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Seasonal variation in abundance and blood meal sources of primary and secondary malaria vectors within Kilombero Valley, Southern Tanzania

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Abstract

Background: Existing control tools have significantly reduced malaria over the past two decades. However, progress has been stalled due to increased resistance in primary vectors and the increasing role of secondary vectors. This study aimed to investigate the impact of seasonal change on primary and secondary vector abundance and host preference. Understanding the impact of seasonal dynamics of primary and secondary vectors on disease transmission will inform effective strategies for vector management and control.

Methods: Vector abundance was measured through longitudinal collection of mosquitoes, conducted monthly during the wet and dry seasons, in Sagamaganga, a village in the Kilombero Valley, Tanzania. Mosquitoes were collected indoors using CDC light traps and backpack aspirators, and outdoors using resting buckets baited with cattle urine. In addition, a direct measure of host preference was taken monthly using human- and cattle-baited mosquito electrocuting traps. A host census was conducted to provide an indirect measure of host preference together with monthly blood meal source analysis. All collected mosquitoes were assayed for *Plasmodium* sporozoites.

Results: A total of 2828 anophelines were collected, of which 78.5% and 21.4%, were primary and secondary vectors, respectively. The abundance of the primary vectors, *Anopheles arabiensis* and *Anopheles funestus*, and of the secondary vectors varied seasonally. Indirect measures of host preference indicated that all vectors varied blood meal choice seasonally, with the direct measure confirming this for *An. arabiensis*. All anopheline mosquitoes tested negative for sporozoites.

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Conclusions: At the study location, the abundance of both primary and secondary vectors changed seasonally. Indirect and direct measures of host preference demonstrated that *An. arabiensis* varied from being zoophilic to being more opportunistic during the wet and dry seasons. A similar trend was observed for the other vectors.

Keywords: Mosquito, Abundance, Blood meal source, Host preference, Sporozoites

Background

The general burden of malaria in sub-Saharan Africa (SSA) has decreased significantly over the last decade [1, 2]. This reduction is largely due to improved coverage of long-lasting insecticidal nets (LLINs) and indoor residual spraying (IRS), as well as early diagnosis and treatment of malaria with artemisinin-based combination therapy [1, 3–6]. This commendable reduction has, however, recently stalled [2], predominantly due to increasing behavioral and physiological resistance in primary vectors [7–9] and the increasing role of secondary vectors in disease transmission. The existing frontline vector control tools are not effective at targeting the resistant primary and the emerging secondary vectors [10, 11].

Historically, primary malaria vectors have been responsible for approximately 95% of the transmission in SSA [12, 13]. Over the past two decades, however, there has been a considerable change in the composition and designation of primary malaria vector species throughout this region [14–16], with secondary vectors now known to contribute to malaria transmission, particularly due to their exophilic and exophagic behaviors [17–19]. Moreover, recent studies, though few in number, indicate that secondary vectors are increasingly reported as contributing to ongoing residual malaria transmission [18, 19]. Current understanding of the ecology of both primary and secondary vectors, and how these respond to seasonal variation, remains limited but is critical for the design and allocation of interventions. Thus, updated regional information on the seasonal activity of, and malaria transmission intensity in, primary and secondary vector populations is necessary to help strengthen knowledge on how to tackle these vectors [20–22].

In Tanzania, and other parts of SSA, *Anopheles arabiensis* and *Anopheles funestus* sensu stricto (*An. funestus* s.s.) are the primary malaria vectors [23–25]. These vectors differ in their feeding preference and malaria transmission efficiency, with *An. funestus* s.s. being more anthropophilic than *An. arabiensis*, which rather are more opportunistic [11, 26, 27]. Due to this difference in host preference, *An. funestus* s.s. is considered to be the predominant malaria vector in the area [24, 28]. Control of these and other vectors is limited, due to increased insecticide and behavioral resistance [8] and a current dearth of knowledge concerning their ecology and population dynamics at a local scale, both of which are

pertinent for improving surveillance and control strategies to achieve malaria elimination. In this study, a longitudinal investigation was performed to determine (i) the seasonal abundance and blood meal sources, as a proxy for host preference, of primary and secondary malaria vector species in Sagamaganga village, Kilombero Valley, Tanzania; and (ii) the potential contribution of secondary malaria vectors in transmitting malaria parasites. We discuss our results in the context of vector ecology and its contribution to future sustainable vector control strategies and management.

