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Implications for malaria transmission: a cross-sectional study on the bionomics and susceptibility of local malaria vectors in urban and periurban settings of Ndola district

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ABSTRACT

Objectives To assess vector behaviour and phenotypic resistance for effective vector control programming.

Design This was a cross-sectional study.

Setting This study was conducted in the urban and periurban areas of Ndola district, Zambia.

Participants/study units A total of 166 houses were selected for adult mosquito collection, and an additional 60 collection efforts were made for larval collection from potential larval habitats.

Primary and secondary outcome measures The primary outcome was the behaviour of the malaria vectors, and the secondary outcome was their phenotypic resistance status.

Results The main breeding sites identified were irrigation trenches (4.67 larvae/dip) and garden ponds (2.72 larvae/dip) created from extensive urban agriculture practices. *Anopheles funestus sensu stricto (An. funestus s.s)* and *Anopheles gambiae sensu stricto (An. gambiae s.s)* were found to coexist in all the four sites, with *An. funestus s.s* identified as the most dominant malaria vector. Densities of *An. gambiae s.s,* seeking a blood meal (χ^2=12.566, df=3, p=0.001) and resting indoors (Z=56.5, p=0.019), were found to be higher in urban than periurban sites compared with *An. funestus s.s*, which had similar distribution across the study sites. Sprayed houses were significantly associated with reduced mosquito numbers (B=−0.956, incidence rate ratio=0.384, p=0.001). *Anopheles gambiae* was fully susceptible to organophosphates and neonicotinoids but highly resistant to pyrethroids, carbamates and organochlorines.

Conclusions The emergence of *An. funestus s.s* in an area previously dominated by *An. gambiae s.s* and its coexistence with *An. gambiae s.s* in the dry season pose a risk of sustaining malaria transmission all year round. Agricultural practices in urban areas resulted in highly productive mosquito breeding sites; thus, there is a need for targeted vector control.

STRENGTHS AND LIMITATIONS OF THIS STUDY

⇒ The study design and sampling strategies used allow for the determination of species composition, abundance, host-seeking and resting behaviour of malaria vectors.
⇒ The presence of *An. gambiae s.s* larvae habitats in the dry season facilitates mosquito breeding, which may drive malaria transmission; thus, there is a need to plan for additional measures.
⇒ The susceptibility of *An. gambiae s.s,* the most efficient malaria vector, was determined against seven different insecticides from five different classes, but not for *An. funestus s.s* due to limited numbers of adult *An. funestus s.s* and difficulty in finding larval habitats or sufficient adults to perform induced oviposition.
⇒ This study was conducted in the dry season, and the entomological indices determined may only be applicable to the dry season.

BACKGROUND

Malaria remains a public health challenge in Zambia, accounting for approximately 1.4% of the global malaria disease burden. It is estimated that about four people die from malaria every day in Zambia.[1 2] High rainfall regions in northern Zambia experience the highest disease burden, while densely populated and arid regions in the south experience lower burden.[3–6]

The primary malaria vectors in Zambia include *An. funestus s.s, An. gambiae s.s* and *An. arabiensis.*[7–9] *An. funestus s.s,* the most abundant and widely distributed malaria vector in the country, thrives during the dry season, whereas *An. gambiae s.s,* the most efficient malaria vector, thrives predominantly in the wet season.[8] Historically, *An. gambiae s.s* has been the dominant malaria vector in Copperbelt Province in the past decades.[9] In contrast, *An. arabiensis,* a more zoophilic mosquito, is the primary malaria vector in the southern regions and a secondary malaria

vector in the eastern parts of the country—a region of moderate transmission.[5 9–11]

Insecticide-treated bednets (ITNs), indoor residual spraying (IRS) and artemisinin combined therapy (ACT) have played a vital role in reducing malaria disease burden in Zambia.[12–14] These interventions have been informed by entomological and parasite surveillance data generated from several parts of the country in the past two decades.[13–15] The Copperbelt province, on the other hand, has implemented IRS since the 1950s and, over the past two decades, has scaled up IRS, ITNs and ACT, which were associated with a significant decrease in the malaria disease burden.[14 16] Furthermore, this success also led to a decline in entomological surveillance in the province. Since 2017, the Copperbelt Province has experienced a rise in the number of malaria cases, indicating a change in the epidemiological landscape, necessitating renewed entomological activities for informed vector control programming.

