

## Dynamics and Characterization of Larval Breeding Sites of *Anopheles gambiae* s.l. in the Department of Saint-Louis (Senegal)

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### Abstract

Malaria remains a major public health problem in Senegal, particularly in areas in the pre-elimination phase, such as the department of Saint-Louis, where the incidence of cases has increased sharply between 2021 and 2024. This study aimed to characterize the larval breeding sites of *Anopheles gambiae* complex the main vectors of malaria, and to identify the species present. Surveys were carried out during the 2024 rainy season in five municipalities of the department. Each deposit was described, georeferenced and subjected to physicochemical measurements (pH, temperature, salinity, conductivity). Larval density was estimated by the dipping method. Out of 143 sites visited, 100 were positive for anopheline larvae, of which 59% were natural and 41% were artificial. Breeding sites smaller than 10 m<sup>2</sup> concentrated 82.3% of the larvae ( $p < 0.001$ ). Mapping showed high activity in the middle of the rainy season, particularly in Cité Niakh (Saint-Louis), Ngallèle (Saint-Louis), Pikine (Saint-Louis) and Diougop (Gandon), with a decrease at the end of the rainy season. PCR identification showed that the majority consisted of *Anopheles arabiensis* (95%), followed by *An. melas* (4%) and *An. gambiae* s.s. (1%). The physicochemical parameters of the deposits had averages of pH 8.12 ± 0.55, temperature 31.6°C ± 4.28, salinity 4.38 g/L ± 3.21 and conductivity 8,135 µS/cm ± 5,990. In conclusion, these results highlight the complexity of the ecological factors influencing the presence of mosquito vectors and emphasize the need to strengthen entomological surveillance and implement targeted actions to limit malaria transmission in this area.

**Keywords:** *Anopheles gambiae* s.l., malaria, breeding sites, Saint-Louis, Senegal.

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### INTRODUCTION

Mosquitoes of the genus *Anopheles* are the only vectors of human malaria. Their wide distribution and high adaptability allow them to occupy a variety of environments, from humid forest areas to arid regions (Robert *et al.*, 2017). The genus includes several complexes of morphologically similar but genetically distinct species, among which the *Anopheles gambiae* complex plays a major role in malaria transmission in Africa (Coetzee *et al.*, 2000). These species exhibit ecological and behavioral variations that directly influence their vectorial capacity and the transmission dynamics of the *Plasmodium* parasite. Malaria remains one of the leading causes of morbidity and mortality in tropical regions. According to the World Malaria Report

(WHO, 2024), about 263 million cases and 597,000 deaths were recorded in 2024, 95% of which were in sub-Saharan Africa. In Senegal, the disease remains endemic, with more than 429,000 cases reported in the same year (WHO, 2024). However, the distribution of malaria is heterogeneous across the country, with some areas showing a recent upsurge in cases despite control efforts. The department of Saint-Louis illustrates this dynamic. Located in a Sahelian zone with high climatic variability, it has seen a notable increase in malaria incidence in recent years, from 489 cases in 2021 to 2695 in 2024 (MSAS, 2025). This evolution could be linked to environmental changes and the ecological adaptation of vectors. In this context, the present study aims to better understand the ecological and biological factors influencing the distribution of *Anopheles* complex in the

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department of Saint-Louis. More specifically, it seeks to map *Anopheles* larval breeding sites during the rainy season, characterize their physicochemical parameters (pH, temperature, salinity, conductivity), and genetically identify the species present in study area.

## MATERIALS AND METHODS

### Study area

The department of Saint-Louis, located in the northwest of Senegal, is bordered to the west by the Atlantic Ocean, to the north by the Senegal River, to the east by the department of Dagana, and to the south by the Louga region. It lies at the 16°02' north latitude and 16°24' west longitude. According to the ANDS (2023), it has a population of 385,368 inhabitants on an area of 879 km<sup>2</sup>, resulting in a density of 427 inhabitants/km<sup>2</sup>, making it the most densely populated department in the region. The department comprises five communes: Saint-Louis (254,171 inhabitants), Gandon (71,080 inhabitants), Mpal (11,025 inhabitants), Ndiébène Gadiol (27,938 inhabitants), and Fass Ngom (23,154 inhabitants).

### Characterization of deposits

The study was conducted during the 2024 rainy season (August - November) in the department of Saint-Louis. Each water point likely to harbor *Culicidae* larvae was inspected. Any positive roosting, i.e. where *Anopheles* larvae were observed, was recorded on a field sheet and georeferenced using a portable GPS (Kobotoolbox). For each roost, several variables were noted: the origin (natural or artificial), the durability of the water (temporary, with a probable drying up in a few days or weeks, or permanent, with water maintained during the season), the presence or absence of vegetation, as well as the estimated surface area of the roost (<10 m<sup>2</sup> or >10 m<sup>2</sup>). GPS coordinates (latitude, longitude) and date were also recorded.

The physicochemical parameters measured on each positive deposit are water temperature (in °C), conductivity (in µS/cm), pH and salinity. These measurements were carried out with a multi-parameter digital water quality tester (YY-400 – 4-in-1 Digital Water Tester). Larval density was estimated using the dipping method with a 350 ml graduated beaker in accordance with the protocols of Service (1993) and Talipouo *et al.* (2017). For deposits larger than 10 m<sup>2</sup>, ten samples were taken at eight strategic points, with two additional dippings in the areas considered to be the most representative. For deposits with a surface area of less than or equal to 10 m<sup>2</sup>, six samples were taken at four points, including repetitions in the most productive areas. Small roosts, such as footprints or hoof prints, were sampled with a ladle in order to recover the entire accessible volume. Each sample was poured into a white container for easy observation, then the larvae were collected and counted. The larval density of *Anopheles* was expressed as the average number of larvae by dipping, calculated according to the following formula:

Larval density (LD) =  $TL / TD$  where LD represents the larval density (larvae/dip), TL is the total number of *Anopheles* larvae collected, and TD is the total number of dips performed.

