Khatib et al. Malaria Journal (2025) 24:92
https://doi.org/10.1186/s12936-025-05333-6

Malaria Journal

RESEARCH

Open Access

Early evening outdoor biting by malaria-infected *Anopheles arabiensis* vectors threatens malaria elimination efforts in Zanzibar

Bakar Khatib[1], Juma Mcha[1], Zamzam Pandu[1], Makame Haji[1], Makame Hassan[1], Huba Ali[1], Ramla Mrisho[1], Kali Abdallah[1], Ali Ali[1], Khadija Ali[1], Talib Said[1], Safia Mohamed[1], Humphrey Mkali[2], Said Mgata[2], Stella Makwaruzi[2], Michael Gulaka[2], Geofrey Makenga[2], Sigsbert Mkude[2], Victoria Githu[3], Victor Mero[3], Naomi Serbantez[4], Sarah-Blythe Ballard[5], Adeline Chan[6], Shija Joseph Shija[1] and Nicodem J. Govella[2,3*]

Abstract

Background The Zanzibar Malaria Elimination Programme relies on insecticide-treated nets as the principal vector control method, supplemented by reactive focal indoor residual spraying. Despite the success, local malaria transmission persists, and the underlying reasons for sustained transmission remain unclear, yet critical to optimizing vector control for elimination. Entomological characterization of transmission dynamics was conducted to identify the gaps with existing interventions and opportunities for complementary interventions.

Methods Adult malaria vectors were collected monthly for two consecutive nights at ten sentinel sites (6 Unguja, 4 Pemba) from October 2022 to September 2023. Hourly indoor and outdoor human landing catch method was used for collecting mosquitoes from 18:00 to 06:00 h.

Results *Anopheles arabiensis* was the predominant malaria vector species across all the sentinel sites, except in the urban district of Unguja, where *Anopheles gambiae* sensu stricto was predominant. Malaria parasite-infected *An. arabiensis* bites were distributed disproportionately between indoors ($n = 4$), 22:00 to 02:00 h, and outdoors ($n = 10$) earlier in the evenings, 1800 to 2100 h.

Conclusion The outdoor catches of malaria-parasite infected mosquitoes before typical sleeping hours highlight the potential risk of human exposure to outdoor transmission.

Keywords outdoor biting, malaria elimination, entomological characterization, malaria vector, malaria transmission, malaria elimination in Zanzibar

*Correspondence:
Nicodem J. Govella
ngovella@psi.or.tz; govella@ihi.or.tz
Full list of author information is available at the end of the article

Background

The Zanzibar Malaria Elimination Programme (ZAMEP) has successfully sustained a low human *Plasmodium falciparum* malaria prevalence of < 1% over the past decade through vector control interventions comprised primarily of insecticide-treated nets (ITNs), complemented with indoor residual spraying (IRS) [1]. Despite this success, local transmission persists, and the underlying reasons for the sustained transmission remain unclear. Understanding the drivers of sustained transmission is critical for optimizing malaria vector control responses and tailoring complementary interventions for malaria elimination. A growing body of evidence indicates the effectiveness of ITNs and/or IRS is being compromised by biting location, biting times of vector [2, 3], human behaviours, particularly outdoor activities that increase biting risk [4, 5], insecticide resistance [6] and low utilization of intervention [7]. The relationship between human behaviour and adapted mosquito behaviours are highly context-specific. Thus, local entomological investigations are important for informing vector control approaches in elimination areas.

Epidemiological data from 2022–2023 in Zanzibar, based on Malaria Case Notification (MCN) system [8], demonstrated a sharp increase in malaria cases: 19,007 from July 2022 through June 2023, compared to 4,603 cases reported over the previous 12 months with majority of cases shown to be local (Zanzibar Malaria Elimination Program, Weekly Malaria Report, Unpublished). Evidence from across sub-Saharan Africa shows that, following wide-spread use of ITNs, the species composition of malaria vectors has changed since 2005, with progressive declining in dominance of the highly anthropophilic, endophagic, and endophilic *Anopheles gambiae* sensu stricto (*s.s.*) to its sibling species *Anopheles arabiensis* [9–11], which is less vulnerable to indoor-targeted interventions [12–14].

Unlike *An. gambiae s.s.*, which has a strong preference for human blood and typically bites indoors late at night, making it highly vulnerable to vector control interventions designed to protect indoor and sleeping spaces, *An. arabiensis*, exhibits flexible feeding and resting behaviours. Some of these behaviours may attenuate the impact of indoor-targeted interventions, including ITNs and/or IRS [2, 3, 15–18]. Such atypical behaviours include early exit from houses, outdoor early evening or morning biting habits, and feeding on non-human hosts [14, 15]. These behaviours increase their likelihood of evading fatal contact with ITNs or IRS, enabling a large proportion of them to survive to an age when they are capable of transmitting the pathogen [19], thereby sustaining residual transmission [3, 16].

While the available epidemiological data provide insight into the burden [20, 21] of malaria including case distribution, classification [22, 23], and human behavioural/occupational risk factors in Zanzibar, they do not fully explain transmission drivers, gaps in existing interventions, or identify opportunities for complementary vector control interventions. Here, entomological investigations were conducted to identify the malaria vector species composition, mosquito biting behaviours, and risk of human indoor and outdoor exposure to infectious bites throughout nighttime hours to inform selection and aid optimization of vector control strategies.

Methods

Study area and sentinel sites

The Zanzibar archipelago measures approximate 2461 sq km and is located off the northeast coast of the Tanzania mainland. It is made up of two large islands, Unguja and Pemba, both aiming for malaria elimination. Zanzibar has great variability in the distribution of malaria cases across the eleven districts. *Anopheles arabiensis*, a member of *An. gambiae* complex, is widely distributed across Zanzibar. Other members of *An. gambiae* complex present include *Anopheles merus* and *An. gambiae s.s.*. The latter two species are known to be focalized in relatively few places [24, 25]. Zanzibar experiences a bi-modal rainfall pattern with the short rainy season typically from October to December and the long rainy season from March to June, with some variation in recent years.

