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Ecology of *Anopheline* (*Culicidae*) larvae in the breeding sites of Matadi-Kibala District, Kinshasa (DR Congo)

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Abstract
This study was conducted in the Matadi-Kibala district, Kinshasa (DRC), to assess the influence of physicochemical water parameters on the larval distribution of Anopheles mosquitoes in various natural and anthropogenic breeding sites during the 2024 dry and rainy seasons. Twelve sites were sampled, and physical parameters (temperature, conductivity, turbidity, pH) as well as chemical parameters (dissolved O_2, BOD_5, COD, NH_4^+, NO_2^-, NO_3^-, PO_4^{3-}) were measured both in situ and in the laboratory.
Results showed that water temperature ranged between 27 °C and 30 °C, with high conductivity in some sites (up to 1311 µS/cm) and turbidity reaching 714 NTU. The pH was slightly neutral to basic (6.6-8.1). COD and BOD_5 levels were generally high, indicating a significant organic load. Three Anopheles species were identified: Anopheles gambiae, Anopheles funestus, and Anopheles coustani. An. gambiae was dominant in most habitats (186 individuals during the rainy season versus 83 in the dry season), followed by An. funestus (72 in the rainy season and 27 in the dry season). An. coustani was found in smaller numbers. Larval abundance was higher during the rainy season, suggesting a direct effect of rainfall on the formation and productivity of breeding sites. Overall analyses indicate that larval distribution is closely linked to variations in abiotic parameters, particularly temperature, conductivity, COD, BOD_5, and dissolved oxygen concentration. These findings confirm that polluted or organic-rich habitats are favorable for An. gambiae, whereas An. funestus prefers more stable and slightly oxygenated environments.

Keywords: Ecology, Anophelinae, larval habitats, physicochemical parameters, Matadi-Kibala, Kinshasa

1. Introduction
Mosquitoes of the genus Anopheles (family Culicidae) are the main vectors of malaria, a parasitic disease that remains one of the world's greatest public health challenges, especially in sub-Saharan Africa. According to the World Health Organization (WHO, 2023), approximately 249 million cases and 608,000 deaths were recorded in 2022, over 94% of which occurred in Africa. In the Democratic Republic of Congo (DRC), malaria accounts for more than 40% of medical consultations and remains the leading cause of morbidity and infant mortality (National Malaria Control Program [PNLP], 2023) [32].
Mosquitoes of the subgenus Anopheles display considerable ecological and adaptive diversity, allowing them to occupy various types of larval habitats. The development of their larvae depends on numerous abiotic factors such as temperature, pH, turbidity, conductivity, and dissolved oxygen, as well as biotic factors such as aquatic vegetation, algae, microorganisms, and predators (Minakawa *et al.*, 2001; Munga *et al.*, 2009; Imbahale *et al.*, 2011) [25, 28, 14]. The quality and stability of these breeding sites directly affect adult mosquito survival, density, and vector competence (Okech *et al.*, 2007; Afrane *et al.*, 2008) [34, 1].
In urban and peri-urban environments, the rapid and often unplanned expansion of settlements, poor management of wastewater and rainwater, as well as agricultural and domestic activities promote the formation of numerous artificial or semi-natural larval habitats (Fillinger *et al.*, 2004; De Silva *et al.*, 2020) [8, 7]. These environmental changes help maintain Anopheles populations even in areas previously considered unsuitable for their breeding (Awolola *et al.*, 2014) [2].

In Kinshasa, the capital of the Democratic Republic of Congo, several studies have reported the presence of *Anopheles gambiae s.l.* and *Anopheles funestus* in various habitats, including puddles, ditches, drains, swamps, and vegetable-growing areas (Mampuya *et al*., 2017; Luntadila *et al*., 2019) [21, 20]. The Matadi-Kibala district, located in the Mont Ngafula commune, is a rapidly expanding urban area characterized by hilly topography, stagnant pools, marshy zones, and temporary springs, particularly during the rainy season. These hydrological and anthropogenic conditions make this area suitable for studying the ecological dynamics of Anopheles larval habitats.

The main objective of this study was to characterize the ecology of Anophelinae (Culicidae) larvae in the breeding sites of Matadi-Kibala district, Kinshasa. Specifically, the study aimed to identify and describe the types of larval habitats used by Anophelinae in the study area, to assess the physicochemical and biological parameters associated with larval presence and abundance, to analyze the relationships between ecological characteristics of the habitats and the spatial and temporal distribution of Anopheles larvae, and to provide practical recommendations for improved environmental management and integrated vector control adapted to the urban context of Kinshasa.

The relevance of this research lies both in its scientific and operational value. It helps fill a knowledge gap on Anophelinae larval ecology in the Congolese urban context, where available data remain fragmented and often outdated. It also improves understanding of the ecological factors determining breeding site productivity, within the framework of malaria ecological epidemiology. The findings could guide local vector control strategies by identifying priority habitats for elimination or larvicidal treatment. Moreover, incorporating environmental variables will strengthen Integrated Vector Management (IVM) actions recommended by WHO, thereby promoting a sustainable and eco-responsible approach to malaria control in Kinshasa.

2. Study Area

The Matadi-Kibala district, located in the Mont-Ngafula commune of Kinshasa, is a semi-urban area undergoing rapid development, with specific geographical, social, and economic characteristics.

Matadi-Kibala is situated at the southwestern end of Kinshasa, along National Road No. 1. The area presents a varied relief alternating between hills, valleys, and plateaus. It serves as a strategic point and crossroads between the capital and surrounding regions (Katalayi, 2008) [16]. The district is bordered to the north by Matadi-Mayo (identified by the high-voltage power pylons), to the south by Matadi-Mayo II (near Malu Garage on Luzolo Avenue, along the Matadi Road via 3rd Republic and Mbuela Avenue), to the east by Mama Mubutu I and II neighborhoods (separated by the Matadi Road), and to the west, along the Matadi Road, by the commune of Ngaliema. The total surface area of Matadi-Kibala is about 20 km², with a population density of 8 inhabitants per km².