Finally, larval samples from *Anopheles*-positive sites were preserved in 95% ethanol. Each bottle had the following information: the code of the lodge, the date and the name of the locality. These specimens were then transferred to the laboratory for further genetic identification. All the data collected in the field was entered via an electronic questionnaire designed with Kobocollect on a tablet. This data was recorded daily and then transmitted to the server for storage and analysis.

### Genetic Identification :

Identification was carried out by polymerase chain reaction (PCR).

### DNA extraction

For each positive roost found, two larvae were taken randomly for genetic identification. For each larva, DNA was extracted using the CTAB 2% (Cetyl trimethyl ammonium bromide) method. Each larva was placed in an individual tube and then ground in 200 µl of 2% CTAB buffer previously prepared and stored at room temperature. After one hour (1 hour) in a water bath at 65 °C, material was mixed by inversion with 200 µL of chloroform and then centrifuged for five minutes at 12,000 revolutions per minute (rpm). The upper phase was removed and put into another identified tube, then 200 µL of isopropanol was added to this supernatant; The whole thing was well mixed by inversion before being centrifuged for fifteen minutes at 12,000 revolutions per minute. Subsequently, the isopropanol was removed and drained, replaced with 70% ethanol and the new mixture was centrifuged for five minutes at 12,000 revolutions per minute. After the ethanol had been removed, the pellet was dried for five minutes in the Speed-vac. Finally, for elution, 200 µl of ultrapure water was added to each tube and the resulting DNA was stored between -4 °C and -8 °C.

### DNA amplification by PCR

The method of Wilkins *et al.* (2006) was used for sample identification using multiplex PCR with the primers IMP-UN, AR-3T, GA-3T, ME-3T, QD-3T, IMP-S1 and IMP-M1 (Table I). This approach is based on the use of IMP (Intentional Mismatch Primers), which allow the detection of single nucleotide polymorphisms, thus facilitating the distinction of species of the *Anopheles gambiae* complex. Amplification of the sequences of interest was performed in a 25 µL reaction volume, including 15.5 µL of ultrapure water, 8 µL of AmpliTaq Gold 360 Master Mix, 0.5 µL of each primer as well as 1 µL of DNA extract. After centrifugation, the reaction mixture was in a thermal cycler and amplified according to the following program: initial denaturation at 95 °C for 5 minutes, followed by 30 cycles including denaturation at 95 °C for 30 seconds, hybridization at 58

°C for 30 seconds, and elongation at 72 °C for 30 seconds. A final elongation phase was then carried out at

72 °C for 5 minutes. The amplicons obtained were stored at a temperature of 4 °C.

**Table I : Primer sequences for the identification of species of the gambiae complex after (Wilkins et al., 2006)**

Primers	Sequences
IMP-UN	5'-GCTGCGAGTTG-3'
AR-3T	5'-GTGTTAACGTGTCCTCTCCgTC-3'
GA-3T	5'-GCTTACTGGTTGGTCGGCAtGT-3'
ME-3T	5'-CAACCCACTCCCTTGACGaTG-3'
IMP-S1	5'-CCAGACCAAGATGGT TCGcTG-3'
IMP-M1	5'TAGCCAGCTTGTCCACTAGTtTT-3'

#### Agarose gel electrophoresis

First, 15g of agarose was dissolved in 100 ml of TAE 1X in an Erlenmeyer flask container. The solution is placed in a microwave for three minutes to ensure complete dissolution. After adding 7 µl of Biothium (a dye that allows DNA to be visualized under UV light), the solution is poured into a mold equipped with a comb forming the wells for the amplicons. Once solidified, the gel is placed in a tank filled with TAE 1X buffer. For each sample, 4 µl of amplicon is mixed with 1 µl of charge blue and then deposited in a gel well. The level of band migration was measured using a 100 bp molecular weight marker. After removing all the amplicons, the tank will be carefully closed and connected to an electric generator set to 100 V and 400 mA to allow the amplicons to migrate for 60 minutes. At the end of the migration, a Gel Doc connected to a computer was then used to visualize the fluorescent DNA bands. The expected bands are as follows: *An. melas*: 528 bp; *An. arabiensis*: 387 bp; *An. coluzzii*: 333 bp and *An. gambiae*: 221 bp.