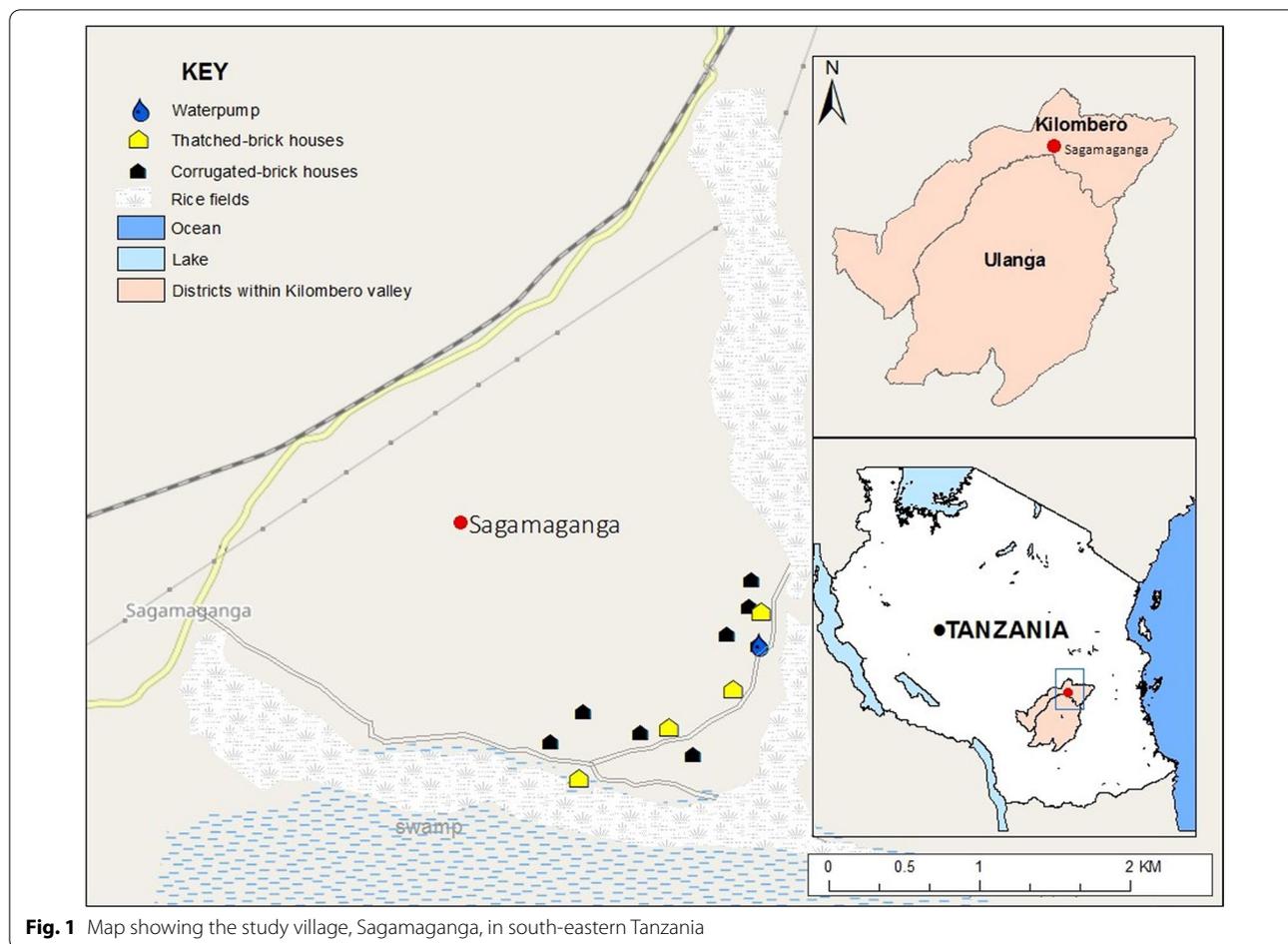
Methods

Study area

This study was conducted in Sagamaganga village (8°3'50.352" S, 36°47'46.254" E), in the Kilombero Valley, south-eastern Tanzania (Fig. 1). The valley lies 300 m a.s.l. The average annual temperature ranges between 20 °C and 32 °C, with the annual rainfall ranging between 1200 and 1800 mm [29]. The general wet and dry seasons are from February to June and July to January, respectively. The main economic activities are agricultural, and include rice cultivation and livestock keeping. The most common domestic animals include cattle, goats, sheep, chickens and dogs. The malaria prevalence in Kilombero valley has generally decreased over the past decade, notably from 14% in 2011 to 11% in 2017 [30, 31].

Selection and characteristics of study households

The global positioning system (GPS) coordinates of all households, in the part of Sagamaganga village where the study was conducted, were recorded using a handheld GPS (Garmin GPSMAP 60CSx; Garmin International Inc., Olathe, USA) before the final selection of study households. Ten study households were selected from the list of 33 houses in the part of the village with high livestock keeping, using a simple random sampling technique by R statistical software (version 3.6.2; [47]). All study households had mud-brick walls and open eaves. Four households had thatched roofs, while the remaining six had corrugated iron roofs. One household had no cattle shed, but was surrounded by cattle sheds from neighboring households. The number of occupants per household varied from one to five. Depending on the number of beds, each household was provided with one to three new LLINs (Olyset; A to Z Textiles Mills,



Arusha, Tanzania). In half of the study households, chickens were kept in the living rooms during the night. In all study households, cooking was done outside.

Host census

The host census data were obtained before the onset of the study by surveying the household owners within the study area regarding the number of household occupants and the number of animals owned and present during the study.

Adult mosquito collections

Mosquitoes were collected from inside and outside the study households. Indoor collections were conducted using one US Centers for Disease Control and Prevention (CDC) light trap (model 512; John W. Hock Company, Gainesville, FL, USA) per household [32], and a CDC backpack aspirator (John W. Hock Company) [33]. Outdoor collections were conducted using resting buckets (black plastic, 25.4 × 43 cm, diameter × height) baited with 24-h-aged cattle urine [34, 35], and two mosquito electrocuting traps (METs) baited with

