white cloth sheets. The door and windows were closed and the rooms were sprayed using payogon (insecticide used in PSC). The insecticide sprayed rooms were left closed for 10 min. After a period of 10 minutes exposure, the knocked down adult mosquitoes were picked up using forceps. The Kd mosquitoes were categorized by blood feeding/digestion stages as unfed, freshly fed, gravid and half gravid and were kept into Petri dishes containing filter paper and transported to laboratory for identification.

**Light trap method**

Light traps were placed at indoor and outdoor using the WHO criteria for light trap collection starting from 06:00 pm to 06:00am. The collected sample from each light trap were preserved for further identification and were used to indicate entomological indices.

**Mosquito Rearing, Processing and Identification**

The field-collected immature stages of *An.* mosquitoes kept in well-labeled vials were transferred separately to paper cups covered with a netting material and fixed with a rubber band. The larvae and pupae were reared in the laboratory to the adult stages. Moreover, the wild collected adults were killed by chloroform and then preserved as described above. The preserved specimens of mosquitoes were examined under dissecting microscope and the species were determined with the taxonomic Keys (Gillies and Coetzee, 1987).

**Entomological Survey**

Entomological surveys were carried out in the selected area for 4 months. The entomological surveys were carried out by a well-trained team of 1Entomologist, 2 public health officers and 3 insect collectors from Elabered malaria entomology laboratory and public health officers from the respective areas of study sites.

**Meteorological Data**

On monthly basis temperature (minimum and maximum), R.H. and rainfall in study areas were recorded at the sentinel sites.

**Data Analysis**

The data were analyzed using SPSS software Program, ANOVA (Ver.20) and descriptive analysis by other computer software.

**Ethical Clearance**

Clearance to conduct the study were obtained from Ministry of Health, Ethical Approval Committee.

## Results

**General description of study sites**

Entomological data were collected from the places that have been serving as a zonal malaria sentinel sites for the last 20 years. These 4 sentinel sites were selected according to the criteria for selection sentinel site and are a true representative of our zone. However, the study was conducted in 3 of these sentinel sites due to transportation problems. Entomological data were collected from three sub zones namely: Elabered, Hagaz and Habero sub zones. Two sites from Elabered sub zone (Balwa and Adi-bosqual), one site from Hagaz sub zone (Adi-alamin) and one site from Habero sub zones (Filfle) were included in the study.

Entomological surveillance was conducted for the consecutive 4 months from August - November, 2020. During these period of study, the average temperature of the study area were 25.4 in Elabered, 27.7 in Hagaz and 26.6 in Haboro, as depicted in table 4.1, the average PH extends from 7.80 in Filfile to 8.50 in Adi-Alamin. Larval density among the study sites was found to be insignificant in respect to the PH differences (Sig P=0.582).

Table 4.1, shows, the average Longitude/latitude/elevation of the study sites and were 38.69/15.69/1649m in Adi-bosqual, 38.70/15.66/1805m in Balwa, 38.39/16.02/1298m in Filfile and 38.23/15.72/924 in Adi-alamin.

**Larval number and larval habitats**

Elabered and Habero sub zones are found along the Anseba River, and More or less the breeding sites of these sites were entirely restricted to this River. On the contrary breeding sites of Adi- alamin (Hagaz sub zone) were found to be water bodies accumulated from the dam constructed nearby.

As depicted in table 4.2, most of the breeding sites were related to the river in which more than 77.5% of larval habitats were found to be stream edges (river edges), very similarly animal foot print were directly represents the river and were 10% of the total. Streams, dams and well collectively were representing only for 7.5% of the habitats found during this study period. The rest 5% of the larval habitat were from swamps. These all larvae were collected from 40 aquatic habitats.

Nature of the larval habitats, its exposure to sun light and presence of vegetation were recorded and it was found that nearly 58% of the habitats were turbid, 87.5% of these habitats were exposed to sun light and 70% of it was with floating vegetation. Exceptionally larval habitat in Adi-bosqual was clean water.