Vector surveillance across Zambia has revealed some level of heterogeneity in the behaviour and susceptibility of malaria vectors within and between selected districts.[9 17] Most active entomological sites are located in areas of high or low malaria transmission, with limited representation in settings of moderate transmission.[18] Additionally, over 95% of all entomological surveillance activities are conducted in rural settings, yet a substantial number of reported malaria cases originate from periurban and urban areas.[19–21] To address these gaps, we conducted entomological studies in Ndola between July 2021 and October 2021 in two ecologically distinct settings representing the periurban and urban areas of Ndola district with a moderate malaria transmission setting to assess vector behaviour and phenotypic resistance for effective vector control programming.

METHODS

Study design and study area

This was a cross-sectional study conducted in the dry season in Ndola district, the provincial capital of the Copperbelt Province. The mean annual temperatures range from 12°C to 25°C, with mean annual rainfall ranging from 200 to 900 mm. The rainy season spans from November to March, followed by a longer dry season from April to October.

Two catchment areas Chipulukusu and Kaniki were selected for their high malaria incidence rates in 2020. The malaria incidences for Chipulukusu and Kaniki health centres were 435 per 1000 people at risk and 971 per 1000 people at risk.[2] Chipulukusu is an urban catchment area with houses constructed with cement blocks, burnt bricks or mud bricks and have iron or grass roofing. Mosquito collection in Chipulukusu was conducted in two zones: Musalu (−12.9524 S, 28.66012 E), a densely populated area with limited road access and extensive vegetable gardening activities, and Mapalo (−12.9374 S, 28.67564 E), an equally densely populated area but with road access and very minimal vegetable gardening activities. Kaniki is a periurban catchment consisting mainly of mud houses with thatched grass or iron-sheet roofs. Mosquito collection in Kaniki was conducted in Kamalasha (−12.8556955 S, 28.5311082 E), a densely populated area near the Sakania border with a swamp on the western side of the Ndola-Mufulira Road, and Pima (−12.77416 S, 28.483865 E), a farming setting with houses organised in clusters. Both catchment areas serve as low-cost residential settings.

Sample size

A total of 166 houses were selected for adult mosquito collection; 56 houses for Centers for Disease Control and Prevention light traps (CDC-LTs), 30 houses for pyrethrum spray catches (PSCs) and 80 houses for aspirations. An additional 60 collection efforts were made for larval collection from potential larval habitats.

Sample size justification

This study used WHO guidelines on mosquito sampling, and the sample size used for this study follows previous modelling studies conducted on the minimum number of houses required to estimate mosquito abundance using a precision of 20% allowable for ecological studies.[22 23]

Inclusion and exclusion criteria

The inclusion criteria for this study were twofold; first, only houses with an adult (16 years and above) were considered and houses where written consent was obtained. Houses where people cook using firewood from inside were excluded from the study.

House selection and adult mosquito collection

House selection was randomly done in each participating zone in the catchment area, maintaining a minimum of 200 m between two participating houses. Mosquito collection in each participating house was only done once, and only one mosquito collection method was employed per house sampled. The collection of mosquitoes was conducted between July and September 2021 from 166 randomly selected houses; 83 houses from Chipulukusu catchment area and the other 83 houses from Kaniki catchment area.

CDC light traps

The CDC-LT was used as a proxy for determining the biting density of mosquitoes to human hosts. The traps were set in randomly selected houses, and mosquito collection occurred from 18:00 hours to 06:00 hours the following morning. Each trap was set at a height of 1.5 m from the ground, adjacent to a sleeping person and near their legs.