Ten pre-established sentinel sites, 6 from Unguja and 4 from Pemba, were used for the routine monitoring of malaria vector bionomics including vector abundance, species composition, vector biting time and biting location (outdoor versus indoor) and sporozoite infection status (Fig. 1). Sentinel site selection was based on criteria that included: a diversity of malaria endemicity, risk of malaria from environmental determinants (agricultural practices such as rice irrigation), topography, and population density (urban versus rural). One sentinel site from each of the 10 districts was selected as shown in Fig. 1.

Mosquito sampling

Mosquitoes were collected by human landing catch method (HLC). With this method, a volunteer sits on a chair while exposing their legs and aspirates mosquitoes attempting to bite them using a mouth aspirator [26–28]. Human landing catch is allowable in Zanzibar; however, written informed consent was obtained from all volunteers participating in the activity. The volunteers conducting HLC were provided with malaria prophylaxis and were all administered deworming albendazole for lymphatic filariasis as part of the annual mass drug administration program conducted in Zanzibar. Research

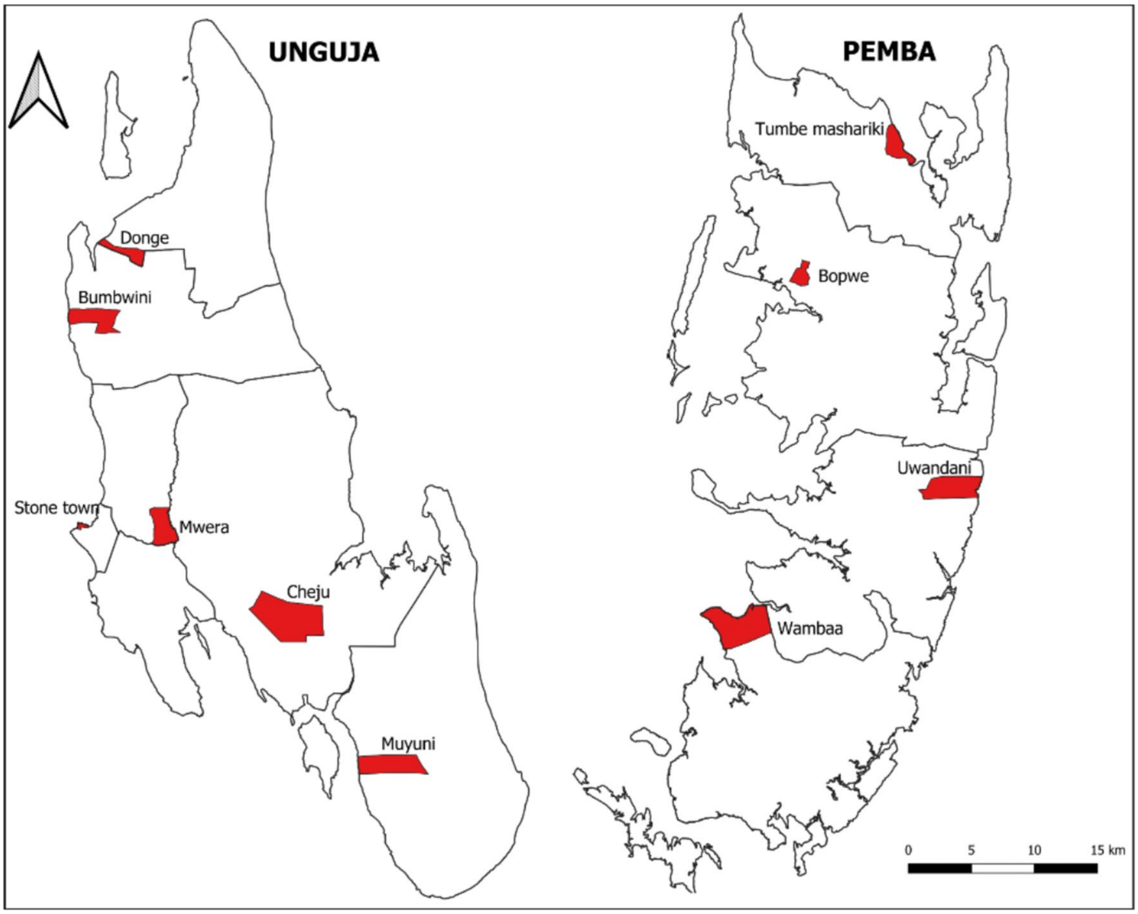

Fig. 1 Distribution of the sentinel sites across Unguja and Pemba (red-represent sentinel site)

indicates that HLC volunteers on prophylaxis are considerably at relatively low risk of malaria than the surrounding population [29]. Whether there is increased risk of HLC volunteers to other vector-borne diseases remain unknown. Human landing catch was conducted hourly from 18:00 to 06:00 h from two randomly selected sentinel houses in each of the sentinel sites. Collections were made from both indoors and outdoors at each house monthly for two consecutive nights from October 2022 to September 2023. Each collection hour was designed to include 45 min for mosquito collection and a 15 min rest-break for coffee and stretching due to ethical consideration The mosquitoes collected in each hour were placed in separate pre-labelled paper cups corresponding to the hour of capture and location (indoor versus outdoor) and stored in a cool box before being transported to the laboratory during the morning hours for sorting and morphological identification. This method was conducted across all sentinel sites to allow assessment of mosquito biting density, species composition, biting time and location phenotype, and investigate the potential of local transmission.

Processing of sample and molecular analysis

Mosquito samples from all field catches were sorted, counted, and morphologically identified as either *An. gambiae* sensu lato (*s.l.*), *Anopheles funestus s.l.*, *Anopheles rufipes*, *Anopheles maculpalpis* or *Anopheles coustani* using a morphological key [30] and with the aid of a dissecting microscope. All specimens of *Anopheles* were individually stored in 1.5-mL tubes containing desiccated silica gel under cotton wool for further molecular speciation confirmation by polymerase chain reaction (PCR). The thorax and head of the same specimen of individual mosquitoes that had undergone PCR testing were subjected to sporozoite detection using enzyme-linked immunosorbent assay (ELISA) [31]. This established ELISA approach was complemented by heating all positive CSP ELISA lysate to 100 °C for 10 min to eliminate false positivity [32].