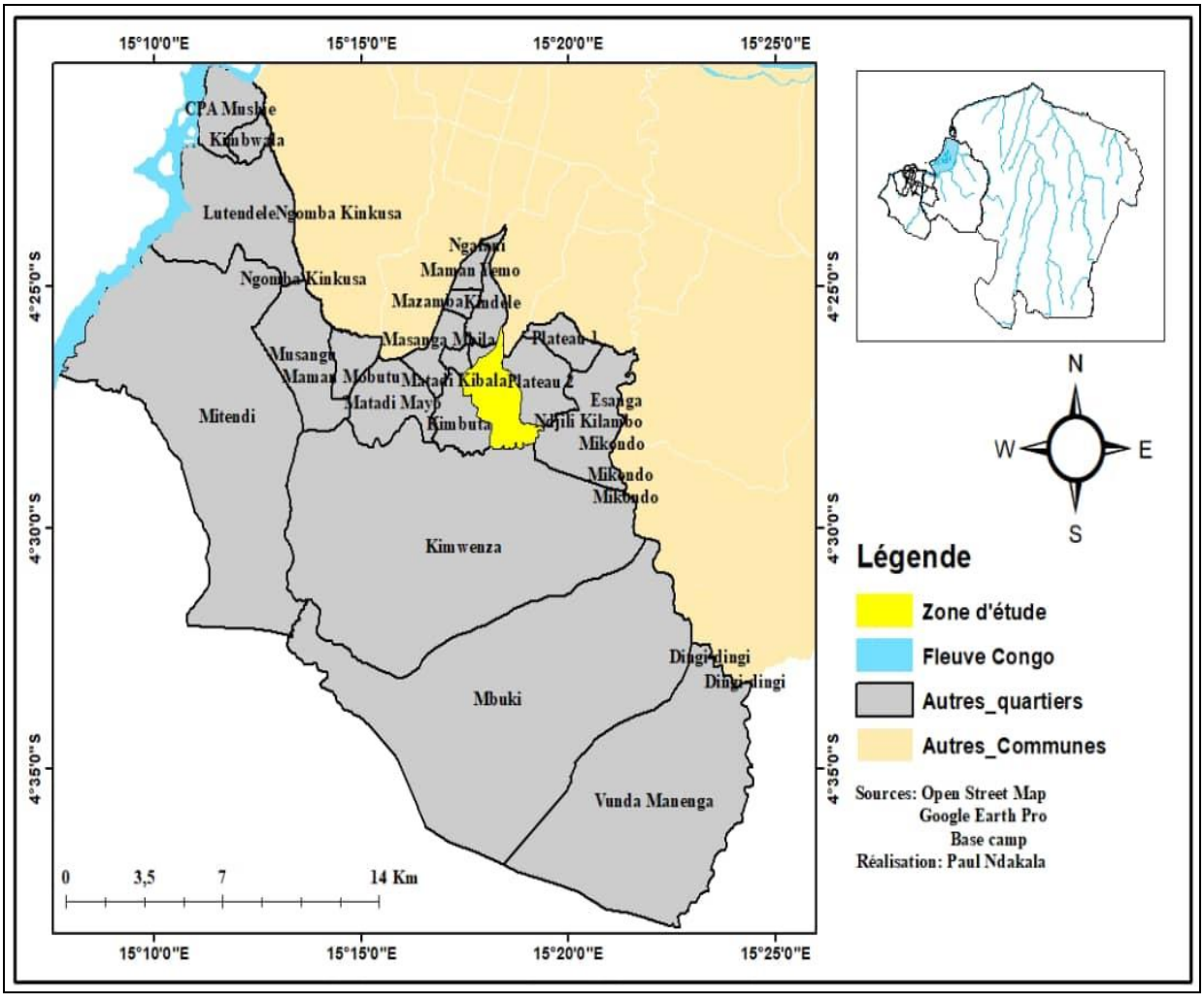

Fig 1: Localization of Matadi-Kibala District in the Mont-Ngafula Commune

From a geomorphological standpoint, the area is built on sandy terrain. Rainwater, diverted by unregulated construction, has caused erosion in several streets. Despite the local community's efforts to carry out small-scale rehabilitation works, three major erosion heads have been identified.

Previous studies on the soil structure of Kinshasa have shown that the region is dominated by sandy and erosive textures. The valley areas are characterized by market gardening on yellow, mineral-rich subsoil. This pedological structure promotes soil degradation on steep slopes, as is the case in the hilly zones of Matadi-Kibala.

As for vegetation, the original forest formations that once covered the area have been replaced by new landscapes dominated by tree planting (fruit and ornamental trees). This transformation is largely due to human activity aimed at meeting socioeconomic needs (housing, land parcelling, firewood collection, crop fields, etc.). Today, the landscape of Matadi-Kibala is largely dominated by urban arboriculture, which fulfills both economic and ecological functions.

The main rivers, Nsaya and Mafumba, are of great economic importance for local residents, providing sites for agriculture and small-scale quarrying. However, during the rainy season, these rivers often overflow, causing significant flooding and damage to nearby households.

3. Methods

3.1. Localization of Larval Habitats

This step consisted of searching for and identifying potential mosquito breeding sites. The types of habitats varied across the area (puddles, ponds, water wells, abandoned septic tanks, irrigation canals, tires, etc.). Natural habitats were distinguished from artificial ones. A breeding site could be shaded by vegetation or fully exposed to sunlight; it could also be permanent (large water bodies) or temporary (puddles, small ponds, etc.).

Anopheles breeding sites are typically small water bodies (puddles) that are sometimes difficult to detect (e.g., tree holes, animal hoofprints). In total, twelve sites were located four along the Nsaya River, four in drainage channels (sites 5-8), and four near the Mafumba River.

Sampling was conducted from January to December 2024, covering both the dry and rainy seasons. Larval habitats were identified through systematic surveys of different natural and artificial aquatic microhabitats (pools, puddles, ditches, drains, footprints, and wet agricultural zones).

