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# Identification and characterization of *Anopheles* Breeding habitats in Dabakala, Central-East Côte d'Ivoire

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### Abstract

Malaria prevention in Côte d'Ivoire is mainly based on the use of long-lasting insecticide-treated nets. Faced with the resistance of vectors to insecticides, the use of anti-larval measures proves to be of interest. This study conducted in the Dabakala health district aims to identify *Anopheles* breeding sites and their physicochemical characteristics influencing the productivity of larvae. Surveys were carried out in the urban and rural areas during the rainy season (July 2020) and the dry season (February 2021). Out of 102 potential breeding sites listed, a total of 53 were positive, including 38 sites of *Anopheles* and 15 of other *Culicinae*. In both urban and rural areas, most *Anopheles* breeding sites were observed during the rainy season, while the density of *Anopheles* larvae was higher in the dry season. These highest densities were observed, in rural areas in rice fields essentially, and in the urban regions in temporary water impoundments, water wells, and rice fields. The temperature, pH, and depth of water were positively correlated with the density of *Anopheles* larvae. This study allowing the identification of favorable periods and sites of *Anopheles* breeding in the Dabakala district can help to design a strategy for the control of *Anopheles* larvae.

Keywords: Anopheles larvae, breeding sites, larval density, Dabakala, rural, urban.

## 1. Introduction

Malaria is a major cause of morbidity and mortality worldwide <sup>[1].</sup> In 2020, more than 600,000 malaria deaths were reported worldwide, 80% of them were children under 5 years of age <sup>[2]</sup>. Africa remains the most affected region <sup>[3, 4]</sup> and continues to bear the highest burden of global malaria burden, accounting for 96% of all malaria deaths <sup>[1]</sup>. In Côte d'Ivoire, malaria is a major public health problem, with 4,725,162 confirmed cases in 2020 and children under 5 years of age accounting for 2,145,870 cases <sup>[5]</sup>. Transmission of the malaria parasite is ensured by three main vectors belonging to species complexes and groups: *An. gambiae s.s., An. funestus s.s.,* and *An. nili s.s.* <sup>[6, 7, 8]</sup>. They have variable biting and living behaviors <sup>[9]</sup>. The abundance and distribution of these vectors are linked to the presence and productivity of suitable breeding sites <sup>[10]</sup>. Species of the *Anopheles gambiae* complex preferentially colonize shallow, sunny, clear, and clean water bodies <sup>[10]</sup>. In contrast, *An. funestus* proliferates in shaded sites with aquatic plants <sup>[11]</sup> and *An. nili* prefers calm coves of rivers and forest streams with shaded edges <sup>[12]</sup>. The physicochemical characteristics of the sites can influence the choice of oviposition site, survival, and abundance of malaria vectors <sup>[13, 14]</sup>. These conditions can affect locally the epidemiology of malaria <sup>[11]</sup>.

The main control method to reduce the burden of malaria in Côte d'Ivoire is the use of Long-Lasting Insecticidal Nets (LLINs)<sup>[15]</sup>. However, the development of resistance to insecticides and the increase of the exophagous behavior of mosquitoes underline the interest in complementary control measures such as anti-larval measures <sup>[16,17]]</sup> as recently recommended by WHO to reduce malaria transmission <sup>[1]</sup>. However, the effective implementation of any anti larval control action requires knowledge of *Anopheles* breeding sites <sup>[18]</sup>. This study was conducted in that context, in one of the four districts most affected by malaria, the health district of Dabakala, selected within the framework of the PMI/Vector-Link project in Côte d'Ivoire for the surveillance of malaria vector bionomics.

### 2. Materials and Methods

### 2.1 Study site

A descriptive cross-sectional survey was carried out in July 2020 and February 2021 in the Dabakala health district. Located in the center-east savannah zone of Côte d'Ivoire, 498 km from Abidjan, the Dabakala health district is the fourth district with a high incidence of malaria after Sakassou, Nassian, and Béoumi 2018<sup>[19]</sup>. In this district, two localities were selected: the city of Dabakala (8°21'48 "N; 4°25'43 "W)

and the village of N'Gala (8°21'36'04 "N; 4°21'51'33 "W) located 5 km from the town (Figure 1). The district's climate is Sudano-Guinean, characterized by two dry seasons and two rainy seasons  $^{[20]}$ . The average monthly rainfall was 102.31 mm, ranging from 22.15 mm in the dry season to 159.28 mm in the rainy season. The monthly average temperature was 28.49 °C with a maximum of 30 °C in the dry season and a minimum of 27.48 °C in the rainy season. The vegetation is dominated by wooded savannah with large trees in places.



