

Entomological study of Anopheles vector bionomics and persistent malaria transmission at National Malaria Control Program Sentinel sites in the Democratic Republic of the Congo

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ABSTRACT

Introduction

In the Democratic Republic of the Congo, the malaria transmission landscape is influenced by diverse bioclimatic conditions, leading to varied transmission patterns across the country.

Purpose

The primary objective of this study was to investigate the bionomics of malaria vectors across 22 NMCP sentinel sites. By analyzing the different malaria transmission facies, we aimed to provide detailed insights into vector behavior and abundance to inform more effective malaria control strategies.

Methods

Mosquitoes were captured at 22 sentinel sites, with sampling conducted in 20 randomly selected households per site. These households were divided into two groups: ten utilized human bait traps, and the remaining ten employed pyrethrum spray catches. The captures were performed over five consecutive nights every six months. A total of 1,723 *Anopheles* mosquitoes were collected for analysis.

Results

The study identified five *Anopheles* species, with *Anopheles gambiae* s.l. being the most abundant and primary vector, representing 75% of the total captures. The other species included *An. funestus* group (15%), *An. paludis* (12%), *An. coustani* (0.3%), and *An. nili* (0.2%). Behavioral observations revealed that *An. gambiae* s.l. is active both indoors and outdoors, with peak biting occurring from 9 p.m. to 4 a.m., and an average biting density of 12 bites per person per night. 13209 mosquitoes (i.e., 88.5%) were captured using the HLC method, while, among the 1,723 mosquitoes captured using the PSC method, 51% were blood-fed, 24% were semi-gravid, 18% were gravid, and 7% were fasting. *An. gambiae* s.l. was found to be present year-round, contributing to continuous malaria transmission.

Conclusion

This study provides crucial data on the prevalence and behavior of malaria vectors, particularly emphasizing the role of *Anopheles gambiae* s.l. as the primary vector in malaria transmission. The findings highlight significant differences in bite rates among species and the year-round activity of *An. gambiae* s.l., underscoring the need for targeted vector control measures. The data will be used to refine and implement effective vector control strategies, such as long-lasting insecticide-treated nets (LLINs) and indoor residual spraying (IRS), to reduce malaria transmission and mortality rates at NMCP sentinel sites.

INTRODUCTION

Malaria remains a critical public health issue, particularly in sub-Saharan Africa, where it significantly impacts morbidity and mortality rates (De Silva & Marshall, 2012; O'Meara et al., 2010; Tusting et al., 2017; Yimam et al., 2021). This disease is caused by parasites of the genus *Plasmodium* and transmitted through the bite of female *Anopheles* mosquitoes (Nundu et al., 2021; Riveron et al., 2018). Despite significant global health efforts, malaria continues to be a leading cause of illness and death worldwide, with an estimated 249 million cases and 608,000 deaths reported in the latest World Malaria Report (World Health Organization [WHO], 2023). The African continent bears the brunt of this burden, accounting for 94% of global malaria cases and 95% of related deaths (WHO, 2021, 2022, 2023). Within Africa, countries like Nigeria, the Democratic Republic of the Congo (DRC), Uganda, and Mozambique report the highest incidence and mortality rates (WHO, 2021, 2022, 2023). In the DRC, the National Malaria Control Program (NMCP) reported 27,296,419 cases and 24,880 deaths in 2022, highlighting a significant increase compared to previous years (Programme National de Lutte contre le Paludisme [PNLP], 2022).

Long-lasting insecticidal nets (LLINs) and indoor residual spraying (IRS) are primary interventions, but their effectiveness is compromised by the rise of insecticide resistance and changes in vector behavior, such as increased outdoor biting (Kouassi et al., 2023; Lelisa et al., 2017). Entomological surveillance is crucial for optimizing malaria control strategies. Accurate data on vector distribution, abundance, and behavior are necessary to adapt interventions to local conditions. Sentinel site-specific studies provide valuable insights into these aspects, but there remains a research gap in understanding vector bionomics at these sites. This lack of detailed, site-specific information hinders the development of targeted and effective vector control measures.

Current strategies, including the introduction of new LLINs with synergistic agents like pyrethroid-PBO and targeted IRS, are informed by entomological data (N'do et al., 2021; Zanga et al., 2022). However, the effectiveness of these interventions varies due to the heterogeneity in malaria transmission and vector resistance patterns (Matubi et al., 2020; Nguiffo-Nguete et al., 2023). Thus,

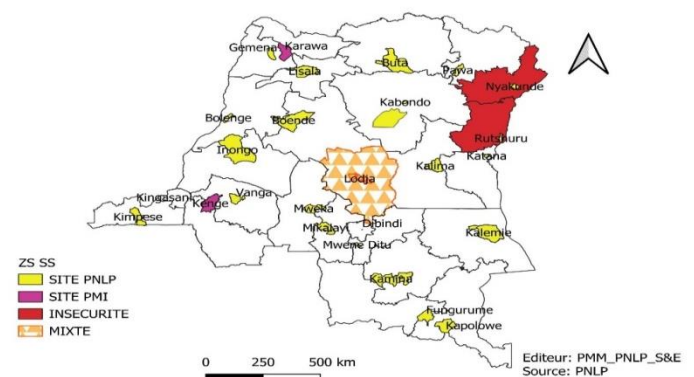
continuous entomological monitoring is essential to adapt strategies to evolving vector dynamics and environmental conditions. This study aims to address the research gap by conducting a comprehensive entomological survey at sentinel sites over three years (2021 to 2023). By examining vector species composition, biting behavior, and resistance profiles, the study will provide crucial data to guide the optimization of malaria control strategies and enhance the effectiveness of intervention programs. Understanding these dynamics will inform more targeted and effective malaria control measures, ultimately contributing to better health outcomes in endemic regions.

METHODS

Study Locations

The study was conducted in 22 Democratic Republic of the Congo (DRC) NMCP sentinel site health zones.

Figure 1:
NMCP/DRC sentinel sites



Random Selection of Households

To ensure a representative sample and reduce selection bias, households were randomly selected from each sentinel site. The process began with a comprehensive household list provided by local health authorities. From this list, households were chosen using a random number generator to ensure that every household had an equal chance of being included. The selection process was stratified to include various neighbourhoods and socio-economic groups within each site to account for potential variability in malaria transmission and vector bionomics.

Criteria for Household Selection and Controls

Inclusion Criteria: Households were included if they were located within the designated sentinel site boundaries and

had been present in the area for at least six months to ensure stability in vector populations.

Exclusion Criteria: Households were excluded if they had recently undergone major construction or renovation, which could potentially alter local vector habitats or behaviours.

Geographical Controls: The random selection was balanced to include households from both urban and rural areas within each sentinel site, aiming for consistency in environmental conditions.

Temporal Controls: Data collection was conducted during the same period across sites to minimize seasonal variations in vector activity and malaria transmission.

Methods for Data Collection

Human Landing Catch (HLC)

To evaluate mosquito biting time, trophic behaviour, and biting rate, and to monitor species composition, the Human Landing Catch method was used (Hii et al., 2000). Trained community relays (Reco) captured adult mosquitoes over five consecutive nights in different dwellings, i.e., a total of 10 dwellings per half-year. One person was placed inside, and another outside each dwelling as capturers, taking turns after six hours. All *Anopheles* mosquitoes collected by HLC were identified to species level using morphological characteristics and a dichotomous key (Gillies & Coetzee, 1987). Quality control was performed by entomologists from the NMCP and the Kinshasa University School of Public Health. The *Anopheles* mosquitoes were preserved in 1.5 ml Eppendorf tubes on silica gel for further molecular analysis by the Kinshasa School of Public Health.

Figure 2:
Human landing catch



Pyrethrum Capture (PC)

Pyrethrum capture (PC) was carried out in the morning from 6:00 to 9:00 a.m. in the same areas as the HLCs but in different houses to estimate the resting density (Thomson, 1947). Before the PCs were conducted, all occupants were asked to leave their homes. The rooms were sprayed with a commercially available aerosol containing pyrethroids and PBO to stun any mosquitoes resting inside the dwellings. Twenty minutes after spraying, all mosquitoes that had fallen on their backs were collected on a white sheet spread over flat surfaces. The female *Anopheles* mosquitoes were classified according to four physiological states of the abdomen (fasting, gorged, semigravid, and gravid). After morphological identification in the field, each collected mosquito was correctly labelled and placed in an Eppendorf tube containing silica gel. Identification was verified by an NMCP entomologist upon returning to Kinshasa.

