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Analysis of the Physicochemical and Biological Parameters of the Larvae Bridges of Malaria Vectors in the Commune of Lemba-Kinshasa, Democratic Republic of Congo (DRC)

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Abstract

Background and objective: The objective of this study was to evaluate the influence of environmental factors on the distribution and proliferation of breeding sites and larvae of Anopheles gambiae and Anopheles funestus in the commune of Lemba. *Methods*: 351 larval breeding sites including were identified and studied from April 15 to June 15, 2019. Five physicochemical parameters (pH, temperature, conductivity, turbidity and oxygen dissolved) were measured using a HI 9812-S brand Combo multiparametric probe. *Results*: The commune of Lemba is generally characterized by water with a basic pH (pH > 7). The pH amplitude of the waters of the Super-Lemba deposits is 2.06, with an average of 8.17 ± 0.42, while in Mbanza-Lemba (ML) the pH amplitude is 2.02, with an average of 7.87 ± 0.51. The breeding grounds of Super-Lemba (SL) larvae (29.12˚C ± 1.5˚C), with an amplitude of 6.60˚C. Turbidity varies with an amplitude of 814 in SL with an average of (328.19 ± 13.3) and 499.00 in ML with an average of (306.06 ± 11.6). Dissolved oxygen has a relatively low amplitude in ML, *i.e.*, 19.80 mg/l. Only the first factorial axis is explained because it expresses 100% of the total inertia. This implies that the abundance of anophelinae larvae in prospected breeding sites at SL can be explained by high values of conductivity, pH, turbidity and dissolved oxygen; while the proliferation of culicinae larvae in ML is linked to a high temperature of the waters of the breeding places. *Conclusion*: The temperature of the roosts, the PH of the roosts greater than 7 and the conductivity are at the basis of the proliferation of mosquito larvae in these two districts studied in the commune of Lemba.

Subject Areas

Preventive Medicine, Public Health

Keywords

Larval Breeding Sites, Anopheles, Environmental Parameters

1. Introduction

The impact of environmental factors, both biotic and abiotic, on mosquito development and behavior is well documented. Previous studies have shown that temperature and larval diet influence development time, survival rates, and the competence of culicidae to transmit infectious agents [1]. Water temperature is a determining factor in the rate of growth and development of immature mosquitoes. The ability to withstand high temperatures differs between species and larval stages [2]. Water temperature is positively correlated with larval density. The rate of development of A.gambiae sl from egg to adult has been shown to be enhanced when the temperature increases from 18˚C to 35˚C [3]. Regarding water turbidity, *A. gambiae* s.l. develop in both clear and turbid deposits. It breeds more abundantly in temporary, turbid water bodies, such as those formed by rain, unlike Anopheles funestus which prefer more permanent water bodies [4]. Taking into account the pH of larval habitats [5] revealed on the one hand that the acidic pH profile was responsible for high mortality for mosquito species in northern Nigeria. And on the other hand, that the cohabitation of mosquito larvae also influences the behavior of mosquitoes.

To date in the DRC, very few publications are focused on this issue. However, it is necessary to establish the physicochemical profile according to the ecological particularities of endemic areas where the disease is permanently present. The objective of this study was to determine the physicochemical and biological characteristics of the larval breeding grounds of malaria vectors in the two sites selected in the commune of Lemba.

2. Materials and Methods

2.1. Site of the Study

The present study was conducted in the city province of Kinshasa, capital of the Democratic Republic of Congo, precisely in the Commune of Lemba from April 15 to June 15, 2019. Larval surveys were carried out in two sites namely, super-Lemba and Mbanza-Lemba.

The commune of Lemba is bounded:

- To the north by the intersection of the Matete River with the axis of Kikwit Avenue up to its intersection with the axis of the circle of the interchange, the axis of the circle in the south and east directions up to its intersection with the axis of the intersection of the Matete River;

• To the east by the Matete River to its source. And a straight line between the source of the river and the southeast axis of the University of Kinshasa concession;

• To the south and west of this southwest point of the University of Kinshasa concession, the road axis which surrounds the said concession up to its intersection with By-pass which in turn goes up to the intersection with a straight line connecting the axis with the eastward bifurcation of the Yolo River. The Yolo River to its intersection with the axis of the Kikwit River.