**Table 2:** Geographical location and PH characteristic of larval habitats by sites in Elabered, Hagaz and Habero sub zones: Anseba Zone, Eritrea (2020).

| Site | Latitude | Longitude | Elevation | PH | |
|---|---|---|---|---|---|
| | | | | Minimun | Maximun |
| Adi-Bosqual | 15.69 | 38.64 | 1649 | 7.69 | 10.30 |
| Balwa | 15.67 | 38.70 | 1637 | 7.98 | 8.70 |
| Adi-Alamin | 15.72 | 38.23 | 831 | 8.00 | 8.80 |
| Filfle | 16.73 | 38.39 | 1296 | 7.78 | 7.82 |

**Table 3:** General characteristics of larval habitats by sites in Elabered, Hagaz and Habero sub zones: Anseba Zone, Eritrea (2020).

| Description | Characteristic | Adi-Bosqual | Balwa | Adi-Alamin | Filfle |
|---|---|---|---|---|---|
| Nature of water | Clean | 54.5% | 22.2% | 0.0% | 14.3% |
| | Turbid | 27.3% | 77.8% | 66.7% | 64.3% |
| | Polluted | 0.0% | 0.0% | 0.0% | 7.1% |
| | Dark | 18.2% | 0.0% | 33.3% | 14.3% |
| Water characteristic | Puddle | 0.0% | 0.0% | 0.0% | 0.0% |
| | Rain Pool | 0.0% | 0.0% | 0.0% | 0.0% |
| | Swamps | 18.2% | 0.0% | 0.0% | 0.0% |
| | River edge | 54.5% | 100.0% | 33.3% | 100.0% |

| | | | | | |
|---|---|---|---|---|---|
| | Animal Foot print | 27.3% | 0.0% | 16.7% | 0.0% |
| | water container | 0.0% | 0.0% | 0.0% | 0.0% |
| | stream/dam/well | 0.0% | 0.0% | 50.0% | 0.0% |
| Exposure to sun light | Shaded | 0.0% | 0.0% | 0.0% | 0.0% |
| | Partially Shaded | 18.2% | 0.0% | 50.0% | 0.0% |
| | Sun Light Expo | 81.8% | 100.0% | 50.0% | 100.0% |
| Presence of Vegetation | emerged | 0.0% | 0.0% | 0.0% | 0.0% |
| | Submerse | 27.3% | 0.0% | 0.0% | 0.0% |
| | Floating | 45.5% | 88.9% | 83.3% | 71.4% |
| | No Vegetation | 27.3% | 11.1% | 16.7% | 28.6% |

**Larval density (LD) per habitat**

A total of 3785 anopheles mosquito larvae were collected from the above mentioned study sites. During sampling; the area were divided in to sections and further the sections were divided in to specific sites. Based on these divisions, sampling was taken from each site. Density of larva is best calculated using larva per dip. Based on this calculation, Filled was found to have the highest LD over the study period of 4months. It was found to be 23.09 larva/dip and an average LD in Adi-Bosqual was found 17.04 larvae/dip. Meanwhile as it's shown in table 4.3, the variation of LD between the months was seen and November was found to be with the highest LD and it was 22.08 Larva/dip. The lowest density was recorded in October, i.e. 12.28 larva/dip. No statistical significance were seen in the larval density between the months (Sig P=0.582).

**Anopheles mosquito species composition**

After rearing the mosquitoes in laboratory a total of 1746 female anopheles mosquitoes were identified to their species level morphologically. As depicted in figure 4.1, *An. gambiae* was the dominant vector zonally. Dominance of the vectors between the sites was not found to be different. Generally a total of 5 species were identified and *An. gambiae* were accounts for 55.9% of the total identified, followed by *An. cineruse* (22.3%), An. pretoriensis (17.7%), *An.pharoensis* (1.98%) and unclear species (2.19).

**Adult anopheles mosquito population and density**

The study covered both the aquatic stages and the adult stages. Hence, adult mosquitoes were collected using PSC, light trap and human landing catch methods. As depicted in Figure 4.2, a total of 395 adult mosquitoes were collected using both PSC and light traps. 298 of them were collected by light trap and the rest 97 were using PSC method. The morphological identification of these mosquitoes shows, 55%, were An. gambiae,, 18% An. Cinerus ,1% An. Pretorensis and unidentified species yet (26%)

**Table 4:** Larval density per habitat by study sites in Hagaz, Habro and Elabered sub zones: Anseba zone, Eritrea (2020).