Figure 3:
Pyrethrum capturing



Limitations of Methods

Human Landing Catch (HLC)

Ethical Concerns: HLC involves direct contact with mosquitoes, posing a risk of malaria transmission to collectors. Proper precautions and antimalarial prophylaxis are essential to mitigate this risk (Hii et al., 2000).

Bias: HLC may not capture mosquitoes that bite outside the sampling period or those that do not bite humans (Gillies & Coetzee, 1987).

Labour-Intensive: The method requires significant manpower and training, which may not be feasible in all settings.

Pyrethrum Spray Catch (PSC)

Limited Coverage: PSC only captures mosquitoes that rest indoors, potentially missing vectors that prefer outdoor resting sites (Thomson, 1947).

Interference from Vector Control: The presence of insecticide-treated surfaces can affect collection efficiency, potentially leading to underestimation of mosquito populations.

Seasonal Variation: The effectiveness of PSC can be influenced by seasonal changes in vector behaviour and environmental conditions (Thomson, 1947).

Entomological Indicators of Malaria Transmission

In specific locations, several factors influence the transmission of malaria to humans. Quantitative estimates of transmission are based on a number of mathematical indices calculated from data collected during entomological surveys.

Period of Aggressiveness

This refers to the proportion of aggressive attempts by adult female vectors inside or outside dwellings at a particular time during their period of activity at night.

Aggressiveness (ma)

The bite rate or aggressiveness represents the number of bites per person per unit of time. The average bite rate per person was calculated by dividing the average number of mosquitoes collected per site by the number of trap nights. The indoor and outdoor ma were expressed per night and disaggregated by mosquito species. It was assessed both inside and outside households. Aggressiveness was determined on the basis of nightly mosquito captures (Initiative Malaria Elimination, 2020).

Place of Aggressiveness

This is the proportion of successful attempts at aggression or blood-feeding by adult female vectors inside and outside dwellings per unit of time.

Aggressiveness Rate

The aggressiveness rate is also known as the product of anopheline density in relation to humans (m) and the anthropophilicity rate (a). It is expressed as the number of *Anopheles* bites per human per unit of time. It is obtained by dividing the total number of *Anopheles* captured by the number of individuals used per unit of time.

Physiological Status

This refers to the four states of the abdomen: fasting, gorged, semigravid, and gravid, of *Anopheles* mosquitoes captured during collection.

Ethical Considerations

Ethical approval for the HLC method was obtained from the relevant research ethics committee before the study. Informed consent was obtained from all participating household members, who were provided with detailed information about the study's objectives, procedures, and potential risks associated with HLC. Consent forms clearly

outlined the measures taken to minimize risks, including the use of prophylactic antimalarial drugs by the collectors and strict adherence to safety protocols. Participants were assured of their right to withdraw from the study at any time without consequence. To ensure confidentiality, all personal data collected were anonymized, and results were reported in aggregate form to protect participants' identities. The study adhered to international ethical guidelines for research involving human subjects, with ongoing assessments to maintain ethical standards throughout the study.

RESULTS

The map (Figure 1) illustrates the distribution of sentinel sites for mosquito species in the Democratic Republic of the Congo (DRC) from 2021 to 2023, showing areas under the National Malaria Control Program (PNLP), regions marked as insecure, areas under the President's Malaria Initiative's Evolve project (PMI-Evolve), and regions with overlapping initiatives. The pie charts represent the composition of different *Anopheles* mosquito species at each site, with *An. gambiae* sl. (green) being the most predominant species across most sites, followed by *An. funestus* grp. (yellow), *An. paludis* (blue), *An. coustani* (turquoise), and *An. nili* (light blue). Regions like North Kivu and Ituri are marked for insecurity, while areas such as Sankuru and parts of Kasai Central show overlapping initiatives from PNLP and PMI-Evolve, indicating coordinated malaria control efforts. The prevalence of *An. gambiae* sl. highlights its significant role in malaria transmission in the DRC.

Figure 4: Diversity of female *Anopheles* species obtained by night capture on humans (HLC) and day capture with pyrethrum in houses (CP), DRC

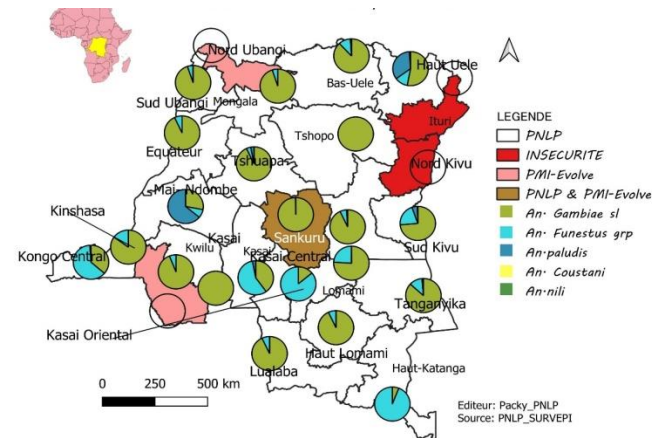


Figure 4 shows the diversity of female *Anopheles* species obtained by night capture on humans (HLC) and day capture with pyrethrum in houses (CP) in the DRC.

Table 1: Number of anopheles collected at sentinel sites by capture on human bite from 2021-2023 inside and outside homes and by pyrethrum

SPECIES	HLC		PC	Total
	INSIDE	OUTDOOR		
<i>An. gambiae</i> sl	5277	4591	1194	11062
<i>An. funestus</i> grp	807	989	322	2118
<i>An. nili</i>	18	11	3	32
<i>An. paludis</i>	663	816	198	1617
<i>An. coustani</i>	16	21	6	49
Total	6781	6428	1723	14932

Table 1 provides the number of *Anopheles* collected at sentinel sites by human bite capture from 2021-2023, inside and outside homes, and by pyrethrum capture.

The study collected a total of 14,932 mosquitoes, with *An. gambiae* sl. being the most prevalent species (11,062 specimens), followed by *An. funestus* grp. (2,118 specimens). *An. nili* was the least collected (32 specimens). Notably, *An. gambiae* sl. was found predominantly indoors, indicating a high risk of indoor malaria transmission. The overall distribution showed 6,781 mosquitoes collected indoors, 6,428 outdoors, and 1,723 by pyrethrum capture (PC). These findings highlight the critical need for targeted vector control strategies, emphasizing indoor interventions such as long-lasting insecticidal nets (LLINs) and indoor residual spraying (IRS) to address the high density of *An. gambiae* sl. Additionally, the low numbers of *An. nili* suggest it may have a less significant role in transmission compared to other species. Public health strategies should consider these species-specific patterns to optimize malaria control efforts and reduce transmission effectively.

The overall analysis of variance (ANOVA) comparing mosquito counts inside and outside revealed an F-statistic of 0.0029 and a p-value of 0.9583, far beyond the 0.05 threshold. This indicates the absence of a statistically significant difference between the mean counts in the two environments for the studied species, suggesting that the observed variations are likely due to chance rather than an actual difference between inside and outside. These results have important public health implications, particularly concerning malaria transmission. The transmission risk

appears similar indoors and outdoors, implying that prevention measures, such as using bed nets and indoor spraying, should also extend to outdoor spaces. It is essential to target mosquito breeding sites outdoors, such as stagnant water, by implementing environmental management actions like draining marshy areas. Furthermore, educating populations about the importance of protecting themselves both indoors and outdoors, especially during mosquito activity hours, is crucial. Continuous monitoring of mosquito populations and their distribution is also necessary to adjust control strategies based on seasonal or regional variations. These findings highlight the importance of an integrated approach to malaria control, combining actions both indoors and outdoors.

Additionally, the calculated t-statistic is approximately 0.996 with 10 degrees of freedom, and the associated p-value is greater than 0.05. Therefore, the null hypothesis cannot be rejected, indicating that there is no statistically significant difference between the means of the two groups (HLC and PSC). The observed differences can be attributed to chance, suggesting that the two methods do not exhibit significant variation in terms of measured averages.

The period of aggressiveness of *Anopheles* captured outdoors and indoors by HLC is shown in **Figure 5**. These graphics show the locations of bites and the period of aggressiveness of *Anopheles* captured by the CAH technique in the 22 sentinel sites of the NMCP/DRC. From these 22 sentinel sites, it emerges that in 8 sites the *Anopheles* exhibited exophagic behavior, while in the remaining 14 sites, they remained endophagic.