The commune of Lemba is made up of 14 districts namely: Echangeur, Kimpwanza, Madrandele, School, Masano, Foire, Salongo, Livulu, Mbanza-Lemba, Kemi, Muli, Gombele, Commercial and Kinshasa.

2.1.1. Climatic Data from the Selected Sites

This is the representation of two curves relating to two main climatic parameters which are precipitation and temperature. A dry period is a period during which the total precipitation of the month expressed in millimeters is less than or equal to twice the temperature of the same months expressed in degrees Celsius (P ≤ double the temperature). The Gaussen ombrothermal diagram established for the study region makes it possible to determine two periods, one dry, it extends from May to September and the other which is humid, it extends from October to May. Indeed, the ombrothermal diagram makes it possible to specify dry and humid periods.

The raw data used come from the N'djili station (Station 64210 of the RVA Aeronautical Meteorology Division, 2013). They cover the period from January 11 to 29, 2019. The N'djili station is located in Kinshasa at coordinates 04˚22'S latitude, 15˚22'E longitude, 310 m altitude.

2.1.2. Biological Material

In order to enable us to carry out our study, the biological material consists of pre-imaginal forms (larvae and nymphs) of Culicidae taken from the predominantly anopheline breeding sites of the selected sites.

2.2. Methods

2.2.1. Prospecting for Lodgings

The prospecting of mosquito larvae breeding grounds was carried out on foot. It first included direct observation of the presence or absence of mosquito larvae [6]. This method made it possible to collect mosquito larvae in their development environment (breeding grounds). This involves collecting larvae from different stages of development.

Our field observations during sampling caught our attention because we noticed that certain sites contained a lot of insects, including mosquitoes. Indeed, the presence of plants in mosquito breeding grounds plays a role as a place of attachment or refuge for insects during bad weather, for example sunstroke, wind, precipitation and other movements. The leaves, for example, especially their lower faces, play the role of protection against intense insolation and wind.

The abundance of grass sometimes also explains the abundance of mosquitoes and therefore their larvae.

This is how we took the presence of plants as factors that could justify the abundance of mosquitoes in certain sites studied.

2.2.2. Collection of Larvae

If these immature stages were present, samples of larvae and nymphs were taken by the dipping method [7] [8] and using a 300 ml ladle. This method consisted of carrying out around ten dipper dives at several locations around the harvest point in order to maximize catches [8]. Thus, the pre-imaginal stages of captured mosquitoes were transferred to trays using transfer pipettes and then transported to coolers in the laboratory. The different larvae collected were then sorted and counted by subfamily (Anophelinae or Culicinae). A total of 200 breeding grounds for mosquito larvae were identified in Mbanza-Lemba and 151 in Super-Lemba.

2.2.3. Physicochemical Analyzes of Data

Five physicochemical parameters (pH, temperature, conductivity, turbidity and dissolved oxygen) were measured in the laboratory after water sampling at each site. These different parameters of the physicochemical analysis of water were measured using the HI 9812-5 multi-parameter device.

2.2.4. Ethical Considerations

The ethical principles linked to respect. The research protocol was presented to the staff and the local ethics committee. He received a favorable opinion.

2.2.5. Data Processing and Analysis

The calculation operations were carried out in Microsoft office Excel office professional version 2019 and, on the other hand, most of the statistical calculations as well as the graphs were carried out using Xlstat 2019. The thematic maps were developed using Arc Gis 10. All the data analyzed were previously subjected to the Shapiro-Wilk (W) test in order to check whether they follow a normal law or not. This test helps determine the type of analysis to perform. Thus, to give credibility to the data from our field observations, we used three types of analyzes and the use of the Chi 2 test.

The variability of larval densities of the different samples was tested by a one-way ANOVA. ANOVAs that proved significant at the threshold of $p < 0.05$ were completed by a Fisher post hoc test.