Data analysis

Data analysis was primarily descriptive, except for the assessment of indoor versus outdoor biting densities and for temporal variation in biting densities between October, 2021 to September, 2022 vs October, 2022 to September, 2023 to ascertain whether the difference was significant. In this case, a generalized linear mixed model (GLMM) effect with a Poisson distribution was applied, fitting biting location (indoor vs outdoor) as fixed effect, and date and site of data collection as random effects, with the catches of *An. arabiensis*, as the response variable. The simple generalized linear model (GLM) with a Poisson distribution was also applied for the comparison of biting densities between data collected in the years 2021/2022 and 2022/2023. Data were stratified to include three months: April, May and June. These months were selected because they coincided with the mid-year malaria surge of 2023 and, therefore, aimed to establish whether density indicators could be used as a proxy for the malaria surge. Year of data collection was treated as fixed effect and mosquito catches as response. A *p*-value less than 0.05 was considered significant.

Results

Catches of Anopheles mosquitoes, distribution and sporozoite infection

A total of 2731 *An. gambiae s.l.,* from Unguja underwent PCR analysis, and 2698 (98.8%) specimens successfully amplified. Of the successfully amplified specimens, 2505 (92.8%), 135 (5.0.%) and 58 (2.1%) were *An. arabiensis*, *An. merus* and *An. gambiae s.s.*, respectively. *Anopheles arabiensis* predominant malaria vector across sites except in Stone Town where *An. gambiae s.s.* were caught in relatively high proportion (Table 1, Fig. 2). Similarly, in Pemba, 1080 *An. gambiae s.l.,* underwent PCR analysis, and 1068 (98.9%) successfully amplified to sibling species, which included 1054 (98.7%) *An. arabiensis*, 13 (1.2%) *An. merus*, and 1 (0.1%) *An. gambiae s.s.* (Table 1, Fig. 2). Other anopheline collected a total of 4 *An. rufipes* and 9 *An. coustanii*.

Mosquitoes belonging to the *An. funestus* group were also analysed by PCR to species level. Of 20 specimens that were collected from Unguja, all 20 were *An. parensis* (Table 1). Of the 21 specimens analysed from Pemba, included 3 *An. funestus s.s.*, 17 *Anopheles parensis*, and 1 *Anopheles vaneedeni* (Table 1).

Sporozoite infection analysis was conducted on a total of 2,731 numbers of *An. arabiensis* and *An. gambiae s.s.* from Unguja. 10 specimens tested positive for *Plasmodium falciparum*, and they were all *An. arabiensis*, leading to a total sporozoite rate of 0.37%. These positive *An. arabiensis* ($n = 6$) were collected outdoors between 19:00 and 21:00 h and ($n = 4$) from indoors between 22:00 and 02:00 h. In Pemba, a total of 1080 *An. arabiensis* mosquitoes were tested for sporozoite infection. Four specimens tested positive for *P. falciparum* sporozoite infection and were all *An. arabiensis*. This represents a total sporozoite rate of 0.37%. Three specimens were collected outdoors between 18:00 and 21:00 h, and one specimen collected outdoors at 23:00 h.

Temporal variation in biting density of *An. arabiensis*

The density of *An. arabiensis* fluctuates over months with two peaks corresponding to short and long rainy seasons (Fig. 3). Between April and June 2023, the density of *An. arabiensis* increased by six-fold compared to the same months in 2022 in Unguja. Such difference was statistically significant [RR = 3.51, 95% CI = 3.11–3.96, $p < 0.001$]. In Pemba and during the same months, the density also increased in 2023 compared to 2022, but the increase did not reach statistical significance [RR = 1.11, 95%CI = 0.99–1.24, $p = 0.05$].

Table 1 Numbers and taxonomic composition of *Anopheles* species mosquitoes collected across sentinel sites

Location	Sites	*An gambiae s.s*	*An arabiensis*	*An merus*	*An funestus*	*An parensis*	*An vaneedeni*	Total
Unguja	Bumbwini	1	236	41	0	0	0	278
Unguja	Mwera	2	364	1	0	0	0	267
Unguja	Cheju	6	1807	7	0	20	0	1840
Unguja	StoneTown	41	21	4	0	0	0	66
Unguja	Donge	1	54	82	0	0	0	138
Unguja	Muyuni	7	23	0	0	0	0	30
Pemba	Bopwe	0	508	2	2	12	1	525
Pemba	Tumbe	1	423	10	0	4	0	438
Pemba	Uwandani	0	65	0	1	1	0	67
Pemba	Wambaa	0	58	1	0	0	0	59