Aspirations

The live adult mosquitoes were collected using a prokopack aspirator from 05:00 hours to 07:00 hours in the morning in houses where people slept. The live adult mosquitoes were then put in bugdom cages where they

were supplied with 10% sugar solution and transported to the laboratory for identification.

Pyrethrum spray catches

Adult mosquitoes resting indoors were collected using PSC from 05:00 hours to 07:00 hours in the morning. Multiple pieces of white linen were spread over the floor, bed and furniture inside the house. Household members were asked to briefly exit the house, and then the house was sprayed to saturation using a pressurised two-in-one pyrethroid insecticide (imiprothrin 1.00 g/kg and deltamethrin 0.51 g/kg) can. After 10 min, all the mosquitoes that were knocked down were picked using a pair of forceps and placed into properly labelled Petri dishes.

Collection of immature mosquitoes (larvae)

Larval collection was carried out in October 2021. Potential larval habitats were initially visually inspected for the presence of larvae using 350 mL capacity standard dippers (BioQuip Products, Inc., California, USA) followed by sampling. The number of dips and number of larvae scooped were recorded. Afterwards, the collected larvae were transported to the Tropical Diseases Research Centre laboratory for rearing in a controlled microenvironment (temperature of 27°C±2°C and a relative humidity of 75%±10%).

Susceptibility testing

Adults, F0 *An. gambiae sensu lacto (An. gambiae s.l)* reared from field-collected larvae from Musalu were exposed to five different classes of insecticides. The mosquitoes aged 2–5 days obtained from wild collected larvae were exposed to pirimiphos-methyl (0.25%), malathion (5%), deltamethrin (0.05%), permethrin (0.75%), bendiocarb (0.01%) and dichlorodiphenyltrichloroethane (4%) (DDT) standard WHO impregnated test paper. The bioassays were conducted in accordance with the WHO guidelines.[24] For clothianidin, CDC bottle bioassay was used as described by Brogdon and Chan.[25] A minimum of 100 female *An. gambiae s.l.* aged 2–5 days old were exposed to each insecticide, and 25 *An. gambiae s.l.* were used as controls for each insecticide tested.

Experimental procedures

1. To determine species composition and abundance of malaria vectors in Ndola: the species composition and abundance were determined by collections from CDC-LT, PSC and aspirations.
2. To assess the biting and resting behaviour of mosquitoes in Ndola: the densities of mosquitoes collected per trap per night (mosquitoes/trap/night) were used to assess the host-seeking behaviour of the malaria vectors. The indoor resting behaviour is indicative of the mosquitoes that rest indoors. This indicator is an important one when considering whether to implement IRS or not. The mean indoor resting densities calculated in this study were determined by mosquito collections from PSC only.
3. To determine the insecticide resistance status of primary malaria vectors: the mosquitoes used for susceptibility testing were the first filial generation from the larvae collected.

Mosquito processing

Morphological identification

The female *Anopheles* mosquitoes collected were initially morphologically identified to the genus level using an identification key for Afrotropical *Anopheles* mosquitoes.[26] Thereafter, the mosquito samples were individually preserved in 1.5 mL Eppendorf tubes containing silica gel. These preserved samples were stored for molecular identification using PCR.

Molecular identification

A subset of adult mosquitoes reared from field-collected larvae and those collected as adults, morphologically identified as *An. funestus s.l.* and *An. gambiae s.l.*, were further subjected to PCR for molecular confirmation of the IDs. DNA extraction for this process was performed using the QIAGEN DNeasy Blood and Tissue kit for insects (QIAGEN Inc., USA). DNA amplification was performed using the Applied Biosystems GeneAmp PCR System 9700 thermocycler. For molecular identification, the methods described by Koekemoer *et al*[27] and Scott *et al*[28] were used for *An. funestus s.l.* and *An. gambiae s.l.*, respectively.

