CHARACTERIZATION OF *ANOPHELES FUNESTUS* LARVAL HABITATS IN KENYA: INSIGHTS INTO MALARIA VECTOR ECOLOGY AND CONTROL

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Abstract.

The breeding of malaria-spreading vectors such as Anopheles funestus *is influenced by various environmental factors that contribute indirectly to the transmission of the Plasmodium parasite. However, there is limited knowledge of larval habitat ecology that hinder prevention and control of mosquito-borne diseases. This study aimed to characterize larval habitats based on physicochemical and habitat characteristics, considering the abundance of* A. funestus. *A cross-sectional survey method was used to collect data on the established transects. Physical parameters (water temperature, pH, conductivity, and total dissolved solids) were measured using a 5-in-1 meter probe. Levels of chemical parameters (sulphate, COD, and BOD) were determined in the laboratory using standard methods. Observations were also made on habitat characteristics (including watercolor, habitat size, and canopy). There was significant effect ($P \leq 0.05$) of conductivity, pH, sulphate, COD, and BOD on the number of* A. funestus *larvae. Water samples with a high population of* A. funestus *larvae were found to have higher conductivity (Me of 470.5), TDS (Me = 235), and pH levels (Me of 6.71). Conversely, water samples with a high population of non-*Anopheles funestus *larvae were found to have higher COD (Me of 843.20), BOD (Me of 367.2), and SO_4 levels (Me of 11.3). A significant correlation ($p < 0.5$) existed between* A. funestus *larvae and physical water parameters. For instance,* Anopheles funestus *larvae was high (Me of 36.85) in stagnant water and in semi-permanent water (Me of 47.37). The study demonstrates that both physicochemical and habitat parameters significantly influence the abundance of* Anopheles funestus *larvae in larval habitats. Parameters such as conductivity, pH, total dissolved solids, sulphate, COD, BOD, watercolor, depth, distance from the homestead, and habitat size were found to be important in determining the presence of* A. funestus *larvae. Therefore, vector control strategies should include larval source management by targeting rivers and other water bodies to prevent the emergence of* Anopheles funestus.

Key Words: *Malaria Vector,* Anopheles funestus*, Habitat-Parameters, Fiyoni, Kenya*

1. Introduction

Mosquitoes are important vectors that spread malaria and other diseases such as dengue and yellow fever (Mereta *et al*., 2013). Mosquito borne infections infects up to 700 million people around the globe every year where close to two million die annually (Mereta *et al*., 2013). *Anopheles funestus* is a major malaria vector in Kenya and therefore targeted for control, biology, insecticide resistance (Mugenzi *et al*., 2022), and behavior studies (Mwema *et al*., 2022. Since *A. funestus* is an opportunistic and anthropophilic, the use of long-lasting and indoor residual spraying methods alone is not enough hence the need to introduce new control strategies (Meza *et al*., 2022). According to Mereta *et al*. (2013), African countries account for most of the mortality, which mostly affects children below five years. For instance, in Ethiopia, approximately 75% of the country are considered malaria endemic zones and as of 2013, roughly 58.3 million individuals resided

in malaria risk zones (Getachew *et al*., 2020a). A study by Kamau *et al*. (2020) in Kenya also revealed that malaria incidence stands at 64-70% among children aged 6 months to four years in the coastal regions of the country. Despite recent efforts to control malaria as a major public health issue within tropical regions, the disease remains a challenge following Plasmodium parasite resistance to antimalarial treatments and insecticides for the vectors. The most common interventions used to curb the malaria vector are long-lasting insecticide treated nets and indoor residual insecticide spraying that target only adult stages of the vector.

Recent studies have linked habitat characteristics to survival of major Anopheline mosquitoes like larval abundance. For example, Getachew *et al*. (2020a) showed that mosquito vectors can be effectively controlled through larval habitat management which has gained more attention in the last few decades as it targets the larval stages

of the mosquitoes. In Iran's malaria prone areas, Anopheline mosquitoes' larval habitats have been characterized using physicochemical parameters (pH, conductivity and temperature) offering a significant step towards planning larval management programs (Soleimani-Ahmadi *et al*., 2014). In Ethiopia, a study has also been done by Getachew *et al*. (2020a) to characterize *Anopheles* breeding habitats as part of malaria control interventions in the country. Mbanzulu *et al*. (2022) conducted a similar study in the Democratic Republic of the Congo to identify the physicochemical traits of *Aedes* mosquito breeding environments as a means of vector control. Characterization of the larval habitats was carried out in a different study by Hessou-Djossou *et al*. (2022) in Benin to ascertain the impact of physicochemical characteristics on the Anopheline larvae population as a method of organizing vector management. To create a habitat-based malaria vector control program, Akeju *et al*. (2022a) conducted a study in Nigeria that also assessed *Anopheles* mosquitoes, including *Anopheles funestus'* larval habitats, utilizing their physicochemical and environmental features. According to a study by Nikookar *et al*. (2017b) in Iran, *Anopheles* larvae are related to temperature, pH, total hardness, sulphate, and conductivity. *Anopheles funestus* larval habitats were described in a prior work by Nambunga *et al*. (2020) in Tanzania utilizing a variety of factors, including distance from the homestead, water depth, and habitat size.

However, in Kenya studies characterizing the larval water ecology and larval habitat of Anopheline mosquitoes like *Anopheles funestus* are scarce despite being a principal malaria vector with reports on possible resistance to control programs. To implement the malaria vector control interventions, it is important to understand the larval habitat ecology to control them at immature stages. Controlling the malaria vector at their immature stages is advantageous as they are concentrated at a specific small site as compared to their adult counterparts which are dispersed and occupy larger areas (Getachew *et al*., 2020a). Hence the knowledge about larval water quality parameters and an understanding of larval habitat characteristics are key to designing effective malaria vector control programs. The aim of this study was therefore to characterize preferred and non-preferred larval sites of *Anopheles funestus* in Fiyoni, Kwale County using their physicochemical parameters and habitat characteristics. Climatic based factors like temperature are key environmental determinants of malaria which is highly conditioned by ecology and affects local dynamics of the disease (Debrah *et al*., 2021a). Understanding insect vectors like mosquitoes, its population dynamics, their interaction with the environment (larval habitat) and Plasmodium parasite is also important in preventing the vector borne disease (malaria) (McCord, 2016). Findings from this study could help in developing habitat-based vector control strategy that may reduce the rate of vector bites and malaria spread. This study further attempts to create an understanding of the ecological factors (water temperature, pH, conductivity, total dissolved solids, watercolor, habitat size, and canopy) that may favor the survival of *A. funestus* larvae. Knowledge of these ecological factors may be necessary in developing sound strategies and policies necessary for mitigating the transmission of malaria.

2. MATERIALS AND METHODS
2.1 Study Site

This study was conducted in the Fihoni villages located in Kwale County, Kenya. The geographical coordinates of the study site range from approximately 39.4857015° E longitude to 4.4011020° S latitude. The elevation of the study site is around 408 meters above sea level. As of 2019, the population in Kwale County was estimated to be 866,820 (KPHC, 2019). The climate in the area is characterized by temperatures ranging from 27°C in May to a maximum of 33°C in July, with an average temperature of 30.0°C (CGoK, 2021). The lowest recorded annual temperature is 24.06°C, while the highest annual temperature is 30.0°C. The average annual rainfall in the region varies between 400 mm and 1680 mm, with May receiving the highest amount of rainfall (131.95 mm) in the county. The primary socio-economic activities in the area are farming, particularly Okra cultivation, and livestock rearing, including goats and cows. The selection of this study site was based on its endemicity to malaria diseases and the presence of perennial rivers within the study area. Sampling for this study was conducted between May and July 2021.