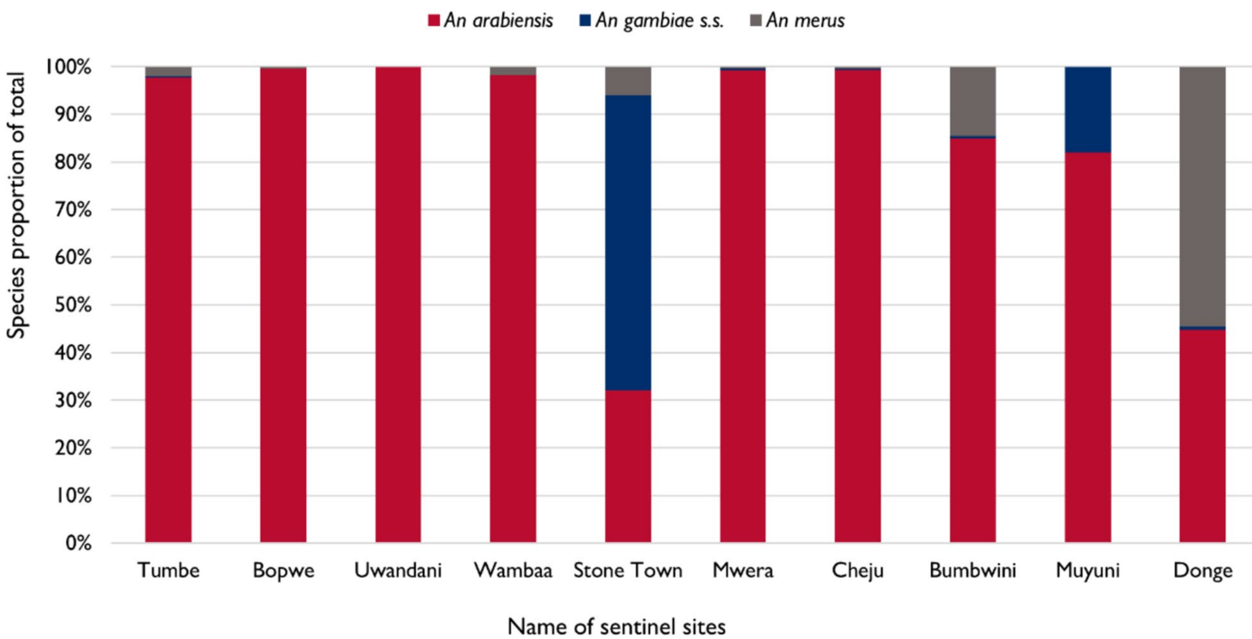

Fig. 2 Sibling species composition of *An. gambiae s.l.* by sentinel sites (deep red = *An. arabiensis*, blue = *An. gambiae* sensu stricto, grey = *An. merus*)

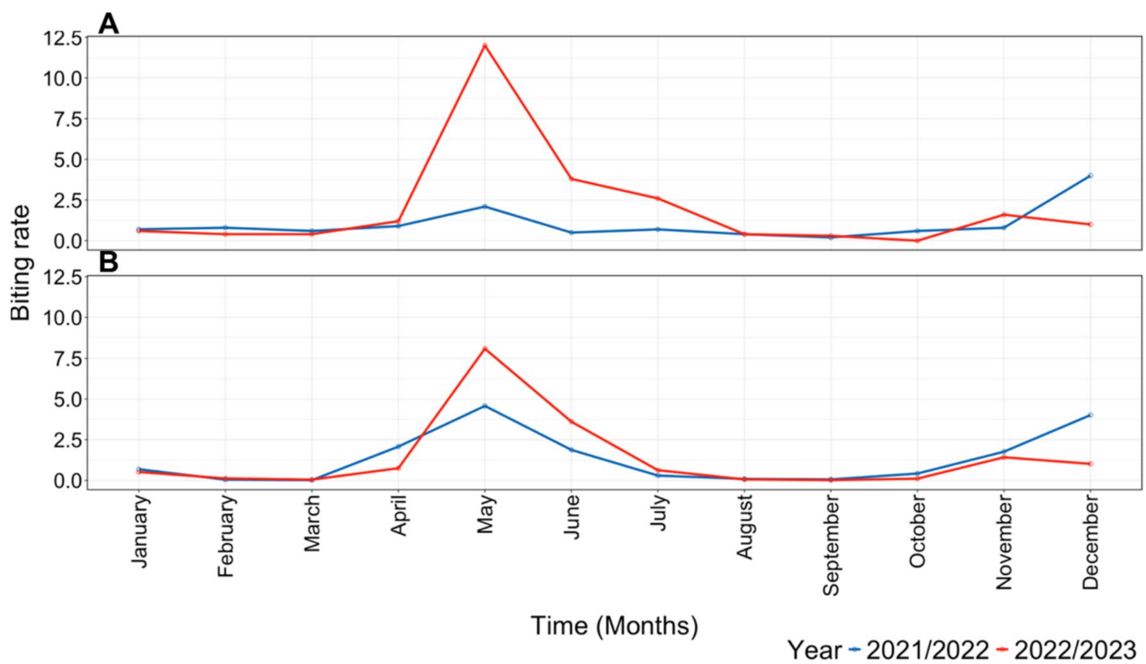

Fig. 3 Monthly biting density of *An. arabiensis* from Unguja and Pemba (**A** represent Unguja and **B** represents Pemba)

Biting behaviours

Overall, *An. arabiensis*, exhibited more outdoor biting than indoor, and this was consistent in Unguja [outdoor = 70% versus indoor = 30%, RR = 2.12, 95% CI = 1.94–2.30, $p < 0.001$] and Pemba (outdoor = 81% versus indoor = 19%, RR = 4.04, 95% CI = 3.43–4.74, $p < 0.001$]. The biting activity started just after sunset and progressed throughout the night until 06:00 h (Fig. 4a, b). This predominantly outdoor biting pattern was generally consistent across sites, except at Muyuni, Bumbwini and Stone Town and Uwandani where pattern was unclear because of the small number of catches.