#### Data analysis

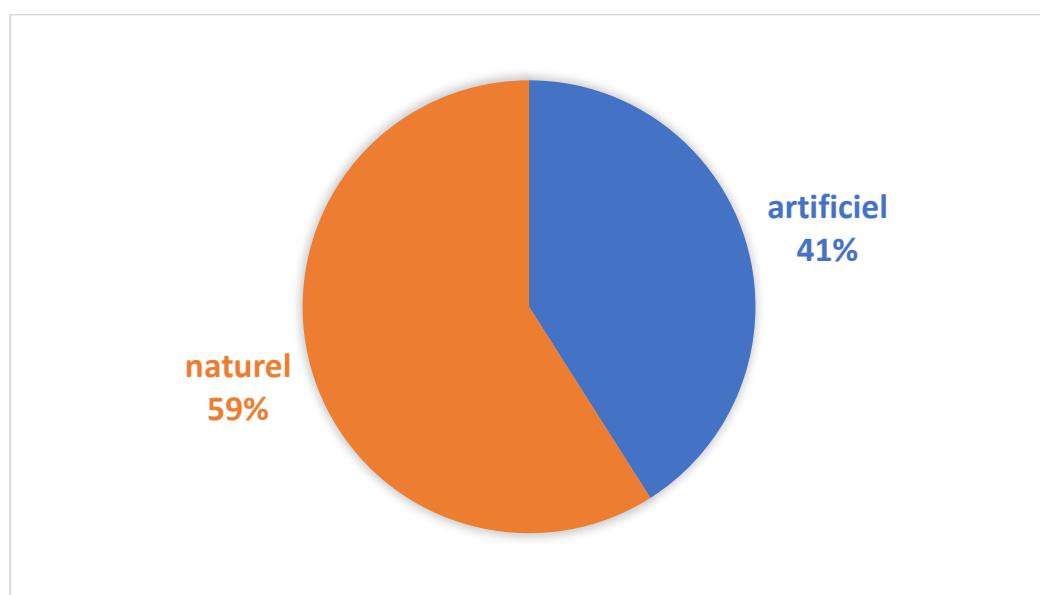
Microsoft Excel was used for data processing in tables and graphs. Indicators were calculated using Epi

Info 7.2 software (CDC, 2019). Statistical analyses were performed using R software version 4.4.3 (R studio Team, 2023). The Chi<sup>2</sup> test was applied to determine if there was a significant association between the presence or absence of larvae and observable roost characteristics (roost size and vegetation). QGIS software version 3.42.1 (QGIS Development Team, 2024) was used for the mapping of larval breeding sites in the study area.

## RESULTS

### Typology and positivity of larval breeding sites

Out of a total of 143 roosts surveyed, 100 were positive for the presence of Anopheles larvae. Natural habitats accounted for 59% of the roosts identified, compared to 41% of artificial roosts (figure 1). Among natural roosts, puddles (40%) were the most frequent, followed by ponds (17%) and streams (11%). Artificial roosts mainly included tire tracks (10%), gutters (5%), basins (4%), barrels (4%), septic tanks (4%), household containers (2%), bricks (1%), swimming pools (1%), and used tires (1%) (Figures 2 and 3).



**Figure 2: Percentage of natural and artificial habitat**



**Figure 3a: Types of natural roosts identified in our study area during the 2024 rainy season (A: pond; B: puddle; C: stream)**

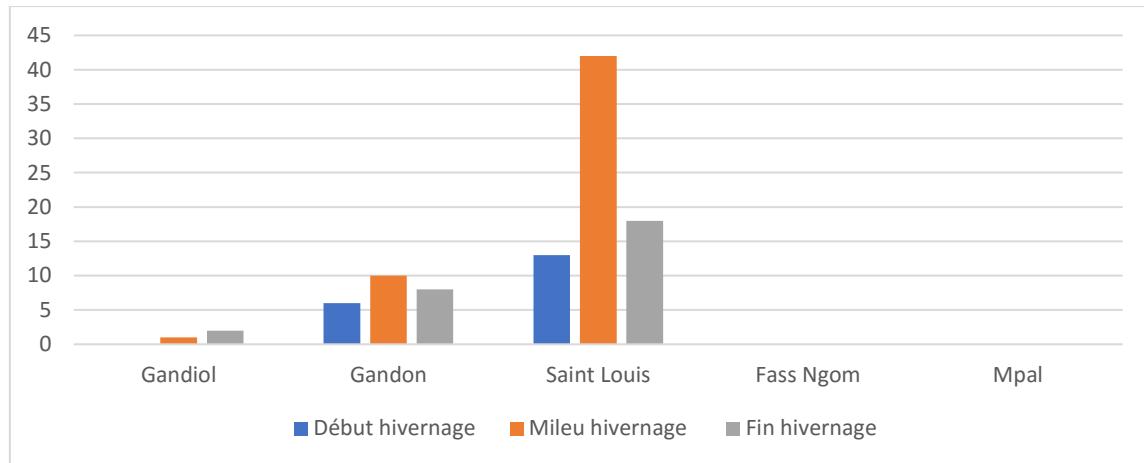


**Figure 3b: Types of artificial roosts identified in our study area during the 2024 rainy season**

D: pelvis; E: brick; F: gutter; G: barrel; H: sewer; I: tire; J: domestic container; K: swimming pool; L: Tire track

Seasonal dynamics show a marked increase in the number of positive roosts in the middle of the wintering (53 sites), compared to the beginning (19 sites) and the end of the season (28 sites). The communes of

Saint-Louis, Gandon and Gondiol concentrated the majority of positive roosts, while none were observed in Fass-Ngom and Mpal (fig 4)