2.2 Survey design, sample collection and storage

The study utilized a cross-sectional survey design to survey mosquito larval habitats along a predetermined transect. Each larval habitat was visited once to collect data and water samples. Along the study area's river, four sampling points were established. At each sampling point, water samples were collected from different corners of the transect using a standard dipper with a long handle (30cm) from Clarke Mosquito Control Products (Rosele, IL) for three days. The individual water samples from each corner were combined to create a composite sample representing that specific sampling point. The composite water samples were then labeled with the date, sample number, and transect area and transported while in cool box to the laboratory at KEMRI in Kwale for further analysis. In the laboratory, the mosquito larvae were extracted and identified soon after arrival.

2.3 Characterization of mosquito Larvae habitat

Larval habitats that contained mosquito larvae either Anopheline or the *Culex* spp (larvae) were identified based on several factors. Some of the factors considered while

choosing mosquito habitat were; distance from homestead, algal presence, vegetation cover, water movement, size of the water body, water depth, water color, habitat type and abundance of vegetation cover. The distance from the homestead was measured using a tape measure and grouped into three categories (<100m, 100-500m, and >500m). Visual observation was used to determine the presence of algae, vegetation cover, water movement, and habitat type. Habitat size (width of the water body sampled) was also measured using a tape measure and categorized as <10m, 10-100m, or >100m. Water movement was assessed visually and categorized as stagnant (with no movement at all) or slow moving. The habitat canopy was categorized as none (no vegetation cover), partial (some vegetation cover), or shade type (more than half of the habitat covered by vegetation).

Vegetation was categorized as submerged (roots and entire plant below the water surface) or emerged (roots at the water bottom with stems or leaves outside the water surface). Water color was visually categorized as clear, colored, or polluted. Water depth was classified into three groups: <10cm, 10-50cm, or >50cm. Water type was categorized as semi-permanent (lasting about two weeks) or small pool (holding water for less than four days). Algae quantity was assessed visually and categorized as none, scarce, moderate, or abundant based on the coverage of the water surface.

2.3. Identification of *Anopheles funestus* larvae and Determination of larval Preferred habitat

To identify mosquito larvae from each water sample collected at various locations, a 100-ml water sample was dispensed into a transparent watch glass. The mosquito larvae were then carefully extracted from the water in the watch glass using a camel-hair brush and placed on Whatman paper. Only the late-stage larvae were kept for analysis, while the early-stage larvae were discarded. To euthanize the late-stage larvae, they were immersed in hot water at 59°C for 2 minutes. The larvae were subsequently identified under a compound microscope by mounting them on a microscopic glass slide, adding a drop of Hoyer's mounting medium, and air drying them (Getachew *et al.,* 2020a). The identification keys provided by Gillies and Coetze were used (Gillies, 1987). The larvae were categorized as either *Anopheles funestus* or other non-*Anopheles funestus* species like Culex spp. The larvae in each category were then counted to determine their respective abundances. The water samples were classified based on the abundance of *Anopheles funestus* larvae. Samples containing more than ten *Anopheles funestus* larvae were categorized as "preferred larval water," while those with fewer than ten *Anopheles funestus* larvae were designated as "non-preferred larval water." Composite water samples from the transects containing more than fifty *A. funestus* larvae were classified as having high density while those

between ten to fifty were treated as moderate. Otherwise, they were categorized as 'low' density. The preserved samples were then stored in vials containing 70% ethanol at the Kwale laboratories.

2.4 Determination of the physicochemical parameters of water samples

The physicochemical parameters of the collected water samples were determined using various methods. In the field, conductivity, temperature, total dissolved solids, and pH were measured directly in the river using a handheld 5-in-1 tester probe (MaiDat, Saudi Arabia). To obtain the readings, the probes were inserted into 2 liters of water collected from the river and gently stirred until the readings on the screen stabilized. Additional physicochemical parameters such as sulfate concentration, chemical oxygen demand (COD), and biological oxygen demand (BOD) were measured in the laboratory using standard methods. Sulfate concentration was determined using a spectrophotometer [(HI 96751 sulfate, Hanna Instruments Inc., Woonsocket, Rhode Island) de Almeida *et al*., 2022]. COD was measured using a COD detector as per the user manual [(Aquasol, APC ODD1, Power Max Engineers, India) Njoroge *et al.,* 2013]. BOD was measured using a H198193 waterproof portable dissolved oxygen BOD meter following the user manual [(Hanna Instruments Inc., Woonsocket, Rhode Island) Hessou-Djossou *et al.,* 2022].

2.5 Data analysis

The data collected on mosquito larvae abundance were analyzed using descriptive statistics to determine whether the collection site was preferred by the *Anopheles funestus* or not and the result presented both in terms of frequency and percentage. To examine the impact of physicochemical parameters on the presence of *Anopheles funestus* and non-*Anopheles funestus* larvae in preferred and non-preferred habitats, data were subjected to a Mann-Whitney test using Scientific Analytical System (SAS) version 9.4 with an (alpha = 0.05). Bonferroni adjustment was applied to address multiple comparisons by setting the adjusted significance level (alpha) at 0.025, obtained by dividing the initial alpha level of 0.05 by 2 to account for the two comparisons made. Data comparing the number of *A. funestus* larvae and non-*Anopheles funestus* larvae with physicochemical parameters were analyzed using the Mann-Whitney test in SAS 9.4 (alpha = 0.05). The association between larval habitat characteristics and the number of *Anopheles funestus* and non-*Anopheles funestus* larvae was assessed using the Chi-square test of association (alpha = 0.05) in SAS 9.4 (alpha = 0.05). Furthermore, the Pearson correlation coefficient was utilized in SAS 9.4 to evaluate the correlation between the number of *Anopheles funestus* larvae, physicochemical parameters, and habitat characteristics (alpha = 0.05).

3. RESULTS
3.1 Larval preference and density in different habitats sampled

Two transects [transect A and C] were majorly preferred by the *Anopheles funestus* larvae with moderate density as compared to transect B which was non-preferred (Table 1). Transect D was preferred with high density of *Anopheles funestus* larvae.

3.2 Effects of physicochemical parameters on the presence of both *Anopheles funestus* and non-*Anopheles funestus* larvae in preferred and non-preferred larval sites

Conductivity had a significant ($p<0.05$) effect on the occurrence of *A. funestus* and non-*Anopheles funestus* larvae. *A. funestus* larvae were significantly higher (Me=470.5) compared to non-*Anopheles funestus* (Me=285). The Mann-Whitney U test statistic results for the two larval sites [U ($N_{A. funestus}$ =10, $N_{Non-A. funestus}$ = 9) = 45, Z= -3.6350, p <.0001) was obtained (Table 2).