Each breeding site was geo-referenced using a Garmin eTrex 30 GPS and classified according to its type, sun exposure, aquatic vegetation presence, substrate nature, and proximity to human dwellings (Munga *et al*., 2009; Imbahale *et al*., 2011) [28, 14].

3.2. Sampling of Larval Habitats

Larvae were collected using a standard 350 mL dipper (World Health Organization, 2013). For each site, ten dips were taken at different points on the water surface. The larvae were sorted, counted, and stored in containers filled with water from the same habitat. Larval instars (L1 to L4) and pupae were identified based on morphological criteria (Gillies & Coetzee, 1987) [10]. Specimens were preserved in 70% ethanol for laboratory identification.

3.3. Taxonomic Identification of Larvae

Larval identification was carried out at the Hydrobiology Laboratory of the National Pedagogical University using a binocular magnifier and the dichotomous keys of Gillies & Coetzee (1987) [10] and Holstein (1952) [13]. Anopheles larvae were distinguished from other Culicidae genera (Culex, Aedes) by their thoracic palmate hairs and the absence of a respiratory siphon.

When larval-stage identification was uncertain, some specimens were reared to adulthood for confirmation based on adult morphological traits.

3.4. Experimental Setup and Larval Rearing

Rearing was performed in plastic containers (trays) filled with dechlorinated or filtered spring water to a depth of 2-5 cm (Gerberg *et al.*, 1994; WHO, 2005) [9]. Containers were placed in a rearing room at the INRB laboratory under controlled temperature (25-28 °C), relative humidity (70-80%), and a 12:12 h light/dark cycle (Benedict *et al.*, 2009) [6]. Each sample was labeled with the site number, habitat type, and collection date.

As filter feeders, Anopheles larvae feed on fine suspended particles. In rearing, their diet consisted of artificial sources of proteins, carbohydrates, and lipids (Merritt *et al.*, 1992; Gerberg *et al.*, 1994) [23, 9]. Finely ground fish meal, shrimp meal, and brewer's yeast were used for feeding.

Feeding was done once or twice daily in small quantities to avoid water pollution. The amount varied with larval stage: early instars (I and II) received fine particles, whereas older larvae (III and IV) were given coarser ones (Benedict *et al*., 2009) [6].

The larval cycle lasted 7-14 days depending on species and temperature. After the fourth instar, larvae transformed into pupae, which were transferred into floating cups or beakers placed in emergence cages (see Figure 2). Adults typically emerged after 1-2 days (WHO, 2005).

Adult mosquitoes were collected using manual aspirators and transferred into net cages (30 × 30 × 30 cm), where they were fed a 10% sucrose solution until used for identification (Benedict *et al*., 2009) [6].

Species identification followed several keys: Gillies & Coetzee (1987) [10], Yadouleton *et al.*, (2018) [48] and Hadjivassilis & Weill (2007) [12]. A binocular magnifier and/or optical microscope were used to observe diagnostic characters on different parts of the mosquito's body.

3.5. Assessment of Physicochemical Parameters

Four physical parameters were measured in situ due to their sensitivity to environmental conditions: temperature (°C), conductivity at 20 °C (µS/cm), turbidity (NTU), and pH. These were measured using a Combo Hanna HI 98130 multiparameter probe.

The following chemical parameters were analyzed: calcium (Ca^{2+} mg/L), iron (Fe^{2+} mg/L), chloride (Cl^{-} mg/L), ammonium (NH_4^{+}), nitrite (NO_2^{-}), nitrate (NO_3^{-}), phosphate (PO_4^{3-}), and sulfate (SO_4^{2-}). Dissolved oxygen was measured in situ using a Hanna HI9146 oxymeter.

Other chemical analyses were performed at the General Commissariat for Atomic Energy (CGEA/CREN-K) using water samples from the studied rivers. A HACH DR/2400 spectrophotometer was used for analysis. Water samples were filtered with StonyLab filter papers (50 µm pore size), and appropriate titrating reagents were added before selecting the

Hach program (PH) for each element or ion.
Biochemical Oxygen Demand (BOD_5, mg/L) and Chemical Oxygen Demand (COD) were determined after a five-day incubation period in a thermostatic chamber (5 days at 20 °C, in the dark and sealed from air).

3.6. Data Analysis
Data were recorded and analyzed using Microsoft Excel 2021 and PAST version 4.03. Physicochemical parameters were subjected to Pearson correlation analysis to examine relationships with larval abundance. Finally, Canonical Correspondence Analysis (CCA) was performed to visualize the distribution of breeding sites according to their ecological characteristics (Munga *et al.*, 2009; Sisa *et al.*, 2025) [28, 41].

4. Results
4.1. Physical Parameters

Table 1: Physical parameters of water in larval breeding sites in Matadi-Kibala district during the two seasons of 2024

Sites	Temperature (°C)		Conductivity (µS/cm)		Turbidity (NTU)		pH	
	RS	**DS**	**RS**	**DS**	**RS**	**DS**	**RS**	**DS**
Site 1	30	29	204	213	114	109	6,67	6,66
Site 2	28,9	28	111	123	64	58	6,92	6,99
Site 3	29,3	29	194	202	104	98	7,1	7,21
Site 4	29,5	28	190	201	120	117	7,13	7,21
Site 5	28	27	1192	1254	674	685	7,51	7,62
Site 6	30	28	671	711	324	331	7,34	7,53
Site 7	29,9	27	1271	1311	707	714	7,62	7,83
Site 8	29	28	568	601	304	299	7,63	8,09
Site 9	28	27	199	115	115	103	8,18	8,15
Site 10	28,6	28	110	67	67	59	7,54	7,67
Site 11	29	29	99	61	61	58	7,23	7,34
Site 12	30	29	100	111	68	71	7,21	7,45
Mean	29,2±0,7	28±0,7	409,1±426,4	414±455	226,8±234	225,2±239,4	7,34±0.4	7,48±0.4