STATISTICAL ANALYSIS

The data collected were entered in Microsoft Excel, and mean densities excluding larval density were derived from log-transformed data using Williams mean $M_w = (X_1 + 1)(X_2 + 1)(X_3 + 1)\ldots(X_n + 1)^{1/n}$ to account for skewed (non-normal distribution) and count data.[20 29]

The dataset was then exported to IBM SPSS Statistics V.25. The Kruskal-Wallis H test was used to compare the means (*Mw*) of malaria vectors seeking a host. The Mann-Whitney U test was used to compare the densities of the malaria vectors resting indoors from the two sites where PSC was conducted. Additionally, a negative binomial model with a log function was used to identify factors associated with counts of malaria vectors in the sampled housing structures. Susceptibility status of *An. gambiae s.s* was determined using WHO mortality scoring guidelines.[22 23]

Patient and public involvement

There was no direct patient and public involvement. The findings from this study will be shared with Ndola District Health Office and the Ministry of Health.

RESULTS

Species composition and abundance from adult mosquito surveys

A total of 166 houses were sampled, and from these, 744 female mosquitoes were collected. *Culex* accounted for

Table 1 Entomological indices

Entomological indices	Chipulukusu catchment		Kaniki catchment		Total
	Mapalo	Musalu	Kamalasha	Pima	
Species composition					
An. funestus s.l	45	23	15	40	123
An. gambiae s.l.	24	71	5	6	106
An. gibbinsi	0	0	1	1	2
Culex	88	181	45	78	392
Mansonia	51	63	1	6	121
Total	208	338	67	131	744
Mean densities of malaria vectors seeking a blood meal (*Mw*)					
An. funestus s.l.	0.97*	0.10*	0.40*	0.40*	–
An. gambiae s.l.	1.83*	1.26*	0.10*	0.14*	–
Mean densities of malaria vectors resting indoors (*Mw*)					
An. funestus s.l.	–	0.72*	–	1.2*	–
An. gambiae s.l.	–	1.31*	–	0.05*	–

**Mw*=densities using Williams mean.

53% (392/744), *An. funestus s.l.* 17% (123/744), *Mansonia* 16% (106/744), *An. gambiae s.l.* 14% (106/744) and *An. gibbinsi s.l.* less than 1% (2/744) of the total mosquitoes collected (table 1). Mosquito abundance by site showed the highest mosquito collections were from Musalu (338/744) and Mapalo (208/744), the two urban sites, followed by Pima (131/744) and Kamalasha (67/744), the two periurban sites. Notably, *Culex* mosquitoes comprised the largest proportion at each of the four sites (table 1). Further, species composition by site shows that periurban sites from Kaniki, Kamalasha and Pima had one more species, A*n. gibbinsi,* not found in the two sites from Chipulukusu (Mapalo and Musalu). *Culex* were the most abundant mosquito collected from each site (Kamalasha 67%, Pima 59%, Musalu 53% and Mapalo 42%). The second most abundant mosquito in Kaniki (Kamalasha 22% and Pima 30%) was *An. funestus s.l.* This was followed by *An. gambiae s.l.* (Kamalasha 7% and Pima 5%). In Chipulukusu, *Mansonia (24%)* and *An. gambiae s.s* (21%) were the second most abundant mosquito species collected from Mapalo and Musalu, respectively (table 1).

Molecular identification of the malaria vectors collected revealed that 89% (67/75) of the female *An. funestus s.l.* analysed amplified as *An. funestus s.s* at 505 base pairs and 85.5% (171/200) of *An. gambiae s.l.* successfully amplified as *An. gambiae s.s* at 390 base pairs.