Temperature of larval water had no significant effect ($p<0.05$) on the occurrence of *Anopheles funestus* and non-*Anopheles funestus* larvae. The *A. funestus* larvae were significantly higher (Me=30.6) compared to non-*Anopheles funestus* larvae (Me=30.48). The Mann-Whitney *U* test statistic results [U ($N_{A. funestus}$ =10, $N_{Non-A. funestus}$ = 9) = 76, Z= -1.1116, p = 0.2490)] was obtained (Table 2).

Total dissolved solids had a significant effect ($p<0.05$) on the occurrence of *A. funestus* and non-*Anopheles funestus* larvae. The *A. funestus* were significantly higher (Me=235.00) compared to non-*Anopheles funestus* larvae (Me=191.00). The Mann-Whitney U test statistic results (U ($N_{A. funestus}$ =10, $N_{None A. funestus}$ =9) = 51, Z= -3.1449, p = 0.0014) was obtained for the two larval sites (Table 2).

Water pH had a significant ($p<0.05$) effect on the occurrence of *A. funestus* and non-*Anopheles funestus* larvae. The *A. funestus* larvae gave a higher median (Me = 7.43) than that of the non-*Anopheles funestus* larvae (Me = 6.45). The Mann-Whitney U test statistic results (U ($N_{A. funestus}$ =10, $N_{None A. funestus}$ =9) = 47, Z= -3.4701, p = 0.0004) was obtained for the two larval sites (Table 2).

Sulphate had a significant effect (p <0.05) on the occurrence of *A. funestus* and non-*Anopheles funestus* larvae. Non-*Anopheles funestus* larvae however gave a higher median (Me = 11.30) than that of *A funestus* larvae (Me = 0.10). The Mann-Whitney *U*-test statistic results (U ($N_{A. funestus}$ =10, $N_{None A. funestus}$ =9) = 130, Z= 3.6298, p = 0.0002) was obtained for the two larval sites (Table 2).

The COD had a significant effect on the occurrence of the *A. funestus* and non-*Anopheles funestus* larvae. *A funestus* larvae gave a higher median (Me= 843.36) compared to that of non-*Anopheles funestus* larvae (Me= 803.20). The Mann-Whitney U test statistic results (U ($N_{A. funestus}$ =10, $N_{None A. funestus}$ =9) = 130.5, Z= 3.7712, p = 0.0001) was obtained for the two larval sites (Table 2).

The BOD had a significant positive effect (p <0.05) on the occurrence of the *A. funestus* and non-*Anopheles funestus* larvae. *A. funestus* larvae gave a higher median (Me = 367.20) compared to that of the non-*Anopheles funestus* larvae (Me = 340.20). The Mann-Whitney U test statistic results (U ($N_{A. funestus}$ =10, $N_{None A. funestus}$ =9) = 130.5, Z= 3.7712, p = 0.0001) was obtained for the two larval sites (Table 2).

3.3 Association between the habitat parameter and the number of *A. funestus* larvae in Fihoni, Kwale county.

There was no significant (p > 0.05) association between water movement and number of mosquito larvae in water body (χ^2 (2, 19) = 4.718, p = 0.0945). However, the number of *A. funestus* mosquito larvae were 0 % and 5.26% in habitats with rapidly flowing and stagnant water respectively. On the other hand, non-*Anopheles funestus* (*Culex* spp) were 10.53% and 31.58% in habitats with rapidly flowing and stagnant water respectively (Table 3).

There was no significant (p > 0.05) association between habitat canopy and number of mosquitos larvae (χ^2

Table 1: *Anopheles funestus* level of water preference in various habitats sampled.

Transect/Larval Site visited	Type of larvae	Frequency (Percentage Counts)	A. *funestus* Larval Density	Habitat Preference by *Anopheles funestus*
Transect A	A. *funestus* larvae Non-A. *funestus*	45 (13.20) 63 (18.48)	Moderate	Preferred
Transect B	A. *funestus* larvae Non-A. *funestus*	8 (2.35) 87 (25.51)	Low	Non-preferred
Transect C	A. *funestus* larvae Non-A. *funestus*	36 (10.55) 45 (13.20)	Moderate	Preferred
Transect D	A. *funestus* larvae Non-A. *funestus*	51 (14.96) 6 (1.75)	High	Preferred
		Total 341(100.00)		

where A, B, C and D =transects along the river, brackets =percentage number of larvae collected from the various transects

Table 2: Effects of physicochemical characteristics (parameters) on *A. funestus* and non-*Anopheles funestus* presence in larval water in Fihoni, Kwale county.

Par.	Type of larvae	Mean	SE	*Me*	Min	Max	*N*	*U*	*Z - score*	*P -Value*
			22.54				10	45	-3.6350	<.0001
Cond.	*A. funestus*	467.20		*470.50*	428.00	496.00				
	Non-*A. funestus*	335.56	65.96	*285.00*	276.00	426.00	9			
Temp	*A. funestus*	30.69	0.48	*30.60*	30.10	31.90	10	76	*-1.1116*	*0.2490*
	Non-*A. funestus*	30.48	0.25	*30.40*	30.10	30.90	9			
TDS	*A. funestus*	234.60	13.90	*235.00*	216.00	254.00	10	51	*-3.1449*	*0.0014*
	Non-*A. funestus*	182.44	34.08	*191.00*	138.00	227.00	9			
pH	*A. funestus*	7.38	0.29	*7.43*	6.71	7.74	10	47	*-3.4701*	*0.0004*
	Non-*A. funestus*	6.51	0.19	*6.45*	6.31	6.85	9			
SO4	*A. funestus*	1.22	3.54	*0.10*	0.10	11.30	10	130	*3.6298*	*0.0002*
	Non-*A. funestus*	11.29	0.03	*11.30*	11.20	11.30	9			
COD	*A. funestus*	807.22	12.70	*803.20*	803.20	843.36	10	130.5	*3.7712*	*0.0001*
	Non-*A. funestus*	843.36	0.00	*843.36*	843.36	843.36	9			
BOD	*A. funestus*	342.90	2.70	*340.20*	340.20	367.20	10	130.5	*3.7712*	*0.0001*
	Non-*A. funestus*	367.20	0.00	*367.20*	367.20	367.20	9			

where Max= Maximum, N= Number of positive samples, U = Mann-Whitney U test, Cond. = Conductivity, Temp = Temperature, Par.= Parameter, TDS= Total Dissolved Solids, pH=potential of hydrogen, SO4=Sulphate, COD= Chemical Oxygen Demand, BOD= Biological Oxygen Demand, Negative values in Z-scores shows that data point is below average. Analysis was done using Scientific Analysis System version 9.4, Replicates=3, SI Units= International System of Units, Me= Median, SE=Standard Error. The p-values (for the *A. funestus and* non-A. *funestus*) in the table were tested using Bonferroni adjustment (0.05/2=0.025) for each physico-chemical parameter.

(2, 19) = 5.2476, p = 0.0725). The *A. funestus* larvae were 21.05% and 10.53% in habitats with no canopy and heavy canopy respectively. On the other hand, non-*Anopheles funestus* larvae were 0.00% and 26.32% in habitats with no canopy and habitats with heavy canopy respectively (Table 3).