The water temperature of the breeding sites varied between 27 °C and 30 °C depending on the sites and seasons. The lowest values were observed in sites 5, 9, and 10 (27-28 °C), while the highest were recorded in sites 1, 6, and 7 (≈30 °C). The mean temperature was 29.2±0.73 °C in the rainy season and 28±0.7 °C in the dry season.
Regarding conductivity, values ranged from 99 µS/cm (site 11) to 1311 µS/cm (site 7). The overall mean was 409.1±426.4 µS/cm in the rainy season and 414±455 µS/cm in the dry season. The high standard deviation indicates substantial variation between sites.
Turbidity fluctuated between 58 NTU and 714 NTU, with the highest levels found in sites 5, 6, and 7. The mean values were 226.8±234 NTU in the rainy season and 225.2±239.4 NTU in the dry season.
As for pH, it varied from 6.66 to 8.15, with mean values of 7.34±0.39 in the rainy season and 7.48±0.43 in the dry season. The low standard deviation (0.39-0.43) indicates that pH remained relatively stable among sites.

4.2. Chemical Parameters

Table 2: Chemical parameters of water in larval breeding sites in Matadi-Kibala district during the two seasons of 2024

Sites	**DO (mg/L)**		**BOD_5 (mg/L)**		**COD (mg/L)**		**NH_4^+ (mg/L)**		**NO_2^- (mg/L)**		**NO_3^- (mg/L)**		**PO_4^{3-} (mg/L)**	
	RS	**DS**	**RS**	**DS**	**RS**	**DS**	**RS**	**DS**	**RS**	**DS**	**RS**	**DS**	**RS**	**DS**
Site 1	2,03	1,89	0,97	1,7	1,46	2,3	0,014	0,014	0,012	0,018	9,14	10,4	2,92	3,52
Site 2	2,12	2,01	1,05	1,46	1,58	3,2	0,014	0,014	0,015	0,017	8,37	9,76	3,58	3,85
Site 3	2,81	2,51	1,09	1,58	1,64	3,1	0,016	0,016	0,012	0,014	10,4	11,56	4,68	4,8
Site 4	1,87	1,67	1,13	1,43	1,7	3,2	0,013	0,014	0,013	0,013	12,67	13,7	4,85	4,8
Site 5	1,9	1,78	8,12	8,95	10,07	13,17	0,074	0,067	0,017	0,017	20,4	23,3	6,04	6,4
Site 6	1,8	1,79	6,87	8,76	12,84	15,2	0,01	0,019	0,012	0,012	19,48	21,7	5,62	5,69
Site 7	2,1	2,09	13,04	14,8	21,18	24,5	0,079	0,061	0,02	0,013	38,6	43,4	5,51	5,71
Site 8	2,81	2,69	11,78	12,8	27,39	31,4	0,061	0,048	0,021	0,017	48,17	51,4	8,1	8,7
Site 9	2,87	2,86	9,87	12,5	22,88	27,8	0,048	0,047	0,028	0,023	44,8	47,5	6,68	6,8
Site 10	2,1	1,98	1,46	2,3	2,3	3,2	0,014	0,014	0,014	0,014	23,45	24,67	6,78	6,8
Site 11	2,56	2,61	1,58	2,13	2,13	2,8	0,014	0,013	0,014	0,014	24,6	25,7	7,34	7,6
Site 12	2,94	2,95	1,64	2,65	2,65	3,12	0,016	0,017	0,016	0,016	27,5	28,67	6,87	6,96
Mean	2,33	2,24	4,88	5,92	8,98	11,08	0,031	0,029	0,016	0,016	23,97	25,98	5,75	5,97
	0,44	0,46	4,72	5,24	9,75	11,09	0,03	0,021	0,005	0,003	13,67	14,5	1,54	1,54

Table 2 shows that dissolved oxygen (DO) values ranged between 1.67 and 2.95 mg/L, with an average of 2.33±0.44 mg/L in the rainy season and 2.24±0.46 mg/L in the dry season.
Biochemical Oxygen Demand (BOD_5) ranged from 0.97 to 13.04 mg/L, with a mean of 4.88±4.72 mg/L in the rainy season and 5.92±5.24 mg/L in the dry season.
Chemical Oxygen Demand (COD) values varied from 1.46 to 31.4 mg/L, with an average of 8.98±9.75 mg/L in the rainy season and 11.08±11.09 mg/L in the dry season.
Ammonium (NH_4^+) values ranged between 0.01 and 0.079 mg/L, averaging 0.031±0.03 mg/L in the rainy season and 0.029±0.021 mg/L in the dry season.
For nitrite (NO_2^-), concentrations varied between 0.012 and 0.028 mg/L, with a stable mean of 0.016 mg/L in both seasons.

Nitrate (NO_3^-) values ranged from 8.37 to 51.4 mg/L, with mean values of 23.97±13.6 mg/L in the rainy season and 25.98±14.5 mg/L in the dry season.
Finally, phosphate (PO_4^{3-}) concentrations ranged from 2.92 to 8.7 mg/L, with mean values of 5.75±1.54 mg/L in the rainy season and 5.97±1.54 mg/L in the dry season.

4.3. Distribution of Anopheles Larvae in Breeding Sites of Matadi-Kibala District

Table 3: Distribution of Anopheles species in breeding sites of Matadi-Kibala during the two seasons of 2024

Sites	Anopheles species						Total
	Anopheles gambiae		*Anopheles funestus*		*Anopheles coustani*		
	SS	SP	SS	SP	SS	SP	
Site 1	4	8	2	6	1	4	25
Site 2	0	0	2	7	3	5	17
Site 3	5	13	0	0	0	1	19
Site 4	3	6	2	8	0	0	19
Site 5	10	18	3	5	4	7	47
Site 6	11	18	5	9	3	7	53
Site 7	13	29	9	23	0	0	74
Site 8	17	35	0	0	0	0	52
Site 9	3	13	0	3	0	0	19
Site 10	5	9	0	0	5	9	28
Site 11	5	16	1	3	0	2	27
Site 12	7	21	3	8	0	3	42
Total	83	186	27	72	16	38	422

The number of *Anopheles gambiae* larvae ranged from 0 to 35 individuals depending on the sites. The highest numbers were observed in sites 7 (29 in the rainy season) and 8 (35 in the rainy season). The total number recorded was 269 individuals for both seasons combined.
For *Anopheles funestus*, larval counts varied from 0 to 23 individuals, with the most populated sites being 6, 7, and 12. A total of 99 individuals were recorded for both seasons.
As for *Anopheles coustani*, larval numbers ranged from 0 to 9 individuals, with the highest counts found in sites 5, 6, and 10. The total was 54 individuals.
Altogether, 422 larvae were recorded across all sites and both seasons.

