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Habitat Characteristics and Seasonal Breeding Dynamics of *Anopheles gambiae s.l* (Diptera: Culicdae) in the Rural Communities of Ebonyi State, Nigeria- Implication for Vector Control

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ABSTRACT

Malaria transmission is to a large extent, determined by the availability of larval breeding habitats and the consequent production and distribution of adult vectors. This study accessed the habitat characteristics and larval abundance of malaria vector in the rural communities of Ebonyi State Nigeria. Larval collection was made on monthly basis between 7:00am and 11:00am on each sampling day, using WHO standard dipper of 350ml capacity. All larvae collected each day were appropriately labeled and taken to the vector research unit of Medical Microbiology laboratory, Ebonyi State University, for identification. Temperature and pH of the breeding water, were directly measured in the field using appropriate instruments. Data generated were analyzed using statistical package for social sciences (SPSS) version 20.0. General linear model multivariate analysis of variance was used to estimate 95% confidence interval in larval densities. Our result showed highest peak of larval abundance in June, 9543(4.49±2.824) in 2020 and July, 3800(2.09±1.069) in 2021. The mean larval density varied among communities and villages with the highest abundance (15,802), recorded in Okposi Umuoghara. Larval abundance was highest in puddle (33,064), habitats with clear water (26,809), and sunlit habitats (35,031). Temperature and pH of the breeding water were positively correlated with anopheline larval density (r=0.19; P>0.05), and (r=0.009; P>0.05), respectively. An understanding of the larval breeding habitat preferences, how the availability of the vectors, influences malaria prevalence would be extremely relevant to design malaria control strategies through vector control approach.

Key words: Malaria, season, breeding, habitats larval abundance, Rural communities, Ebonyi State.

RÉSUMÉ

Caractéristiques de l'habitat et dynamique de reproduction saisonnière d'*Anopheles gambiae* S.L (Diptera : Culicdae) dans les communautés rurales de l'État d'Ebonyi, Nigéria - Implications pour la lutte antivectorielle

La transmission du paludisme est en grande partie déterminée par la disponibilité des habitats de reproduction larvaire et la production et la distribution des vecteurs adultes qui en résultent. Cette étude a analysé les caractéristiques de l'habitat et l'abondance larvaire du vecteur du paludisme dans les communautés rurales de l'État d'Ebonyi, au Nigéria. La collecte des larves a été effectuée mensuellement entre 7h00 et 11h00 chaque jour d'échantillonnage, à l'aide d'une louche standard de l'OMS d'une capacité de 350 ml. Toutes les larves collectées chaque jour ont été correctement étiquetées et transportées à l'unité de recherche sur les vecteurs du laboratoire de microbiologie médicale de l'Université d'État d'Ebonyi, pour identification. La température et le pH de l'eau de reproduction ont été mesurés directement sur le terrain à l'aide d'instruments appropriés. Français Les données générées ont été analysées à l'aide du progiciel statistique pour les sciences sociales (SPSS) version 20.0. Une analyse de variance multivariée du modèle linéaire général a été utilisée pour estimer l'intervalle de confiance à 95 % des densités larvaires. Nos résultats ont montré que le pic d'abondance larvaire le plus élevé a été atteint en juin, 9 543 (4,49 ± 2,824) en 2020 et en juillet, 3 800 (2,09 ± 1,069) en 2021. La densité larvaire moyenne variait selon les communautés et les villages, l'abondance la plus élevée (15 802), enregistrée à Okposi Umuoghara. L'abondance larvaire était la plus élevée dans les flaques d'eau (33 064), les habitats avec de l'eau claire (26 809) et les habitats ensoleillés (35 031). La température et le pH de l'eau de reproduction étaient corrélés positivement à la densité larvaire d'anophèles (r = 0,19 ; p > 0,05) et (r = 0,009 ; p > 0,05), respectivement. Une compréhension des préférences des larves en matière d'habitat de reproduction et de l'influence de la disponibilité des vecteurs sur la prévalence du paludisme serait extrêmement pertinente pour concevoir des stratégies de lutte antivectorielle.

Mots clés : Paludisme, saison, reproduction, habitats, abondance larvaire, communautés rurales, État d'Ebonyi.