Table 2 provides an overview of *Anopheles* mosquito collection efforts using Human Landing Catches (HLC) and night traps across 22 sites in various regions. A total of 14,366 mosquitoes were collected, with the highest numbers found in Kamina (1,604) and the lowest in Kimpese (105). The number of traps varied by site, with most regions using 40 to 80 traps. The mosquito aggression rate toward humans, measured as the Human Biting Rate (HBR), ranged from 2 to 20 bites per night, with Kamina recording the highest HBR (20) and Inongo the lowest (2). This variability highlights significant

regional differences in mosquito activity and human exposure to bites, emphasizing areas where malaria prevention efforts may be most needed.

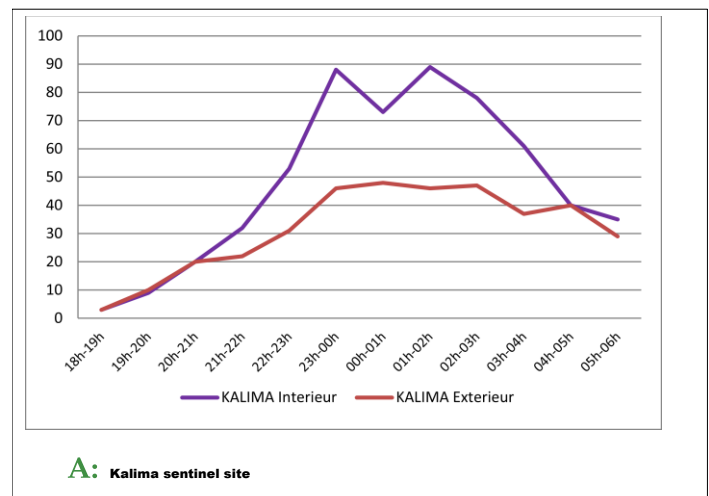
Table 2 shows the average number of bites per person per night at the 22 sentinel study sites. The *Anopheles* bite rate was particularly high at 8 sites, namely, Kamina (20), Inongo (17), Kalima (13), Mikalayi (13), Kapolowe (12), Lodja (11), Mweka (11), and Kalemie (11). The average *Anopheles* bite rate was less than 11 at the remaining 14 sites. The average for all sites was 12 bites/night/person.

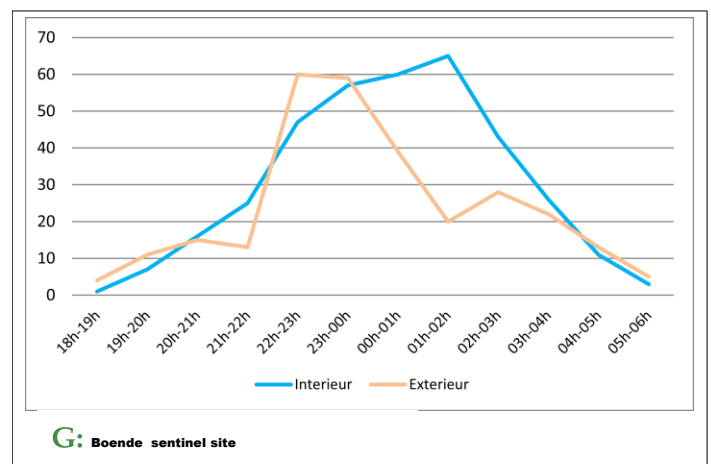
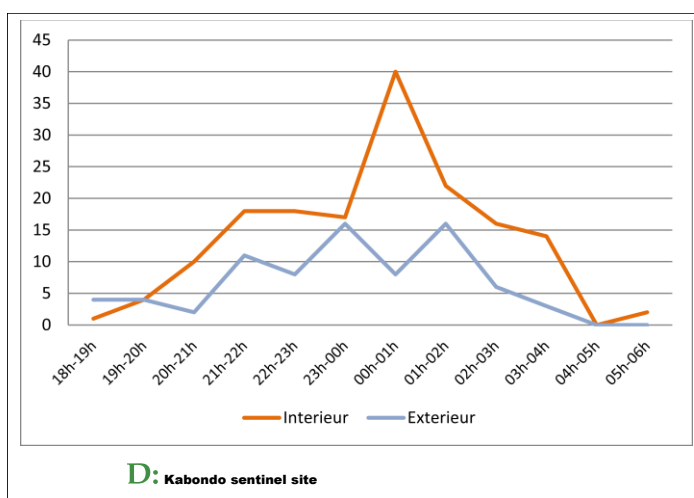
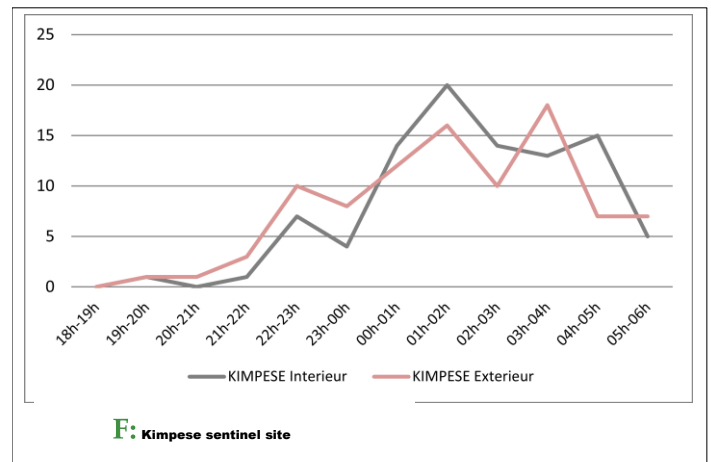
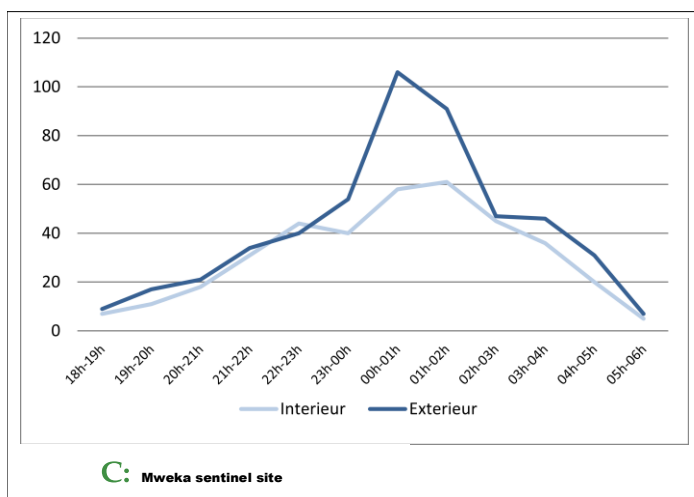
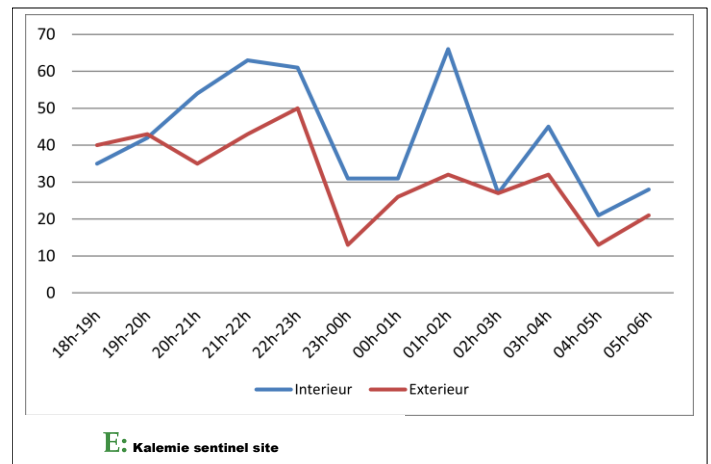
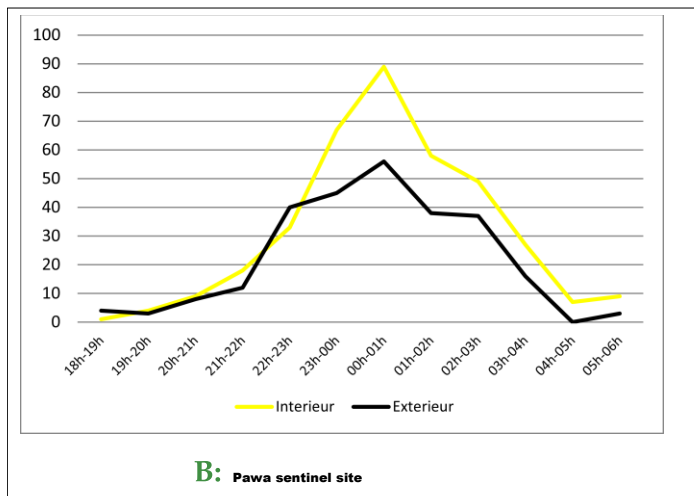
Figure 6 shows the physiological status of the abdomen of *Anopheles* captured by PC. At all sites, the majority of the *Anopheles* collected by CP were blood-gorged (50%, 874/1723), followed by semi-gravid (24%, 411/1723), gravid (19%, 321/1723), and fasted (7%, 117/1723) individuals. This suggests strong contact between humans and vectors.