4.4. Pearson Correlation Between Physicochemical Parameters and Larval Abundance

Table 4. Pearson correlation between physicochemical parameters and larval abundance by Anopheles species in Matadi-Kibala district (2024)

Physical and Chemical Parameters	*A. gambiae (r,p)*	*A. funestus (r,p)*	*A. coustani (r,p)*
Temperature	0,72; <0,01	0,58; <0,05	0,36; 0,12
Conductivity	0,41; 0,08	0,29; 0,17	0,61; <0,05
Turbidity	0,63; <0,01	0,48; 0,06	0,27; 0,20
pH	0,33; 0,14	0,21; 0,28	0,19; 0,33
DO	-0,67; <0,01	-0,51; 0,07	-0,59; <0,05
DBO_5	0,56; <0,05	0,38; 0,10	0,62; <0,05
DCO	0,68; <0,01	0,64; <0,01	0,49; <0,05
NH_4^+	0,44; 0,09	0,33; 0,15	0,55; <0,05
NO_2^-	-0,31; 0,16	-0,46; <0,05	-0,22; 0,25
NO_3^-	-0,18; 0,34	-0,24; 0,21	-0,17; 0,36
PO43-	0,29; 0,18	0,51; <0,05	0,32; 0,15

The analysis of correlations between water physico-chemical parameters and the occurrence of Anopheles larvae reveals species-specific relationships. In *A. gambiae*, temperature (r = 0.72; $p<0.01$), turbidity (r = 0.63; $p<0.01$), and chemical oxygen demand (COD) (r = 0.68; $p<0.01$) show strong and highly significant positive correlations. These results indicate that this species develops preferentially in warm, slightly turbid waters rich in organic matter, conditions typically found in domestic or anthropogenically influenced habitats (Afrane *et al.*, 2008) [1]. The negative correlation with dissolved oxygen (r = -0.67; $p<0.01$) also suggests that *A. gambiae* tolerates poorly oxygenated environments, characteristic of intense biological activity and organic decomposition.
For *A. funestus*, significant relationships are observed with COD (r = 0.64; $p<0.01$) and phosphate (PO_4^{3-}) (r = 0.51; $p<0.05$). These correlations indicate an affinity of this species for waters with moderate organic loads and nutrient enrichment, often associated with semi-permanent habitats containing aquatic vegetation (Gillies & De Meillon, 1968; Awolola *et al.*, 2007) [3].
Regarding *A. coustani*, several parameters show significant correlations, including conductivity (r = 0.61; $p<0.05$), biochemical oxygen demand (BOD_5) (r = 0.62; $p<0.05$), COD (r = 0.49; $p<0.05$), and ammonium (NH_4^+) (r = 0.55; $p<0.05$). These findings indicate this species' tolerance to ion-rich and organically loaded environments, often corresponding to stagnant or slightly polluted waters. Similarly, the negative correlation with dissolved oxygen (r = -0.59; $p<0.05$) supports this preference for habitats with low water renewal.
Overall, the three Anopheles species studied exhibit variable sensitivity to physico-chemical parameters, but the dominant trend is a positive association with indicators of organic pollution (COD, BOD_5, NH_4^+) and a negative relationship with dissolved oxygen. These observations confirm that larval habitats favorable to Anopheles mosquitoes are generally

eutrophic, warm, and poorly oxygenated waters, where organic inputs enhance nutrient availability necessary for larval development (Ndenga *et al.*, 2011; Minakawa *et al.*, 2002) [33, 24].