The canonical correspondence analysis made it possible to compare the behaviors of the breeding sites due to their respective physicochemical compositions in relation to the larval densities of the mosquitoes. This ACC was carried out to detect possible groupings between larval densities and the different explanatory variables which influence their occurrences in the sites studied in this work.

S = Number of species contained in the sample P_i = frequency of species i n_i: number of individuals of a species of rank i. This index has the unit of the bit, its value depends on the number of species present, their relative proportions and the logarithmic base H' is minimal when it is equal to zero, that is to say when the sample contains a single species. It is maximal (theoretically infinite) when all individuals belong to different species, in this case H' is equal to log2(S).

3. Results

3.1. Physicochemical Characterization of the Deposits Studied

3.1.1. Hydrogen Potential (pH)

Figure 1 indicates that most of the deposits identified at Super-Lemba and Mbanza-Lemba are generally characterized by waters with a basic pH ($pH > 7$). Indeed, the pH amplitude of the waters of the Super-Lemba deposits is 2.06, with an average of 8.17 ± 0.42, while in Mbanza-Lemba the pH amplitude is 2. 02, with an average of 7.87 ± 0.51. However, it appears from the Student's test that the waters of the larval breeding sites of Super-Lemba have a significantly higher pH than that of Mbanza-Lemba ($t = 5.90$; $p < 0.001$) (Figure 1).

3.1.2. Temperature

Figure 2 indicates that, the low average temperature value is observed in the breeding grounds of Super-Lemba larvae (29.12˚C ± 1.5˚C), with an amplitude of 6.60˚C; and the largest is recorded at Mbanza-Lemba (31.59˚C ± 3.4˚C), with an amplitude of 16.00˚C. This reflects a significant variation in temperature at the level of the larval breeding grounds of Mbanza-Lemba. Indeed, the Student test attests to a significant difference between the temperature averages observed in the two neighborhoods considered in this study ($t = 8.47$; $p < 0.001$). This different information is provided in Figure 2 bellow.

3.1.3. Conductivity

As shown in Figure 3, it can be seen that the conductivity varies considerably within each district, with an amplitude of 1674.40 in Super-Lemba with an average of (645.07 ± 25.8) and 999.00 in Mbanza-Lemba with an average of (614.44 ± 22.5). However, the Student's t test does not indicate a significant difference between these two districts regarding the conductivity of the water of the mosquito larval breeding grounds ($t = 1.20$; $p = 0.230$). The role of water conductivity is evident on the distribution of mosquito species. Thus, species will choose between environments with low conductivities or high conductivities. The induction of hatching for certain species would be conditioned by the conductivity of the water of the roost.

3.1.4. Turbidity

As shown in Figure 4, it shows that turbidity varies considerably within each district, with an amplitude of 814 in Super-Lemba with an average of (328.19 ± 13.3) and 499.00 in Mbanza-Lemba with an average of (306.06 ± 11.6). However, the Student test does not reveal any significant difference between these two neighborhoods in terms of the turbidity of the water in the mosquito larval breeding grounds ($t = 1.73$; $p = 0.084$). Concerning turbidity, several studies carried out in Africa show that this factor indirectly affects larvae and cannot be discussed in relation to larval densities.

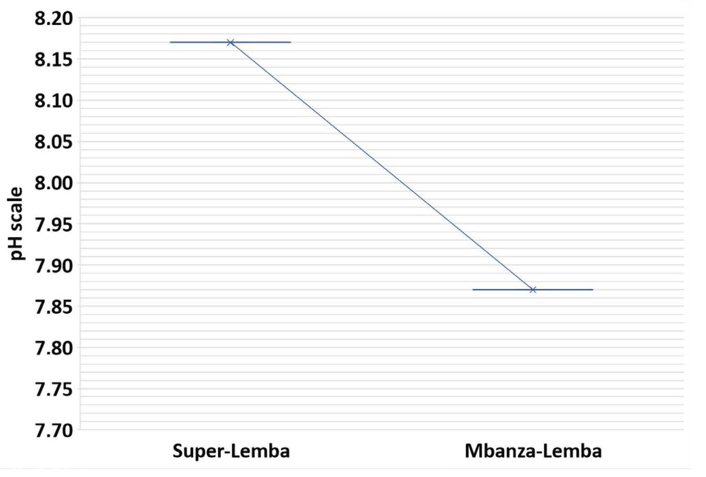

Figure 1. Hydrogen potential (pH).