| Description | Villages | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Adi-Bosqual | | | | Balwa | | | | Adi-Alamin | | | | Filfle | | | |
| | Oct. | Nov. | Dec. | Total | Oct. | Nov. | Dec. | Total | Oct. | Nov. | Dec. | Total | Oct. | Nov. | Dec. | Total |
| Number of dips | 50 | 16 | 6 | 72 | 50 | 11 | 9 | 70 | 16 | 2 | 5 | 23 | 31 | 19 | 22 | 72 |
| Total No. of anopheles | 715 | 342 | 170 | 1227 | 217 | 199 | 184 | 600 | 214 | 20 | 61 | 295 | 659 | 499 | 505 | 1663 |
| Total No. of Culicine | 16 | 0 | 0 | 16 | 0 | 0 | 0 | 0 | 106 | 50 | 89 | 245 | 17 | 11 | 33 | 61 |
| Anopheles density per dip | 14.3 | 21.4 | 28.3 | 17.0 | 4.34 | 18.1 | 20.4 | 8.6 | 13.4 | 10 | 12.2 | 12.8 | 21.3 | 26.3 | 23.0 | 23.1 |

**Table 5:** B: Correlation between the PH value os the study sites and the larva density

| | | PH | Larval density |
|---|---|---|---|
| PH | Pearson correlation | 1 | **0.109** |
| | Significance | | **0.582** |
| | N | 30 | **28** |

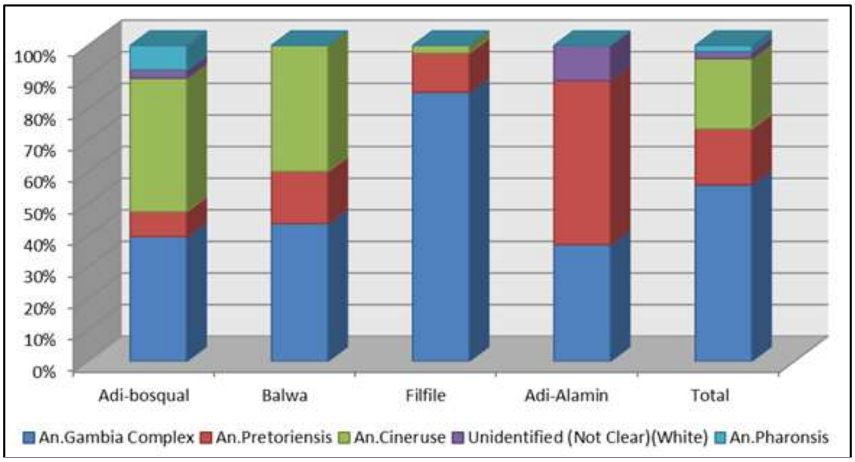


**Fig 1:** Composition of anopheles species by sites in Hagaz, Elabered and Habero sub zones: Eritrea (2020)

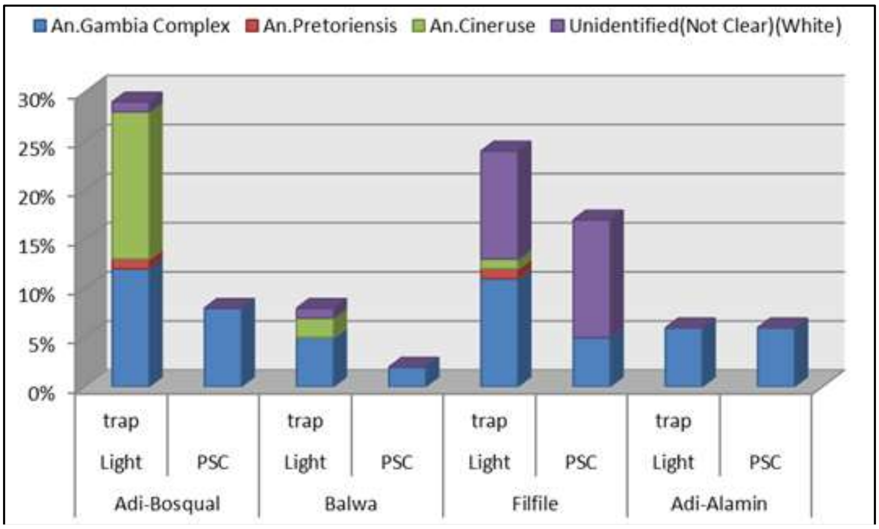


**Fig 2:** Adult anopheles composition and by adult mosquito collection methods and site, in Hagaz, Habero and Elabered, Anseba zone Eritrea (2020)

**Adult density**

Throughout the study period the results of monthly spray catches and trap of female *anopheles* mosquitoes indicated the presence of the insect in all the study locations. The numbers caught exhibited marked seasonal fluctuations but not in parallel with the densities of the immature stages. As depicted in table 4.4 the highest number of adults was recorded in October (229) followed by November (103) and the lowest density was recorded in December 63). Likewise the difference of adult mosquito density between the sites were recorded, and it was found to be high in Filfle in both methods of collection, light trap=133 and PSC=39, and the lowest were recorded from Balwa.