either a male human volunteer (MET_h) or a calf (MET_c) [36]. All collections were done in 2019, over a 6-month period across the wet and dry seasons, on 6 consecutive nights per month. Indoor collections using CDC light traps and backpack aspirator as well as outdoor collections using resting buckets were conducted during the first 5 nights per month, with MET trapping done on the sixth night of each month to assess host preference. The CDC light traps were suspended approximately 1.2 m above the ground next to a person sleeping under an LLIN and were turned on at 18:00 h and off at 06:00 h by a trained house occupant. The CDC backpack aspirator was used to collect mosquitoes resting on inner walls and ceilings of each house in the morning (06:00–09:00 h) for approximately 10 min. Resting buckets, lined with wet black cloth, one per household, were placed 5 m from the household and cattle shed(s), to collect outdoor resting mosquitoes. Each bucket was baited with 250 ml of 24-h-aged cattle urine collected in plastic cups. Mosquitoes inside the resting buckets were collected using a CDC backpack aspirator between 06:00 h and 09:00 h. The METs, baited with a human volunteer or a calf, were

placed 20 m apart and approximately 100 m upwind of the households (Fig. 2). Both the human and calf weighed approximately 70 kg, with the same individuals used throughout the study. The METs were placed on wooden platforms raised 20 cm above the ground. The stand legs of each platform were kept inside water-filled plastic basins to prevent ants from interfering with the collected mosquitoes. During each experimental night, trapping with METs was conducted for 12 h from 18:00 h to 06:00 h. The traps were turned off every 45 min to allow 15 min for the collection of electrocuted mosquitoes.

Mosquito species, blood meal and sporozoite analyses

Collected mosquitoes were killed with chloroform, identified using morphological identification keys [37, 38] and sorted based on their sex and abdominal status (fed and unfed). Mosquito species identified as *Anopheles gambiae* sensu lato (*An. gambiae* s.l.; $n=1713$) and *An. funestus* group ($n=507$) were individually preserved in 1.5-ml Eppendorf tubes containing silica gel and submitted to the laboratory for further species-specific identification by multiplexed PCR [39–42].