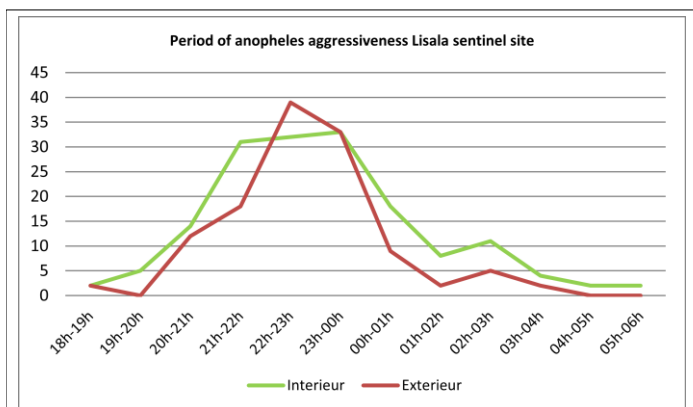
Period of aggressiveness of anopheles captured outdoors and indoors by HLC

The various figures below (**F.5 A-S**) show the periods of aggressiveness of anopheles captured outdoors and indoors by HLC.

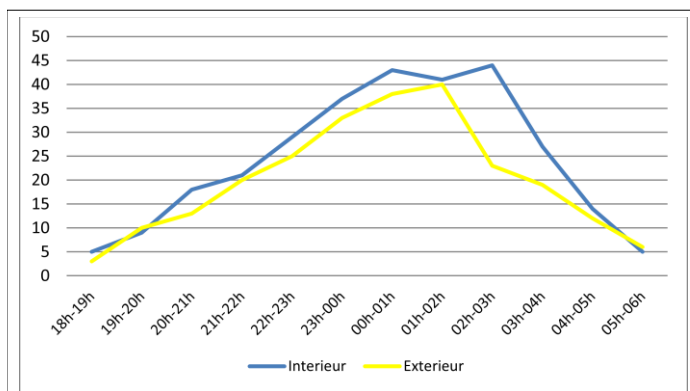
Figure 5:
Period of aggressiveness of anopheles caught per site by HLC