Comparison of densities among the collection methods were shown in table 4.5, and its found that, PSC collection was high in adi-Bosqual from Elabered sub zone which is 1.8 adults per HH, followed by Filfle, 1.26 adult/HH. Likewise adult density by light trap was very high in Filfle (5.4 adults/trap and followed by adi-bosqual (5 adult /trap)

**Physiological status of mosquitoes and bed net utilization of HHs**

As noted in figure 4.3**,** among the adult mosquitoes collected, the majority were found to be unfed (75.2%), 24.8% were fed and the rest few were half gravid and no gravid mosquito were recorded. During this study period, members of the HHs participated in both PSC and light traps collection were questioned and observed for bed net availability and utilization. Table 4.6 shows more than 94% of them were slept under bed net on the previous night.

**Mosquito Biting Behavior at indoor and outdoor**

Another method of density measurement and prediction of epidemic in an area can be easily known using human biting index (HBI). HBI is calculated as anopheles bite per person per hour. As depicted in Fig 4.4, the highest HBI were found in Adi-Bosqual (4.9 anopheles/person/hour), followed by Filfle (3 anopheles/person/hour), Balwa (1.1 anopheles/person/hour) and Adi-Alamin (0.3 anopheles/person/hour). During this type of collection, the mosquitoes were more dominantly bite at the outdoor site in the three areas of collection.

**Mosquito Night-Biting Cycle**

Human biting catch is best used to indicate the pick biting hours of anopheles mosquitoes in an area. With all its difficulties and ethical considerations, this method of collection was conducted at all the study sties. As shown in figure 4.5, during the collection hours of 12hrs (from 06:00pm to 06:00am), the pick biting hours were found to be from 08:00pm-12:00pm, most high at 10:00pm to 12:00pm. Nearly 95% of all adult mosquitoes collected using human landing catch was between these hours.

**Table 4:** Adult mosquito collection by PSC and CDC light trap by study sites at Hagaz, Elabered and Habero sub zones: Anseba zone, Eritrea (2020)

| Description | Adi-Bosqual | | | | Balwa | | | | Adi-Alamin | | | | Filfle | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | PSC | | Light trap | | PSC | | Light trap | | PSC | | Light trap | | PSC | | Light trap | |
| Total number of HH | +ve | -ve | +ve | -ve | +ve | -ve | +ve | -ve | +ve | -ve | +ve | -ve | +ve | -ve | +ve | -ve |
| | 9 | 6 | 7 | 3 | 4 | 9 | 4 | 6 | 7 | 23 | 9 | 15 | 14 | 16 | 20 | 4 |
| Anopheles | 28 | | 100 | | 4 | | 15 | | 24 | | 45 | | 38 | | 133 | |
| Culicidae | 0 | | 0 | | 2 | | 0 | | 0 | | 0 | | 1 | | 5 | |
| No. of PSC and CDC light traps | 15 | | 10 | | 15 | | 10 | | 30 | | 24 | | 30 | | 24 | |
| Density per PSC/and light trap | 1.9 | | 10 | | 0.3 | | 1.5 | | 0.8 | | 1.9 | | 1.3 | | 5.5 | |

**Table 5:** Physiological condition and bed net utilization by study site at Hagaz, Elabered and Habero sub zones: Anseba zone Eritrea (2020)

| Description | Adi-Bosqual | | Balwa | | Adi-Alamin | | Filfle | |
|---|---|---|---|---|---|---|---|---|
| | PSC | Light trap | PSC | Light trap | PSC | Light trap | PSC | Light trap |
| No. of bedrooms | 10 | 15 | 8 | 13 | 25 | 30 | 24 | 30 |
| No. of people in previous night | 26 | 51 | 18 | 42 | 115 | 129 | 86 | 90 |
| No. of people slept under bed net in the previous night | 24 | 37 | 17 | 33 | 109 | 128 | 82 | 87 |