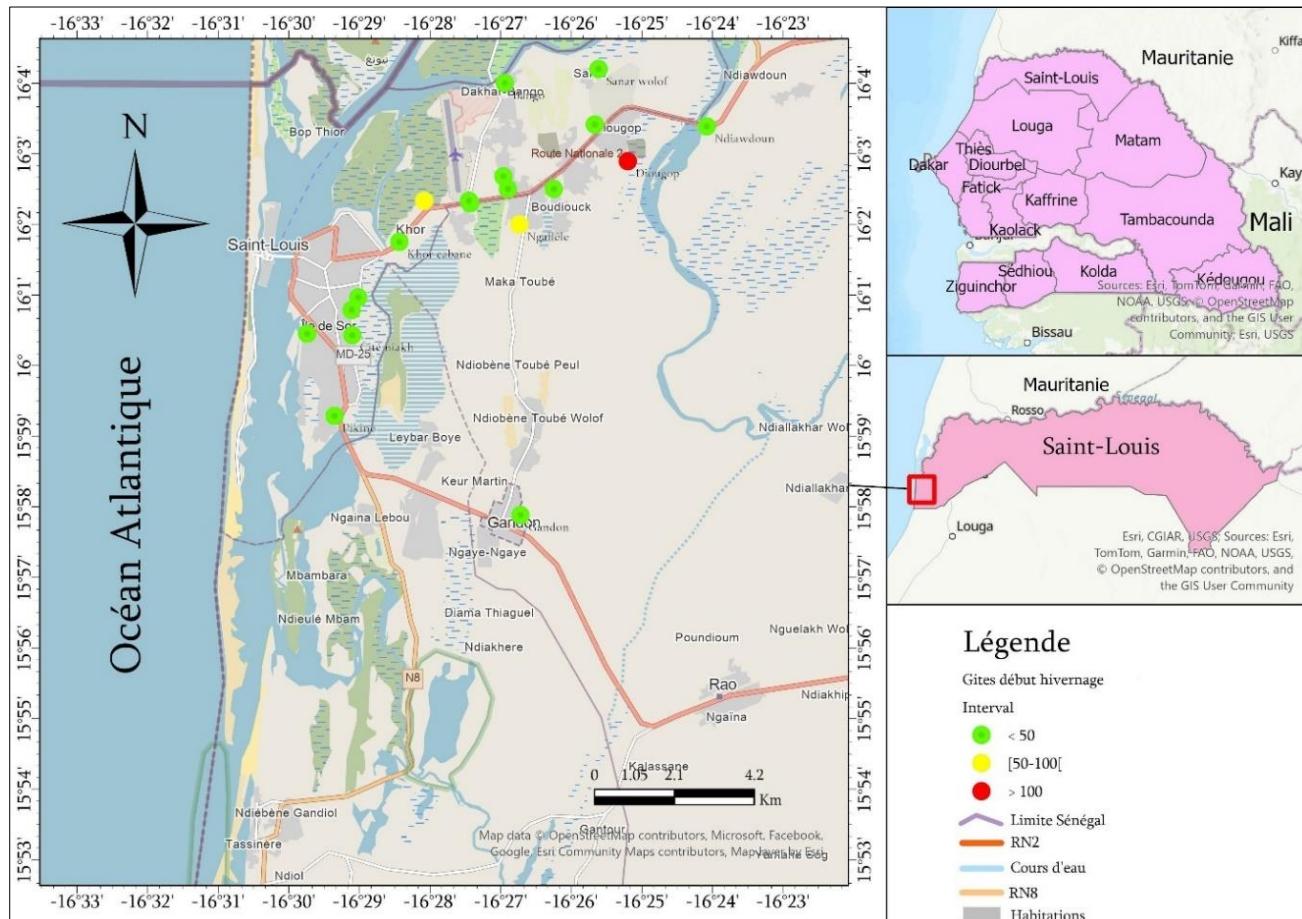


**Figure 4: Positivity of larval sites surveyed in the department of Saint-Louis during the 2024 wintering season**

#### Mapping and spatial dynamics of larval breeding sites

The spatial distribution of Anopheles positive roosts showed strong heterogeneity between municipalities and during the rainy season (Figures 5, 6 and 7).

At the beginning of the winter, the roosts were few in number and concentrated mainly in the urban areas of Saint-Louis (Cité Niakh, Ngallèle, Pikine, Khar Yalla, Vauvert, Khor Cabane, Bango.) and semi-urban areas of Gandon (Diougop, Boudiouk, Sanar Wolof, Ndiawdoun) with moderate densities ( $\leq 100$  larvae/dip).



**Figure 5: Mapping of larval habitats of early rainy season**

In the middle of the winter, the density and distribution of roosts reached their maximum, with 53 positive sites spread over a large portion of the territory. The districts of Cité Niakh, Pikine, Ngallèle, Boudiouk,

Sanar Wolof, and Diougou had the highest densities, sometimes exceeding 300 larvae/dip. This expansion corresponds to the period of maximum rainfall, favouring the formation of many temporary roosts.