There was no significant association (p > 0.05) between distance from homestead and number of the mosquitoes' larvae in the stream at Fihoni (χ^2 (2, 19) = 5.4386, p = 0.0659). The *A. funestus* larvae were 26.32% and 21.05% in habitat with distance that are less than 50 m from homesteads and distances which are more than 100 m from homesteads respectively. On the other hand, non-*Anopheles funestus* larvae were 5.26% and 15.79% in habitat with distances that are less than 50 m from homesteads and distances which are more than 100 m from homesteads (Table 3).

There was no significant (p > 0.05) association between water depth and the number of mosquito larvae in water bodies (χ^2 (2, 19) = 0.6929, p = 0.7072). *A. funestus* larvae were 36.84% and 10.53 in habitat with water depth of less than 50 cm and more than 100 cm respectively. On the other hand, non-*Anopheles funestus* larvae were 31.5% and 5.26% in habitat with water depth which is less than 50 cm and more than 100 cm respectively (Table 3).

There was no positive association between water type and the number of *A. funestus* larvae (p>0.05) (χ^2 (1, 19) = 0.5322, p = 0.4657). *A. funestus* larvae were 47.37% and 5.26% in habitats with semi-permanent and small pools respectively. On the other hand, non-*Anopheles funestus*

larvae were 36.84% and 10.53% in habitats with semi-permanent and small pools respectively (Table 3).

Association between water color and the number of mosquito larvae showed no positive significant difference (p > 0.05) between the *A. funestus* and the *Culex* spp (χ^2 (2, 19) = 1.3177, p = 0.5174). The *A. funestus* larvae were 15.79% and 21.05% in habitats with clear and polluted water respectively. However, non-*Anopheles funestus* larvae were 10.53% and 26.32% in habitats with clear and polluted water respectively (Table 3).

Association between algae quantity and the number of mosquito larvae revealed no significant difference between the *Anopheles funestus* larvae and those of *Culex* spp (χ^2 (3, 19) = 6.2981, p = 0.0980). *A. funestus* larvae were 15.79% and 21.05% in habitats with scarce algae density and habitats with abundant algae density. However, non-*Anopheles funestus* larvae were 21.05% and 10.53% in habitats with scarce and abundant algae quantities respectively (Table 3).

Association between habitat size and the number of mosquito larvae showed no significant difference between the *A. funestus* larvae and those of *Culex* spp (χ^2 (2, 19) = 0.4820, p = 0.7858). The *A. funestus* larvae were 36.84% and 5.26% in habitats with a water body of diameter less than 10 m in size and those with a diameter more than 100 m in size respectively. On the other hand, non-*Anopheles funestus* larvae were 26.32% and 5.26% in water body size with less than 10 m water body size and more than 100 m water body size respectively (Table 3).

Table 3: Effects of habitat parameters on the abundance of *Anopheles funestus* and non-*Anopheles funestus* larvae

Habitat Characteristics Assessed	Frequency (Percentage Larvae Abundance)		Total
	A. funestus	Non-*A. funestus*	
Water movement			
Stagnant water	5 (26.32)	6 (31.58)	11 (57.89)
Slow flow	5 (26.32)	1 (5.26)	6 (31.58)
Rapid flow	0 (0.00)	2 (10.53)	7 (36.58)
Total	10 (52.63)	9 (47. 37)	19 (100)
Habitat canopy			
None	4 (21.05)	0 (0.00)	4 (21.05)
Scarce	4 (21.05)	4 (21.05)	8 (42.10)
Heavy	2 (10.53)	5 (26.32)	7 (36.85)
Total	10 (52.63)	9 (47.37)	19 (100)
Distance from homesteads			
<50m	5 (26.32)	1 (5.26)	6 (31.58)
50-100m	1 (5.26)	5 (26.32)	6 (31.58)
>100m	4 (21.05)	3 (15.79)	7 (36.84)
Total	10 (52.63)	9 (47.37)	19 (100)
Water depth			
< 50cm	7 (36.84)	(6 (31.58)	13 (68.42)
50-100cm	1 (5.26)	2 (10.53)	3 (15.79)
>100cm	2 (10.53)	1 (5.26)	3 (15.79)
Total	10 (52.63)	9 (47.37)	19 (100.00)
Water type			
Semi-permanent	9 (47.37)	7 (36.84)	16 (84.21)
Small pool	1 (5.26)	2 (10.53)	3 (15.79)
Total	10 (52.63)	9 (47.37)	19 (100.00)
Water color			
Clear	3 (15.79)	2 (10.53)	5 (26.32)
Colored	3 (15.79)	2 (10.53)	5 (26.32)
Polluted	4 (21.05)	5 (26.32)	9 (47.37)
Total	10 (52.63)	9 (47.37)	19 (100.00)
Algae density			
Scarce	3 (15.79)	4 (21.05)	7 (36.84)
Moderate	3 (15.79)	3 (15.79)	6 (31.58)
Abundant	4 (21.05)	2 (10.53)	6 (31.58)
Total	10 (52.63)	9 (47.37)	19 (100.00)
Water body size			
<10m	7 (36.84)	5 (26.32)	12 (63.16)
10-100m	2 (10.53)	3 (15.79)	5 (26.32)
>100m	1 (5.26)	1 (5.26)	2 (10.53)
Total	10 (52.63)	9 (47.37)	19 (100.00)

where brackets represent percentage abundance of *Anopheles funestus* larvae and non-*Anopheles funestus* larvae in various habitat parameters assessed

3.3.9 Correlation between number of *Anopheles funestus* larvae, physicochemical parameters and habitat parameters

There was a significant negative correlation (r=-0.822, p<0.0001) between the number of *Anopheles funestus* and non-*Anopheles funestus* larvae and conductivity levels in the water sampled (Table 4). There was none significant positive correlation (r=-0.276, p=0.253) between the number of *Anopheles funestus* and non-*Anopheles funestus* larvae and temperature readings in the sampled water analyzed. Number of *Anopheles funestus* and non-*Anopheles funestus* larvae had a strong positive correlation (r=-0.734, p=0.000) with the total dissolved solids (Table 4). Number of *Anopheles funestus* and non-*Anopheles funestus* larvae had negative significant correlation (r=-0.877, p<0.0001) with the values of pH recorded in the sampled water analyzed. Number of *Anopheles funestus* and non-*Anopheles funestus* larvae had a strong positive significant correlation with sulphate concentration (r=0.900, p<0.0001), COD (r=0.900, p<0.0001) and BOD (r=0.900, p<0.0001) in the water sample (Table 4). Similarly, total dissolved solids had a stronger negative correlation (r=-0.619, p=0.005) with habitat's water depth. pH had a stronger negative correlation with habitat's water depth (r=-0.829, p<0.0001) and distance from homestead (r=-0.567, p=0.011). Sulphate has a stronger positive correlation with habitat's water depth (r=0.857, p<0.0001) and distance from homesteads (r=0.548, p=0.015). COD had a stronger positive correlation with water depth (r=0.858, p<0.0001) and distance from homestead (r= 0.549, p=0.015). BOD had a stronger positive correlation with habitat's water depth (r=0.858, p<0.0001) and distance from homestead (r= 0.549, p=0.015) (Table 4).

4. RESULTS

A basic understanding of the mosquito larval ecology is necessary to plan and develop effective malaria control strategies. The present study found that physicochemical characteristics and habitat characteristics are the main variables that characterize *Anopheles funestus* larval habitat.