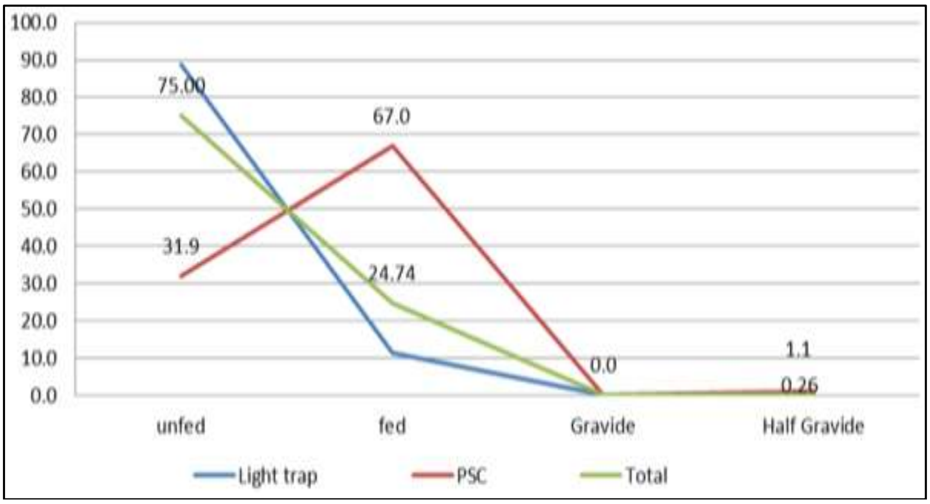


**Fig 3:** Physiological condition of adults collected from all study sites at Hagaz, Elabered and Habero sub zones: Anseba zone, Eritrea (2020)

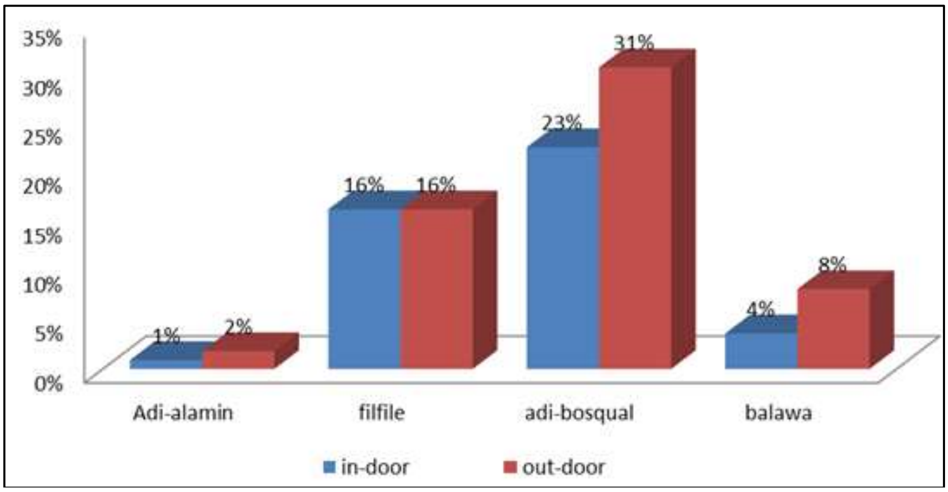


**Fig 4:** Indoor and outdoor mosquito biting behavior by study site at Hagaz, Elabered and Habero sub zons: Anseba zone, Eritrea (2020)