4.5. Canonical Correspondence Analysis (CCA) Between Anopheles Species and Physicochemical Parameters

4.5.1. Dry Season

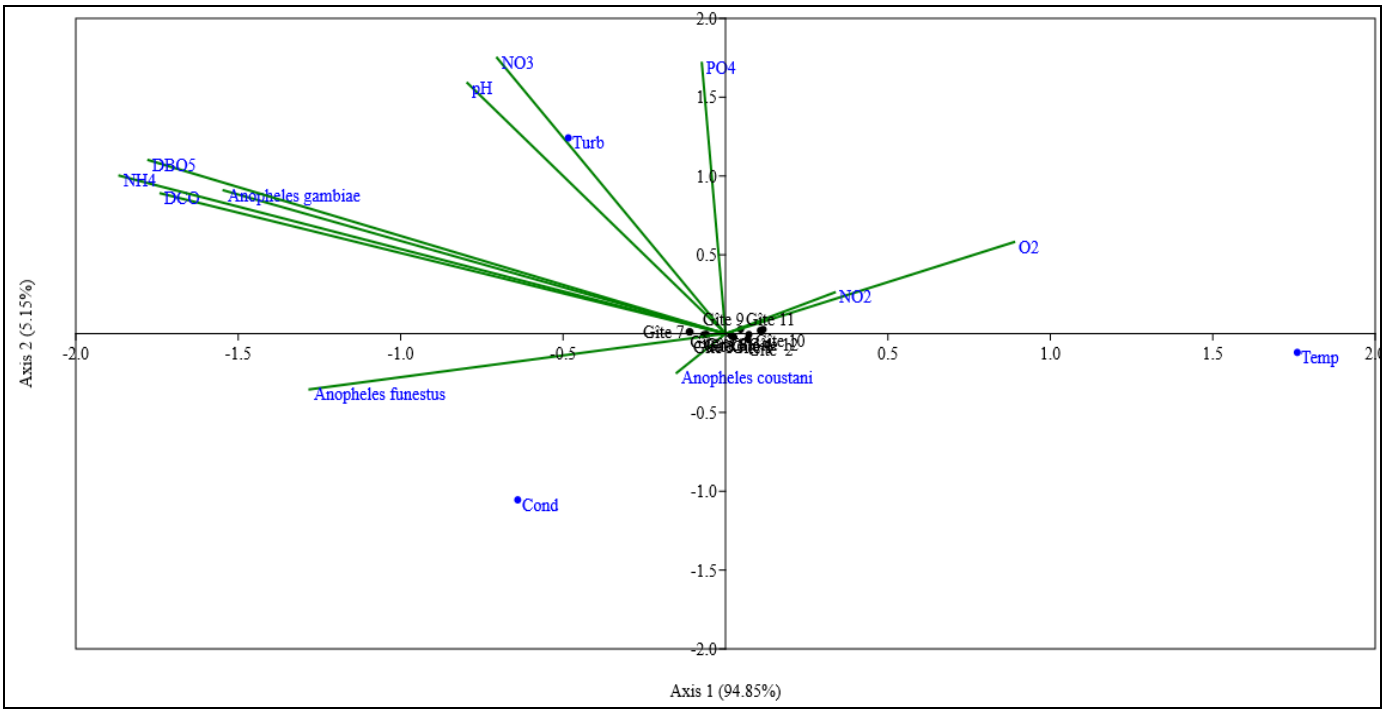

Fig 2: Correlations between Anopheles species and physicochemical parameters in breeding sites during the dry season

The first axis of the CCA explained 94.85% of the total variance, while the second explained 5.15%. Together, both axes accounted for 100% of the variance (Figure 2).

Strong positive correlations were observed between *Anopheles gambiae* and the physicochemical parameters ammonium, BOD_5, COD, pH, nitrate, turbidity, and phosphate.

Other positive correlations were noted between *Anopheles funestus* and *Anopheles coustani* with conductivity, ammonium, BOD_5, and COD.

4.5.2. Rainy Season

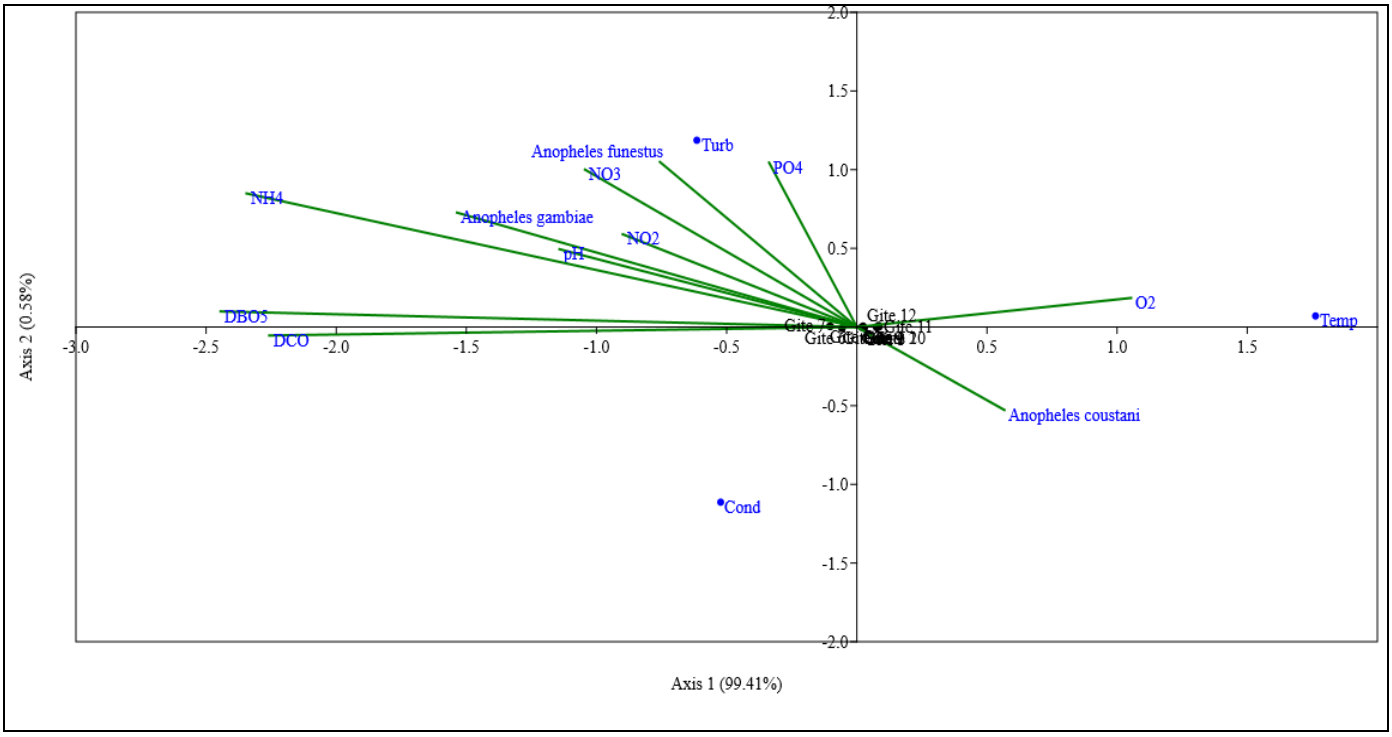

Fig 3: Correlations between Anopheles species and physicochemical parameters in breeding sites during the rainy season

Figure 3 shows that the first axis explained 99.41% of the total variance, while the second explained 0.58%, accounting

together for 100% of the total variance.

Anopheles gambiae and *Anopheles funestus* showed strong associations with turbidity, BOD_5, COD, NH_4^+, pH, NO_2^-, NO_3^-, and PO_4^{3-}.

Larvae of *Anopheles coustani* were strongly correlated with water temperature and dissolved oxygen levels.