4.1 Relationship between physicochemical parameters and the presence of *Anopheles funestus* or non-*Anopheles funestus* larvae in preferred and non-preferred larval sites

Anopheles funestus larvae were higher while non-*Anopheles funestus* larvae were lower where conductivity was highest. These findings are different from Nambunga *et al*. (2020) who reported that non-*Anopheles funestus* larvae were highest where conductivity was highest. This can be explained by the presence of mobile ions, mineral salts and dissolved salts that carry electric charges hence increasing conductivity in the larval water (Akeju *et al*., 2022b). Such minerals (for example, magne-

sium and calcium) provide a conducive environment for larval growth *(Tchigossou et al*., 2017). Findings of this study suggest that higher temperatures were also associated with lesser numbers of *Anopheles funestus* larvae while the number of non-*Anopheles funestus* larvae were higher. This finding agrees with Nikookar *et al*. (2017a) who suggested that *Culex* spp grows better at higher temperatures. These results could be explained by the fact that higher temperatures could be affecting the enzyme catalyzed reactions in Anopheline mosquitoes, hence reducing their population significantly (Nambunga *et al*., 2020).

There was a significant association between total dissolved solids and the number of *Anopheles funestus* larvae compared to non-*Anopheles funestus* larvae. These findings are similar to those of Emidi *et al*. (2017) who found that there is a higher amount of total dissolved solids in Anopheline dominated larval sites. This may be due to the higher usage of inorganic fertilizers on the nearby farms (Siddiqua *et al*., 2022).

Anopheles funestus larvae were numerous compared to non-*Anopheles funestus* larvae in larval habitats with higher pH values. This finding disagrees with Marrelli *et al*. (2021) who reported that non-Anopheline species such as *Culex* may thrive in various pH including alkaline environment. This difference in results can be explained by the fact that alkaline environment may favor the growth of larvae while lowering hatching rates while acidic larval environment may reduce the growth and survival of species at their larval stages but enhances the rate at which the eggs hatch (Oyewole *et al*., 2009).

Anopheles funestus larvae were many where Sulphate, COD and BOD levels were lower compared to the non-*Anopheles funestus* larvae. These findings are similar to those of Oyewole *et al*. (2009) who found out that sulphate negatively affects anopheline larval growth and survival. The effect of sulphate may be due to fertilizer use which reduce the turbidity of the larval water thus making it suitable for breeding by culicine mosquitoes (Mbanzulu *et al*., 2022).

4.2 Effects of larval habitat parameters on *Anopheles funestus* and non-*Anopheles funestus* larvae presence in preferred and non-preferred larval sites.

Stagnant water and water movement with slow flow reported a higher number of *Anopheles funestus* larvae compared to non- *Anopheles funestus* larvae. Similar findings were seen in Nambunga *et al*. (2020) who reported that *Anopheles funestus* larvae were more abundant in larval water with slow and stagnant flow as compared to non-*Anopheles funestus* larvae. This may be due to high levels of aerations because of dissolved oxygen in aquatic habitats making them preferable to *Anopheles funestus* (David *et al*., 2021).

Larval sites that had no habitat canopy and those that had a scarce canopy reported high numbers those of

Table 4: Correlation matrix between the Number of *Anopheles funestus* and non-*Anopheles funestus* larvae and the physicochemical and habitat parameters in Fihoni Kwale County.

	WB	WM	HC	DH	WD	WT	VT	WC	VQ	AlQ	HS	MLN	Min	Max	SI Units	
															SI Units	Mean ± Std
Cond	-0.779 (<.0001)	-0.531 (0.019)	-0.403 (0.087)	-0.573 (0.010)	0.069 (0.780)	-0.062 (0.800)	0.094 (0.701)	0.025 (0.919)	-0.246 (0.311)	0.100 (0.685)	-0.179 (0.463)	-0.822 (<.0001)	276	496	S/m	404.8 ±82.1
Temp	-0.433 (0.064)	-0.029 (0.907)	-0.363 (0.127)	-0.337 (0.158)	-0.183 (0.452)	-0.364 (0.125)	-0.371 (0.118)	-0.537 (0.018)	-0.367 (0.122)	-0.218 (0.358)	-0.224 (0.253)	-0.276			°C	30.6 ±36.5
TDS	-0.619	-0.262 (0.215)	-0.298	-0.397 (0.488)	0.170	-0.076 (0.757)	0.217 (0.373)	0.017 (0.946)	-0.254 (0.295)	0.262 (0.278)	0.024 (0.923)	-0.734 (0.000)	138	254	ppm	209.9 ±36.5
pH	-0.829 (0.005)	-0.311 (0.279)	-0.405 (0.092)	0.090 (0.488)	-0.123 (0.757)	0.158 (0.373)	-0.053 (0.946)	-0.254 (0.295)	-0.064 (0.923)	0.211 (0.278)	-0.064 (0.923)	-0.877 (<.0001)	6.31	7.74	-	6.9 ±0.51
SO₄	0.857 (<.0001)	0.295 (0.196)	0.342 (0.086)	-0.104 (0.714)	0.122 (0.616)	-0.276 (0.518)	-0.013 (0.828)	0.322 (0.293)	-0.317 (0.387)	0.039 (0.794)	0.900 (<.0001)	0.900 (<.0001)	1	11.3	Mg/L	5.9 ±5.7
COD	0.858 (<.0001)	0.297 (0.220)	0.343 (0.152)	-0.103 (0.672)	0.122 (0.618)	-0.275 (0.252)	-0.013 (0.958)	0.323 (0.179)	-0.316 (0.186)	0.041 (0.875)	0.900 (<.0001)	0.900 (<.0001)	803.2	843.4	Mg/L	20.6
COD	0.858 (<.0001)	0.297 (0.216)	0.343 (0.151)	-0.103 (0.674)	0.122 (0.620)	-0.275 (0.254)	-0.013 (0.957)	0.323 (0.178)	-0.316 (0.188)	0.041 (0.868)	0.900 (<.0001)	0.900 (<.0001)	803.2	843.4	Mg/L	20.6
BOD	0.858 (<.0001)	0.297 (0.216)	0.343 (0.151)	-0.103 (0.674)	0.122 (0.620)	-0.275 (0.254)	-0.013 (0.957)	0.323 (0.178)	-0.316 (0.188)	0.041 (0.868)	0.900 (<.0001)	0.900 (<.0001)	340.2	367.2	Mg/L	354.4 ±3.9

where WB = Water body, WM = Water movement, HTC = Habitat Canopy, DHM = Distance from homestead, WD = Water depth, WT = Water type, VT = Vegetation type, WC= Water color, VQ = Vegetation quantity, AlQ= Algae quantity, HS = Habitat Size, ML = Mosquito larvae, Max = Maximum, Min= Minimum, BOD = Biological oxygen demand, COD = chemical oxygen demand, TDS = Total dissolved solids, Temp = Temperature, cond = Conductivity, SI Units= International Systems Units, S/m=Siemens per metre, °C=Degree Celsius, ppm=parts per million, -= no units, mg/L= milligrams per Litre, Values in parenthesis are *p*-values, Negative values shows a negative correlation, Analysis was done using Statistical Analytical System (SAS) version 9.4

Anopheles funestus compared to the non-*Anopheles funestus* larvae. These findings differ from Debrah *et al*. (2021) who indicated that *Anopheles funestus* prefers larval habitats covered with a thicker vegetation. The differences in results may be due to the role of aquatic macrophytes which plays a role in survival and larval development (Nambunga *et al*., 2020).