INTRODUCTION

Malaria is a serious vector-borne disease affecting hundreds of millions of people in Africa (Emami *et al.*, 2017). The factor that contributes to spread and transmission of malaria depend on the interaction among the human host, *Anopheles* vectors, malaria parasite, and environmental conditions (Arora and Arora, 2009). Natural transmission of malaria infection occurs by exposure to the bites of infected female *Anopheles* mosquitoes. The prevalence of malaria is higher in rainy season than in dry season due to availability of breeding habitat of mosquitoes such as water, ponds, pot hole, puddles, and uncovered ditches, sometimes occasioned by the flat table nature of most rural communities in Nigeria with the capacity to hold water after rainfall (Olowe *et al.*, 2014; Amadi, 2016). The availability of breeding sites of *Anopheles* mosquito is largely determined to an extent by the availability of host for blood meals (Minakawa *et al.*, 1999). *Anopheles gambiae* prefer breeding in sites close to house with typical breeding habitat such as puddles, shallow ponds, brick-pits, ditches, human foot, tyre tracks, and edge of boreholes which are often created by human activities (Koenradt *et al.*, 2004; Mwangangi *et al.*, 2007). These habitats are usually open, containing little or no aquatic vegetation, often with clear or turbid water that may be exposed to sunlight or in a partially shaded environment. Production of adult *A. gambiae s.l* occurs in small, temporary, sunlit, turbid pools of water (Koenraadt *et al.*, 2004; Mwangangi *et al.*, 2007). It has been suggested that the main reason that *A. gambiae* larvae are mostly found in open temporary aquatic habitats is that the mortality of *A. gambiae* larvae is lower in these habitats than in large and shaded habitats (Minakawa *et al.*, 2004; 2005). Larval predation is less prevalent in temporary habitats than in large, permanent habitats (Sunahara *et al.*, 2002). *A. gambiae* exploits the increased resources in warmer, open breeding habitats that produce more algae, which is the main food source for *A. gambiae* larvae than shaded habitats (Gimnig *et al.*, 2002). The warmer temperatures encountered in open habitats also shorten larval-to-pupal developmental time and consequently reduce the larval mortality associated with desiccation and predation (Bayoh and Lindsay, 2004; Gimnig *et al.*, 2002). Knowledge of vector breeding preferences in water collections is essential for effective vector control strategies. Information on vector breeding habitat preferences and seasonal breeding dynamics is limited in the study area and the knowledge is insufficient to achieve effective malaria control through vector approaches. An understanding of the larval ecology and at what period of the year the vector breeds most, would be extremely relevant to design vector control programmes.

Material and Methods

Study Area

Ebonyi State is located in the Southeastern part of Nigeria. It shares boundaries with Benue State to the North, Enugu State to the Northeast, Abia State to Southeast and Cross River State to the East (**Fig. 1**). It is located between latitude 6º15'N and longitude 8º05'E. Ebonyi State is made up of three senatorial zones and thirteen Local Government Areas with a total population of 2,968,698 people estimated in 2016 (National Population Commission (NPC) web, (2017). The climate of the area is tropical and the vegetation typical of the rain forest type with an average annual rainfall of 1300mm and average monthly temperature of 30 ±5ºC. There are two distinct seasons, the wet and the dry seasons, the former takes place between April and October, while the later occurs from November to March each year. The topography of most rural communities in Ebonyi State is mainly flat tableland with the capacity to hold water after rainfall and these usually creates breeding ground for mosquito vectors. A number of streams and rivers traverse most rural communities in Ebonyi State, which constitute the major sources of water supply to the inhabitants of the communities. The occupation of most rural communities in Ebonyi State is mainly farming with major crops such as yam, cassava, and rice produced across all parts of the state.