Blood meal content in mosquitoes was analyzed by enzyme-linked immunosorbent assay (ELISA; Carramore International Ltd, Holmfirth, UK) using the abdomens of all blood-fed primary and secondary malaria vectors ($n=562$) [43, 44]. Antisera from humans, cattle, goats, chickens, dogs (immunoglobulin G [IgG] identifiers; KPL, Gaithersburg, MD, USA) and sheep (VWR, Stockholm, Sweden) were used. Circumsporozoite ELISA (IgG identifiers, KPL) of heads and thoraces of all primary

and secondary malaria vectors ($n=2828$) were also conducted [45]. To avoid false positives, the ELISA lysate was heated to 100 °C for 10 min, to ensure total elimination of heat-labile non-*Plasmodium falciparum* antigens [46].

Statistical analysis

Monthly variations in mosquito collections with CDC light traps, resting buckets and one backpack aspirator were analyzed using generalized linear mixed models (GLMMs) with R statistical software version 3.6.2 [47]. Since the data were zero-inflated and over-dispersed, as confirmed by the Shapiro test, a GLMM was used with the Template Model Builder package *glmmTMB* and a negative binomial distribution, as well as extensions to accommodate the zero-inflation and over-dispersion of the data [47, 48]. Following an initial modeling including “Month,” “Household IDs,” “Trap type,” “Location of the trap,” “Roof type,” “Feeding status,” “Number of cattle sheds,” “Distance of cattle sheds from the house,” “Number of cattle,” “Number of goats,” “Number of sheep,” “Number of chickens,” “Presence of chickens inside the house,” “Use of bed nets,” “Number of occupants in the house,” “Insecticide applied to cattle” and “Repellents applied to children” as fixed effects and “Date” treated as a random effect, those effects without significance and no interaction with other effects were removed until a low and stable second-order Akaike information criterion (AICc) was achieved, and the residual deviance approached the degrees of freedom. Please note that as all houses included in this study had open eaves and mud brick walls, these characters were not included in the analyses. In the final model addressing seasonal change in abundance, “Month,” “Location of the trap,” “Trap type” and “Household IDs” were treated as fixed effects, while “Date” was treated as a random effect. Separate analyses were performed for primary and secondary malaria vectors. In the final model addressing host preference, which included only blood-fed females, host preference was estimated as the percentage of fully fed mosquitoes for a given blood meal. A GLMM (*lme4* package, [47]) was used with a negative binomial distribution and accounting for over-dispersion to analyze the variation in host preference of fed mosquitoes across seasons, as well as across the locations (indoor/outdoor). The fixed effects were “Month,” “Location of the trap,” “Trap type” and “Household IDs,” while “Date” was treated as a random effect. No statistical analysis was conducted for sporozoite assays, as all mosquitoes tested negative.

Ethics approval and consent to participate

The ethics approval to conduct this study was obtained from the Institutional Review Board of the Ifakara Health Institute (Ref. IHI/IRB/EXT/No: 23 – 2019), and

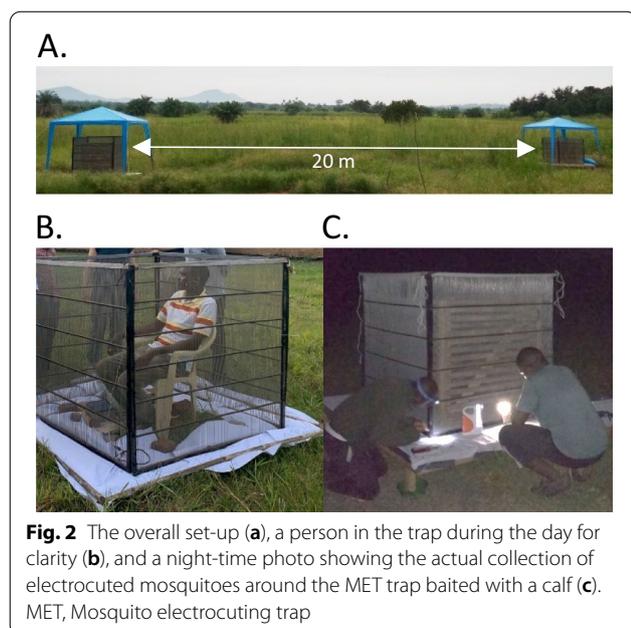


Fig. 2 The overall set-up (a), a person in the trap during the day for clarity (b), and a night-time photo showing the actual collection of electrocuted mosquitoes around the MET trap baited with a calf (c). MET, Mosquito electrocuting trap