Fig 1: Geographical location of the study sites

## 2.2 Larvae sampling

Mosquito breeding site surveys were conducted during the rainy season (July 2020) and the dry season (February 2021). They consisted of exploring all the water collections likely to harbor mosquito larvae within a 100 m radius. Breeding sites were categorized according to the presence or absence of larvae (negative/positive), their nature: temporary water impoundments, water wells, rice fields, natural breeding sites, containers, tires, abandoned objects, water storage construction material, and sanitation material. In addition, parameters such as depth, surface area, sunlight, water turbidity, vegetation around the site, and permanence of the site were determined. The surface and depth of the site were measured with a tape measure. The surface of the site was classified into three categories: i) small (< 1 m<sup>2</sup>); ii) medium  $(1-10 \text{ m}^2)$  and iii) large (> 10 m<sup>2</sup>). Similarly, three modalities were used for the water turbidity: i) clear when the bottom of the dipper was visible in the water; ii) turbid when the bottom of the dipper was barely visible, and iii) muddy when the bottom of the dipper was not visible. The permanence of the breeding site was assessed according to the length of time it contained water by interviewing residents. The breeding site is considered temporary if it contains water for three months and permanent if it contains water for more than three months. In addition, using a portable multi-parameter (HANNA HI 98129), physicochemical parameters of the water were measured in situ: temperature, pH, Total Dissolved Solids (TDS), and electrical conductivity.

# **2.3** Collection and determination of density of mosquito larvae and pupae

Larvae and pupae were collected with a dipper at positive sites for breeding using the method described by Taliopouo *et al.* <sup>[21]</sup>. and Coffinet *et al.* <sup>[22]</sup>. That method consisted of sampling water at the breeding site in several locations and without repetition. Depending on the amount of water the rate sampling can vary from 1 to 10 dipper strokes. Larvae and pupae were collected in labeled tubes and transported to the laboratory for density determination and rearing. Larvae of stages 3 and 4 were enumerated by genus and the density of the breeding sites was determined by the ratio of the number of larvae collected divided by the number of dipper strokes <sup>[24]</sup>.

### 2.4 Data analysis

Data were entered into an Excel 2013 spreadsheet and analyzed using STATA version 14.2 (Stata Corporation; College Station, TX, USA). The chi2 or Fischer exact test was used to compare the proportions of the sites. Multivariate logistic regression analysis was used to identify factors influencing breeding site positivity. Mean larval densities of sites were compared between seasons and locations using the Mann-Whitney test. A principal component analysis determined the relationship between physicochemical parameters and larval density.

### 3. Results

### 3.1 Nature and importance of mosquito breeding sites

A total of 102 potential breeding sites were identified during the study period. They are grouped into 9 categories and distributed in 74 (72.55%) and 28 (27.45%) urban and rural settings, respectively (Table 1). Temporary water impoundments and natural breeding sites represented each more than 20% of the breeding sites. Watering wells (n= 17) and rice fields (n=16) represented 16.67% and 15.69% of the sites respectively. Among the sites identified in urban areas, Temporary water impoundments (n=25; 33.78%) were dominant, followed by watering wells (n=17; 22.97%). However, in rural areas, abandoned objects (n=8; 28.57%) and rice fields were predominant (n=6; 21.43%).

Table 1: Nature and importance of potential breeding sites in urban and rural areas of Dabakala in July 2020 and February 2021

Notive of breading sites	Urban area	Rural area	Total
Nature of breeding sites	n (%)	n (%)	n (%)
Abandoned objects	0	8 (28.57)	8 (7.84)
Objects of sanitation	0	1 (3.57)	1 (0.98)
Objects of construction	1 (1.35)	0	1 (0.98)
Tires	2 (2.70)	1 (3.57)	3 (2.94)
Water wells	17 (22.97)	0	17 (16.67)
Water storage containers	1 (1.35)	3 (10.71)	4 (3.92)
Temporary water impoundments	25 (33.78)	5 (17.86)	30 (29.41)
Rice fields	10 (13.51)	6 (21.43)	16 (15.69)
Natural breeding sites	18 (24.32)	4 (14.29)	22 (21.57)
Total	74 (100)	28 (100)	102 (100)