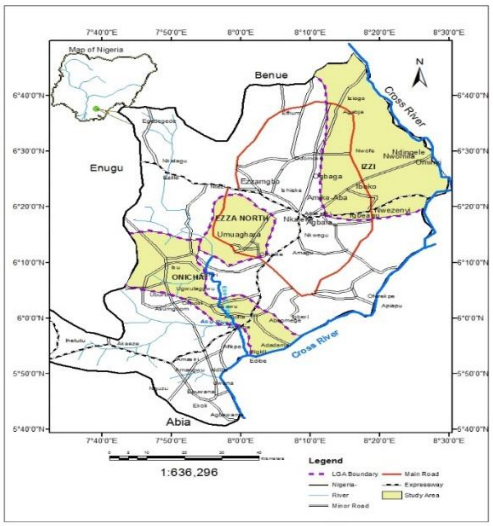

Figure 1: Map of Ebonyi State, showing the study LGAs

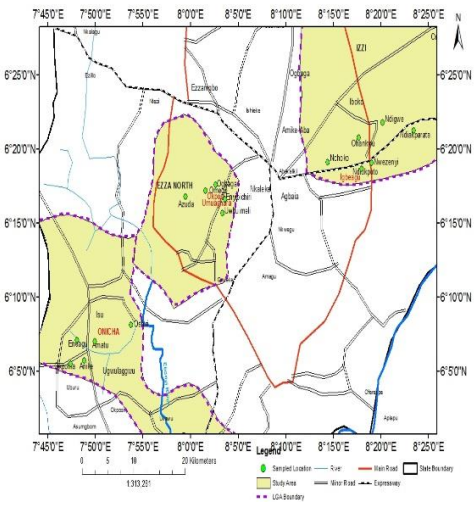

Figure 2: Map of the study areas (LGAs) showing the sampling locations.

Table 1. Showing the Geographical Coordinates of the larvae collection sites

LGA/Locality	Latitude	Longitude
Ezza North		
Ogbaga	6.33953700	8.07101390
Enyibichiri	6.32738000	8.07149000
Omege	6.32001700	8.06179000
Azuda	6.32231000	8.00624000
Uwhuimeli	6.30887010	8.09260570
Izzi		
Ndigwe	6.32157666	8.19808000
Nchoko	6.32132333	8.21946000
Ohiankwu	6.32487900	8.25356160
Nwezenyi	6.32976600	8.26703500
Ndiokpoto	6.33368330	8.27480666
Ndiakparata	6.37152500	8.27226600
Onicha		
Oshiri	6.13864000	7.89006000
Okpoma	6.08896666	7.81187333
Amautu	6.08895000	7.81186660
Enuagu	6.08523666	7.81153187
Anike	6.08540333	7.80769000
Isu Road	6.08924333	7.80837166

Source: Author's recording using hand held GPS device (GARMIN)

Three (3) communities (one per LGA) were randomly selected from three LGAs, one from each of the three (3) senatorial zones that make up Ebonyi State (**fig. 2**). A preliminary survey of suitable *Anopheles* mosquito breeding habitats was carried out from April to May 2020 by searching for areas where water stagnates in Igbeagu, Onicha Igboeze and Okposi Umuoghara communities. Suitability of the breeding sites was determined by closeness to residential houses (2-5m) as well as presence of larval exuriae or immature stages of mosquito larvae (USAID/CDC, 2011). GPS Coordinate of each identified breeding site was measured using hand held device (**table 1**) and was used to located each identified site throughout the sampling period.

Environmental Characterization of Larval Habitat

The body of the water identified in these areas was classified according to their stability as temporal. Based on the nature of the water collection, they were classified as puddle, tyre tracks, borehole channel, quarry pit, and ditch. The characteristic of the water was recorded as clear, turbid (when the breeding water is not transparent or muddy) or polluted (when the breeding water contain waste material that produces offensive odour). Based on the exposure of the breeding water bodies to sunlight, the habitat was classified as shaded (when the habitat is not exposed to sunlight), partially shaded (when the habitat is not fully exposed to sunlight, either partly covered by a tree), or sunlit (when the habitat is fully under sun exposure) while based on the emerging vegetation; the habitats were classified as emergent, submerged, floating or clear of weed as described by USAID/CDC, (2011). Other environmental variables such as temperature and pH of the breeding water were also measured and recorded on each sampling day during the sampling hours (7am-11am). Temperature of the breeding water was measured with digital thermometer while the pH of the water was measured with digital Hannah pH meter (pHep).

Anopheles Mosquito Larval Collection (Monthly Abundance)

Monthly survey was conducted to establish the availability of stagnant water, habitat characteristics and larval densities on monthly basis from May 2020 to October 2021 in all habitat types (puddle, tyre tracks, borehole channel, quarry pit, and ditch) identified in the three communities. Larval specimen collection was done using standard dipper (350ml capacity) according to WHO procedures (WHO, 2013). When *Anopheles* mosquito larvae were present, 10 -30 dips were taken depending on the size of the larval habitat at intervals along the edge. Where dipping was not possible (e.g. when larvae are seen on small water collection), pipette was used to pick up the larvae into the collection container. Sampling was carried out in the morning between 7:00am to 11:00am for about 10-30 mins at each larval habitat depending on the abundance of the larvae in such habitat. Anopheline larvae were identified in the field based on their resting position on the surface of the water. The density of the larvae from each habitat was expressed as the number of larvae per dip (total number of larvae/number of dips). All *Anopheles* larvae collected each day of sampling, from each site, were labeled appropriately and transferred to the USAID/PMI/VECTORLINK Ebonyi sentinel site insectary in the Department of Medical Microbiology Vector Research Unit, Ebonyi State University for further identification using keys provided by USAID/CDC, (2011).