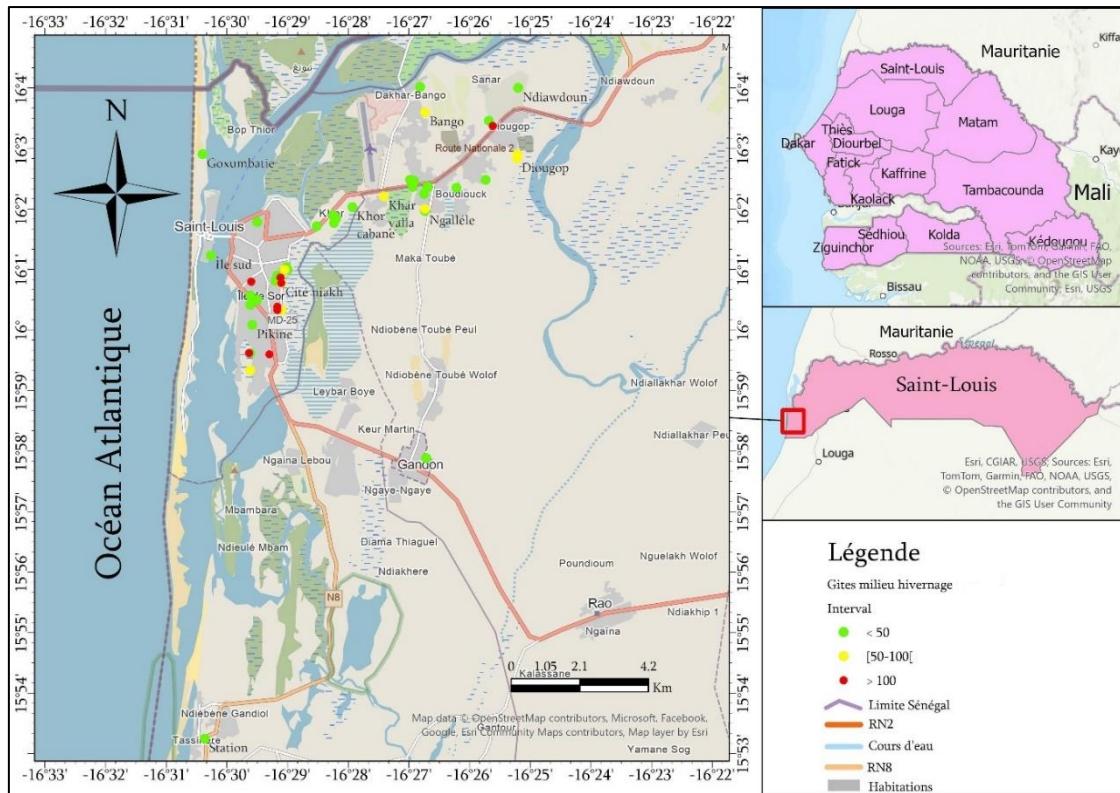


Figure 6: Mapping of larval habitats in the middle of the rainy season

At the end of the winter, the total number of positive roosts decreased, mainly in Saint-Louis and Gandon. However, some artificial roosts (septic tank,

containers) remained active, maintaining the reproduction of Anopheles despite the decrease in rainfall.

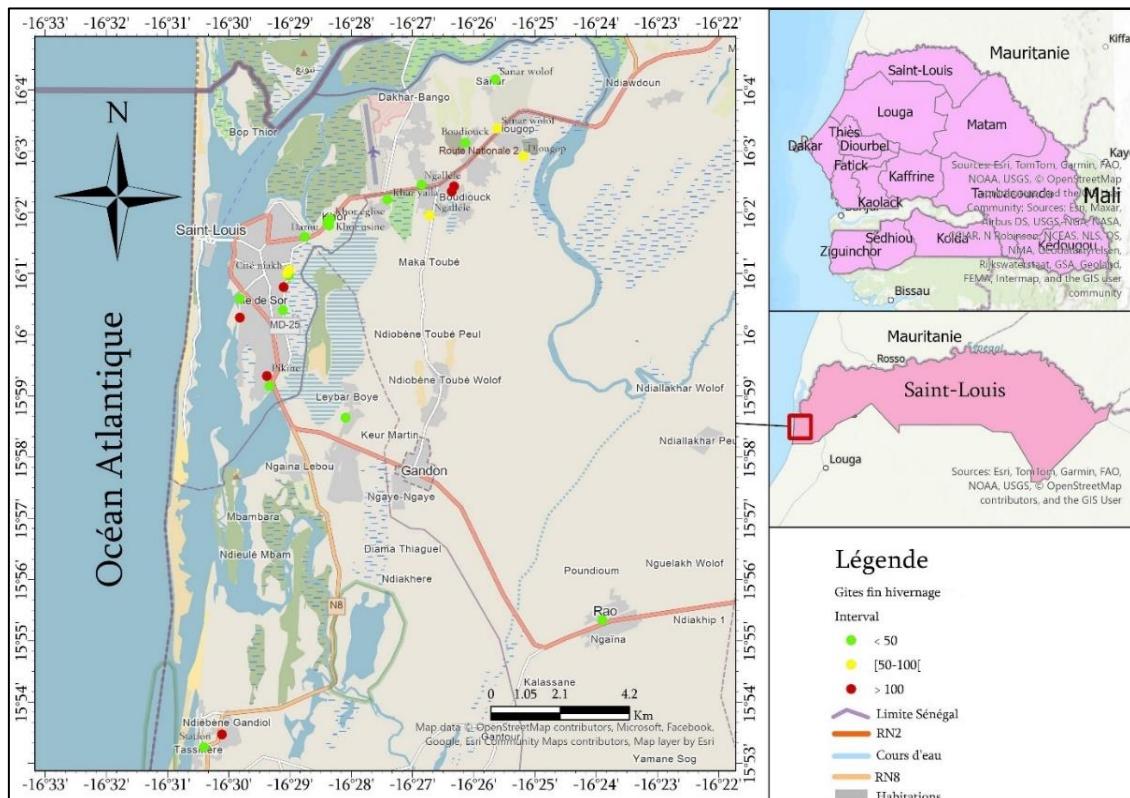


Figure 7: Mapping of larval habitats at the end of the rainy season

The maps (Figures 5, 6 and 7) illustrate these spatio-temporal variations, highlighting a strong correlation between the distribution of roosts and urbanized or peri-urban areas with permanent water points.

#### Productivity of the roosts according to their size

Analysis of the distribution of larvae according to the surface area of the roosts shows that 82.3% of the total larval density comes from roosts of less than 10 m<sup>2</sup>,

with an average density of 51.78 larvae per dipping. Breeding sites of more than 10 m<sup>2</sup> contribute 17.7%, with an average density of 47.38 larvae per dipping (Table II). In this table, N represents the number of deposits sampled by surface category. The average density corresponds to the average number of larvae collected per sample (dip) in each category. Statistical analysis by the Chi<sup>2</sup> test shows a significant difference between the two groups ( $p < 0.001$ ).