Habitats that are less than 50 m from homesteads within the study sites had higher numbers of *Anopheles funestus* larvae compared to non-*Anopheles funestus* larvae. Similar findings were reported in Mwingira *et al*. (2020); Schoelitsz *et al*. (2020) who indicated that homesteads near larval habitats have a higher number of *Anopheles* mosquitoes. This may be because Anopheline mosquitoes have a higher demand for blood meal from humans in their adult stage *(Nambunga et al*., 2020).

Larval sites that had a water depth of less than 50 cm showed higher number of *Anopheles funestus* compared to non-*Anopheles funestus*. The findings contradict the findings of Nambunga *et al*. (2020) who found out that *Anopheles funestus* prefers larval habitats with more than 50 cm of depth. This can be explained by the fact that *Anopheles* species lack a siphon to breathe under the water and hence may need less deeper water bodies (Ondiba *et al*., 2019). *Anopheles funestus* were also higher in water depth more than 100 cm as compared to non-*Anopheles funestus* larvae. This finding concurs with Kahamba *et al*. (2022) who found out that the survival of *Anopheles funestus* depends on several climatic factors. This may be because *Anopheles funestus* tends to avoid adverse climatic factors like direct sunlight, predation and crowding which affects the rate of their larval development (Debrah *et al*., 2021b).

There were more *Anopheles funestus* larvae in larval sites with semi-permanent stream compared to the non-*Anopheles funestus* larvae. A different result was reported by Imbahale *et al*. (2011), who suggested that *Anopheles* larvae could be found in permanent water bodies. The difference in results could be explained by the fact that in the permanent streams, the water flow lasts longer during which they are colonized by culicine larvae unlike the seasonal water bodies which dry up faster (Omolade, 2018).

More *Anopheles funestus* larvae were present in larval sites with clear color as compared to non-*Anopheles funestus* larvae. These findings are in line with Nambunga *et al*. (2020) who showed that *Anopheles funestus* breeds in clear larval habitats. This could be because *Anopheles* species prefers clear larval water where eggs laid can float freely horizontally (Kahamba *et al*., 2022).

Larval habitats with a water body size of less than 10 m showed a higher population of *Anopheles funestus* larvae than non-*Anopheles funestus* larvae. Different findings were reported in Imbahale *et al*. (2011) who hinted that water bodies with less than 10 m size have more Culicine species. That may be attributed to the ability of non-*Anoph-*

eles funestus to thrive faster under such conditions due to higher temperatures (Carlson *et al*., 2009). Larval sites that were between 10 m and 100 m had lower numbers of *Anopheles funestus* larvae as compared to non-Anopheline larvae. The findings disagree with Keating *et al*. (2004) who found that Anopheline may prefer smaller habitats. These difference in results may be due to possible crowding of larvae and competition of available resources in the larval habitats (Akeju *et al*., 2022b).

4.3 Correlation between the number of *Anopheles funestus* larvae and, physicochemical and habitat parameters

The number of *Anopheles funestus* and non-*Anopheles funestus* larvae is negatively correlated with the conductivity and temperature of water in the larval habitat. Different results were reported by Akeju *et al*. (2022b) who found that electrical conductivity significantly correlates positively with *Anopheles funestus* larvae. The differences in results may be due to the role of temperature in mediating mosquito physiology and metabolic rate since metabolic rates would increase exponentially with an increase in temperature within exothermic animals (Akeju *et al*., 2022b). Total dissolved solids and pH are weakly correlated with water depth. This finding conforms to Subba Rao (2008) who reported that total dissolved solids decrease with water depth. This may be due to human activities like long-term irrigation and the application of agricultural fertilizers which increases salinity in the top surface water (Akeju *et al*., 2022b).

Level of sulphates, COD and BOD strongly correlated with water depth and habitat distance from homesteads, water movement, habitat canopy, water type, vegetation quantity and habitat size. This finding conforms to Nambunga *et al*. (2020), who reported that *Anopheles funestus* larvae correlates with vegetation cover. This may be due to the vegetation's role in the larvae's ecology. This finding agrees with Tang *et al*. (2019), who reported that COD has a correlation with the distance from homesteads. Probably, this is due to human dwellings' proximity to the rivers which leads to degradations resulting from biological changes and pollution (Abba & Elkiran, 2017). The BOD correlates strongly with distance from homesteads. Similar findings were reported by Siddiqua *et al*. (2022), who explains how closeness to human dwellings may lead to deposition of organic materials which increases BOD. This may be due to the deposition of organic materials in the water, which leads to odor and color, which are associated with higher BOD (Nyandwaro, 2017; Ferronato & Torretta, 2019).

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References

Abba, S. I., & Elkiran, G. (2017). Effluent prediction of chemical oxygen demand from the wastewater treatment plant using artificial neural network application. *Procedia Computer Science*, *120*, 156–163. https://doi.org/10.1016/j.procs.2017.11.223

Akeju, A. V., Olusi, T. A., & Simon-Oke, I. A. (2022a). Effect of physicochemical parameters on *Anopheles* mosquitoes larval composition in Akure North Local Government area of Ondo State, Nigeria. *The Journal of Basic and Applied Zoology*, *83*(1). https://doi.org/10.1186/s41936-022-00298-3

Akeju, A. V., Olusi, T. A., & Simon-Oke, I. A. (2022b). Effect of physicochemical parameters on *Anopheles* mosquitoes larval composition in Akure North Local Government area of Ondo State, Nigeria. *The Journal of Basic and Applied Zoology*, *83*(1). https://doi.org/10.1186/s41936-022-00298-3

Carlson, J. C., Dyer, L. A., Omlin, F. X., & Beier, J. C. (2009). Diversity Cascades and malaria vectors. *Journal of Medical Entomology*, *46*(3), 460–464. https://doi.org/10.1603/033.046.0307

Christiansen-Jucht, C., Parham, P. E., Saddler, A., Koella, J. C., & Basáñez, M. G. (2014). Temperature during larval development and adult maintenance influences the survival of *Anopheles gambiae* s.s. *Parasites and Vectors*, *7*(1). https://doi.org/10.1186/s13071-014-0489-3

David, M. R., Dantas, E. S., Maciel-de-Freitas, R., Codeço, C. T., Prast, A. E., & Lourenço-de-Oliveira, R. (2021). Influence of Larval Habitat Environmental Characteristics on Culicidae Immature Abundance and Body Size of Adult *Aedes aegypti*. *Frontiers in Ecology and Evolution*, *9*. https://doi.org/10.3389/fevo.2021.626757

de Almeida, N. C. V., Louzada, J., Neves, M. S. A. S., Carvalho, T. M., Castro-Alves, J., Silva-do-Nascimento, T. F., Escalante, A. A., & Oliveira-Ferreira, J. (2022). Larval habitats, species composition and distribution of malaria vectors in regions with autochthonous and imported malaria in Roraima state, Brazil. *Malaria Journal*, *21*(1), 1–16. https://doi.org/10.1186/s12936-021-04033-1