Data Analysis

Data generated from survey was analyzed using statistical package for social sciences (SPSS) version 20.0. General linear model (GLM) multivariate analysis of variance was used to calculate the estimated marginal means and 95% confidence interval (C. I.) in larval densities within the different habitat types (puddle, tyre track, borehole channel, quarry pit, and ditch).

RESULTS

Monthly abundance and mean density of anopheline larvae across the breeding sites

The monthly abundance and mean larval density is presented in **Fig. 3 and Table 2**. The highest number of larvae, 9543(4.49±2.824) was collected in the month of June and the least 295(1.36±0.714) in December 2020 while in 2021, the highest and the least abundance and mean larval densities of 3800 and 132 (2.09±1.069 and 1.24±0.501) were recorded in July and February respectively. There was a significant difference in the larval density among the months [χ^2= 6.652, df= 824, P= 0.000 (P<0.05). Similarly, there was significant difference in the anopheline larval density between 2020 and 2021 [χ^2= 84.201, df= 2, P= 0.000 (P<0.05). Seasonal collections indicates higher anopheline larval abundance

in the rainy season (wet months) (39,551) than dry season (dry months) (**fig. 4)**.

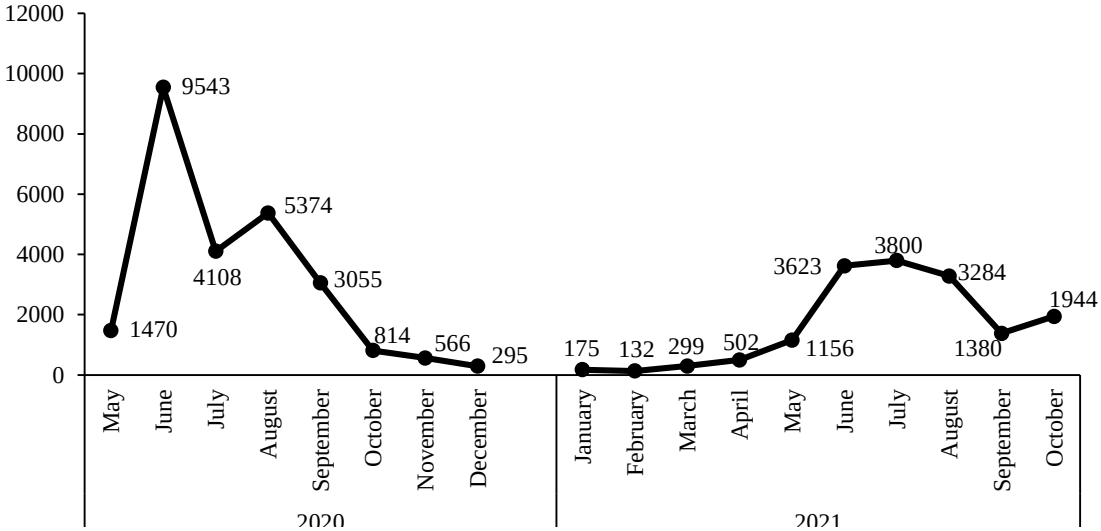

Figure 3. Monthly abundance of anopheline larvae across the study sites.

Table 2. Monthly abundance and mean density of anopheline larvae from 2020 - 2021.

Year	Month of collection	Number of Larvae Collected	Mean±SE
2020	May	1470	1.40 ±0.682
	June	9543	4.49±2.824
	July	4108	2.30±1.428
	August	5374	2.86±1.843
	September	3055	2.07±1.383
	October	814	1.41±1.076
	November	566	1.75±0.719
	December	295	1.36±0.714
2021	January	175	1.24±0.501
	February	132	1.24±0.522
	March	299	1.70±0.576
	April	502	1.96±0.369
	May	1156	1.31±0.680
	June	3623	1.99±0.964
	July	3800	2.09±1.069
	August	3284	1.94±0.925
	September	1380	1.40±0.857
	October	1944	1.71±0.779