**Table II : The variation in the presence of larvae and the average density as a function of the surface area of the roosts**

Surface area of the gîtes in m <sup>2</sup>	N	Presence of larvae (%)	Density (larvae/dip)
Less than 10 <sup>m<sup>2</sup></sup>	81	82,3	51,78
More than 10 <sup>m<sup>2</sup></sup>	19	17,7	47,38

#### Breeding productivity according to the presence of vegetation

Analysis of roost productivity by vegetation presence shows that 35.5% of total larval density was observed in vegetated roosts, compared to 64.5% in non-vegetated roosts (Table III). Although this trend suggests

that vegetation may negatively influence the presence of Anopheles larvae, the p-value ( $0.8231 > 0.05$ ), from a Chi<sup>2</sup> test, indicates that this difference is not statistically significant. N represents the number of deposits sampled by surface category.

**Table III : Distribution of positive roosts according to vegetation cover**

Vegetation	N	Presence of larvae (%)
Presence	33	35,5
Absence	67	64,5
<b>Total</b>	<b>100</b>	<b>100</b>

#### Physico-chemical parameters

The larval habitats studied in the department of Saint-Louis show a high physico-chemical variability depending on the site. The pH of the waters, between 6.35 and 9.27, indicates a predominance of slightly alkaline waters, while the temperature varies from 20.2°C to 41°C, reflecting thermal conditions favourable to larval development. The salinity, ranging from 0.15 to 10.45 g/L, highlights a diversity of habitats ranging from

fresh water to brackish or salt water, which is confirmed by the electrical conductivity values, which are widely dispersed between 275 and 19,590 µS/cm. Despite this heterogeneity, statistical analysis revealed no significant correlation between the mean density of Anopheles larvae and the measured parameters (pH, temperature, salinity, conductivity;  $p > 0.05$ ). Although slight positive trends were observed, these variables do not directly influence larval density (figure 7).

**Table IV : Chemical parameters of larval habitats in the department of Saint Louis**

Variables	pH	Temperature	Salinity g/L	Conductivity
Min	6,35	20,2	0,15	275
Max	9,27	41	10,45	19590
Average	8,1198	31,6475	4,3836	8135,14
Median	8,11	31,5	3,62	6883,5
Standard deviation	± 0,5459492	± 4,281537	± 3,21249	± 5990,01
Variance	0,2980606	18,33156	10,32012	35880215

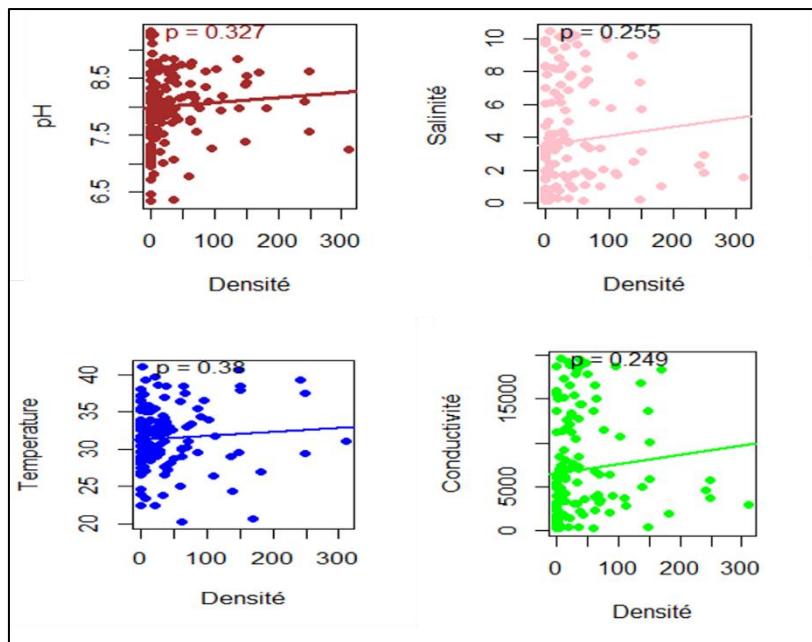


Figure 7: Effect of pH, salinity, conductivity, and temperature on mean larval density

#### Species identification by PCR

Molecular identification was made with the molecular weight marker: *An. melas* 528pb, *An. arabiensis* 387pb, *An. coluzzi* 333pb and *An. gambiae*

221pb (Wilkins et al., 2006). PCR revealed the presence of 3 species, all from the *Gambiae* complex: *Anopheles arabiensis* 95%, *Anopheles gambiae* s.s. 1% and *Anopheles melas* 4% (Figure 8).