Biting and resting behaviour of malaria vectors

The mean number of *An. funestus s.s* seeking a blood meal from Mapalo was 2.42 times higher (*Mw*=0.97/trap/night) than in Kamalasha (0.4/trap/night) and Pima (0.4/trap/night) and 9.7 times higher than in Musalu (0.1/trap/night) (table 1). For *An. gambiae s.s,* the mean number of mosquitoes seeking a blood meal from Musalu (1.26/trap/night) was 1.5 times more than that in Mapalo (0.83/trap/night) and higher than that in Kamalasha (0.1/trap/night) and Pima (0.14/trap/night), with differences of 12.6 and 9 times, respectively. Despite these variations in mean densities, the Kruskal-Wallis H test revealed no statistical difference in the host-seeking behaviour of *An. funestus s.l.* (χ^2=4.598, df=3, p=0.204) across the four sites. However, a statistical difference was observed in the host-seeking behaviour of *An. gambiae s.l.* (χ^2=12.566, df=3, p<0.001).

Indoor resting density of malaria vectors—PSC

The indoor resting density of *An. funestus s.l.* in Pima (1.2 mosquitoes per house) was 1.67 times higher than that in Musalu (0.72 mosquitoes per house), whereas for *An. gambiae s.l.* in Musalu (1.31 mosquitoes per house), the indoor resting density was 262 times higher than that in Pima (0.05 vectors per house) (table 1). The Mann-Whitney U test indicated no statistical difference in the resting densities of *An. funestus s.l.* between Pima and Musalu (Z=143.5, p=0.202), but a statistical difference in the resting densities of *An. gambiae s.l* (Z=56.5, p<0.019) was observed between the two sites.

Anopheles mosquito larval habitats

A total of 43 potential *anopheline* larval habitats were identified, and 55.81% (n=*24*; 95 CI: 40% to 71%) of these were found to contain larvae. All the larval habitats found to contain larvae were from either Musalu (70.83%; 95% CI: 49% to 87%) or Mapalo (29.12%; 95% CI: 13% to 51%) sites. Seven different categories of potential larval habitats identified included blocked trenches, foundation trenches, garden ponds, irrigation canals (channels), shallow wells, streams and tyre marks. From the different larval habitats, 2643 larvae were collected from a total of 914 dips. The proportion of larvae collected from

Table 2 Mosquito larval habitats

Site	Type of larval habitat	No. of dips	Number of larvae collected (%)	Larval habitats identified	Larval habitats with larvae	Mosquito genera	
						Anopheles larvae	*Culex larvae*
Mapalo	Foundation trenches	71	89 (3.37)	5	4	3	4
	Tyre marks	86	31 (1.17)	2	2	2	2
	Blocked drainages	18	13 (0.49)	1	1	1	1
	Subtotal	175	133 (5.03)	8	7	6	7
Musalu	Irrigation canals	362	1690 (63.94)	19	11	9	11
	Tyre marks	147	258 (9.76)	3	2	2	2
	Garden ponds	203	553 (20.92)	4	3	3	3
	Blocked drainages	27	9 (0.34)	1	1	1	1
	Subtotal	739	2510 (94.97)	27	17	15	17
Kamalasha	Tyre marks	-*	0 (0.0)	5	0	0	0
	Subtotal	-*	0 (0.0)	5	0	0	0
Pima	Shallow wells	-*	0 (0.0)	2	0	0	0
	Stream	-*	0 (0.0)	1	0	0	0
	Subtotal	-*	0 (0.0)	3	0	0	0
Total from all sites		914	2643 (100)	43	24	21	24

*No larvae found after visual inspection followed by 10 dips.

Musalu was 94.97% (2,510/2,643; 95% CI: 94% to 96%), whereas the remaining 5.03% (133/2,643; 95% CI: 4.2% to 5.9%) were collected from Mapalo (table 2). Additionally, 63.94% (1,690/2,643; 95% CI: 62% to 66%) of the collected larvae were from irrigation canals, 20.92% (553/2,643; 95% CI: 19% to 22%) were from garden ponds, 10.93% (289/2,643; 95% CI: 9.7% to 12%) were from tyre marks, 3.37% (89/2,643; 95% CI: 2.7 to 4.1%) were from foundation trenches and 0.83% (22/2,643%; 95% CI: 0.52% to 1.3%) were from blocked trenches.