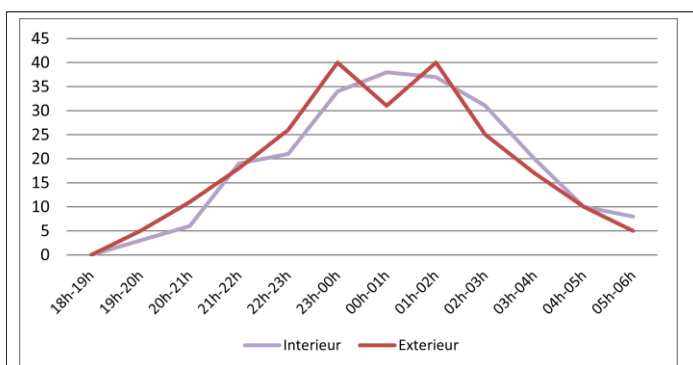




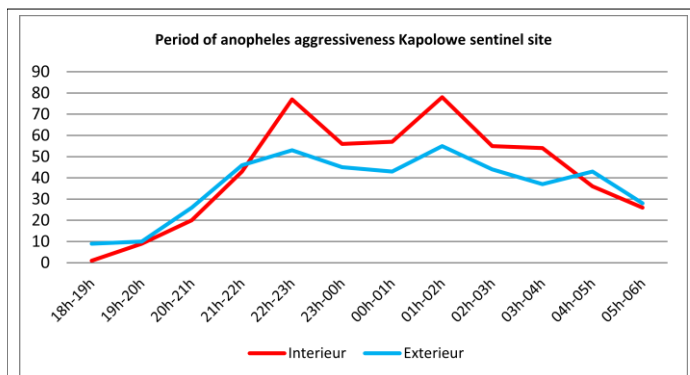
H: Lisala sentinel site



K: Katana sentinel site



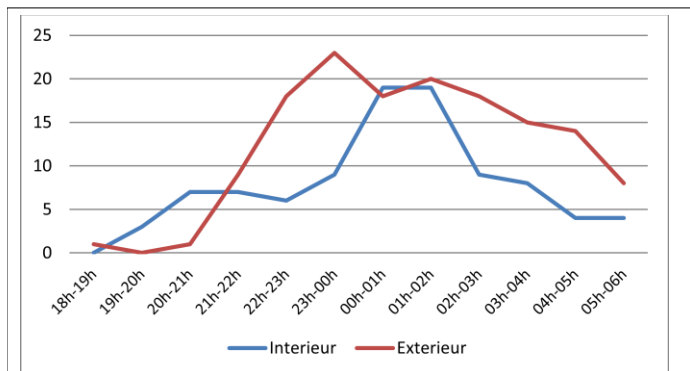
I: Gemena sentinel site



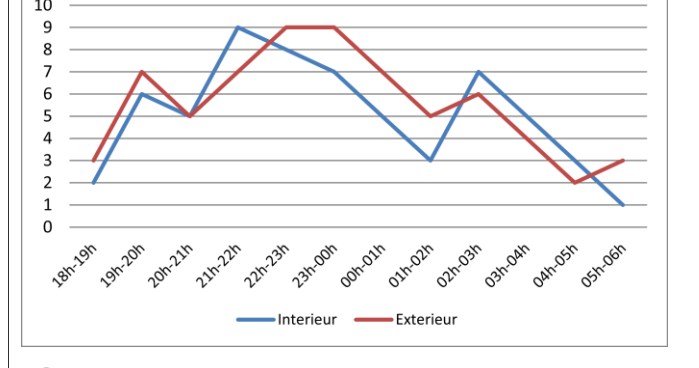
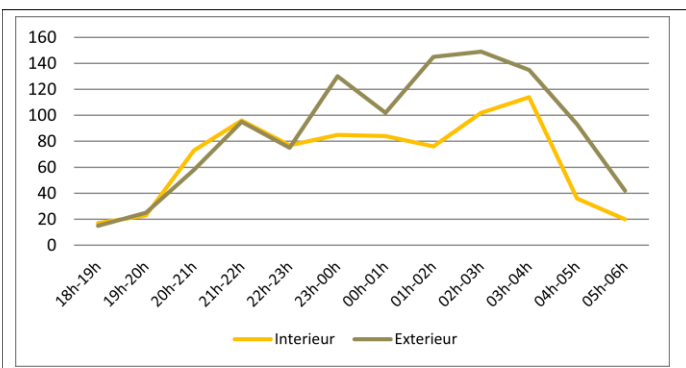
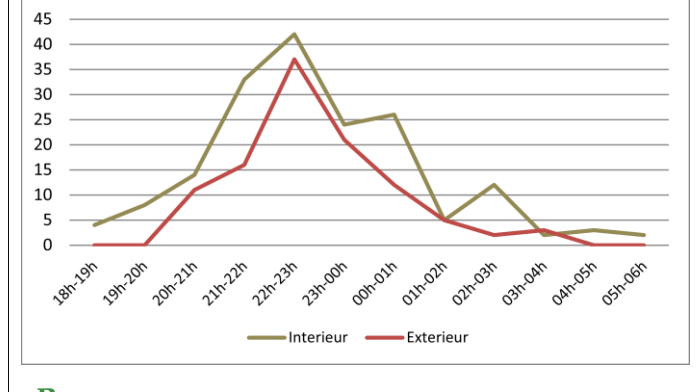
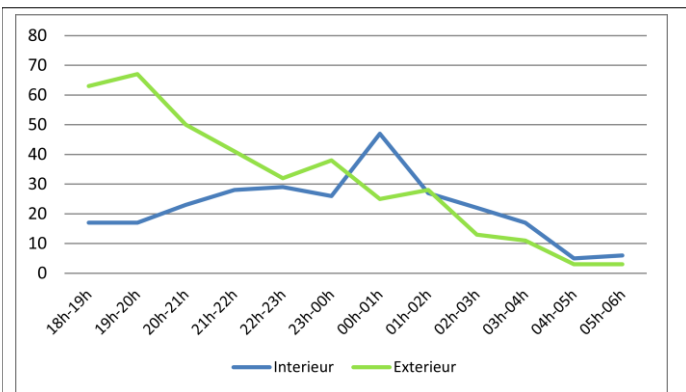
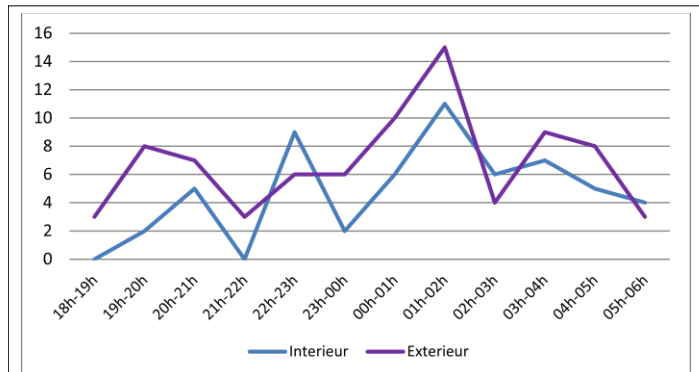
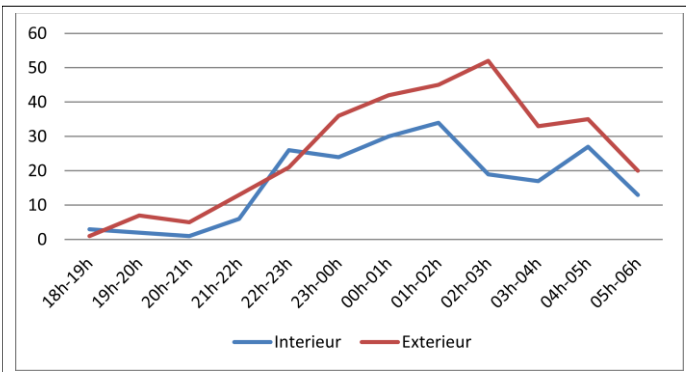
L: Kapolowe sentinel site

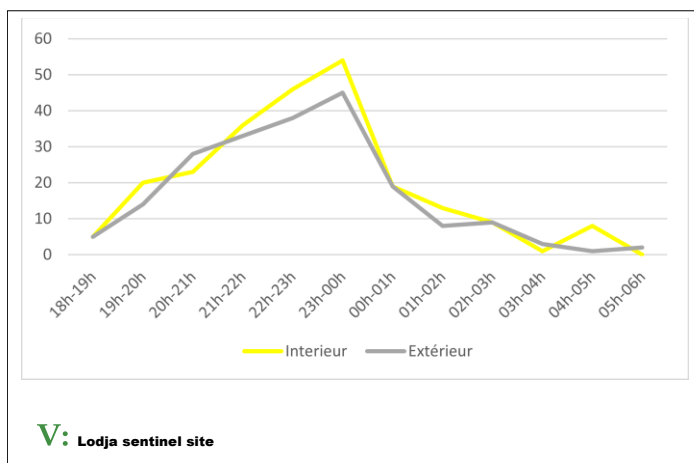
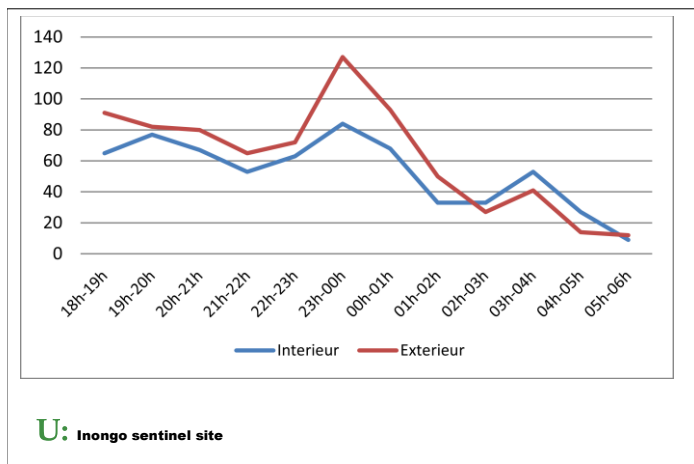
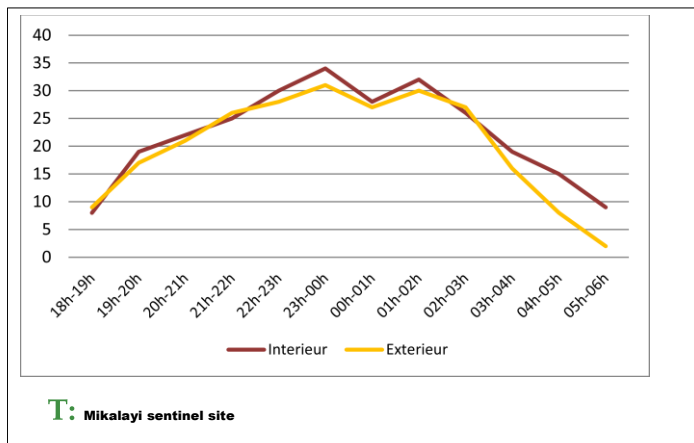


J: Fungurume sentinel site



M: Kapolowe sentinel site





These graphics above show the locations of bites and the period of aggressiveness of anopheles captured by the CAH technique in the 22 sentinel sites of the NMCP/DRC. From these 22 sentinel sites, it emerges that in 8 sites the anopheles have shown exophagic behavior, and in the remaining 14 sites the anopheles are still endophagic.

The **Table 2** provides an overview of *Anopheles* mosquito collection efforts using Human Landing Catches (HLC) and night traps across 22 sites in various regions. A total of 14,366 mosquitoes were collected, with the highest numbers found in Kamina (1,604) and the lowest in Kimpese (105). The number of traps varied by site, with most regions using 40 to 80 traps. The mosquito aggression rate towards humans, measured as the Human Biting Rate (HBR), ranged from 2 to 20 bites per night, with Kamina recording the highest HBR (20) and Inongo the lowest (2). This variability highlights significant regional differences in mosquito activity and human exposure to bites, emphasizing areas where malaria prevention efforts may be most needed.

Table 2: *Anopheles* aggressiveness rate at 22 sentinel sites

	1. Kabondo	2. Pawa	3. Buta	4. Kalima	5. Katana	6. Kalemie	7. Kapolowe	8. Kamina	9. Fungurume
Total Anopheles collected by HLC	259	633	240	1052	535	879	951	1604	312
HLC night traps	60	80	40	80	60	80	80	80	40
Aggression rate towards humans per night (HBR)	3	8	6	13	7	11	12	20	8

	10. Mikalayi	11. Dihinzi	12. Mwaka	13. Mweneditu	14. Lodja	15. Lisala	16. Boende	17. Gemena	18. Bolenge	19. Inongo	20. Vanga	21. Kingasani	22. Kimpese
Total Anopheles collected by HLC	502	139	878	512	439	608	662	463	136	1386	632	282	105
HLC night traps	40	40	80	80	40	80	80	60	80	80	80	40	40
Aggression rate towards humans per night (HBR)	13	4	11	6	11	8	8.3	6	2	17	8	7	3

Table 2 shows the average number of bites per person per night at the 22 sentinel study sites. The *Anopheles* bite rate was particularly high at 8 sites, namely, kamina (20), Inongo (17), kalima (13), mikalayi (13), kapolowe (12), lodja (11), mwaka (11) and kalemie. The average *Anopheles* bite rate was less than (11) at the remaining (14) sites. The average for all sites was 12 bites/night/man.

Figure 6 below shows the physiological status of the abdomen of anopheles captured by a PC (pyrethrum capture).