5. Discussion

The average temperatures recorded in the larval habitats of Matadi-Kibala ranged between 27 °C and 30 °C, with a mean of 29.2±0.73 °C during the rainy season (RS) and 28±0.7 °C during the dry season (DS) (Table 1). These values indicate a thermal environment favorable to the larval development of Anopheles mosquitoes, whose optimal life cycle occurs between 25 and 32 °C (Paaijmans *et al*., 2009) [38]. The slight decrease in temperature observed during the dry season could be attributed to reduced sunlight and increased evaporation, lowering heat levels in shallow habitats.

Electrical conductivity varied widely among sites, ranging from 99 µS/cm to 1311 µS/cm, with averages of 409±426.4 µS/cm (RS) and 414±455 µS/cm (DS). This large variability reflects the diversity of water origins, from weakly mineralized natural habitats to polluted waters derived from domestic activities and urban runoff (drainage ditches). High values (> 600 µS/cm) in habitats 5, 6, 7, and 8 suggest a high ionic load associated with anthropogenic pollution (detergents, washing, household discharges). Although these conditions are unfavorable for some species, they can still be tolerated by *Anopheles gambiae s.l*., known for its ecological plasticity (Klinkenberg *et al*., 2008) [18].

Average turbidity values were 226.8±234 NTU in RS and 225.2±239.4 NTU in DS, indicating little seasonal variation. Some habitats (especially 5, 6, and 7) showed very high values (> 300 NTU), reflecting high organic matter loads. Previous studies (Okogun *et al*., 2003) [35] have shown that slightly turbid water favors Anopheles larval proliferation by reducing visual predation; however, excessive turbidity may limit photosynthesis and thus oxygenation.

The pH values ranged between 6.6 and 8.1, with slightly basic means (7.34±0.39 in RS and 7.48±0.43 in DS). These values fall within the optimal range for mosquito larval development (6.5-8.5; Mutero *et al*., 2010) [29]. The slightly higher pH during the dry season could be due to increased mineral salt concentration following water evaporation.

Overall, these physical parameters indicate an environment with limited seasonal variation but strongly influenced by local human activities (washing, drainage, wastewater discharges). These features are typical of semi-natural urban habitats found in the outskirts of Kinshasa.

The physical measurements (temperature, turbidity, current velocity, and depth) in Matadi-Kibala's larval habitats showed significant variations between the two seasons. The Kruskal-Wallis test revealed statistically significant differences ($p<0.05$) for temperature, turbidity, and depth, indicating strong seasonal fluctuations. These variations align with typical hydrological changes in humid tropical regions (Manguin *et al*., 2008) [22].

During the rainy season, water temperature was slightly higher, promoting greater biological activity in the habitats. Turbidity also increased due to runoff transporting organic and inorganic materials into breeding sites. These conditions particularly favor Anopheles larval development, maintaining high humidity and increased food availability (Gimnig *et al*., 2001; Kweka *et al*., 2012) [19].

Dissolved oxygen (DO) concentrations ranged from 1.8 to 2.9 mg/L, with means of 2.33±0.44 mg/L (RS) and 2.24±0.46 mg/L (DS). These low values reflect eutrophic environments rich in organic matter. Nevertheless, Anopheles larvae tolerate hypoxic conditions due to their aerial respiration (Kaboré *et al*., 2023) [15]. Biological oxygen demand (BOD_5) varied considerably (0.97-13 mg/L), indicating moderate to high organic pollution, confirmed by high chemical oxygen demand (COD) values (> 30 mg/L) in some sites. Such environments, rich in organic matter, can provide food for larvae through microorganism proliferation (Imbahale *et al*., 2011) [14].

Ammonium (NH_4^+) levels remained generally low (< 0.08 mg/L), while nitrate (NO_3^-) concentrations averaged 23.97±13.6 mg/L (RS) and 25.98±14.5 mg/L (DS), indicating a consistent nitrogen input of domestic origin. These moderate concentrations promote phytoplankton and algal growth, providing larval food sources (Bamba *et al*., 2021) [4].

Phosphate (PO_4^{3-}) concentrations averaged 5.75±1.53 mg/L (RS) and 5.97±1.54 mg/L (DS), exceeding the eutrophication threshold (≥ 0.5 mg/L per WHO). These high levels indicate strong phosphorus pollution, likely linked to detergents and household discharges. Such nutrient-rich environments favor high biological productivity, explaining the high larval densities observed in habitats 5 to 8.

These findings confirm that the water quality of Matadi-Kibala larval habitats is generally degraded and strongly influenced by anthropogenic factors. Such contexts are typical of African urban areas where stagnant domestic waters form suitable habitats for mosquito vectors (Kayembe *et al*., 2021).

Chemical parameters (pH, conductivity, DO, ammonium, nitrates, phosphates) also showed notable seasonal variations. The Kruskal-Wallis test indicated that conductivity and DO values differed significantly between seasons ($p<0.05$). Higher conductivity in the dry season suggests increased dissolved salts due to evaporation (Ndenga *et al*., 2011) [33].

The pH remained generally neutral to slightly alkaline in most habitats, a condition favorable for Anopheles larvae survival (Muturi *et al*., 2008) [30]. Dissolved oxygen was lower in the rainy season due to organic-laden runoff increasing biochemical oxygen demand. These results align with those of Oyewole *et al*. (2009) [37] in Nigeria, showing that slightly alkaline and moderately oxygenated waters are optimal for *Anopheles gambiae* and *Anopheles funestus* development.

A clear dominance of *Anopheles gambiae s.l.* larvae (63.6%, 269/422 individuals) was observed, followed by *Anopheles funestus* (23.5%) and *Anopheles coustani* (13%). This dominance occurred in both seasons but was more pronounced in the rainy season (RS = 296 individuals vs DS = 126).

This seasonal variation was directly linked to the increase in temporary habitats and water availability during the rainy season (Yadouleton *et al*., 2010) [49]. Habitats 7 and 8 recorded the highest larval densities (74 and 52 individuals, respectively), correlated with high conductivity, turbidity, and organic loads conditions typical of semi-permanent, nutrient-rich habitats with limited competition and predation (Mwangangi *et al*., 2007) [30].