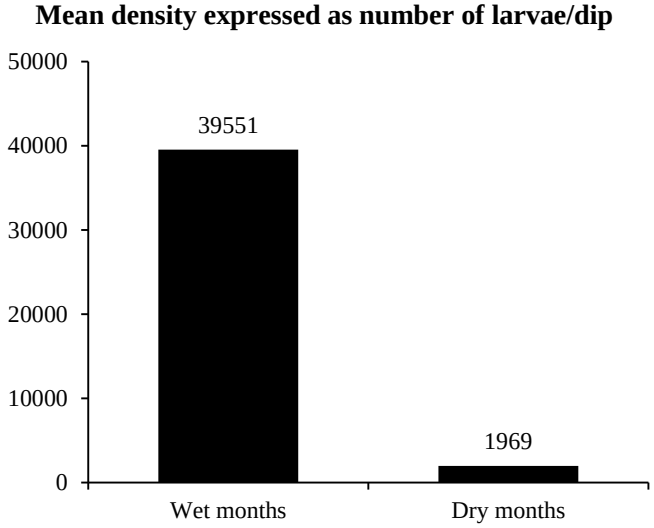

Fig. 4. Abundance of anopheline larvae during the sampling seasons

The Anopheline larval abundance and mean density between the study communities and villages

The anopheline larval abundance and mean density differed significantly between the villages in the three communities (**Table 3**). In Igbeagu community, the larvae were more abundant in Nchoko (3472) with mean larval density (2.08±0.979) while the least abundance (1202) with mean density (1.24±0.604) was collected in Ohiankwu. In Okposi Umuoghara community, the highest abundance and mean larval density 5308(2.82±2.819) was recorded in Ogbaga village while the least abundance and mean larval density 1321(2.38±1.268) occurred in Omege village. However, in Onicha Igboeze community, more larvae (4322) with mean larval density (3.04±2.012) were collected in Isu Road while the least 583(1.28±0.644) larvae/dip were recorded in Amautu. There was significant difference in the mean larval density among the villages [F=4.300, df= 16, P= 0.000 (P<0.05)].

Abundance and mean anopheline larval density among habitat types

Anopheline larvae were more abundant in puddles (33,064) with mean larval density of 2.09±1.573 while the least abundance (143) was recorded in quarry pits with mean larval density of (1.79±0.127) (Table 4). There was significant difference in the mean larval density among the habitat type [F=4.918, df= 4, P= 0.001 (P<0.05)].

Table 3. Abundance and mean anopheline larval density among the sampled villages.

Community/Villages	Number of larvae Collected	Mean±SE
Onicha Igbeze		
Oshiri	2316	2.48±1.416
Enuagu	1471	1.76±1.246
IsuRoad	4322	3.04±2.012
Anike	2239	2.46±1.558
Okpoma	1080	1.56±1.037
Amautu	583	1.28±0.644
Total	**12,011**	**2.097±1.319**
Umuoghara		
Ogbaga	5308	2.82±2.819
Enyibichiri	1791	1.91±0.947
Omege	1321	2.38±1.268
Azuda	3882	2.31±2.024
Uwhuimeli	3500	2.10±1.603
Total	**15,802**	**2.304±1.7322**
Igbeagu		
Ndigwe	3381	2.07±1.222
Nchoko	3472	2.08±0.979
Ohiankwu	1202	1.24±0.604
Ndiakparata	2422	1.86±1.497
Nwezenyi	2911	2.02±1.206
Ndiokpoto	1415	1.77±1.011
Total	**14,803**	**1.84±1.0865**

Table 4. Abundance and mean anopheline larval density among habitat types

Habitat type	No. of Larvae Collected	Mean±SE
Puddle	33064	2.09±1.573
Tyre Track	7569	2.55±1.776
Ditch	243	1.55±0.554
Quarry pit	143	1.79±0.127
Borehole channel	531	1.38±0.580
Total	**41550**	**2.14±1.592**

Abundance and mean anopheline larval density in relation to habitat characteristics

The highest anopheline larval abundance (26,809) with mean larval density of 2.26±1.534 was recorded among habitats with clear water while the least larval abundance was recorded among habitats with polluted water (281) with mean larval density of 8.03±0.000 (**Table 5**). There was a significant difference in the mean larval density among the habitat characteristics [F=10.951, df= 2, P= 0.000 (P<0.05)]. Sunlit breeding habitats recorded the highest number of larvae (35031) with mean larval density of 2.16±1.595 than shaded or partially shaded habitat (7585) with mean density of 2.07±1.580. There was no significant difference in the larval density based on light exposure [F=0.550, df= 824, P= 0.459 (P<0.05)]

The mean temperature of the breeding water ranged between 29.12±0.904 and 30.55±19.030 while the pH ranges between 7.02 and 8.42 (**Table 6**). There is a

positive correlation between anopheline larval density and temperature (r=0.19; P>0.05), as well as pH (r=0.009; P>0.05) of the breeding water.