Abandoned objects: cut can, abandoned bucket, abandoned mortar, plastic bag, water trough; objects of sanitation: septic tanks; objects of construction: electricity pole holes; Water storage containers: jar, barrel, abandoned well; temporary Water impoundments: puddles, animal footprints, water between the furrows of market gardens; Rice fields: rice field irrigation channel, rice paddy; natural breeding sites: ponds, streams, rock hollow

# **3.2** Nature and importance of *Anopheles* breeding sites identified in Dabakala

Overall, 53 positive sites were identified, of which 38 (71.30%) contained at least one *Anopheles* larva and 15 (28.30%) *Culicinae* larvae. In general, *Anopheles* larvae were found more in temporary water impoundments and rice fields in proportions of 36.84% (n=14) each. In urban areas,

Anopheles breeding sites constituted 73.68% (n=28) against 26.32% (n=10) in rural areas. Anopheles larvae colonized more temporary water impoundments 42.86% (n=12) in urban areas, followed by rice fields 32.14% (n=9). In contrast, in rural areas, rice fields were colonised 50% (n=5), followed by temporary water impoundments 20% (n=2) and abandoned objects 20% (n=2) (Table 2).

**Table 2:** Number and proportion of positive breeding sites according to their nature in an urban and rural area at Dabakala in July 2020 and February 2021.

	Ur	bain	Rural		Rural Total	
Nature of the breeding site	Positive breeding sites	Anopheles breeding sites	Positive breeding sites	Anopheles breeding sites	Positive breeding sites	Anopheles breeding sites
	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)
Abandoned objects	0 (0)	0 (0)	5 (33.33)	2 (20)	5 (9.43)	2 (5.26)
Objects of sanitation	0 (0)	0 (0)	1 (6.67)	1(10)	1(1.89)	1 (2,63)
Objects of construction	1 (2.63)	0 (0)	0 (0)	0 (0)	1(1.89)	0 (0)
Tires	1 (2.63)	0 (0)	1 (6.67)	0 (0)	2 (3.77)	0 (0)
Water wells	7(18.42)	5 (17.86)	0 (0)	0 (0)	7(13.21)	5 (13.16)
Water storage containers	1 (2.63)	0 (0)	0 (0)	0 (0)	1 (1.89)	0 (0)
Temporary water impoundments	13 (34.21)	12 (42.86)	2 (13.33)	2 (20)	15 (28.30)	14 (36.84)
Rice fields	9 (23.68)	9 (32.14)	5(33.33)	5 (50)	14 (26.42)	14 (36.84)
Natural breeding sites	6 (15.79)	2 (7.14)	1 (6.67)	0 (0)	7 (7.55)	2 (5.26)
Total	38 (100)	28 (100)	15 (100)	10 (100)	53 (100)	38 (100)

Abandoned objects: cut can, abandoned bucket, abandoned mortar, plastic bag, water trough; Objects of sanitation: septic tanks; Objects of construction: electricity pole holes; water Water storage containers: jar, barrel, abandoned well; Temporary Water impoundments: puddles, animal footprints, water between the furrows of market gardens; Rice fields: rice field irrigation channel, rice paddy; natural breeding sites: ponds, streams, rock hollow

# **3.3** Nature and importance of the *Anopheles* breeding sites identified according to the seasons and the urban and rural areas in Dabakala

During the survey, most *Anopheles* breeding sites were identified in the rainy season. These sites were 0.5 and 0.89 times higher in the rainy season than in the dry season in urban and rural areas respectively. In the urban area, rice fields (OR = 72; CI= 5.70 - 908.90) and temporary water impoundments (OR = 7.38; CI= 1.40 - 39.08) were positively

associated with the presence of *Anopheles* larvae. They were respectively 71 and 6.38 times more associated with the presence of *Anopheles* larvae than natural breeding sites and abandoned objects. In rural areas, the presence of *Anopheles* larvae was positively associated with rice fields (OR = 15; CI= 1.03 - 218.30), which were 14 times more associated with the presence of *Anopheles* larvae than abandoned objects (Table 3).