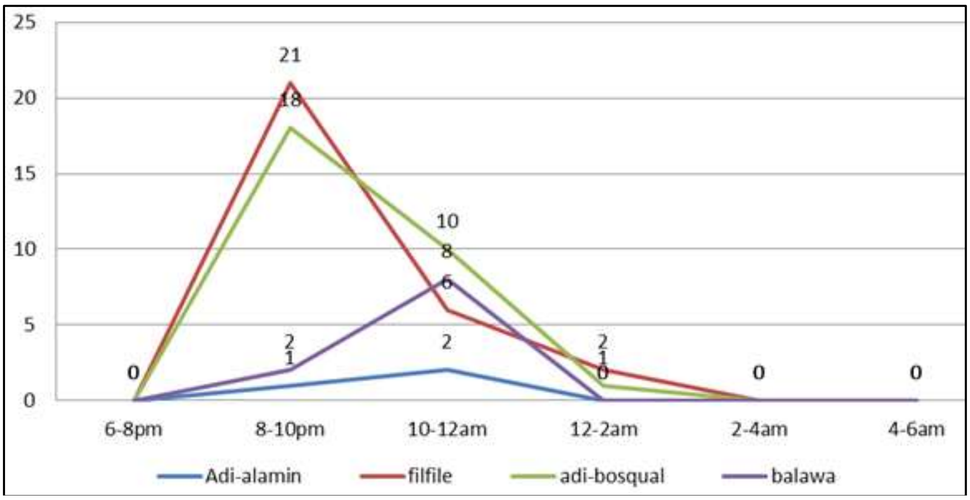


**Fig 5:** Anopheles mosquito night biting cycle by site at Habero, Elabered and Hagaz sub zones: Anseba zone, Eritrea (2020)

## Discussion

The result of the present study showed abundance, distribution, and bionomics or composition of potential malaria vectors in Hagaz, Elabered and Haboro sub zones, Anseba zone Eritrea. It includes the comparison of anopheles mosquito population of two surveys. The first survey was conducted in 1999-2001, in 5 zone of the country and the findings of the present study. In the previous study by Shilulu *et al*., (2003), among the 13 anopheles species found in Eritrea 7 were from Anseba zone. They were *An. gambiae s.l*., *An. d'thali*, *An. cinereus*, *An. rhodesiensis*, *An. rupicolus*, *An. demeilloni* and *An. garnhami*. It is obvious that changes on the bionomics and abundance of anopheles may occur due to many reasons encountered to our surrounding. For example, subsequent investigations discover the invasion of an Asian mosquito species know to call *An. stephensi*, was established in all the countries we shared a border with (*Vis*. Ethiopia, Sudan and Djibouti). Especially in Djibouti, severe out breaks has been reported annually since 2012 [17]. The present study was found a total of 5 *anopheles species vis*. *An. gambaie*, *An. cinereus*, *An. pharoensis* (*anopheles spp*. previously found in the highlands of Eritrea in a very small number), *An. pretoriensis* and *1 unidentified species*, with a dominant number of *An. gambiae*. Zonally only two species were found to be similar to the previous study but the rest *species* were new to our zone. *An. pretoriensis* was a new finding to our zone and to the country. This species were long been established in Ethiopia and Sudan [18, 19].

The present study revealed that, *An. gambaie* was found to be the most dominant malaria vector in the study areas. Similarly the study by Shilulu was found the same result that among the identified 2,513 malaria species, nearly 85% were found to be *An. gambie s.l.* Likewise, *An. gambiae* (said to be the most dangerous animal in the world), is the most dominant and efficient malaria vectors in Africa [13].

Different anopheles mosquito collection options have different purposes. PCS for example provide the information on indoor resting vector species irrespective of the resting surface. Some of its purposes are it used to determine indoor resting vectors, mosquito density and seasonal fluctuations. In addition to this, it also used to assess the degree of endophilly. Similarly light traps which calibrate at the indoor also used for the same purposes. Based on this understanding, during the present study, *An. gambiae* was found to be more endophilic and endophagic mosquito species. The same result was revealed from Kenya that *An. gambiae* was found to feed predominantly at the indoor site [20]. Feeding preference of this mosquito is the main reason for its vectorial capacity and transmission competence. Different studies were found that more than 95% of this species were exclusively feed in humans [21, 22]. The present study was failed to measure the human biting index of the vectors for different reasons.

Anseba River is among the major Rivers of the country, and this river touches more than half of the zone. 75% of the study sites included in the present study was found along this river. As a result the breeding sites recorded during the study were entirely restricted to this river. The study was designed to conduct at the end of the summer time starting from late August, thus the degree of rainfall was reduced and consequently the flood was reduced to its minimum level. The reduction of the flow of the water along this River was created temporary flood pools and in addition, animal foot prints were created. Hence, these two (*vis*. temporary flood pools and animal foot prints) was found to be the most suitable mosquito breeding habitats in the three study area. Exceptionally Adi-Alamin is located outside the river catchment area and the main mosquito breeding habitat were water accumulated from the nearby dam. A study conducted in Sudan along the Nile River were come up with similar result, that the maximum density of *An. gamiae* complex were directly related to the temporary flood pools (river edge) created by the River [23, 24]. On the contrary *An. pharoensis* was found from swamps. Swamps were visibly dominated by this mosquito species. So it can be said that swamps are the favorable breeding habitat for *An. pharoensis*. A study from the south western Ethiopia, by Getachew *et al.*, (2020), was found that, *An. pharoensis* was typically found from swamps [18]. Mosquitoes found in swamps are more likely present in smallest number/density compared to the other mosquito breeding habitats because these type of larval habitat harbor different competitors and predators which affects the development of mosquito larva [25]. In such breeding habitats it is difficult to complete the aquatic life without prayed by predators [26, 27].