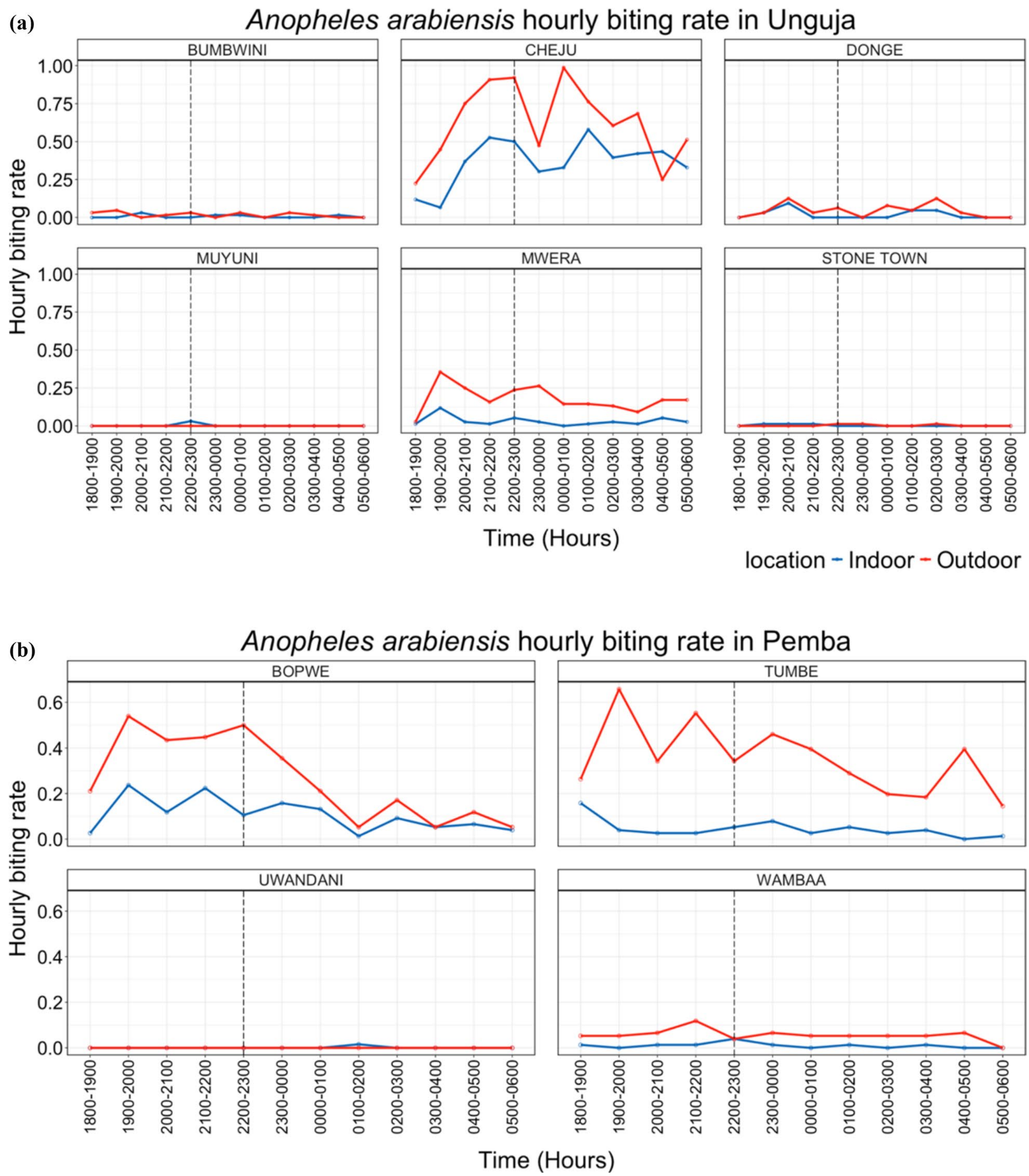

Fig. 4 **a** Hourly biting rate by sentinel site and location (outdoor vs indoor) for *An. arabiensis* in Unguja (The dashed line represent the time when most local residents are likely to be indoors and asleep under ITNs). **b** Hourly biting rate by sentinel site and location (outdoor vs indoor) for *An.arabiensis* in Pemba (The dashed line represent the time when most local residents are likely to be indoors and asleep under ITNs)

Discussion and conclusion

Anopheles arabiensis is the predominant malaria vector in Zanzibar, and its density increased substantially during the long rainy season between April and June of 2023, compared to the same period of 2022. This malaria vector exhibit more outdoor biting habit with more malaria parasite-infected mosquitoes found attempting to bite outdoors and before the likely typical sleeping hours (22:00 h) of the most local residents of this location. Despite the dominance of *An. arabiensis*, one site in Urban district (Stone Town), has relatively more *An. gambiae s.s*.., a highly efficient malaria transmitting mosquito vector. Within the *An. funestus* group, the primary and most competent *An. funestus s.s*., was detected only in Pemba, however, the secondary malaria vector species from this group, *An. parensis* is spread across the two islands..

The predominant outdoor and early biting behaviour by *An. arabiensis* supports previous findings in Zanzibar and elsewhere across sub-Sarahan Africa [15, 25]. These behaviours coupled with the catches of malaria-parasite infected mosquitoes outdoors before the likely typical sleeping hours suggest the existence of the risk of human exposure to outdoor transmission in Zanzibar. This aligns perfectly well with the findings of the recent case–control epidemiological study in Zanzibar, designed to determine populations at high risk for malaria due to occupational or behavioural characteristics. Evidence from this study indicates that people engaging in nighttime outdoor behaviours or activities including urban individuals in night watchmen or police occupations and/or individuals in rural and urban areas taking nighttime meals outdoors, were at higher risk for malaria than controls who did not report engaging in these activities (HRP study 2023, unpublished).

Therefore, while the widespread use of ITNs has proven highly effective [33] and must be sustained, interventions specifically targeting populations at high risk for malaria due to occupational or behavioural factors, which include outdoor activities where ITN use is not practical [4, 34], are necessary to interrupt malaria transmission [2, 3]. Human exposure to outdoor biting infected mosquitoes represents a period of risk against which ITNs and/or IRS are less effective. Modelling studies indicate that 20% exposure to the outdoors in areas where malaria transmission occurs may result into 10.6 million additional cases of malaria annually across Africa, regardless of sustained universal coverage of ITNs and IRS [3]. This is because ITNs and IRS prevented transmission are mediated primarily by mosquitoes biting when people are indoors or under ITNs [13]. Despite the fact that indoor-delivered insecticide-based interventions can still have an effect against outdoor-biting mosquitoes at communal level, due to repeated exposure of mosquitoes to ITNs during the gonotrophic cycle [35], thus reduces the proportion of mosquitoes surviving to the age required to transmit the pathogen [36, 37], complementary interventions targeting outdoor biting should be considered [12, 15] to contribute towards malaria elimination [38] in Zanzibar. To this end, it could be beneficial to evaluate the feasibility, cost, and effectiveness of interventions aimed at tackling outdoor biting in Zanzibar.