Figure 6:
Physiological status of the abdomen of anopheles captured by a PC

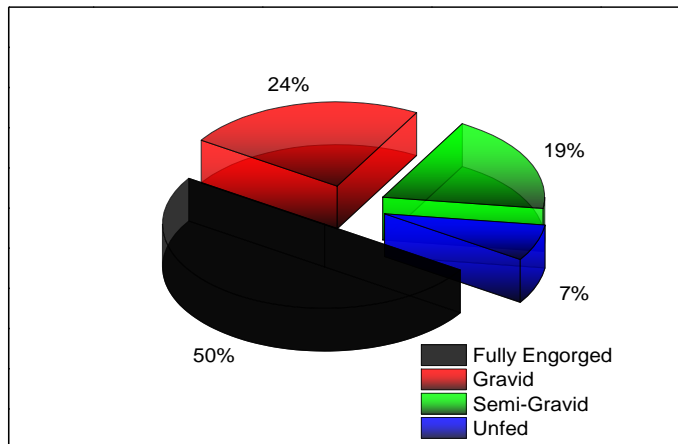


Figure 6 shows that at all sites, the majority of the *Anopheles* collected by CP were blood-gorged (50%, 874/1723), followed by semi-gravid (24%, 411/1723), gravid (19%, 321/1723), and fasted (7%, 117/1723) individuals. This suggests strong contact between humans and vectors.

DISCUSSION

Studying the bionomics of malaria vectors and their role in the transmission of infection is critical not only for vector and parasite surveillance but also for identifying effective control strategies tailored to the elimination of malaria (Lobo et al., 2015; Soma et al., 2020). The objectives of this study, conducted at the NMCP sentinel sites, were to highlight the behaviors and essential parameters of malaria transmission by the vectors found at these sites and to assess the level of transmission. Entomological monitoring, carried out over three years with two visits per site annually, enabled us to identify five *Anopheles* species, two of which are already implicated in malaria transmission in the DRC (Loonen et al., 2020; Wat'Senga et al., 2018b). Similar results have been reported in several studies conducted across multiple provinces of the DRC (President's Malaria Initiative [PMI], 2020; Basosila et al., 2022; PMI, 2018; PNLN, 2023). This diversity of species can be attributed to various ecological and climatic factors that shape the biotopes, providing specific breeding grounds for the larvae of each species, such as permanent streams, puddles, small dams, and abandoned ponds.

The *Anopheles gambiae* s.l. complex was found at all study sites, confirming its dominance in malaria transmission in

the DRC (Bobanga et al., 2016; Nardini et al., 2017; Zanga et al., 2024). Regarding the period of aggressiveness, the results (Fig. 3A-V) show that at these 22 sentinel sites, *Anopheles* exhibited exophagic behavior at eight sites (Mweka, Lisala, Gemena, Butu, Mweneditu, Kamina, Dibindi, and Inongo), while at the remaining 14 sites (Kalima, Pawa, Kabondo, Kalemie, Kimpese, Boende, Fungurume, Katana, Kapolowe, Vanga, Kingasani, Mikalayi, Bolenge, and Lodja), the mosquitoes exhibited endophagic behavior, with the period of aggression starting from 10 p.m. to 4 a.m. at several sites.

A study by Basosila et al. (2022) revealed that *Anopheles gambiae* s.l. was more exophagous than endophagous, with an indoor peak starting slightly later between 11 p.m. and 2 a.m., whereas the outdoor peak started slightly earlier, at 7 p.m., in two health zones in Nord-Ubangi Province. This confirms the change in malaria vector behavior and highlights the need for complementary methods to the use of long-lasting insecticidal nets (LLINs) for targeted vector control.

The results in Table 2 illustrate the aggressiveness of *Anopheles* at the 22 sentinel sites. The bite rate was particularly high at eight sites, namely, Kamina (20), Inongo (17), Kalima (13), Mikalayi (13), Kapolowe (12), Lodja (11), Mweka (11), and Kalemie (11). The average *Anopheles* bite rate was below 11 at the remaining 14 sites. The average across all sites was 12 bites per night per person. This could be attributed to the population's incorrect use of LLINs and/or limited access to this preventive tool, coupled with *Anopheles* resistance to the pyrethroid insecticides with which LLINs are impregnated. A study conducted in 360 households in the Bonzola health zone in Mbuji-Mayi reported that 61.39% of households had good practices regarding LLINs and their benefits (Promesse & Kaniki, 2020). The rest of the population, unaware of the proper use of these nets, is therefore seriously exposed to mosquito bites and consequently to malaria. Moreover, the three-year LLIN distribution cycle allows a long time for the physical and chemical integrity of the tool to degrade (PNLN, 2023).

Regarding the physiological state of the mosquitoes, 50% were found to be engorged, reflecting strong human-vector attraction. The aggressiveness inside dwellings was

greater than outside, which could be explained by the endophagic behavior of *An. gambiae* s.l. However, exophagic behavior is beginning to expand at some PNLP sites in the DRC. This study shows that in the DRC, *An. gambiae* s.l. plays a major role in malaria transmission, as also revealed by the work of Wat'senga et al. (2020).

This vector is particularly effective due to favorable climatic conditions and the prevalence of suitable breeding habitats, contributing to a high malaria burden. In comparison, in other Sub-Saharan African countries such as Nigeria and Ghana, although *An. gambiae* s.l. is also a major vector, infection rates vary depending on control strategies and environmental factors (Ebhodaghe et al., 2024; Takken et al., 2022). These differences in epidemiological profiles highlight the importance of adapting interventions to local vector-specific and environmental contexts to better combat malaria across different geographical settings.

The results of this study on the behavior of *Anopheles* mosquitoes in various regions of the DRC confirm previous observations regarding the variability in mosquito biting rates, influenced by ecological and geographical conditions. Regions such as Kamina and Kalemie exhibit high human biting rates (HBR), highlighting an increased risk of malaria transmission, while other areas like Kimpese and Dibindi show lower rates. This regional diversity, combined with emerging behaviors such as exophagic biting, poses a challenge for current malaria control strategies, such as Indoor Residual Spraying (IRS) and LLINs, which primarily target nocturnal and endophagic mosquitoes.

The shift towards outdoor biting reduces the effectiveness of these measures, necessitating the integration of new approaches such as larval source management and spatial repellents. To improve malaria control programs, it is recommended to include outdoor control strategies, adjust IRS/LLIN interventions according to local vector behaviors, and enhance continuous surveillance of mosquito behavior. Additionally, better community education on personal protection measures and the development of innovative tools to combat outdoor transmission are essential. However, the study has limitations, such as a restricted geographical sample and a

lack of seasonal data, which should be addressed in future research to better adapt malaria control strategies in the DRC.

CONCLUSION AND RECOMMENDATIONS

The entomological surveillance conducted across 22 sentinel sites in the PNLP/RDC has provided crucial insights into the behavior of malaria vectors over a three-year period. The findings identify *Anopheles gambiae* s.l. as the dominant species, exhibiting strong endophagic behavior, underscoring its significant role in indoor malaria transmission. This data directly informs policy and decision-making for malaria control in the Democratic Republic of the Congo (DRC). Specifically, it is recommended that sentinel site monitoring be conducted biannually to capture seasonal variations in vector behavior and resistance patterns. The National Malaria Control Program (NMCP) should adjust its strategies based on these findings by tailoring Long-Lasting Insecticidal Net (LLIN) distribution and Indoor Residual Spraying (IRS) campaigns to more effectively target the predominant indoor-biting vectors. LLIN distribution should be reinforced in areas with high indoor mosquito density, and IRS should be strategically timed and localized based on the peak periods of vector activity identified. These targeted interventions, guided by up-to-date entomological data, will enhance the effectiveness of malaria control efforts and contribute to a significant reduction in transmission rates across the country.