Table 3: Nature and importance of the Anopheles breeding sites identified according to the seasons and the urban and rural areas in Dabakala

Area	Characteristics of the breeding sites	Anopheles breeding sites	OR	IC à 95%	Р
		Season			
	Dry	10 (35.71)	1		
	Rainy	18 (64.29)	1.5	(0.575 - 3.97)	0.402
Urbon	N٤	ature of the breeding site			
UIUali	Natural breeding sites	2 (7.14)	1		
	Water wells	5 (17.86)	3.33	(0,55 - 20,22)	0.19
	Rice fields	9 (32.14)	72	(5.70 - 908.90)	0.001
	Temporary water impoundments	12 (42.88)	7.38	(1.40 - 39.08)	0.019
		Season			
	Dry	1 (10)	1		
	Rainy	9 (90)	1.89	(0.11 – 33.89)	0.666
Durol	Na	ature of the breeding site			•
Kulai	Abandoned objects	1 (10)	1		
-	Rice fields	5 (50)	15	(1.03 - 218.30)	0.047
	Temporary water impoundments	2(20)	2	(0.18 – 22.06)	0.571
	Objects of construction	1 (10)			

Abandoned objects: cut can, abandoned bucket, abandoned mortar, plastic bag, water trough; objects of construction: electricity pole holes; water storage containers: jar, barrel, abandoned well; temporary water impoundments: puddles, animal footprints, water between the furrows of market gardens; Rice fields: rice field irrigation channel, rice paddy; Natural breeding sites: ponds, streams, rock hollow

# **3.4** *Anopheles* density according to the nature of the site and the seasons

The variation in mean larval density between seasons is presented in Table 4. Mean larval densities were significantly higher ( $\chi^2 = 4.861$ ; p = 0.027) during the dry season (3.23 larvae/dipper) compared to the rainy season (1.28 larvae/dipper). In urban areas, mean larval densities were estimated at 3.22 larvae/dipper in the dry season compared to 1.59 larvae/dipper in the rainy season ( $\chi^2 = 1.389$ ; p = 0.238).

During the dry season, in the area urban, the highest larval density with more than 3 larvae/layer was observed in each of the breeding sites constituted of the temporary water impoundments, water wells, and rice fields. In rural areas, the average larval densities recorded in the dry and rainy seasons were 3.3 and 0.67 larvae/dipper respectively ( $\chi^2 = 1.939$ ; p = 0.164). Only rice fields showed a high larval density with 3.3 larvae/dipper in the dry season.

Table 4: Larval density of Anopheles according to the breedings sites' nature and the seasons in Dabakala in July 2020 and February 2021

	Noture of the breeding site	Larval density (larvae / dipper)		
	Nature of the breeding site	Rainy Season	Dry Season	
	Water wells	0	3.18±4.36	
Urban	Temporary water impoundments	1.97± 3.74	3.3±5.13	
	Rice fields	1.25±0.87	3,1	
	Natural breeding sites	0.9	0	
	Total 1	1.59 ±2.65	3.22± 4.15	
	Abandoned objects	$0.4 \pm 0.28$	0	
Durol	Objects of sanitation	0,2	0	
Kulai	Temporary water impoundments	0.25 ± 0.21	0	
	Rice fields	$1.15 \pm 1.43$	3.3	
	Total 2	0.67 ±0.99	3.3	
	Total	$1.28 \pm 2.26$	3.23± 3.94	

Abandoned objects: cut can, abandoned bucket, abandoned mortar, plastic bag, water trough; Objects of sanitation: septic tanks; objects of construction: electricity pole holes; storage containers: jar, barrel, abandoned well; Temporary water impoundments: puddles, animal footprints, water between the furrows of market gardens; Rice fields: rice field irrigation channel, rice paddy; Natural breeding sites: ponds, streams, rock hollow

# **3.5** Determination of physicochemical parameters of *Anopheles* larval sites

The physicochemical parameters of the larval habitats studied are reported in Table 5. The pH of the breeding sites was slightly basic with a mean value of 7.71. It varied from 7.41 in the dry season to 7.83 during the rainy season with a significant difference between the seasons ( $\chi^2 = 3.982$ ; p=

0.045). The mean values of electrical conductivity, temperature, TDS, and depth of the breeding sites recorded in the rainy season were  $520.96\mu$ S/cm,  $30.09^{\circ}$ C, 276.85mg/l, and 0.16m against  $568.63 \mu$ S/cm,  $30.11^{\circ}$ C, 283.91mg/l and 0.27m in the dry season respectively. These values show no significant difference between seasons (P > 0.05).