the Medical Research Coordination Committee at the National Institute for Medical Research (NIMR) (Ref. NIMR/HQ/R.8a/Vol.IX/3085). Before data collection, community meetings were conducted to explain the purpose and data collection procedures. Signed informed consent was obtained from the heads of all study households. All study households were provided with a sufficient number of LLINs (Olyset® nets).

Results

Overall mosquito abundance

Combined across all the trapping collections, a total of 19,586 mosquitoes were caught and identified, of which 5877 (2828 females, 3049 males) were anophelines and 13,709 (9967 females, 3742 males) were culicines. The 2828 female primary and secondary vectors assayed by PCR were identified to be *An. arabiensis*, *An. funestus* s.s., *An. lesoni*, and *An. rivulorum* (Table 1). The primary vectors, *An. arabiensis* and *An. funestus* s.s., collected with CDC light traps, backpack aspirators, and outdoor resting boxes were more abundant than the secondary vectors ($P=0.020$). Of the secondary vectors, the dominant species were *An. coustani* and *An. squamosus*. The overall abundance of the anophelines (Table 1) varied across seasons ($\chi_1^2=104.06$, $P<0.001$), with significantly higher numbers collected during the wet season compared to the dry season, irrespective of the trap type (GLMM: OR=5.10, $P<0.001$, Fig. 3). During the wet season, the abundance of vectors outdoors increased by two-fold relative to indoors (GLMM: OR=2.42, $P<0.001$). Roof type was found to have no effect of mosquito abundance in the GLMM.

Seasonal change in the abundance of primary and secondary malaria vectors

Anopheles arabiensis mosquitoes were collected in significantly higher numbers throughout the seasons (GLMM: odds ratio [OR]=5.03, $P=0.015$) compared to that of other vectors, peaking during May ($\chi_1^2=2.84$, 95% confidence interval [CI]: 1.72–3.97) and June ($\chi_1^2=3.37$, 95% CI: 0.05–7.67) (Fig. 3a). The abundance of *An. funestus* s.s. and *An. coustani* showed similar seasonal patterns as *An. arabiensis*, peaking in May ($\chi_1^2=1.62$, 95% CI: 1.2–2.0; $\chi_1^2=0.78$, 95% CI: 0.4–1.2) and June ($\chi_1^2=1.17$, 95% CI: 0.1–2.5; $\chi_1^2=0.43$, 95% CI: –0.14 to 1.01) (Fig. 3b, c). In contrast, the abundance of the remaining secondary vectors declined significantly in June, to a level maintained throughout the dry season (Fig. 3d). The rate of increase in abundance of all secondary vectors appeared constant from March to May, whereas the abundance of the primary vectors significantly increased at a faster rate later in the season between April and May (GLMM: OR=3.33, $P<0.001$) (Fig. 3).

The effect of host prevalence on mosquito abundance

The host census revealed an unequal abundance (Table 2) and distribution of potential human and animal hosts in the study village. Households with > 30 cattle had significantly higher numbers of primary (by approx. twofold) and secondary (by approx. fivefold) vectors compared to those with zero to 30 cattle (GLMM: OR=1.98, $P=0.006$ and OR=4.64, $P=0.003$, respectively). Moreover, households with 11–50 sheep had a significantly higher number of primary and secondary vectors compared to those without sheep (GLMM: OR=2.81, $P=0.006$ and OR=4.77, $P=0.037$, respectively). Approximately 61% (95% CI: 56.7–64.7%) of the human-fed primary and secondary vectors were collected in households with > 30 cattle, while the remaining vectors (95% CI: 35.6–43.6%) were collected in households with a lower number of cattle. Neither the presence nor the abundance of any of the other remaining hosts, including chickens, were found to affect mosquito abundance.