The dominance of *An. arabiensis* relative to its sibling species *An. gambiae s.s.* across sentinel sites supports existing findings from elsewhere in sub-Saharan Africa [9–11]. Following the wide-spread use of standard ITNs, the proportion of *An. arabiensis* has been progressively increasing relative to its sister species *An. gambiae s.s.* [10, 11, 39], which was previously the predominant malaria vector across SSA [40]. In some settings, the population of *An. gambiae s.s.* has been reduced to undetectable levels [41, 42]. This is because the use of standard ITNs has been highly effective against *An. gambiae s.s*., due to its behaviour of feeding predominantly indoor and later at night, thus increasing its vulnerability to fatal contact with ITNs [13, 15]. The persistence of *An. gambiae s.s.* in relatively higher proportion to the *An. arabiensis* at one sentinel site, Stone Town, merits consideration. Two possible reasons could explain this: (1) unlike other sites, this urban site had no history of IRS implementation until reactive focal IRS was implemented as a response to the malaria upsurge in December, 2023, and (2) there is consistently low utilization of ITNs, which is similar to other urban settings like Dar es Salaam [43, 44].

While *An. funestus s.s*. was very rare, *An. parensis* appears to be widespread. In the context of malaria elimination, this suggests the need for close monitoring, as these species are known to carry and potentially transmit human *P. falciparum* parasite, albeit to a lesser extent. For example, there are three independent reports from Mainland Tanzania confirming *An. parensis* as a carrier for malaria-causing parasites [45].

The substantial increase of malaria vector densities associated with the long rainy season in 2023 was unusual and coincided with the malaria surge in Unguja. While the specific contributors to this increase are not fully characterized, the 2023 weather patterns were associated with the El Nino Southern Oscillation events, which are considered to be associated with broader climate change [46–48]. Monitoring climatic events—particularly rainfall—and how they affect disease-vector transmission dynamics and vector control intervention efficacy should be considered to allow timely adjustment of interventions strategies.

This routine malaria vector surveillance is typically led by national malaria programmes and is not without limitations. First, the conventional measurement of mosquito behaviours ignores the importance of human movements indoors and outdoors. This is therefore, prone to misleading where and when human exposure to bites are mostly occurring and underestimation of the personal protective efficacy provided by the indoor-delivered interventions such as ITNs [3, 35, 49, 50]. This survey design prevented quantification of human exposure and estimate of protective efficacy of intervention delivered to human because of the lack of human behaviour survey in Zanzibar. Human behaviours, including time spent outdoor in the evening, time spent sleeping indoors and under ITNs and time waking up in the morning, integrated with human biting rate over time and space, are critical for determining human exposure and impact of interventions [3, 35, 49, 50]. This, is therefore, recommended to ZAMEP to integrate human behaviour survey in the routine entomological monitoring. Second, the entomological data collection remains paper-based and stored in staff computers, lacking essential key links to other ZAMEP's data platforms. This raises data security issues, limits timely data processing, and is difficult to effectively manage quality and triangulate with other data due to lack of integration with other systems. An electronic data system that allows any entomological data to be stored in entomology-specific data tables with predefined relational linkages, enabling rapid, even automated synthesis and analysis of data [51] is recommended.

Despite these limitations, this study strengthens the evidence for outdoor malaria transmission risk and calls for complementary interventions to accelerate malaria elimination efforts in Zanzibar.

Acknowledgements
We are grateful to the invaluable contributions and support from various people and institutions. We are grateful to the Ministry of Health (MOH) through Zanzibar Malaria Elimination Program (ZAMEP) for their collaboration and logistic support. We are also thankful to the Shehas from the respective study shehia for their invaluable co-operation in allowing the team for the access to the shehia and the head of households for allowing the team to conduct mosquito capturing in their houses.

Author contributions
BK, JM, ZP, MH, HA, RM, KA, AA, KA, TS and SM implemented the study. BK and NJG drafted the manuscript. NJG, VG and VM supported data analysis. HM, SM, GM, SM, NS, SB, AC, SJS and NJG conceived the study. NJG, NS, SB, and AC reviewed and edited the draft manuscript. All authors reviewed and approved the final version of the manuscript.

Funding
This study was made possible by the generous financial support of the American people through the U.S. President's Malaria Initiative (PMI) under the terms of USAID/PSI Cooperative Agreement number 72062122CA0008. Part of the study, especially, analysis and development of manuscript was jointly supported by UKRI-Medical Research Council (under the African Research Leaders Award number MR/T008873/1) and the UK Foreign, Commonwealth & Development office (FCDO) under the MRC/FCDO concordant agreement which is also part of the EDCTP2 programme supported by the European Union.

Availability of data and materials
No datasets were generated or analysed during the current study.

Declarations

Conflict of interests
The authors declare no competing interests.

Disclaimer
The findings and conclusions in this report are those of the authors and do not necessarily represent the official position of the U.S. Centers for Disease Control and Prevention or the U.S. Agency for International Development.

Author details
[1]Zanzibar Malaria Elimination Program, Ministry of Health of Zanzibar, Zanzibar, Tanzania. [2]PMI Dhibiti (Control) Malaria Project, Population Services International, Dar Es Salaam, Tanzania. [3]Environmental Health and Ecological Sciences Department, Ifakara Health Institute, Dar Es Salaam, Tanzania. [4]U.S. President's Malaria Initiative, U. S. Agency for International Development, Dar Es Salaam, Tanzania. [5]U.S. President's Malaria Initiative, US Centers for Disease Control and Prevention, Dar Es Salaam, Tanzania. [6]Entomology Branch, Division of Parasitic Diseases and Malaria, U.S. President'S Malaria Initiative, U.S. Centers for Disease Control and Prevention, Atlanta, GA, USA.