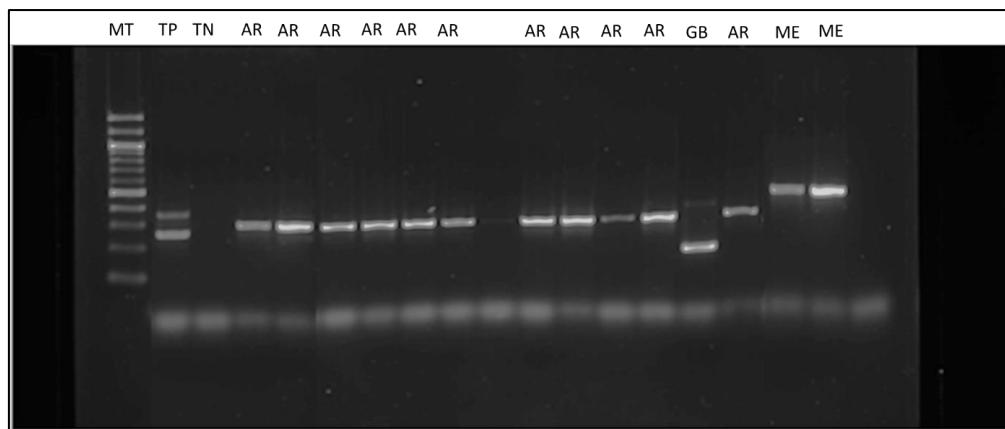


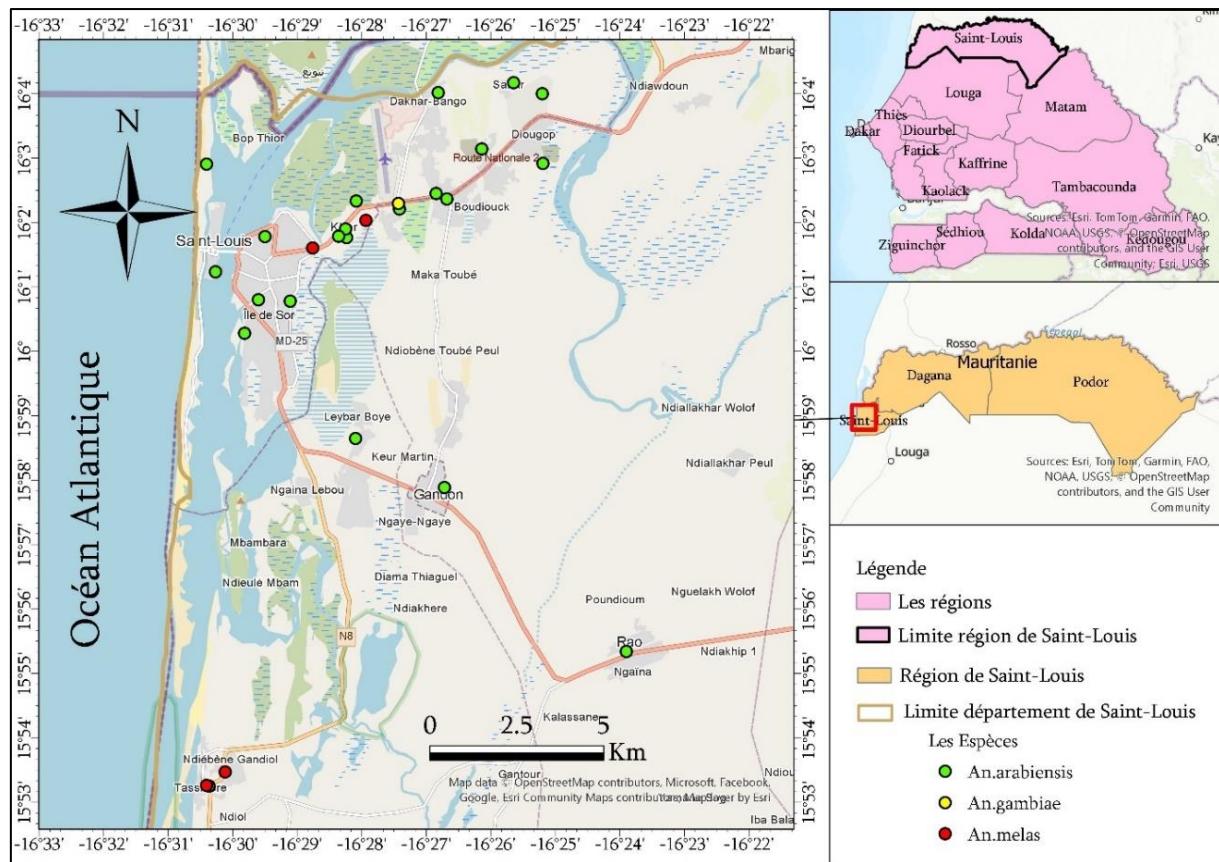
Figure 8: Photo of an agarose gel after migration. MT: size marker, TP: positive control, TN: negative control, AR: *An. arabiensis*, GB: *An. Gambiae*, ME: *An. Melas*

#### Map of the distribution of identified species

Figure 9 presents the spatial distribution of the different *Anopheles* species identified in the department of Saint-Louis during the 2024 wintering season

The identification of species of the *Anopheles gambiae* complex was carried out from the larvae collected in the positive roosts by the dipping method, with n samples taken from each roost according to the size of the habitat. A representative sample of 2 larvae

per roost was selected for molecular analysis (species-specific PCR). The results show that *Anopheles arabiensis* is the dominant species in all the communes and at all periods of wintering. *An. melas* has been observed only in the localities of Darou and Khor Cabane (Saint-Louis), as well as in Ndiebène and Tassinère (Gandioli), mainly in the middle and end of the season. As for *An. gambiae*, it was only detected in the locality of Khar Yalla (Saint-Louis), at the beginning of the wintering period.