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REFERENCES

- Bamou, R., Kopya, E., & Djamouko-Djonkam, L. (2020).** Resistance of local malaria vectors in the forest region of southern Cameroon: Assessment of the Anophelinae blood-seeking bionomic and pyrethroids resistance of local malaria vectors in the forest region of southern Cameroon. *Journal of Entomology and Zoology Studies*, 8(1), 1054–1062.
- Basosila, N., Ngbolua, J.-P., Eric, M., Mawunu, M., Irish, S., Basimike, M., & Jonas, N. (2022).** Study of the behavior and entomological parameters of *Anopheles* in two health zones of the North-Ubangi Province, Democratic Republic of Congo. *Egyptian Academic Journal of Biological Sciences, E. Medical Entomology & Parasitology*, 14(2), 57–63. <https://doi.org/10.21608/eajbse.2022.259846>
- Bobanga, T., Umesumbu, S. E., Mandoko, A. S., Nsibu, C. N., Dotson, E. B., Beach, R. F., & Irish, S. R. (2016).** Presence of species within the *Anopheles gambiae* complex in the Democratic Republic of Congo. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, 110(6), 373–375. <https://doi.org/10.1093/trstmh/trw035>
- Chinappi, M., Via, A., Marcatili, P., & Tramontano, A. (2010).** On the mechanism of chloroquine resistance in *Plasmodium falciparum*. *PLoS ONE*, 5(11), e14064. <https://doi.org/10.1371/journal.pone.0014064>
- Collins, W. E., & Jeffery, G. M. (2005).** *Plasmodium malariae*: Parasite and disease. *Clinical Microbiology Reviews*, 18(3), 570–581. <https://doi.org/10.1128/CMR.00027-07>
- De Silva, P. M., & Marshall, J. M. (2012).** Factors contributing to urban malaria transmission in sub-Saharan Africa: A systematic review. *Journal of Tropical Medicine*, 2012, 1–10. <https://doi.org/10.1155/2012/819563>
- Dondorp, A. M., Nosten, F., Yi, P., Das, D., Physo, A. P., Tarning, J., Lwin, K. M., Ariey, F., Hanpithakpong, W., Lee, S. J., Ringwald, P., Silamut, K., Herdman, T., An, S. S., Yeung, S., Socheat, D., & White, N. J. (2009).** Artemisinin resistance in *Plasmodium falciparum* malaria. *Drug Therapy*, 361(5), 455–467. <http://www.ncbi.nlm.nih.gov/pubmed/21543403>
- Ebhodaghe, F. I., Sanchez-Vargas, I., Isaac, C., et al. (2024).** Sibling species of the major malaria vector *Anopheles gambiae* display divergent preferences for aquatic breeding sites in southern Nigeria. *Malaria Journal*, 23, 60. <https://doi.org/10.1186/s12936-024-04871-9>
- Ermert, V., Fink, A. H., Morse, A. P., & Paeth, H. (2012).** The impact of regional climate change on malaria risk due to greenhouse forcing and land-use changes in tropical Africa. *Environmental Health Perspectives*, 120(1), 77–84. <https://doi.org/10.1289/ehp.1103681>
- Gillies, M. T., & Coetzee, M. (1987).** A supplement to the Anophelinae of Africa South of the Sahara. *South African Institute for Medical Research*, 55, 1–145.
- Hawadak, J., Dongang Nana, R. R., & Singh, V. (2021).** Global trend of *Plasmodium malariae* and *Plasmodium ovale* spp. malaria infections in the last two decades (2000–2020): A systematic review and meta-analysis. *Parasites & Vectors*, 14(1), 294. <https://doi.org/10.1186/s13071-021-04797-0>
- Hii, J. L. K., Smith, T., Mai, A., Ibam, E., & Alpers, M. P. (2000).** Comparison between anopheline mosquitoes (Diptera: Culicidae) caught using different methods in a malaria endemic area of Papua New Guinea. *Bulletin of Entomological Research*, 90(3), 211–219. <https://doi.org/10.1017/s000748530000033x>
- Initiative Malaria Elimination. (2020).** *Outil de planification de la surveillance entomologique*.
- Kalala, D.-J. M., & Fyama, J. N. M. (2019).** Crises alimentaires et mesures d'atténuation en République démocratique du Congo. *Revue des stratégies et promotion de bonnes pratiques*. In Konrad Adenauer Stiftung. www.kas.de
- Karch, S., & Mouchet, J. (1992).** *Anopheles paludis*: Important vector of malaria in Zaire. *Bulletin de la Société de Pathologie Exotique (1990)*, 85(5), 388–389.

- Kouassi, B. L.,** Edi, C., Ouattara, A. F., Ekra, A. K., Bellai, L. G., Gouaméné, J., Kacou, Y. A. K., Kouamé, J. K. I., Béké, A. H. O., Yokoli, F. N., Gbalegba, C. G. N., Tia, E., Yapou, R. M., Konan, L. Y., N'Tamon, R. N., Akéré, M. A., Koffi, A. A., Tanoh, A. M., & Chabi, J. (2023). Entomological monitoring data driving decision-making for appropriate and sustainable malaria vector control in Côte d'Ivoire. *Malaria Journal*, 22(1), 14. <https://doi.org/10.1186/s12936-023-04439-z>
- Lelisa, K.,** Asale, A., Taye, B., Emanu, D., & Yewhalaw, D. (2017). Anopheline mosquitoes behaviour and entomological monitoring in southwestern Ethiopia. *Journal of Vector Borne Diseases*, 54(3), 240–248. <https://doi.org/10.4103/0972-9062.217615>
- Lobo, N. F.,** St. Laurent, B., Sikaala, C. H., Hamainza, B., Chanda, J., Chinula, D., Krishnankutty, S. M., Mueller, J. D., Deason, N. A., Hoang, Q. T., Boldt, H. L., Thumloup, J., Stevenson, J., Seyoum, A., & Collins, F. H. (2015). Unexpected diversity of *Anopheles* species in Eastern Zambia: Implications for evaluating vector behavior and interventions using molecular tools. *Scientific Reports*, 5, 17952. <https://doi.org/10.1038/srep17952>
- Loonen, J. A. C. M.,** Dery, D. B., Musaka, B. Z., Bandibabone, J. B., Bousema, T., Lenthe, M. Van, Stefanija, B. P., Fesselet, J. F., & Koenraadt, C. J. M. (2020). Identification of main malaria vectors and their insecticide resistance profile in internally displaced and indigenous communities in Eastern Democratic Republic of the Congo (DRC). *Malaria Journal*, 19(425), 1–14. <https://doi.org/10.1186/s12936-020-03497-x>
- Matubi, E. M.,** Kaounga, G. I., Zanga, J., Mbuku, G. B., Maniania, J. N. K., Mulenda, B., Sodi, J. N. M., Tamfum, J. J. M., & Masiangi, P. (2020). Insecticide susceptibility of *Anopheles gambiae* S.L and identification of some resistance mechanisms in Kwilu province in the Democratic Republic of Congo. *Pan African Medical Journal*, 37, 79. <https://doi.org/10.11604/pamj.2020.37.79.18635>
- Metelo-Matubi, E.,** Zanga, J., Binene, G., Mvuama, N., Ngamukie, S., Nkey, J., Schopp, P., Bamba, M., Irish, S., Nguya-Kalembe-Maniania, J., Fasine, S., Nagahuedi, J., Muyembe, J. J., & Mansiangi, P. (2021). The effect of a mass distribution of insecticide-treated nets on insecticide resistance and entomological inoculation rates of *Anopheles gambiae* s.l. in Bandundu City, Democratic Republic of Congo. *Pan African Medical Journal*, 40(118). <https://doi.org/10.11604/pamj.2021.40.118.27365>
- N'do, S.,** Bandibabone, J. B., Soma, D. D., Musaka, B. Z., Prudhomme, J., Habamungu, C. C., Namountougou, M., Sangaré, I., Kientega, M., Kaboré, D. A. P., Bayili, K., Yerbanga, R. S., Diabate, A., Dabire, R. K., Ouedraogo, J. B., Belem, A. M. G., Boëte, C., Guardiola-Claramonte, M., & Chimanuka, B. (2021). Insecticide resistance profiles in malaria vector populations from Sud-Kivu in the Democratic Republic of the Congo. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, 115(11), 1339–1344. <https://doi.org/10.1093/trstmh/trab116>
- Nardini, L.,** Hunt, R. H., Dahan-Moss, Y. L., Christie, N., Christian, R. N., Coetzee, M., & Koekemoer, L. L. (2017). Malaria vectors in the Democratic Republic of the Congo: The mechanisms that confer insecticide resistance in *Anopheles gambiae* and *Anopheles funestus*. *Malaria Journal*, 16(448). <https://doi.org/10.1186/s12936-017-2099-y>
- Nguiffo-Nguete, D.,** Mugenzi, L. M. J., Manzambi, E. Z., Tchouakui, M., Wondji, M., Tekoh, T., Watsenga, F., Agossa, F., & Wondji, C. S. (2023). Evidence of intensification of pyrethroid resistance in the major malaria vectors in Kinshasa, Democratic Republic of Congo. *Scientific Reports*, 13, 14711. <https://doi.org/10.1038/s41598-023-41952-2>
- Nundu, S. S.,** Culleton, R., Simpson, S. V., Arima, H., Muyembe, J. J., Mita, T., Ahuka, S., & Yamamoto, T. (2021). Malaria parasite species composition of *Plasmodium* infections among asymptomatic and symptomatic school-age children in rural and urban areas of Kinshasa, Democratic Republic of Congo. *Malaria Journal*, 20, 389. <https://doi.org/10.1186/s12936-021-03919-4>
- O'Meara, W. P.,** Mangeni, J. N., Steketee, R., & Greenwood, B. (2010). Changes in the burden of malaria in sub-Saharan Africa. *The Lancet Infectious Diseases*, 10(8), 545–555. [https://doi.org/10.1016/S1473-3099\(10\)70096-7](https://doi.org/10.1016/S1473-3099(10)70096-7)