Received: 1 October 2024 Accepted: 11 March 2025
Published online: 20 March 2025

References

1. Ministry of Health. Zanzibar malaria elimination programme. In: Zanzibar malaria elimination strategic plan IV 2018/19–2022/23; December 2017.
2. Russell TL, Beebe NW, Cooper RD, Lobo NF, Burkot TR. Successful malaria elimination strategies require interventions that target changing vector behaviours. Malar J. 2013;12:56.
3. Sherrard-Smith E, Skarp JE, Beale AE, Fornadel C, Norris LC, Moore J, et al. Mosquito feeding behavior and how it influences residual malaria transmission across Africa. Proc Natl Acad Sci USA. 2019;116:15086–95.
4. Monroe A, Moore S, Koenker H, Lynch M, Ricotta E. Measuring and characterizing night time human behaviour as it relates to residual malaria transmission in sub-Saharan Africa: a review of the published literature. Malar J. 2019;18:6.
5. Finda M, Moshi IR, Monroe A, Limwagu AJ, Nyoni AP, Swai JK, et al. Linking human behaviours and malaria vector biting risk in south-eastern Tanzania. PLoS ONE. 2018;14: e0217414.
6. Ranson H, Nguessan R, Lines J, Moiroux N, Nkuni Z, Corbel V. Pyrethroid resistance in African anopheline mosquitoes: what are the implications for malaria control? Trends Parasitol. 2011;27:91–8.
7. Lindsay SW, Thomas MB, Kleinschmidt I. Threats to the effectiveness of insecticide-treated bednets for malaria control: thinking beyond insecticide resistance. Lancet Glob Health. 2021;9:e1325–31.
8. Mkali HR, Reaves E, Lalji SM, Al-mafazy A-W, Joseph JJ, Ali AS, et al. Risk factors associated with malaria infection identified through reactive case detection in Zanzibar, 2012–2019. Malar J. 2021;20:485.
9. Derua YA, Alifrangis M, Hosea KM, Meyrowitsch DW, Magesa SM, Pedersen E, et al. Change in composition of the *Anopheles gambiae* complex and its possible implications for the transmission of malaria and lymphatic filariasis in north-eastern Tanzania. Malar J. 2012;11:188.
10. Msugupakulya BJ, Urio NH, Jumanne M, Ngowo H, Selvaraj P, Okumu F, et al. Changes in contributions of different *Anopheles* vector species to malaria transmission in east and southern Africa from 2000 to 2022. Parasit Vectors. 2023;16:408.
11. Bayoh MN, Mathias DK, Odiere MR, Mutuku FM, Kamau L, Gimnig JE, et al. *Anopheles gambiae*: historical population decline associated with regional distribution of insecticide-treated bed nets in western Nyanza Province, Kenya. Malar J. 2010;9:62.
12. Killeen GF, Kiware SS, Okumu FO, Sinka ME, Moyes CL, Massey NC, et al. Going beyond personal protection against mosquito bites to eliminate malaria transmission: population suppression of malaria vectors that exploit both human and animal blood. BMJ Glob Health. 2017;2: e000198.
13. Killeen GF. Characterizing, controlling and eliminating residual malaria transmission. Malar J. 2014;13:330.
14. Kitau J, Oxborough RM, Tungu PK, Matowo J, Malima RC, Magesa SM, et al. Species shifts in the *Anopheles gambiae* complex: do llins successfully control *Anopheles arabiensis*? PLoS ONE. 2012;7: e31481.
15. Killeen GF, Marshall JM, Kiware SS, South AB, Tusting LS, Chaki PP, et al. Measuring, manipulating and exploiting behaviours of adult mosquitoes