Blood meal source

As a proxy for host preference, all blood-fed *Anopheles* mosquitoes ($n = 562$) were analyzed for meal source (Table 3). Most blood-fed mosquitoes were caught in the resting buckets and the CDC light traps. The majority of the identified blood meals in *An. arabiensis* were bovine (42%) and sheep (52%), with only 2.8% of the mosquitoes having fed on a human (Table 3). In contrast, the majority of *An. funestus* s.l. fed on humans, while the rest fed on cattle or both human and cattle (Table 3). The blood meal analysis of the secondary vectors identified bovine as the primary blood meal source, with few blood meals from humans and other hosts (Table 3). Neither primary nor secondary vectors were found to contain chicken blood, despite this being the second most abundant vertebrate in the area (Table 3).

Blood meal source varied significantly over the seasons ($\chi_1^2=18.81$, $P=0.016$), and the number of fed mosquitoes was approximately fivefold higher during the wet season than during the dry season (GLMM: $P<0.001$) (Fig. 4). There was no association between location (indoor/outdoor) and blood meal source imbibed by fed mosquitoes ($\chi_1^2=4.86$, $P=0.772$). The balance between the blood meal sources in *An. arabiensis* changed across the seasons, with bovine blood meals dominating during the late wet and beginning of the dry seasons (May to August), while before and after this period sheep blood was predominant (Fig. 4). Although only a low number of blood-fed *An. funestus* s.s. were caught (Table 3), a large proportion of these were found to have human blood meals between May and August, while prior to this period the dominant blood source was bovine (Fig. 4). The majority of secondary vector species were only found

Table 1 Malaria vectors collected indoors and outdoors during the wet and dry seasons

Season (months)	Location	Collection/trapping method	Primary <i>Anopheles</i> vector species		Secondary <i>Anopheles</i> vector species									
			<i>An. arabiensis</i>	<i>An. funestus sensu stricto</i>	<i>An. leesonii</i>	<i>An. rivulurum</i>	Unknown <i>An. funestus</i>	<i>An. coustani</i>	<i>An. squamosus</i>	<i>An. pharoensis</i>	<i>An. ziemanni</i>	<i>An. rufipes</i>	<i>An. tenebrosus</i>	<i>An. welcomei</i>
Household collections (March-June)	Indoor	CDC	658 (332 ^a)	123 (109 ^b)	84	36	23	155 (438)	153 (22)	105 (700)	13 (34)	9 (0)	6 (1)	2 (0)
	Indoor	BP	30 (21 ^a)	13 (2 ^b)	16	2	8	15 (78)	13 (0)	10 (16)	-	0 (1)	-	-
Dry (July-September)	Outdoor	RB	118 (98 ^a)	9 (23 ^b)	21	4	12	20 (23)	4 (0)	24 (87)	-	0 (3)	4 (3)	-
	Indoor	CDC	211 (209 ^a)	52 (58 ^b)	34	16	5	19 (12)	-	-	-	-	-	-
Host preference collection (March-June)	Indoor	BP	40 (16 ^a)	0 (2 ^b)	6	1	1	-	-	-	-	-	-	-
	Outdoor	RB	39 (21 ^a)	1 (1 ^b)	3	2	0	1	-	-	-	-	-	-
Total mosquitoes collected	Outdoor	METc	293 (283 ^a)	0 (18 ^b)	1	5	0	25 (71)	-	7 (43)	1 (0)	-	1 (0)	-
	Outdoor	METH	189 (176 ^a)	6 (8 ^b)	1	1	0	7 (26)	-	0 (40)	-	-	2 (0)	-
Total mosquitoes collected	Outdoor	METc	114 (14 ^a)	1 (13 ^b)	5	5	1	5 (15)	-	4 (24)	-	-	-	-
	Outdoor	METH	21 (4 ^a)	5 (1 ^b)	3	1	0	2 (1)	-	1 (2)	-	-	-	-
Total mosquitoes collected		2828	1713 (1174 ^a)	210 (235 ^b)	174	73	50	249 (664)	170 (22)	151 (912)	14 (34)	9 (4)	13 (4)	2 (0)

Values in table are the number of mosquitoes collected, with the values in parentheses indicating the number of male mosquitoes