 Table 5: Physicochemical parameters of Anopheles breeding sites according to seasons in Dabakala in July 2020 and February 2021

Demonstern	Sea	son	Total amount and	2	р
Parameters	Rainy	Dry	i otal averages	<i>x</i> -	
Temperature (°C)	30.09 ±30,04	30.11±5,12	30.10±3.69	0.084	0.772
рН	7.83±0,61	7.41±0,52	7.71±0.61	3.982	0.045*
Electrical conductivity (µS/cm)	520.96±655,44	568.63±258.03	534.76±566	3,249	0.07
TDS (mg/l)	276.85±346.22	283.91±126.71	281.02±297.62	2.855	0.091
Depth	0.16±0.26	0.27±0.23	0,2±0,25	0.996	0.318

# **3.6** Sites' characteristics influencing the presence of *Anopheles* larvae in Dabakala

Multi-variate logistic regression analysis showed that the presence of *Anopheles* larvae was associated with the nature of the site. They were more present in temporary water reservoirs (OR= 1.77; CI= 1.29 -2.43; p < 0.001), rice fields (OR= 16.21; CI= 6.71 - 39.09; p < 0.001), market garden irrigation wells (OR= 1.31; CI= 1.17 -1.46; p < 0.001) and natural sites (OR= 0.36; CI= 0.25 -0.52, p < 0.001) (Table 6). In addition, the stability of the site, the vegetation around the

sites, the season and the turbidity of the water in the sites influenced the presence of *Anopheles* larvae. The probability of their presence was significantly associated with shaded sites (OR= 4.52; CI= 3.38 - 6.04; p < 0.001), and temporary sites (OR= 6.10; CI= 4.01 - 9.29; p < 0.001). The rainy season (OR= 1.32; CI= 0.85- 2.05; p = 0.223), breeding sites with a surface area between 1 -10 m<sup>2</sup> (OR= 0.58; CI= 0.47 - 7.22; p= 0.673) and breeding sites < 1 m<sup>2</sup> (OR= 1.38; CI= 0.41- 3.0; 0.805; P= 0.805) also influenced the presence of larvae but with no significant difference.

Table 6: Relationship between the characteristics of the breeding sites and the presence of Anopheles larvae in the Dabakala sites.

		Multivariate regression analysis			
Characteristics	Anopheles breeding sites n (%)	Adjusted OR	95% CI	P-values	
	Season				
Dry	11 (28.95)	1			
Rainy	27 (71.05)	1.32	(0.85-2.05)	0.223	
	Turbidity				
Full of mud	11 (28.95)	1			
Clear	15 (39.47)	0.67	(0.29 -1.56)	0,352	
Cloudy	12 (31.58)	3.31	(0.64 - 17.24)	0,154	
	Nature of the breeding sites				
Abandoned objects	2 (5.56)	1			
Temporary water impoundments	14 (36.84)	1,77	(1.29 - 2.43)	< 0.001*	
Rice fields	14 (36.84)	16.21	(6,71 – 39.09)	< 0.001*	
Water wells	5 (13,16)	1.31	(1.17 -1.46)	< 0.001*	
natural breeding sites	2 (5,56)	0.36	(0.25 - 0.52)	< 0.001*	
	Permanence of site				
Permanent	1 (2.63)	1			
Temporary	37 (97.37)	6.1	(4.01-9.29)	< 0.001*	
	Vegetation around the site				
Shaded	8 (21.05)	1			
Not shaded	30 (78.95)	4.52	(3.38 – 6.04)	< 0.001*	
	Sunlight exposure				
No		1			
Yes	38 (100)	ND	-	-	
	Size of the site (m <sup>2</sup> )		-	-	
> 10	11 (28.95)	1			
1 -10	9 (23.68)	0.58	(0.47 – 7.22)	0.673	
< 1	18 (47.37)	1.13	(0.41-3.08)	0.805	

CI 95% confidence interval, OR odds ratio, ND: Not determined

# 3.7 Correlation between *Anopheles* larval density and physicochemical parameters

The principal component analysis (PCA) shows correlations between physicochemical parameters and the larval density of *Anopheles*. These data, represented on two axes F1 and F2 explain 55.13% of the total variation (Table 7). Temperature, pH, depth of the breeding site, and *Anopheles* larval density are correlated with the F2 axis (R coefficients = 0.816; 0.643; 0.41 and 0.13 respectively). Larval density is influenced by temperature, depth, and pH (Figure 2). Electrical conductivity and TDS are strongly associated with the F1 axis (R coefficients = 0.983 and 0.979 respectively). These two parameters negatively influence the larval density of *Anopheles*.