to optimise malaria vector control impact. BMJ Glob Health. 2017;2: e000212.
16. Durnez L, Mao S, Denis L, Roelants P, Sochantha T, Coosemans M. Outdoor malaria transmission in forested villages of Cambodia. Malar J. 2013;12:329.
17. Govella NJ, Johnson PCD, Killeen GF, Ferguson HM. Heritability of biting time behaviours in the major African malaria vector *Anopheles arabiensis*. Malar J. 2023;22:238.
18. Taylor B. Changes in the feeding behaviour of a malaria vector, *Anopheles farauti* Lav., following use of DDT as a residual spray in houses in the British Solomon Islands Protectorate. Trans R Entomol Soc Lond. 1975;127:277–92.
19. Smith DL, Battle KE, Hay SI, Barker CM, Scott TW, Mckenzie FE. Ross, Macdonald, and a theory for the dynamics and control of mosquito-transmitted pathogens. PLoS Pathog. 2012;8: e1002588.
20. Thawer SG, Chaky F, Runge M, Reaves E, Mandike R, Lazaro S, et al. Sub-national stratification of malaria riskin mainland Tanzania: a simplified assembly of survey and routine data. Malar J. 2020;19:177.
21. Chacky F, Runge M, Rumisha SF, Machafuko P, Chaki PP, Massaga JJ, et al. Nationwide school malaria parasitaemia survey in public primary schools, the United Republic of Tanzania. Malar J. 2018;17:452.
22. Le Menach A, Tatem AJ, Cohen JM, Hay SI, Randell H, Patil AP, et al. Travel risk, malaria importation and malaria transmission in Zanzibar. Sci Rep. 2011;1:93.
23. Fakih BS, Holzschuh A, Ross A, Stuck L, Abdul R, Al-Mafazy A-W, et al. Risk of imported malaria infections in Zanzibar. A cross-sectional study. Infect Dis Poverty. 2023;12:80.
24. Bjorkman A, Shakely D, Ali A, Morris U, Mkali H, Abbas AK, et al. From high to low malaria transmission in Zanzibar—challenges and opportunities to achieve elimination. BMC Med. 2019;17:14.
25. Musiba RM, Tarimo BB, Monroe A, Msaky D, Ngowo H, Mihayo K, et al. Outdoor biting and pyrethroid resistance as potential drivers of persistent malaria transmission in Zanzibar. Malar J. 2022;21:172.
26. Govella NJ, Maliti DF, Mlwale AT, Masallu JP, Mirzai N, Johnson PCD, et al. An improved mosquito electrocuting trap that safely reproduces epidemiologically relevant metrics of mosquito human-feeding behaviours as determined by human landing catch. Malar J. 2016;15:465.
27. Service MW. Mosquito ecology: field sampling methods. 2nd ed. London and New York: Elsevier Applied Science; 1993.
28. Mboera LEG. Sampling techniques for adult Afrotropical malaria vectors and their reliability in the estimation of entomological inoculation rate. Tanzania J Health Res. 2005;7:117–24.
29. Gimnig JE, Walker ED, Otieno P, Kosgei J, Olang G, Ombok M, et al. Incidence of malaria among mosquito collectors conducting human landing catches in Western Kenya. Am J Trop Med Hyg. 2013;88:301–8.
30. Coetzee M. Key to the females of Afrotropical *Anopheles* mosquitoes (Diptera: Culicidae). Malar J. 2020;19:70.
31. Wirtz RA, Duncan JF, Njelesani EK, Schneider I, Brown AE, Oster CN, et al. ELISA method for detecting *Plasmodium falciparum* circumsporozoite antibody. Bull World Health Organ. 1989;67:535–42.
32. Durnez L, Van Bortel W, Denis L, Roelants P, Veracx A, Trung HD, et al. False positive circumsporozoite protein ELISA: a challenge for the estimation of the entomological inoculation rate of malaria and for vector elimination. Malar J. 2011;10:195.
33. Bhatt S, Weiss DJ, Cameron E, Bisanzio D, Mappin B, Dalrymple U, et al. The effect of malaria control on *Plasmodium falciparum* in Africa between 2000 and 2015. Nature. 2015;526:207–11.
34. Monroe A, Asamoah O, Lam Y, Koenker H, Psychas P, Lynch M, et al. Outdoor-sleeping and other night-time activities in northern Ghana: implications for residual transmission and malaria prevention. Malar J. 2015;14:35.
35. Govella NJ, Okumu FO, Killeen GF. Insecticide-treated nets can reduce malaria transmission by mosquitoes which feed outdoors. Am J Trop Med Hyg. 2010;82:415–9.
36. MacDonald G. The epidemiology and control of malaria. London: Oxford University Press; 1957.
37. Garrett-Jones C, Shidrawi GR. Malaria vectorial capacity of a population of *Anopheles gambiae*. Bull World Health Organ. 1969;40:531–45.
38. WHO. Global technical strategy for malaria 2016–2030. Geneva: World Health Organization; 2016.
39. Derua YA, Kweka EJ, Kisinza W, Githeko AK, Mosha FW. Bacterial larvicides used for malaria vector control in sub-Saharan Africa: review of their effectiveness and operational feasibility. Parasit Vectors. 2019;12:426.
40. Sinka ME, Bangs MJ, Manguin S, Coetzee M, Mbogo CM, Hemingway J, et al. The dominant *Anopheles* vectors of human malaria in Africa, Europe and the Middle East: occurrence data, distribution maps and bionomic précis. Parasit Vectors. 2010;3:117.
41. Kaindoa EW, Matowo NS, Ngowo H, Mkandawile G, Mmbando A, Finda M, et al. Interventions that effectively target *Anopheles funestus* mosquitoes could significantly improve control of persistent malaria transmission in south–eastern Tanzania. PLoS ONE. 2017;12: e0177807.
42. Mwalimu CD, Kiware S, Nshama R, Derua Y, Machafuko P, Gitanya P, et al. Dynamics of malaria vector composition and *Plasmodium falciparum* infection in mainland Tanzania: 2017–2021 data from the national malaria vector entomological surveillance. Malar J. 2024;23:29.
43. Killeen GF, Govella NJ, Mlacha Y, Chaki PP. Suppression of malaria vector densities and human infection prevalence associated with scale-up of mosquito-proofed housing in Dar es Salaam, Tanzania: re-analysis of an observational series of parasitological and entomological surveys. Lancet Planet Health. 2019;3:e132–43.
44. NBS. Tanzania demographic and health survey and malaria indicator survey 2022 key indicators report. Tanzania, Dodoma; 2023.
45. Mapua SA, Samb B, Nambunga IH, Mkandawile G, Bwanaly H, Kaindoa EW, et al. Entomological survey of sibling species in the *Anopheles funestus* group in Tanzania confirms the role of Anopheles parensis as a secondary malaria vector. Parasit Vectors. 2024;17:261.
46. Ferguson HM, Govella NJ. Impacts of climate change on malaria vector control in Africa. In: Fornace K, Conn J, Mureb MA, Chaves LSM, Logan J, editors. Planetary health approaches to understand and control vector borne diseases, Chap. 13. Netherlands: Wageningen Academic Publ; 2024.
47. Giesen C, Roche J, Redondo-Bravo L. The impact of climate change on mosquito-borne diseases in Africa. Pathog Glob Health. 2020;114:287–301.
48. Piovezan-Borges AC, Valente-Neto F, Urbieta GL, Laurence SGW, de Oliveira RF. Global trends in research on the effects of climate change on *Aedes aegypti*: international collaboration has increased, but some critical countries lag behind. Parasit Vectors. 2022;15:346.
49. Namango IH, Moore SJ, Marshall C, Saddler Am Kaftan D, Tenywa FC, et al. A matter of timing: biting by malaria-infected *Anopheles* mosquitoes and the use of interventions during the night in rural south-eastern Tanzania. PLoS Glob Public Health. 2024;4: e0003864.
50. Monroe A, Moore S, Okumu F, Kiware S, Lobo NF, Koenker H, et al. Methods and indicators for measuring patterns of human exposure to malaria vectors. Malar J. 2020;19:207.
51. Kiware SS, Russell TL, Mtema ZJ, Malishee AD, Chaki PP, Lwetoijera DW, et al. A generic schema and data collection forms applicable to diverse entomological studies of mosquitoes. Source Code Biol Med. 2016;11:4.

Publisher's Note