The larval density was the highest in irrigation canals, with 4.67 larvae per dip; this was followed by garden ponds with 2.72 larvae per dip, tyre marks with 1.30 larvae per dip, foundation trenches with 1.25 larvae per dip and blocked drainages with 0.49 larvae per dip.

Factors affecting mosquito counts in housing structures

Seven predictors were used to identify associations with mosquito counts of *An. Funestus s.s* and *An. gambiae s.s* in households, and only the spray status with Fludora Fusion (B=−0.956, incidence rate ratio (IRR)=0.384, p=0.001) was found to be statistically significant (table 3). While three other predictors were associated with reduced mosquito counts, including the number of people who slept in a house the previous night (B=−0.023, IRR=0.978, p=0.714), housing structures with a thatched roof (B=−0.060, IRR=0.942, p=0.870) and the number of long-lasting insecticide nets (LLINs) in a housing structure (B=−0.085, IRR=0.918, p=0.489), these predictors were not statistically significant. On the other hand, the other three predictors, number of animals that slept in a house the previous night (B=0.004, IRR=1.004, p=0.937), housing structures plastered with mud walls or unburnt bricks (B=0.234, IRR=1.264, p=0.559) and housing structures with open eaves (B=0.203, IRR=1.225, p=0.557), were associated with increased mosquito counts but were not statistically significant.

Susceptibility status of *An. gambiae s.s*

The study showed full susceptibility 24 hours postexposure (100% mortality) to organophosphate (malathion 5% and pirimiphos-methyl 0.25%) and neonicotinoids (clothianidin). Conversely, resistance was confirmed to bendiocarb 0.1%, permethrin 0.75%, deltamethrin 0.75% and DDT with the corresponding mortalities of 23%, 14%, 18% and 4%, respectively (table 4). The area where the larvae used for susceptibility testing were collected from Musalu is an area predominantly known for urban agriculture practices.

DISCUSSION

This study reveals that the two main malaria vectors in Zambia, *An. funestus s.l.* and *An. gambiae s.l.*, were found in all four sites, and these were molecularly identified as *An. funestus s.s* and *An. gambiae s.s*, respectively. In Zambia, these mosquitoes have been implicated as the main vectors responsible for malaria transmission and have been found to exist in sympatry.[19 20 30 31] Surprisingly, *An. funestus s.s* was found to be the most abundant malaria vector in Ndola. Historically, the province has been dominated by *An. gambiae s.s*, but this study found *An. funestus s.s* as the dominant malaria vector in Ndola. This finding is similar to other entomological findings in

Table 3 Predictors affecting mosquito counts of *An. funestus s.s* and *An. gambiae s.s*

Parameter	Regression coefficient (B)	Hypothesis test			IRR	95% Wald CI for exp(B)	
		Wald χ^2	df	Sig.		Lower	Upper
(Intercept)	1.919	3.876	1	0.049	6.816	1.009	46.067
Number of people	−0.023	0.134	1	0.714	0.978	0.866	1.104
Number of animals	0.004	0.006	1	0.937	1.004	0.912	1.105
Type of roof	−0.060	0.027	1	0.870	0.942	0.462	1.921
Type of wall	0.234	0.342	1	0.559	1.264	0.577	2.769
Type of eaves	0.203	0.345	1	0.557	1.225	0.622	2.412
Number of LLINs	−0.085	0.478	1	0.489	0.918	0.721	1.169
Spray status	−0.956	10.513	1	0.001	0.384	0.216	0.685
(Scale)	1[a]						
(Negative binomial)	0.798						