Table 5. Abundance and mean anopheline larval density in relation to habitat characteristics

Habitat Characteristic	No. of Larvae Collected	Mean±SE
Nature of Breeding Water		
Clear	26,809	2.26±1.534
Turbid	15526	1.94±1.633
Polluted	281	8.03±0.000
Total	42616	4.08±0.07
Exposure to Light		
Sunlit	35031	2.16±1.595
Partially Shaded	7585	2.07±1.580
Total	**42616**	**2.115±1.588**

Table 6. The mean monthly temperature and pH of the larval breeding water

Months	Temperature (Mean±SE)	pH (Mean±SE)
May	29.12±0.904	8.42±0.380
June	29.17±1.116	8.33±0.500
July	30.55±19.030	8.34±0.476
August	29.47±1.032	8.36±0.625
September	29.13±1.057	8.34±0.468
October	29.99±0.834	8.42±0.509
November	29.27±0.738	7.81±0.477
December	29.53±0.563	7.47±0.431
January	29.33±0.683	7.02±0.210
February	29.33±0.798	7.14±0.430
March	29.15±0.680	7.36±0.287
April	29.31±0.961	7.59±0.401

Discussion

Monthly/Seasonal Breeding of *Anopheles* Mosquitoes and their Habitat Characteristics

The present study collected *Anopheles gambiae s.l* larvae in all the three communities with their populations relatively abundant throughout the period with peak populations observed in June 2020 and July 2021 respectively (**Table 2**). Breeding was predominantly in puddles and tyre tracks (formed by various activities of man and vehicular movements) which was abundant in all the study communities especially during the rainy months. However, other breeding habitats such as ditch, quarry pit, and borehole channel were also identified. Quarry pit and borehole channel formed the main breeding sites during the dry months. Puddles and tyre marks on the road and within residential areas in the communities were observed to be largely responsible for the abundance of *Anopheles* mosquitoes in all the three LGAs (**Table 4**). Similar observations has been reported by other researchers (Onyido *et al.* 2014; Kweka *et al.*2012)

The mean larval densities varied significantly between the study communities and villages (P<0.05) with highest mean larval density recorded in Okposi Umuoghara followed by Igbeagu community. Both Igbeagu and Okposi Umuoghara communities had a preponderance of mosquito breeding sites, accounting for more *Anopheles gambiae s.l* larval abundance, and this may be attributed to the more human and vehicular activities within the two communities. The study communities were characterized by lack of good road, usually with pools of water occasioned by human, and vehicular movements, which forms potential breeding sites for *An. gambiae s.l.* mosquitoes that can colonize a breeding habitat within a few days as the site is created (Minakawa *et al.* 2005).

Larvae of *A. gambiae s.l* normally inhabit diverse small water bodies that are often numerous, scattered, sunlit, turbid, temporary, and close to human dwellings (Gimnig *et al.*, 2001). These habitats differ in physical as well as biological characteristic, which directly influence the distribution and abundance of larval mosquito populations in nature (Kabula *et al.*, 2011). *A. gambiae s.l* larvae was found breeding in shallow, open, temporary water bodies across the study sites in this present study. Other studies have reported similar findings (Koenraadt *et al.* 2004; Dzorgbe *et al.* 2017). Breeding of *A. gambiae s.l* larvae only in temporary habitats as reported in this study, explains why malaria transmission in the study area is seasonal, usually peaking from the outset of raining season. Dzorgbe *et al.* (2017 asserted that whereas permanent habitats continuously support the breeding of *Anopheles* mosquitos, breeding in temporary habitats curtails when they dry up due to intermittent supply of water