River edges or temporary flood pools, animal foot prints were found to be the most favorable mosquito breeding habitat by different studies. In Sudan, Sennar and West Kordofan states for example, flood pools and animal hoof prints were found to be suitable breeding place for different mosquito species [28, 9, 29]. And similar finding from Ethiopia, animal foot print was among the preferred breeding habitat [18]. The present study was found river edges and animal foot print to be the most preferable anopheles mosquito breeding habitats.

Timing of sampling in related to water temperature, presence of vegetation either emergent, submerse or floating are important factors for mosquito density in an area. *An. gambiae* density was associated with places exposed to sun light, lack of emerged, submerged and floating vegetation.[30] Habitats exposed to sunlit have high water temperature thus shorten the time of development of aquatic mosquito stages to the flying adults. On the contrary, in shaded habitats, the temperature of the water would be very low and resulted to elongated aquatic development which exposes the larva/pupa to predator [31]. In addition to this, these variables probably influence attractiveness of the sites for oviposition [32]. The present study found that, the habitats fully or partially exposed to sun light were found to be the most suitable habitat. Presence of vegetation and size of water were also other factors that limit the presence of anopheles larva in a mosquito habitats. According to WHO (1982), mosquito larva are not found on open surface of large bodies of deep fresh water (e.g. Lakes, ponds, rivers or reservoirs) [33]. In the present study the larval habitats were with vegetation; either submersed or floating. Vegetation is believed to be suitable for the larvae/pupa since it serves as a shelter.

Temperature has a very big impact on the development of mosquitoes from larva-pupa-adult and the vectorial capacity as well [34]. Very specifically the development of larval stage is governed by internal and external factors. According to CDC (2014), mosquito larva are typically best at a water temperature of 25-27$^{o}$c [35]. Another study by UNICEF/UNDP/World Bank/WHO revealed that at a water temperature of 24-25$^{o}$c, hatching rate approaches 98%. During the present study, the average temperature over the period of study were extended from 23.5 $^{o}$c to 31.9 $^{o}$c in Habero sub zone, 21.6 $^{o}$c to 30.9 $^{o}$c in Elabered sub zone and 20.9 $^{o}$c to 33.2 $^{o}$c in Hagaz sub zone.

Biting cycle (mosquito biting hours) is another important factor on the transmission of malaria. *An. gambiae* was believed to bite at mid night when humans are slept to the dead. This time allow the vector to feed or to bite for enough time to transmit the diseases without interruption. A study from Cameron showed that most of the anopheles detected in the area was occurred to bite on the second part of the night (12:01am-06:00am).[36] On the contrary, the present study found that more than 90% of the anopheles mosquitos caught by human landing catch were found to bite at the first part of the night (08:00pm to 11:59pm) [36].

**Conclusion**

The study concluded:

- 5 *anopheles species (vis. An. gambaie*, *An. cinereus*, *An. pharoensis*, *An. pretoriensis* and 1 unidentified species) were identified and *An. gambiae* were found to be the most dominant vector.
- *An.gambiae* was found to be more endophilic and endophagic.
- *An. pretoriensis* was a new vector to the country and *a single An. Pharonesis* was found at the highlands of Eritrea before 2 decades but now, it founds to established at the lowlands.
- Majority of the breeding sites were found along the Anseba River.
- Generally, river edges, swamps, animal footprint were among the dominant breeding habitats. And all in all larval density was very high during the whole study period

**Recommendation**

- The role of the identified anopheles species in disease transmission should be incriminated using ELISA test.
- Malaria vector distribution and mapping need to be done at continuous bases which might assist in decision-making to adapt appropriate control measures.
- These days, a container breeding *An. stephensi* is getting inhibited in the neighboring countries and has been created a burden especially in the urban settings. So that the malaria control program of the state of Eritrea need to increase vector surveillance at the urban areas of malaria endemic zones.

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