Dependent variable: no. of malaria vectors model: (intercept), no. of people, no. of animals, type of roof, type of wall, type of eaves, no. of LLIs and spray status.
[a]Fixed at the displayed value.
df, degree of freedom; IRR, Incidence rate ratio; LLINs, long lasting insecticide nets.

other districts within the Copperbelt Province where *An. funestus s.s* is the more dominant vector.[9 15] However, *An. gambiae s.s* remained the more dominant malaria vector in urban areas, whereas *An. funestus s.s* was more abundant in periurban areas, consistent with earlier studies conducted in sub-Saharan Africa.[17 20] This disparity in vector abundance could be attributed to variations in ecological habitats. *Anopheles gambiae* prefers to breed in man-made water habitats such as drainages, tyre tracks, small pools and agriculture sites, while *An. funestus s.s* prefers to breed in permanent and semipermanent water habitats with some vegetative cover.[32 33] An earlier study in the northern parts of the country identified *An. funestus s.s* as the primary driver of malaria transmission in the dry season, whereas *An. gambiae s.s* was the primary driver in the wet season.[30] Nonetheless, the existence of breeding grounds for *Anopheles gambiae s.s* in urban areas implies that even during the dry season, *An. gambiae s.s* will continue to be the primary driver of malaria transmission. The coexistence of these two malaria vectors poses an increased year-round risk of malaria transmission in the area. The recent increase in the incidences of malaria reported in Ndola could be attributed to the changing vector bionomics that now includes *An. funestus s.s* not reported previously in the area.

Mosquito diversity was observed to be higher in periurban than urban sites with the inclusion of *An. gibbinsi,* a potential secondary malaria vector. This vector has been reported in other parts of the country as a potential secondary malaria vector.[34–36] Secondary malaria vectors have not been adequately considered in most vector control programming, yet they contribute to 5% of malaria transmission in the southern African region.[37] Their contribution to transmission is significant, making the need to incorporate interventions targeting secondary malaria vectors into vector control toolkits inevitable.

The host-seeking behaviours of *An. funestus s.s* and *An. gambiae s.s* were different. The host-seeking behaviour

Table 4 Susceptibility status of *An. gambiae s.s* from Musalu

Insecticide tested	Number of mosquitoes exposed	Knockdown at 60 min		Knockdown at 24 hours		Final mortality (24 hours)
		Dead	Alive	Dead	Alive	
Bendiocarb 0.1%	108	14	94	25	83	23%
DDT 4%	113	0	113	5	108	4%
Deltamethrin 0.05%	100	13	87	18	82	18%
Permethrin 0.75%	113	6	107	16	97	14%
Pirimiphos-methyl 0.25%	110	101	9	110	0	100%*
Malathion 5%	104	94	10	104	0	100%*
Clothianidin	107	104	3	107	0	100%*

*Fully susceptible
DDT, dichlorodiphenyltrichloroethane.

of *An. funestus s.s* was found to be homogeneous across the four sites, whereas the host-seeking behaviour of *An. gambiae s.s* was found to be much higher in urban sites with vast larval habitats. This heightened host-seeking behaviour of *An. gambiae s.s* indicates an increased risk of disease transmission in urban sites compared with periurban sites.[38] As such, the need for enhanced vector control methods in urban settings with extensive larval habitats due to the elevated risk cannot be overemphasised.

The mean densities of *An. funestus s.s* found resting indoors were generally low across the periurban and urban sites. However, the indoor resting density of *An. gambiae s.s* in the urban site was much higher than that in the periurban site. Variations in the indoor resting behaviour of *An. funestus s.s* and *An. gambiae s.s* could be influenced by the presence of vast *An. gambiae s.s* breeding sites in urban sites. Therefore, vector control interventions such as IRS and LLINs in such settings may need to be supplemented with larval source management.[39]