Oviposition behaviour of gravid female mosquitoes is an important factor that influenced mosquito species composition. Generally, *A. gambiae s.l* larvae prefer clear, open sunlit waters (Minakawa *et al.*, 2002). In the present study, *A. gambiae s.l* larvae were collected in both clear and turbid (muddy) water bodies that are either exposed to sunlight or partially shady. This finding is supported by Mutuku *et al.*, (2006), who reported *Anopheles* larvae breeding in turbid, sunlit water. Female *Anopheles* mosquitos use open habitats, which are directly under the sun for oviposition because they are warm and the warmth reduces the larval and pupae development time (Minakawa, 1999). In addition, there is less predation and more algal growth, which serves as food for the larvae. Some of the breeding waters were very turbid (muddy), containing humus, yet numerous *A. gambiae s.l* larvae were collected from such site. This observation was

supported by previous report Munga *et al.* (2006) that *A. gambiae* prefer turbid water to clear water. Sattler *et al.* (2005), observed that *A. gambiae s.l* and *A. arabiensis* were associated with habitats that are high in turbidity and that both species increased in larval densities with increasing water turbidity. However, this contrasted former studies (Rohani *et al.*, 2010; Mwangangi *et al.*, 2007; Minakawa *et al.*, 2002), which asserted that *Anopheles* mosquito prefer clear water for ovipositing. Coinciding with the present study, Gimnig *et al.* (2001) also found higher *A. gambiae* larval densities with increasing turbidity. This consequently, is an indication that some physical qualities of a water body may not play a significant role in their proliferation.

The mean water temperatures in all larval positive habitats recorded in this study, ranged from 29.12-30.55 (29-31^{0}C). The results showed that *Anopheles* species had a wide range of observed water temperature, indicating that species, which use open habitats, can show a wider range of tolerance against habitats water temperature. The *A. gambiae s.l.* larval density was positively correlated with habitat water temperature (r=0.19), although there was no significant difference between the water temperature and the sampling months (F= 0.314; df= 11; P= 0.983(p>0.05). Tuno *et al.* (2005), asserted that higher temperatures can be detrimental to the presence of many other aquatic arthropods including predators which subsequently increase the survivorship of anopheline larvae. Similar studies have also reported positive correlation between water temperature and larval density (Kenea *et al.*, 2011; Munga *et al.*, 2006).

There was also positive correlation between the *Anopheles* larval densities and pH of the breeding water (r=0.009). The result however contrasted the earlier study of Adebote *et al.* (2008), which reported negative relationship between pH and *Anopheles* species. However, there was significant difference between larval density and the pH of the breeding water (F=25.218; df=11; P=0.000 (P<0.05). This however, contrasted the results of Rohani *et al.* (2010) who reported that there was no significant difference between pH and *Anopheles* larval densities. The pH of water is dependent on the concentrations of anions, cations, salts and synthetic compounds, which indicates its acidic or basic character (Bos, 1991). Therefore, it may be direct or indirect determinant of any aquatic life. Apart from direct effect, which is not clearly known anions and cations may also indirectly affect mosquito breeding by favouring certain types of aquatic vegetation or organism on which mosquito larvae feed or by affecting potential biological control agents of mosquito larvae (Bos, 1991). The later supports the result of this study since predators were not recorded in any of the study sites.

Conclusion

The present study has found *A. gambiae s.l* breeding mostly in puddles, tyre marks on the road during the rainy months while quarry pits and borehole channels formed the oviposition sites during the dry months. Larval breeding was in shallow, temporal, open sunlit habitats. *A. gambiae s.l* were collected in both clear and turbid water collections, an indication that physical quality of water may not play significant role their proliferation. The breeding sites were predominantly around residential areas, which shows an increased risk of malaria transmission in the study area.

Author Contributions:

"We declare that this work was done by the authors named in this article and all liabilities pertaining to claims relating to the contents of this article will be borne by the authors". Conceptualization, NDE ; Methodology, NDE, IGA, UOES and EMO; Validation, NDE and UOES; Formal Analysis, NDE, IGA, UOES and OCS; Resources, NDE, UOES and OCS; Data curation, UOES and IGA; Original Draft Preparation, NDE; Writing-review and editing, OCS and EMO; Supervision, OIO and IMO

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Data availability

The data presented in this study are available on request from the corresponding author. The data are not publicly available due to inability of the data curator to publish such in a research repository but can be made available if needed.

Conflict of Interest

The authors hereby declare that there is no conflict of interest among them.

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APPENDIX 1:

Plate 1: Typical Anopheline breeding habitats where larvae were collected

Key: A=Tyre track ; B=Puddle; C=Tyre track