**Figure 9: Spatial distribution of species identified in the department of Saint Louis during the 2024 winter season**

## DISCUSSION

This study, conducted in the department of Saint-Louis during the 2024 wintering season, made it possible to characterize the larval habitats and the spatio-temporal distribution of the species of the *Anopheles gambiae* complex. These results are part of a context of a resurgence of malaria, amplified by climate change, rapid urbanization and environmental changes, which require a detailed understanding of the ecology of vectors. Results of this study revealed that, natural roosts represent the majority of the sites surveyed (59%), compared to 41% of artificial roosts. This distribution, closely related to environmental conditions and human activities, is consistent with observations made in other Sahelian areas, where temporary natural roosts appear quickly after the first rains (Sy et al., 2016; Koumba et al., 2018; Iro et al., 2020; Kouadio et al., 2023; Noura et al., 2023; Ndiaye et al., 2025a). Puddles (39%), ponds (16%) and streams (11%) are the most colonized habitats. Septic tanks, anthropogenic breeding sites, become more frequent at the end of the winter, probably because of the lack of sanitation and unplanned urbanization. This finding is in line with the conclusions of studies conducted in urban and peri-urban areas (Kutomy et al., 2022; Ndiaye et al., 2024), who underline the importance of artificial breeding sites in the persistence of larval populations in times of climate transition.

The mapping of the breeding sites revealed a particularly high larval density in the commune of Saint-Louis, particularly in Cité Niakh, Pikine and Ngallèle. Stable outbreaks were also identified in Gandon (Diougop, Boudiouk, Sanar Wolof), while Gadiol, Fass-Ngom and Mpal had few roosts, with the exception of a few isolated outbreaks in Tassinère and Ndiebène (Gadiol). These differences contrast with the results of Kutomy et al., (2022), who observed a greater abundance of the genus *Anopheles* in rural areas. This divergence could be explained by the variability of local contexts: population density, rapid urbanization and particular hydrological conditions.

The latter, marked by the rise of rainwater and river water, favor the formation of temporary pockets of water conducive to larval development. The proximity of these roosts to homes considerably increases the risk of transmission, as shown by other studies in African urban areas, including Dakar (Machault et al., 2009), Cocody (N'Dri et al., 2022), Niamey (Noura et al., 2024) and several other cities in West Africa (Matah et al., 2017; Koumba et al., 2018). A recent study in Djilakh, central Senegal, confirmed that the proximity of breeding sites to dwellings (less than 500 m) is strongly associated with a higher incidence of malaria, which decreases with distance (Ndiaye et al., 2025b). Topographic and soil features also play a key role, influencing stormwater retention and puddle formation, which are known to be preferred roosts for *Anopheles* (Haileselassie et al.

2021). This link is confirmed by a study conducted by Morlighem *et al.*, (2025), which combined human vulnerability and environmental variables to produce a national mapping of malaria risk in Senegal.

Molecular identification revealed the presence of three species of the *An. gambiae* complex: *An. arabiensis*, *An. gambiae* and *An. melas*. *An. arabiensis*, a ubiquitous and thermotolerant species (Collins *et al.*, 2019), has been detected in all types of roosts in Saint-Louis, Gandon and Gadiol, confirming its wide adaptation (Doucouré *et al.*, 2020). *An. gambiae* has been observed only at the beginning of wintering at Khar Yalla, while *An. melas* has been detected in some coastal areas of Saint-Louis and Gadiol. These observations are consistent with those of Petrarca and Coluzzi (1987) and Diop *et al.* (2002), who had previously reported the coexistence of these species in the Senegal River basin, particularly in brackish and mangrove areas.

Physicochemical analyses show a great variability in environmental parameters. The pH, between 6.35 and 9.27 (mean: 8.12), reflects slightly alkaline waters, favourable to larval development, as shown by Ndiaye *et al.* (2024), Ebhodaghe *et al.* (2024) and Noura *et al.* (2024). The average temperature (31.65°C) reflects the warm conditions in the region and promotes the reduction of the aquatic cycle of larvae, increasing vector density (Iro *et al.* 2020). *An. arabiensis*, which is more tolerant of heat and aridity (Collins *et al.*, 2019), seems to have an advantage in this context. The salinity of the waters, ranging from 0.15 to 10.45 g/L (mean: 4.38 g/L), indicates a diversity of habitats ranging from fresh to moderately salty. This variability favours the coexistence of halotolerant species such as *An. melas* and *An. arabiensis*, an observation already confirmed in Nouakchott where *An. arabiensis* can survive up to 17.5 g/L NaCl (Ould Lemrabott *et al.*, 2020). The high conductivities recorded (275 to 19,590 µS/cm) confirm the significant mineralization of certain environments, probably due to the saline influence and the presence of halophilic plants such as *Sueda maritima*. The lack of correlation between larval density and parameters such as pH, temperature, salinity or conductivity suggests that these factors are not major limiting factors for larval development in the study area. This finding is consistent with several studies carried out in Sahelian or Sudano-Sahelian areas, where the larvae of *Anopheles gambiae* s.l. show a high ecological tolerance allowing them to occupy a wide range of habitats, whether permanent or temporary, fresh or brackish (Ould Lemrabott *et al.*, 2020; Sy *et al.*, 2023).

Finally, physical observations indicate that *Anopheles* colonize both large and small roosts, with higher densities in small, weakly vegetated habitats, as confirmed by Iro *et al.* (2020), Ould Lemrabott *et al.* (2020) and Noura *et al.* (2024). The simultaneous presence of *Culex* larvae and maggots, as well as signs of pollution and high organic load, suggest a degradation

of water quality that may influence species composition and larval productivity.

## CONCLUSION

The study highlighted a high spatial variability of *Anopheles* larval sites in the department of Saint-Louis, with a marked concentration in urbanized areas. Species of the *Anopheles gambiae* complex, especially *An. arabiensis*, show a high ecological tolerance to physicochemical variations in their habitats. This plasticity underlines the need for regular environmental monitoring and continuous adaptation of vector control strategies to the local context.

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