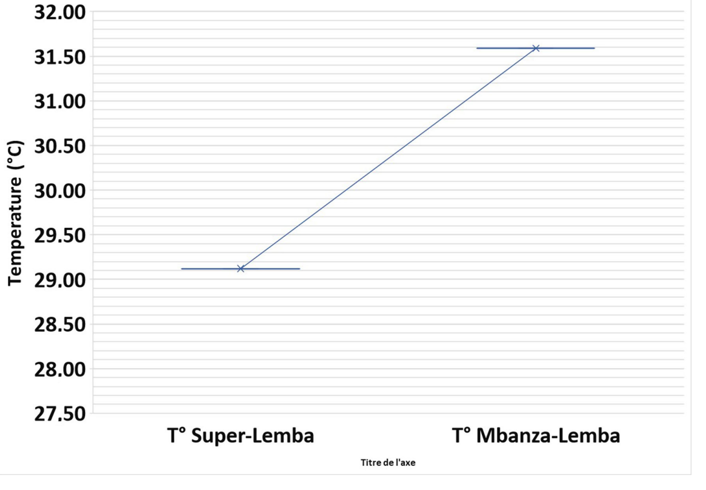

Figure 2. Temperature variation depending on the sites.

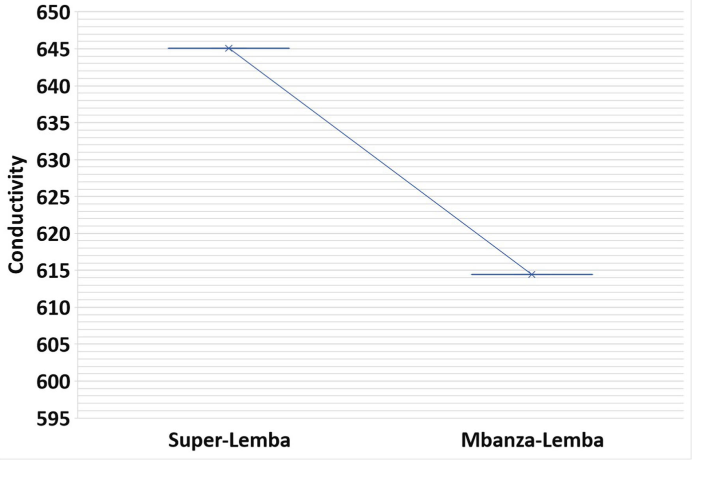

Figure 3. Variation in conductivity depending on the sites.

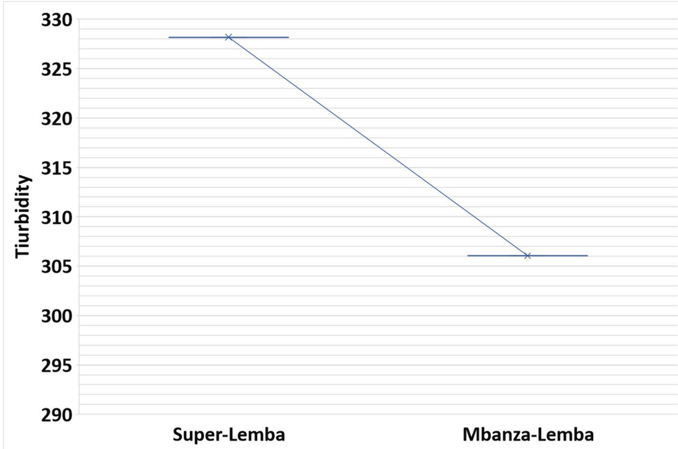

Figure 4. Variation in turbidity depending on the sites.