Table 7: Correlations	between	variables	and factors.	
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	F1	F2
Temperature	-0.083	0.816
pH	-0.167	0.139
Electrical conductivity	0.983	0.083
TDS	0.979	0.120
Depth	-0.232	0.413
larval density of Anopheles	-0.019	0.646

![](_page_5_Figure_2.jpeg)

Fig 2: Relationship between Anopheles larval density and physicochemical water parameters

### 4. Discussion

The objective of this study was to identify breeding sites of the Anopheles and their physicochemical characteristics influencing the presence of Anopheles larvae in the Dabakala health district. The work enabled us to determine nine categories of sites. The most common breeding sites in urban areas were temporary water impoundments (33.78%) and water wells (22.97%). In the rural area, abandoned objects (28.57%) and rice fields (21.43%) were dominant. The importance of temporary water impoundments and abandoned objects could be explained by the precipitation of the rains and the absence of hygiene measures in these areas. The abundance of watering wells during the dry season would be associated with the lack of water in the rice-growing lowlands, which leads people to dig wells to collect water for their market gardening activities. These wells could constitute a source of Culicidian nuisance and a malaria risk factor for local populations, as observed by Dambach et al. [24] in Burkina Faso and Tia et al. [3] in Côte d'Ivoire. These results confirm those of Koumba et al. [25] and El Joubari et al. [26] who highlighted the role of human activities in the creation of mosquito breeding sites. Similar studies carried out by Tia et al.<sup>[3]</sup> in northern Côte d'Ivoire showed a dominance of temporary impoundments, market gardeners' water wells in urban areas, the importance of rice fields, and abandoned jars in area rural. Our results are contrary to those of Akono et al. <sup>[27]</sup> who showed a predominance of water ponds in the area urban while water storage containers were in the rural area. The reason for this difference is that their study was in the coastal region of Cameroun.

The majority of *Anopheles* breeding sites were identified during the rainy season. These breeding sites were 0.5 and 0.89 times more important in the rainy season than in the dry

season, in urban and rural areas respectively. The low proportion of positive sites breeding sites identified in the dry season is due to the drying out of sites suitable for mosquito larval development due to the lack of rainfall, resulting in a scarcity of breeding sites. However, rainfall creates small enclaves of water colonized by mosquitoes in the rainy season as soon as the rain stops <sup>[28]</sup>. These results are consistent with those recorded by Hessou *et al.* <sup>[29]</sup> in Benin. These authors identified more *Anopheles* breeding sites in the rainy season than in the dry season. They linked this observation to the high temperature of the city in the dry season, which would be the cause of the drying up of the breeding sites.

Among the positive sites identified in the urban area, Anopheles larvae were found more in rice fields (OR = 72; CI = 5.70 - 908.90) and temporary water impoundment (OR = 7.38; CI = 1.40 - 39.08). In rural area, rice fields (OR = 1.40 - 39.08). 1.03-218.30) 15: CI = were more favorable for Anopheles larvae. The preference of Anopheles for rice fields can be explained by the fact that rice fields are evolutionary environments where different types of biotopes are more or less favorable to Anopheles, particularly to An. gambiae s.l [30]. Indeed, in the early stages of rice development, the water in the rice paddies is shallow and sunny, which constitutes ideal conditions for the development of An. gambiae s.l. larvae. These observations were made by Robert et al. [31] and by Mouchet et al. [32]. These larvae of An. gambiae s.l. area succeeded by those of An. funestus with the development of rice plants in the boxes <sup>[33]</sup>. Our observations corroborate those of Musiime et al. [34] who reported a preference for Anopheles in rice fields in rural Kanungu (OR= 0.192; CI = 0.078 - 0.523) and Tororo (OR= 4.212; CI =1.225 - 14.557) in Uganda

The density of larval habitats varies with the seasons [35, 36].