Debrah, I., Afrane, Y. A., Amoah, L. E., Ochwedo, K. O., Mukabana, W. R., Zhong, D., Zhou, G., Lee, M. C., Onyango, S. A., Magomere, E. O., Atieli, H., Githeko, A. K., & Yan, G. (2021a). Larval ecology and bionomics of *Anopheles funestus* in highland and lowland sites in western Kenya. In *PLoS ONE* (Vol. 16, Issue 10 October). Public Library of Science. https://doi.org/10.1371/journal.pone.0255321

Debrah, I., Afrane, Y. A., Amoah, L. E., Ochwedo, K. O., Mukabana, W. R., Zhong, D., Zhou, G., Lee, M. C., Onyango, S. A., Magomere, E. O., Atieli, H., Githeko, A. K., & Yan, G. (2021b). Larval ecology and bionomics of *Anopheles funestus* in highland and lowland sites in western Kenya. *PLoS ONE*, *16*(10 October), 1–20. https://doi.org/10.1371/journal.pone.0255321

Emidi, B., Kisinza, W. N., Mmbando, B. P., Malima, R., & Mosha, F. W. (2017). Effect of physicochemical parameters on *Anopheles* and *Culex* mosquito larvae abundance in different breeding sites in a rural setting of Muheza, Tanzania. *Parasites and Vectors*, *10*(1), 1–13. https://doi.org/10.1186/s13071-017-2238-x

Fazeli-Dinan, M., Azarnoosh, M., Özgökçe, M. S., Chi, H., Hosseini-Vasoukolaei, N., Haghi, F. M., Zazouli, M. A., Nikookar, S. H., Dehbandi, R., Enayati, A., Zaim, M., & Hemingway, J. (2022). Global water quality changes posing threat of increasing infectious diseases, a case study on malaria vector *Anopheles stephensi* coping with the water pollutants using age-stage, two-sex life table method. *Malaria Journal*, *21*(1). https://doi.org/10.1186/s12936-022-04201-x

Ferronato, N., & Torretta, V. (2019). Waste mismanagement in developing countries: A review of global issues. In *International Journal of Environmental Research and Public Health* (Vol. 16, Issue 6). MDPI AG. https://doi.org/10.3390/ijerph16061060

Getachew, D., Balkew, M., & Tekie, H. (2020a). *Anopheles* larval species composition and characterization of breeding habitats in two localities in the Ghibe River Basin, southwestern Ethiopia. *Malaria Journal*, *19*(1), 1–13. https://doi.org/10.1186/s12936-020-3145-8

Getachew, D., Balkew, M., & Tekie, H. (2020b). *Anopheles* larval species composition and characterization of breeding habitats in two localities in the Ghibe River Basin, southwestern Ethiopia. *Malaria Journal*, *19*(1). https://doi.org/10.1186/s12936-020-3145-8

Gillies, M. and M. C. (1987). A supplement to the Anophelinae of Africa South of the Sahara. *South African Institute for Medical Research*, *55*. http://www.metoffice.gov.uk/public/weather/climate/u1214qgj0

Hessou-Djossou, D., Djègbè, I., Ahadji-Dabla, K. M., Nonfodji, O. M., Tchigossou, G., Djouaka, R., Cornelie, S., Djogbenou, L., Akogbeto, M., & Chandre, F. (2022). Diversity of larval habitats of *Anopheles* mosquitoes in urban areas of Benin and influence of their physicochemical and bacteriological characteristics on larval density. *Parasites and Vectors*, *15*(1), 1–17. https://doi.org/10.1186/s13071-022-05323-6

Imbahale, S. S., Paaijmans, K. P., Mukabana, W. R., Van Lammeren, R., Githeko, A. K., & Takken, W. (2011). A longitudinal study on *Anopheles* mosquito larval abundance in distinct geographical and environmental settings in western Kenya. *Malaria Journal*, *10*, 1–13. https://doi.org/10.1186/1475-2875-10-81

Kahamba, N. F., Finda, M., Ngowo, H. S., Msugupakulya, B. J., Baldini, F., Koekemoer, L. L., Ferguson, H. M., & Okumu, F. O. (2022). Using ecological observations to improve malaria control in areas where *Anopheles funestus* is the dominant vector. *Malaria Journal*, *21*(1), 1–15. https://doi.org/10.1186/s12936-022-04198-3

Kamau, A., Mtanje, G., Mataza, C., Mwambingu, G., Mturi, N., Mohammed, S., Ong'ayo, G., Nyutu, G., Nyaguara, A., Bejon, P., & Snow, R. W. (2020). Malaria infection, disease and mortality among children and adults on the coast of Kenya. *Malaria Journal*, *19*(1). https://doi.org/10.1186/s12936-020-03286-6

Keating, J., Macintyre, K., Mbogo, C. M., Githure, J. I., & Beier, J. C. (2004). Characterization of potential larval habitats for *Anopheles* mosquitoes in relation to urban land-use in Melindi, Kenya. *International Journal of Health Geographics*, *3*, 1–13. https://doi.org/10.1186/1476-072X-3-9

Maina, D. M., Ndirangu, D. M., Mangala, M. M., Boman, J., Shepherd, K., & Gatari, M. J. (2016). Environmental implications of high metal content in soils of a titanium mining zone in Kenya. *Environmental Science and Pollution Research*, *23*(21), 21431–21440. https://doi.org/10.1007/s11356-016-7249-1

Marrelli, M., Vatandoost, H., Ralph Medeiros De Sousa, A., Wang, H., Wang, Y., Cheng, P., Wang, H., Wang, H., Liu, H., Zhang, C., & Gong, M. (2021). The Larval Density of Mosquitos (Diptera: Culicidae) in Jiaxiang County, Shandong Province, China: Influence of Bacterial Diversity, Richness, and Physicochemical Factors. *Front. Ecol. Evol*, *9*, 616769. https://doi.org/10.3389/fevo.2021.616769

Mbanzulu, K. M., Mboera, L. E. G., Wumba, R., Engbu, D., Bojabwa, M. M., Zanga, J., Mitashi, P. M., Misinzo, G., & Kimera, S. I. (2022). Physicochemical Characteristics of *Aedes* Mosquito Breeding Habitats in Suburban and Urban Areas of Kinshasa, Democratic Republic of the Congo. *Frontiers in Tropical Diseases*, *2*(January), 1–9. https://doi.org/10.3389/fitd.2021.789273

McCord, G. C. (2016). Malaria ecology and climate change. *European Physical Journal: Special Topics*, *225*(3), 459–470. https://doi.org/10.1140/epjst/e2015-50097-1

Mereta, S. T., Yewhalaw, D., Boets, P., Ahmed, A., Duchateau, L., Speybroeck, N., Vanwambeke, S. O., Legesse, W., De Meester, L., & Goethals, P. L. (2013). Physico-Chemical and biological characterization of anopheline mosquito larval habitats Diptera: Culicidae: Implications for malaria control. *Parasites and Vectors*, *6*(1), 1–16. https://doi.org/10.1186/1756-3305-6-320

Meza, F. C., Muyaga, L. L., Limwagu, A. J., & Lwetoijera, D. W. (2022). The ability of *Anopheles funestus* and *A. arabiensis* to penetrate LLINs and its effect on their mortality. *Wellcome Open Research*, *7*, 265. https://doi.org/10.12688/wellcomeopenres.18242.1