Habitats showing extreme physicochemical values (e.g., conductivity > 1000 µS/cm, PO_4 > 6 mg/L) favored *An. gambiae* and *An. funestus*, known for their pollution tolerance (Kweka *et al*., 2012) [19]. In contrast, *An. coustani*, a more zoophilic species, occurred in less polluted habitats (sites 2, 10, 11), appearing less dependent on stagnant urban waters.

These observations confirm that Anopheles mosquitoes exploit a wide range of habitats in urban Matadi-Kibala, with a marked preference for slightly turbid, basic, and nutrient-rich waters. These results are consistent with those of Klinkenberg *et al*. (2008) [18] in Accra and Minakawa *et al*. (2005) [28] in Kenya, showing that Anopheles proliferation in urban environments is favored by declining water quality and increasing artificial habitats.

Pearson correlation analyses revealed significant relationships between some environmental parameters and larval abundance. A positive correlation was found between water temperature and larval density (r = 0.68; $p<0.01$), indicating that higher temperatures promote faster larval growth and survival, as reported by Piyaratne *et al*. (2005) [39].

Turbidity also showed a positive correlation (r = 0.54; $p<0.05$) with larval abundance, probably due to organic enrichment providing food. Conversely, dissolved oxygen exhibited a negative correlation (r = -0.61; $p<0.05$), suggesting that hypoxic habitats were more favorable for larval survival, as noted by Muturi *et al*. (2007) [30].

pH, conductivity, and nitrates showed weak and non-significant correlations ($p > 0.05$) with larval abundance, confirming their secondary role in larval population dynamics in this setting. The results indicate that Anopheles larvae prefer habitats with moderate to high temperature, medium turbidity, and low dissolved oxygen conditions typical of stagnant, lightly shaded, and organic-rich waters ideal for malaria vector reproduction (Minakawa *et al*., 2001; Klinkenberg *et al*., 2008) [27, 18].

The positive correlation between temperature and larval density confirms that warmth accelerates embryonic and larval development (Bayoh & Lindsay, 2003) [5], whereas the negative relationship with DO reflects larval tolerance to eutrophic or polluted environments.

Thus, the combination of Kruskal-Wallis and Pearson correlation tests demonstrates that the most influential factors on larval dynamics in Matadi-Kibala were temperature, turbidity, and dissolved oxygen variables closely linked to seasonality.

Canonical Correspondence Analysis (CCA) between physicochemical parameters and Anophelinae species revealed a clear structuring of larval communities according to environmental conditions and seasonal patterns. The first two canonical axes explained most of the total cumulative variance, showing strong correlations between species distribution and environmental gradients.

During the dry season, CCA indicated that temperature, conductivity, and pH were the main factors influencing Anopheles larval distribution. Species such as *Anopheles gambiae s.l.* and *An. funestus* grouped within habitats characterized by high temperatures (25-30 °C) and low turbidity, indicating a preference for stable, sun-exposed waters (Okogun *et al*., 2003; Kweka *et al*., 2012) [36, 19].

Conductivity was positively correlated with *An. funestus* abundance, suggesting greater tolerance of this species to slightly mineralized waters, often associated with temporary habitats near dwellings (Imbahale *et al*., 2011) [14].

In contrast, *An. coustani* was located in habitats with slightly acidic pH and higher dissolved oxygen, corresponding to shaded, vegetated, semi-permanent habitats (Sinka *et al*., 2010) [40].

During the rainy season, larval distribution became more heterogeneous. CCA revealed a marked influence of turbidity, nutrients (nitrates, ammonium), and dissolved oxygen on species composition. Heavy rainfall increased runoff, altering the chemical and physical composition of breeding sites. *An. gambiae s.l.* remained dominant, benefiting from the proliferation of organic-rich temporary habitats (Mwangangi *et al*., 2010) [30]. Conversely, *An. funestus* showed negative correlations with turbidity and ammonium, preferring clearer, more oxygenated habitats (Sogoba, 2007) [42].

Overall, CCA highlighted that major malaria vector species (*An. gambiae s.l.*, *An. funestus*) are associated with habitats showing intermediate temperature and conductivity values, while their abundance decreases with high turbidity and ammonium indicators of heavy organic pollution.

This relationship emphasizes the importance of environmental management and habitat control in reducing vector proliferation. Manipulating physicochemical conditions (shading, removal of stagnant water, organic matter reduction) can limit breeding site capacity for Anopheles larvae (WHO, 2013; Tchouassi *et al*., 2020) [43].

6. Conclusion

The study conducted in Matadi-Kibala revealed a close relationship between water physicochemical quality and Anophelinae larval dynamics. Results showed that physical parameters (temperature, conductivity, turbidity, pH) and chemical ones (COD, BOD_5, dissolved oxygen, phosphates, nitrates) vary seasonally and directly influence the presence and abundance of Anopheles species.

Habitats with higher temperatures, higher conductivity, and high organic loads (elevated COD and BOD_5) hosted the greatest densities of *Anopheles gambiae*, the dominant species in the area. Conversely, *An. funestus* and *An. coustani* were found in more stable and slightly better-oxygenated waters, indicating differentiated ecological adaptations to local environmental conditions.

The rainy season proved most favorable for larval proliferation due to numerous temporary, human-origin habitats (puddles, drains, stagnant water), while the dry season showed a marked decline in larval densities linked to reduced breeding site availability.

Thus, seasonal variations and local abiotic factors play a key role in the spatial distribution of Anopheles larvae. These results underscore the importance of integrated environmental management of larval habitats in urban and peri-urban areas particularly through stagnant water drainage, community awareness, and regular entomological monitoring.

From a malaria control perspective, identifying the most productive breeding sites provides a scientific basis for targeted interventions, especially before and during the rainy season. Implementing combined larval management strategies including biological, environmental, and health-based approaches could significantly reduce malaria transmission risk in Matadi-Kibala and similar urban zones of Kinshasa.

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