3.1.5. Dissolved Oxygen

As shown in **Figure 5**, most Dissolved oxygen has a relatively low amplitude in Mbanza-Lemba, *i.e.*, 19.80 mg/l, with an average of 80.90 mg/l ± 3.6 mg/l. On the other hand, at Super-Lemba, it was high, *i.e.*, 81.88 mg/l, with an average of 79.48 mg/l ± 3.8 mg/l. **Figure 6** reveals that there are some larval breeding sites in Super-Lemba that have low and atypical dissolved oxygen values. This is the reason why a significant amplitude of dissolved oxygen was observed there. However, the Student's test does not reveal any significant difference between Super-Lemba and Mbanza-Lemba in terms of the dissolved oxygen content of the waters of the mosquito breeding grounds ($t = 1.49$; $p = 0.138$).

3.2. Influence of Physicochemical Parameters on the Distribution of Mosquito Larvae in Different Types of Breeding Sites Vegetation Analysis

The Canonical Correspondence Analysis (CCA) which explains the influence of physicochemical parameters on the larval density of mosquitoes in the two neighborhoods studied. Only the first factorial axis is explained because it expresses 100% of the total inertia. Indeed, this axis reveals that the abundance of anophelinae larvae in prospected breeding sites at Super-Lemba is explained by high values of conductivity, pH, turbidity and dissolved oxygen; while the proliferation of culicinae larvae in Mbanza-Lemba is linked to a high temperature of the waters of the breeding places.

3.3. Vegetation Analysis

Vegetation plays a role as a biotic and physical indicator, taking into account its relationships with hydromorphy, the structure and texture of the soil but also its links with the different larval breeding sites to which the distribution of anopheles is dependent to clearly illustrate the variations of this floristic composition, we have represented it both in number of species and in number of families. The family Cyperaceae and Onangaceae predominate with the same identical specific abundance (2 species) as well as the same species richness 12.5%.

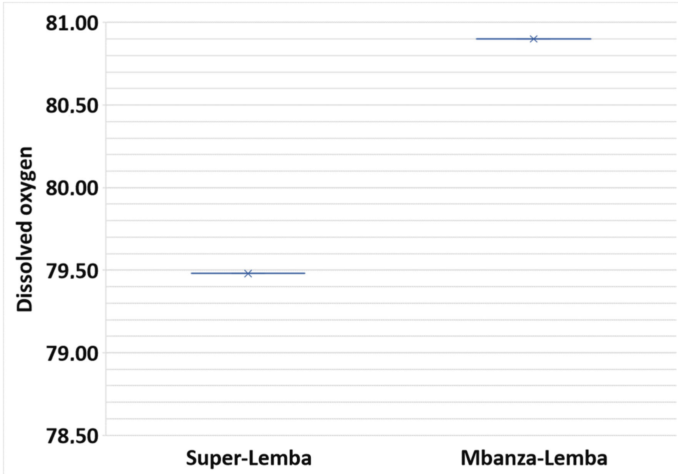

Figure 5. Variation in dissolved oxygen depending on the sites.

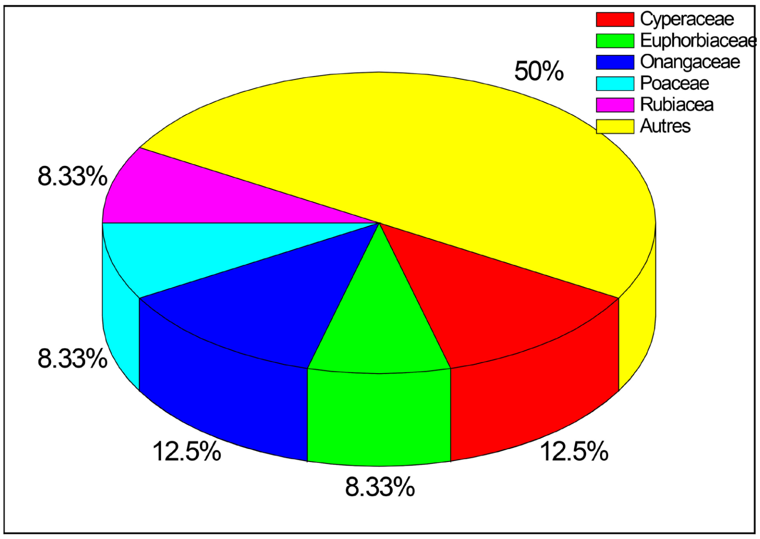

Figure 6. Specific wealth.