The larval habitats that were active breeding sites were all from the two urban sites adjacent to a dambo. The larval habitats identified included irrigation canals (or irrigation channels), garden ponds, tyre marks, foundation trenches and blocked drainages. However, irrigation channels and garden ponds were found to be the main mosquito breeding sites, similar to studies conducted in Ghana, Tanzania, Cote d'Ivoire and China.[40] However, the larval densities found in this study were higher than those found in China, possibly due to differences in the climatic conditions and variations in the bacterial diversity and physicochemical composition of the larval habitats.[41] These factors have been found to influence mosquito oviposition, survival and development into competent malaria vectors, thereby potentially impacting malaria incidence.[3 42] Unfortunately, this study only identified the different types of larval habitats; future research is needed to fully characterise larval habitats in order to generate additional information valuable for an effective and targeted larval source management programme.

The four predictors associated with reduced counts of malaria vectors in housing structures were the number of people who slept in a housing structure, housing structures with a thatched roof, the number of LLINs used the previous night and housing structures sprayed with Fludora Fusion. However, only housing structures sprayed were found to be statistically associated with reduced counts of malaria vectors, similar to what was found in Sao Tome and Principe.[39] Individuals who sleep in sprayed houses experience a lower vector-to-host contact, which entails reduced exposure to infectious mosquito bites, unlike those sleeping in unsprayed houses. Additionally, maximum benefit is derived when at least 85% of houses are sprayed with an efficacious insecticide to kill host-seeking mosquitoes that rest indoors.[43] On the other hand, the number of animals in a housing structure, housing structures with mud wall surfaces and open eaves were associated with increased counts of malaria vectors but were not statistically significant. Elsewhere, a study conducted in Cameroon associated open eaves and holes in the walls with increased mosquito counts.[44] Another study in Gambia also found that closing the eaves reduces mosquitoes entering thatched houses but increases mosquito entry into metal-roofed houses.[45]

Susceptibility tests in this study reveal that *An. gambiae s.s* was fully susceptible to organophosphates (malathion and pirimiphos-methyl) and neonicotinoids (clothianidin). This was also observed in several other districts in Zambia, where *An. funestus s.s* and *An. gambiae s.s* were found to be susceptible to these two classes of insecticides.[19 21] In that regard, organophosphates and neonicotinoids could be effective for controlling mosquito populations of *An. gambiae s.s* in Ndola and several other districts in Zambia, with evidence of susceptibility. However, resistance of *An. gambiae s.s* to pyrethroids (permethrin and deltamethrin) and carbamates (bendiocarb) was confirmed, and this could be attributed to the extensive use of pesticides and insecticides for agriculture and public health purposes. These results align with previous studies that found extensive insecticide resistance to pyrethroids and carbamates in the Copperbelt Province.[9 17] In the wake of widespread resistance to pyrethroids and carbamates, there is reduced efficacy of the malaria vector control tools used and lowered community protection where carbamate and pyrethroid-only active ingredients are used. As such, this has the potential to drive transmission in Ndola District despite implementing these interventions.

Limitations of this study

The study was conducted in the dry season, and the entomological indices determined may only apply for the dry season. Additionally, due to the scarcity of *An. funestus s.s* larval habitats, this study did not determine the susceptibility status of *An. funestus s.s.*

CONCLUSION

The two primary malaria vectors *An. funestus s.s* and *An. gambiae s.s* were found to coexist in the two ecologically distinct settings, with *An. funestus s.s* being the dominant malaria vector. This coexistence has the potential of sustaining high malaria transmission throughout the year, especially in urban areas. Urban agriculture practices created *An. gambiae s.s* breeding sites during the dry season and contributed to the high host-seeking and indoor resting behaviour in the urban sites. Sprayed housing structures were associated with reduced counts of malaria vectors. *An. gambiae* was found to be susceptible to organophosphates and neonicotinoids, but resistance to pyrethroids, carbamates and organochlorides was confirmed. Additional studies are needed to investigate the different mechanisms of pyrethroid resistance in the area.

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