- PMI.** (2018). The President's Malaria Initiative (PMI)/Africa Indoor Residual Spraying (AIRS) Project. Democratic Republic of the Congo 2017 Entomological Monitoring Report. PMI AIRS Project Indoor Residual Spraying (IRS 2) Task Order Six, Abt.
- PMI.** (2020). PMI VectorLink Project: The Democratic Republic of Congo annual entomological monitoring report.
- PNLP.** (2022). *Rapport annuel 2022*.
- PNLP.** (2023). *Plan stratégique nationale de lutte contre le paludisme 2020-2023*. <https://doi.org/10.3138/9781442656505-toc>
- Promesse, C., & Kaniki, K.** (2020). Long-lasting insecticide-treated mosquito nets in the Democratic Republic of the Congo: Knowledge, attitudes and practices among households in Bonzola. *Research Square*. <https://doi.org/10.21203/rs.3.rs-30044/v1>
- Riveron, J. M., Watsenga, F., Irving, H., Irish, S. R., & Wondji, C. S.** (2018). High *Plasmodium* infection rate and reduced bed net efficacy in multiple insecticide-resistant malaria vectors in Kinshasa, Democratic Republic of Congo. *Journal of Infectious Diseases*, 217, 320–328. <https://doi.org/10.1093/infdis/jix570>
- Soma, D. D., Zogo, B. M., Some, A., Tchiekoi, B. N. C., De Sales Hien, D. F., Pooda, H. S., Coulibaly, S., Gnambani, J. E., Ouari, A., Mouline, K., Dahounto, A., Ouedraogo, G. A., Fournet, F., Koffi, A. A., Pennetier, C., Moiroux, N., & Dabire, R. K.** (2020). *Anopheles* bionomics, insecticide resistance, and malaria transmission in southwest Burkina Faso: A pre-intervention study. *PLoS ONE*, 15(8). <https://doi.org/10.1371/journal.pone.0236920>
- Takken, W., Charlwood, D., & Lindsay, S. W.** (2024). The behaviour of adult *Anopheles gambiae*, sub-Saharan Africa's principal malaria vector, and its relevance to malaria control: A review. *Malaria Journal*, 23, 161. <https://doi.org/10.1186/s12936-024-04982-3>
- Thomson, R. C. M.** (1947). The effects of house spraying with pyrethrum and with DDT on *Anopheles gambiae* and *A. melas* in West Africa. *Bulletin of Entomological Research*, 449–464. <https://doi.org/10.1017/S0007485300022276>
- Tusting, L. S., Bottomley, C., Gibson, H., Kleinschmidt, I., Tatem, A. J., Lindsay, S. W., & Gething, P. W.** (2017). Housing improvements and malaria risk in sub-Saharan Africa: A multi-country analysis of survey data. *PLoS Medicine*, 14(2), e1002234. <https://doi.org/10.1371/journal.pmed.1002234>
- Wat'senga, F., Agossa, F., Manzambi, E. Z., Illombe, G., Mapangulu, T., Muyembe, T., Clark, T., Niang, M., Ntoya, F., Sadou, A., Plucinski, M., Li, Y., Messenger, L. A., Fornadel, C., Oxborough, R. M., & Irish, S. R.** (2020). Intensity of pyrethroid resistance in *Anopheles gambiae* before and after a mass distribution of insecticide-treated nets in Kinshasa and in 11 provinces of the Democratic Republic of Congo. *Malaria Journal*, 19(1), 1–13. <https://doi.org/10.1186/s12936-020-03240-6>
- Wat'senga, F., Manzambi, E. Z., Lunkula, A., Mulumbu, R., Mampangulu, T., Lobo, N., Hendershot, A., Fornadel, C., Jacob, D., Niang, M., ... Oxborough, R. M.** (2018a). Nationwide insecticide resistance status and biting behaviour of malaria vector species in the Democratic Republic of Congo. *Malaria Journal*, 17(129). <https://doi.org/10.1186/s12936-018-2285-6>
- Soma, D. D., Zogo, B. M., Some, A., Tchiekoi, B. N. C., De Sales Hien, D. F., Pooda, H. S., Coulibaly, S., Gnambani, J. E., Ouari, A., Mouline, K., Dahounto, A., Ouedraogo, G. A., Fournet, F., Koffi, A. A., Pennetier, C., Moiroux, N., & Dabire, R. K.** (2020). *Anopheles* bionomics, insecticide resistance, and malaria transmission in southwest Burkina Faso: A pre-intervention study. *PLoS ONE*, 15(8), e0236920. <https://doi.org/10.1371/journal.pone.0236920>
- Sow, A., Poudiougou, I. K., Zongo, M., Nignan, T., Kone, A., Ouedraogo, J. B., Diallo, S. M., Traore, H., & Konate, S.** (2023). Resistance to insecticides and its impact on malaria transmission in *Anopheles* mosquitoes in Bobo-Dioulasso, Burkina Faso. *Malaria Journal*, 22, 58. <https://doi.org/10.1186/s12936-023-04559-1>
- Tusting, L. S., Bottomley, C., Gibson, H., Kleinschmidt, I., Tatem, A. J., Lindsay, S. W., & Gething, P. W.** (2017). Housing improvements and malaria risk in sub-Saharan Africa: A multi-country analysis of

- survey data. *PLoS Medicine*, 14(2), e1002234. <https://doi.org/10.1371/journal.pmed.1002234>
- Viana**, M., Da Silva, D. C. P., De Almeida, M. L., Goulart, M. M., & Lima, D. B. S. (2022). *Anopheles* mosquitoes in the Brazilian Amazon: Current status of insecticide resistance and its implications for malaria transmission. *Acta Tropica*, 231, 106455. <https://doi.org/10.1016/j.actatropica.2022.106455>
- Watsenga**, F., Agossa, F., Manzambi, E. Z., Illombe, G., Mapangulu, T., Muyembe, T., Clark, T., Niang, M., Ntoya, F., Sadou, A., Plucinski, M., Li, Y., Messenger, L. A., Fornadel, C., Oxborough, R. M., & Irish, S. R. (2020). Intensity of pyrethroid resistance in *Anopheles gambiae* before and after a mass distribution of insecticide-treated nets in Kinshasa and in 11 provinces of the Democratic Republic of Congo. *Malaria Journal*, 19(1), 1–13. <https://doi.org/10.1186/s12936-020-03240-6>
- Watsenga**, F., Manzambi, E. Z., Lunkula, A., Mulumbu, R., Mampangulu, T., Lobo, N., Hendershot, A., Fornadel, C., Jacob, D., Niang, M., ... Oxborough, R. M. (2018a). Nationwide insecticide resistance status and biting behaviour of malaria vector species in the Democratic Republic of Congo. *Malaria Journal*, 17(129). <https://doi.org/10.1186/s12936-018-2285-6>
- Wondji**, C. S., & Ndo, S. (2020). *Anopheles* species complex and their malaria vectorial capacity in the Democratic Republic of Congo. *Tropical Medicine and Infectious Disease*, 5(3), 103. <https://doi.org/10.3390/tropicalmed5030103>
- Zhou**, Z., Zhan, J., Yang, W., Liu, J., & Jiang, R. (2022). Resistance of *Anopheles* mosquitoes to insecticides: A review of current status and prospects for control in China. *Environmental Science and Pollution Research*, 29, 30788–30803. <https://doi.org/10.1007/s11356-021-17888-5>