This larval density was high in the dry season (3.23 larvae/dipper) compared to the rainy season (1.28 larvae/dipper). In fact, in the dry season, egg-laying sites are scarce due to the low rainfall. That would have the consequence of a high ratio between the number of gravid females and the number of available breeding sites. In contrast, in the rainy season breeding sites are leached away by rain, which contributes to a decrease in their larval density <sup>[16, 37]</sup>. Our results are similar to those of Mbida *et al.* <sup>[37]</sup> who showed a higher larval density in the dry season than in the rainy season in the Wouri estuary, Cameroun. The larval density could be influenced by the physicochemical characteristics of the breeding sites <sup>[38]</sup>.

Temperature values concord with those recorded by Munga et al [39] and Imam et al. [40]. These authors showed larval abundance with a temperature between 28 and 32 °C. Likewise, a positive association was observed between temperature and larval density of Anopheles. This positive association could be justified by the high temperature that favors the development of larvae and the growth of microorganisms which are food sources for the larvae <sup>[41]</sup>. These results comply with those obtained in Niger by Souleymane et al. [42] and in Benin, by Hessou et al. These authors showed a positive correlation between temperature and larval density of Anopheles. The average pH value (7.71) of the breeding sites was slightly basic with a maximum of 7.83 in the rainy season. These pH values were relatively high compared to those recorded by Olusi et al. <sup>[43]</sup> in northern Nigeria, which was 6.93. These results corroborate those of Abdullah et al. <sup>[44]</sup> and Kudom *et al.* <sup>[45]</sup> who showed that *Anopheles* larvae thrive in breeding sites with neutral or slightly alkaline pH. This fact could justify the positive association between pH and larval density observed in our study. These results are similar to those recorded in Cameroon by Djamoukou et al. <sup>[46]</sup> and in Ethiopia by Getachew et al. <sup>[47]</sup>, who found a positive correlation between Anopheles larval density and pH. The mean electrical conductivity of the larval sites was 534.76  $\mu$ S/cm, with a maximum of 568.63  $\mu$ S/cm during the dry season. The average total dissolved solids (TDS) value was 281.02 mg/l with a maximum of 283.91 mg/l in the dry season. The high electrical conductivity and TDS in the dry season can be explained by the accelerated degradation of organic matter in the water, resulting in a dissolved oxygen deficit Berchi et al. [48]. In addition to this, there is the use of agricultural pesticides and herbicides. These averages of electrical conductivity and TDS are relatively high compared to those recorded by Olusi et al. [42], who obtained average values of 250 µS /cm and 16.60 mg/l for electrical conductivity and TDS respectively. Furthermore, a negative correlation was observed between electrical conductivity. TDS, and Anopheles larval density. This negative correlation could be justified by the importance of electrical conductivity and TDS as limiting factors for the development of Anopheles larvae. These observations were made by Alaoui et al. [49] who showed that Anopheles larvae thrive in poorly mineralized water, well-oxygenated, and low in organic matter.

Our results indicate a preference of *Anopheles* for temporary (OR= 6.10; p < 0.001) and non-shaded breeding sites (OR= 4.52; p < 0.001). Their presence in unshaded breeding sites could be explained by adequate exposure to sunlight, which leads to an increase in water temperature of sites favorable for larval development <sup>[10,50]</sup>. The preference of *Anopheles* larvae for temporary rather than permanent breeding sites is explained by the presence of a diversity of predators and

invertebrate the competitor's in permanent breeding sites. Their presence would reduce the density of mosquito larvae <sup>[51,52]</sup>. Several studies have shown that aquatic insects belonging to the orders Coleoptera, Odonata, and Hemiptera are responsible for significant reductions in mosquito populations and could be considered in integrated vector management programs <sup>[53, 54]</sup>. Our results confirm the work carried out in Ethiopia by Mareta *et al.* <sup>[55]</sup>, who observed a high probability of the presence of *Anopheles* larvae at temporary breeding sites.

## 5. Conclusion

Entomological surveys conducted in the Dabakala health district in July 2020 (rainy season) and February 2021 (rainy season) have identified nine (9) categories of breeding sites. Temporary water impoundments and rice fields have been the most colonized by *Anopheles* larvae. The majority of *Anopheles* larvae sites were found during the rainy season. These larvae were found more in unshaded and temporary breeding sites. However, the dry season constitutes the period of the high larval density of the sites, which is favorable to all anti-larval activities against malaria vectors. The larval density of these breeding sites is influenced by temperature, pH, and the depth of the breeding site.

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