They are followed by the family Euphorbiaceae, Rubiaceae and Poaceae with identical specific abundances (2 species) as well as the same species richness (8.33%). The remaining families are very weakly represented with fewer individuals (one species). The cumulative specific wealth of these families represents 50% of the flora collected. As for auto-ecological characteristics, MPH and TD predominate with a richness of 13.9% each. They are followed respectively by GRH and TDH with 11.1% and 8.33%. Autoecological characteristics represent approximately 52%.4. This different detail is provided in **Figure 6** bellow.

3.4. Diversity Indices

The results relating to the Shannon-Wever diversity indices and Pielou's Equitability (E) applied to the species trapped using the Barber pots in each of the study stations are given in this subparagraph. The Shannon-Wever H' index is 2.8 bits on the Mbanza Lemba site and 2.5 bits on the Super Lemba site. The low diversity index (H’) found in the two sites coincides, on the one hand, with the low numbers of families and the low specific richness. The difference observed between the values of this index on the two sites seems due to the dominance of certain families, in particular: Poaceae, Asteraceae, Euphorbiaceae, Fabaceae and Onagraceae. However, it is difficult to establish a direct link between floral diversity and the abundance of mosquito larvae per breeding site. The reduced number of species, as well as the very strict distribution of individuals in space, reflect the extreme conditions that reign in these environments (specific adaptation). Regarding the J’de Piélou fairness index, the spatial evolution of the J’de Piélou fairness varies very little between the two sites, it is 0.94 bits on the Mbanza Lemba site and 0.98 bits on the Super Lemba site. These values also testify to a regularity of populations at the level of all the facies of the sites explored. However, equitability seems slightly higher for the Super lemba site while the diversity index is equivalent to the lowest. Indeed, in most sites the evolution of fairness seems to follow the opposite direction of the evolution of the diversity index H’. This different information is provided in **Figure 7** bellow.

4. Discussion

The larval surveys carried out in this work showed that the study area has a significant density and diversity of larval breeding sites. The majority of these larval breeding sites were created by humans due to their activities (agricultural activities, artificial water surfaces, etc.). These breeding sites, particularly those with at least one larva, are found in the immediate environment of human populations. In addition, these breeding sites are conducive to the development of Anopheles mosquitoes which are major vectors of numerous pathogens responsible for numerous pathologies including malaria [9]. Identical results were obtained by Saotoing *et al.* [9] who noted that the immature stages of anophelinae have great ecological elasticity and are capable of colonizing different environments. These results Tia *et al.* [5] who showed the responsibility of residents in establishing conditions conducive to the development and maintenance of mosquitoes through the creation of their larval habitats. Also, the proximity of mosquito development sites to homes could constitute a health risk and nuisance for surrounding human populations. The presence of these larval breeding sites could

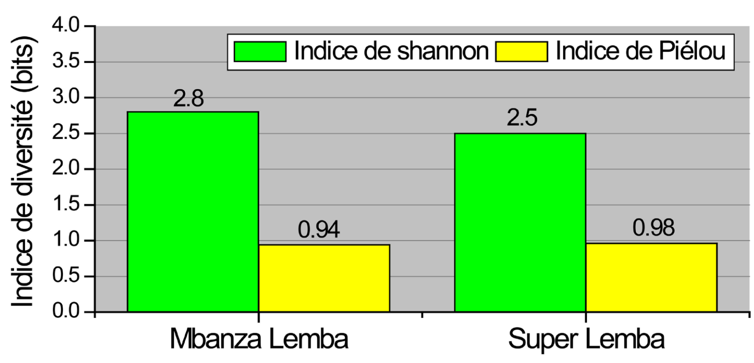

Figure 7. Diversity indices.