Mugenzi, L. M. J., Akosah-Brempong, G., Tchouakui, M., Menze, B. D., Tekoh, T. A., Tchoupo, M., Nkemngo, F. N., Wondji, M. J., Nwaefuna, E. K., Osae, M., & Wondji, C. S. (2022). Escalating pyrethroid resistance in two major malaria vectors *Anopheles funestus* and *Anopheles gambiae* (s.l.) in Atatam, Southern Ghana. *BMC Infectious Diseases*, *22*(1). https://doi.org/10.1186/s12879-022-07795-4

Mwema, T., Lukubwe, O., Joseph, R., Maliti, D., Iitula, I., Katokele, S., Uusiku, P., Walusimbi, D., Ogoma, S. B., Tambo, M., Gueye, C. S., Williams, Y. A., Vajda, E., Tatarsky, A., Eiseb, S. J., Mumbengegwi, D. R., & Lobo, N. F. (2022). Human and vector behaviors determine exposure to *Anopheles* in Namibia. *Parasites and Vectors*, *15*(1). https://doi.org/10.1186/s13071-022-05563-6

Mwingira, V., Mboera, L. E. G., Dicke, M., & Takken, W. (2020). Exploiting the chemical ecology of mosquito oviposition behavior in mosquito surveillance and control: a review. *Journal of Vector Ecology*, *45*(2), 155–179. https://doi.org/10.1111/jvec.12387

Nambunga, I. H., Ngowo, H. S., Mapua, S. A., Hape, E. E., Msugupakulya, B. J., Msaky, D. S., Mhumbira, N. T., McHwembo, K. R., Tamayamali, G. Z., Mlembe, S. V., Njalambaha, R. M., Lwetoijera, D. W., Finda, M. F., Govella, N. J., Matoke-Muhia, D., Kaindoa, E. W., & Okumu, F. O. (2020). Aquatic habitats of the malaria vector *Anopheles funestus* in rural south-eastern Tanzania. *Malaria Journal*, *19*(1), 1–11. https://doi.org/10.1186/s12936-020-03295-5

Nikookar, S. H., Fazeli-Dinan, M., Azari-Hamidian, S., Mousavinasab, S. N., Aarabi, M., Ziapour, P., Esfandyari, Y., & Enayati, A. (2017a). Correlation between mosquito larval density and their habitat physicochemical characteristics in Mazandaran Province, northern Iran. https://doi.org/10.1371/journal.pntd.0005835

Nikookar, S. H., Fazeli-Dinan, M., Azari-Hamidian, S., Mousavinasab, S. N., Aarabi, M., Ziapour, S. P., Esfandyari, Y., & Enayati, A. (2017b). Correlation between mosquito larval density and their habitat physicochemical characteristics in Mazandaran Province, northern Iran. *PLoS Neglected Tropical Diseases*, *11*(8). https://doi.org/10.1371/journal.pntd.0005835

Njoroge, S., Muigai, A. W. T., Njiruh, P. N., & Kariuki, S. (2013). Molecular characterisation and antimicrobial resistance patterns of *Escherichia coli* isolates from goats slaughtered in parts of Kenya. *East African Medical Journal*, *90*(3), 72–83.

Nyandwaro, E. O. (2017). The Impacts of Solid Waste on Ground and Surface Water Quality in Kisii Municipality, Kenya.

Omolade, O. O. (2018). Oviposition and Breeding Water Sites Preferences of Mosquitoes within Ojo area, Lagos State, Nigeria. *Biomedical Journal of Scientific & Technical Research*, *7*(5), 6139–6145. https://doi.org/10.26717/bjstr.2018.07.001565

Ondiba, I. M., Oyieke, F. A., Athinya, D. K., Nyamongo, I. K., & Estambale, B. B. A. (2019). Larval species diversity, seasonal occurrence and larval habitat preference of mosquitoes transmitting Rift Valley fever and malaria in Baringo County, Kenya. *Parasites and Vectors*, *12*(1), 1–14. https://doi.org/10.1186/s13071-019-3557-x

Oyewole, I. O., Momoh, O. O., Anyasor, G. N., Ogunnowo, A. A., Ibidapo, C. A., Oduola, O. A., Obansa, J. B., & Awolola, T. S. (2009). Physico-chemical characteristics of *Anopheles* breeding sites: Impact on fecundity and progeny development. In *African Journal of Environmental Science and Technology* (Vol. 3, Issue 12). http://www.academicjournals.org/AJEST

Schoelitsz, B., Mwingira, V., Mboera, L. E. G., Beijleveld, H., Koenraadt, C. J. M., Spitzen, J., van Loon, J. J. A., & Takken, W. (2020). Chemical Mediation of Oviposition by *Anopheles* Mosquitoes: a Push-Pull System Driven by Volatiles Associated with Larval Stages. *Journal of Chemical Ecology*, *46*(4), 397–409. https://doi.org/10.1007/s10886-020-01175-5

Siddiqua, A., Hahladakis, J. N., & Al-Attiya, W. A. K. A. (2022). An overview of the environmental pollution and health effects associated with waste landfilling and open dumping. In *Environmental Science and Pollution Research* (Vol. 29, Issue 39, pp. 58514–58536). Springer Science and Business Media Deutschland GmbH. https://doi.org/10.1007/s11356-022-21578-z

Siwiendrayanti, A., Anggroro, S., & Nurjazuli. (2021). Characteristic differences between breeding places with and without *Culex* sp. larvae on lymphatic filariasis patient surroundings in an endemic area. *IOP Conference Series: Earth and Environmental Science*, *896*(1). https://doi.org/10.1088/1755-1315/896/1/012079

Soleimani-Ahmadi, M., Vatandoost, H., & Zare, M. (2014). Characterization of larval habitats for anopheline mosquitoes in a malarious area under elimination program in the southeast of Iran. *Asian Pacific Journal of Tropical Biomedicine*, *4*(Suppl 1), S73–S80. https://doi.org/10.12980/APJTB.4.2014C899

Subba Rao, N. (2008). Factors controlling the salinity in groundwater in parts of Guntur district, Andhra Pradesh, India. *Environmental Monitoring and Assessment*, *138*(1–3), 327–341. https://doi.org/10.1007/S10661-007-9801-4

Tang, L., Pan, X., Feng, J., Pu, X., Liang, R., Li, R., & Li, K. (2019). Experimental investigation on the relationship between COD degradation and hydrodynamic conditions in urban rivers. *International Journal of Environmental Research and Public Health*, *16*(18). https://doi.org/10.3390/ijerph16183447

Tchigossou, G., Akoton, R., Yessoufou, A., Djegbe, I., Zeukeng, F., Atoyebi, S. M., Tossou, E., Moutairou, K., & Djouaka, R. (2017). Water source most suitable for rearing a sensitive malaria vector, *Anopheles funestus* in the laboratory [version 1; referees: 2 approved]. *Wellcome Open Research*, *2*(0), 1–16. https://doi.org/10.12688/wellcomeopenres.12942.1

Yokoly, F. N., Zahouli, J. B. Z., Small, G., Ouattara, A. F., Opoku, M., de Souza, D. K., & Koudou, B. G. (2021). Assessing *Anopheles* vector species diversity and transmission of malaria in four health districts along the borders of Côte d'Ivoire. *Malaria Journal*, *20*(1). https://doi.org/10.1186/s12936-021-03938-1