increase the risks of transmission of other infectious agents by these mosquitoes. During this study, larval production was higher because the study was conducted in the rainy season. This abundance is probably linked, on the one hand, to the high quantity of permanent larval breeding sites present in the study area and, on the other hand, to the fact that the rainy season offers opportunities for choice of breeding sites for females of different species of mosquitoes both in quality and quantity. The abundance of Anopheles could also be explained by the level of the nature of the larval breeding sites and by the resilience capacity of the larvae. These observations corroborate those made by Korba *et al.* [10] in Algeria who showed that anophelines have strong adaptation capacities allowing them to develop in several types of habitats. Identical results were obtained by Saotoing *et al.* [9] who noted the great ecological elasticity of Anopheles and their ability to colonize different environments. Moreover, the larval breeding sites colonized during this study varied (puddles, ponds, ponds, water storage containers). These breeding sites were mostly characterized by the presence of papers, bottles, plant debris, etc. According to, Alami *et al.* [11], the proliferation of mosquito species is conditioned by the nature of the larval breeding ground. The nature of the site favors one or other species depending on the characteristics of the site: stagnant or current, polluted or not, devoid of or rich in vegetation [11] [12]. Also, the physicochemical parameters showed a positive correlation with the development and proliferation of mosquito larval populations. Indeed, the development of mosquitoes is conditioned by the temperature and the biological or chemical composition of the water [13]. The results obtained reveal the importance of these two parameters (temperature and pH) on the appearance of fluctuation in mosquito larval populations. Likewise, temperature remains essential in terms of increasing the speed of development of the mosquito [14].

According to the work carried out by Betsi *et al.* [14] and Sy *et al.* [15], females of *A. gambiae* s.l. prefer to lay their eggs in sunny, vegetation-free water collections. These females also have a positive tropism for clear fresh water and brackish water for the deposition of their eggs.

According to the WHO [16], larval development can be affected by temperature. For *A. gambiae* s.l., A. funestus, for example, the larvae grow better at temperatures ranging from 15.7˚ to 36˚C [16] [17]. Which agrees with our observations. Concerning conductivity, the results showed that the induction of hatching for certain species would be conditioned by the conductivity of the water in the roost. These results corroborate those found by Kasereka [18] confirming the existence of a significant correlation between larval density and conductivity. Furthermore, the speed of larval development was significantly correlated with the quantity of dissolved oxygen in the waters of the roosts. According to Berchi [19], dissolved oxygen plays a fundamental role in the dynamics of mosquito larval densities. This confirms our results because the amounts of dissolved oxygen in all larval breeding sites are high.

5. Limitations of Study

The non-inclusion of the sociodemographic characteristics of the surrounding inhabitants because the proximity of mosquito development sites to homes could constitute a health risk and nuisance for human populations: the presence of these larval breeding sites could increase the risks of transmission of other infectious agents by these mosquitoes. The absence of an entomological and parasitological analysis to assess the level of malaria transmission within the local population.

6. Conclusion

This study whose main objective was to highlight the influence of environmental parameters on the development of *Anopheles gambiae* s.l. larvae. shows that the average temperature of all larval breeding sites is 30˚C and the larvae develop in environments with a basic pH, which conditions the dynamics of larval populations. According to the ACC, the high temperature of the waters of the breeding sites turns out to be the explanatory parameter for the proliferation of culicinae larvae in Mbanza-Lemba while the abundance of anophelinae larvae in prospected breeding sites in Super-Lemba is explained by significant values of conductivity, pH, turbidity and dissolved oxygen.

Additional Information

Disclosures

All authors have confirmed that this study did not involve animal sub-jects or tissue.

Contribution of Authors

All authors confirmed having participated.

Conflicts of Interest

The authors declare no conflicts of interest.

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