CDC CDC light traps, BP CDC backpack aspirator, RB Resting buckets, METc mosquito electrocuting trap with cattle as bait, METH MET with human as bait

^a Males of the *An. gambiae* complex

^b Males of the *An. funestus* complex

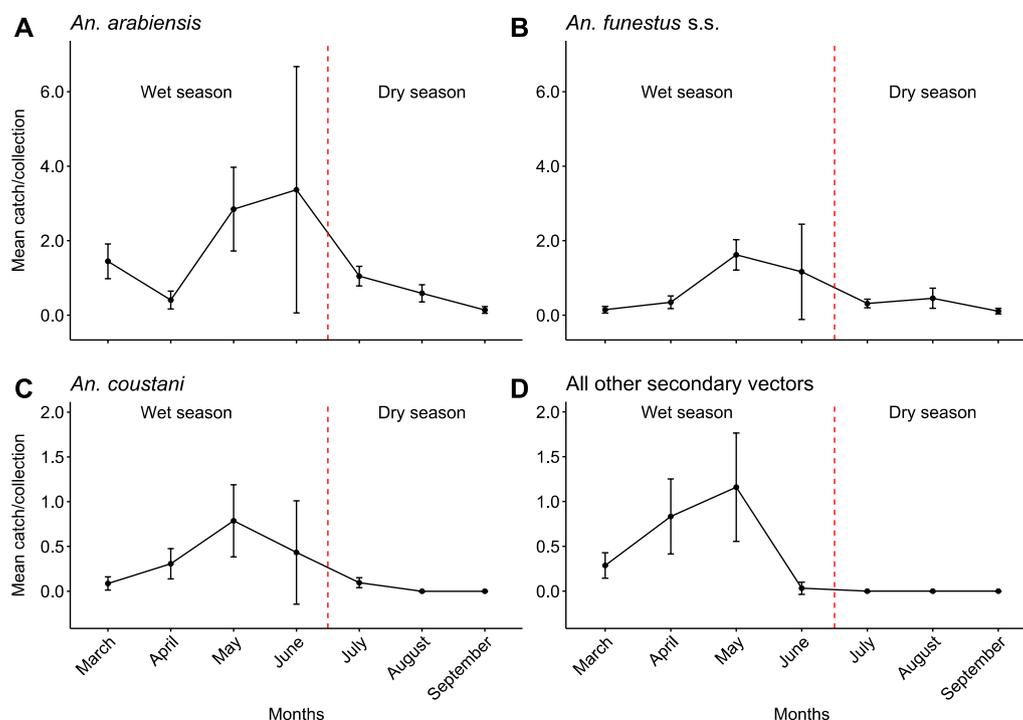


Fig. 3 Seasonal change in the abundance of primary and secondary malaria vectors during the study period. **a, b** The primary vectors, **c** the main secondary vector, **d** other secondary *Anopheles* vector species (e.g. *An. squamosus*, *An. pharoensis*, *An. ziemanni*, *An. rufipes*, *An. wellcomei*, *An. tenebrosus*)

to contain blood meals between March and May, except for *An. ziemanni*, which was only present in June (Fig. 4). While the majority of these blood meals were bovine, other sources, including human, were found (Fig. 4). The number of household occupants and insecticide treatment of the livestock (3–6 times per month, every 3 months) had no effect on mosquito blood meal source in the GLMM.

Host preference analysis

Mosquito electrocuting traps, baited with either a human or a calf, were used to directly assess host preference. Of the 707 *Anopheles* mosquitoes collected using METs outdoors, the highest number of primary vectors were identified as *An. arabiensis* (94.6%, $n = 617$; 95% CI: 92.5–96.2%) with only a few *An. funestus* s.l. (5.3%, $n = 35$; 95% CI: 3.8–7.4%). Among the collected secondary vectors, *An. coustani* was the most abundant (70.9%, $n = 39$, 95% CI: 56.9–81.9%) followed by *An. pharoensis* (21.8%, $n = 12$; 95% CI: 12.2–35.4%). The proportion of *An. arabiensis* collected in the calf-baited MET was twofold higher than that in the human-baited MET (GLMM: OR = 2.36, $P = 0.013$). For *An. funestus* s.s., the proportion of blood-fed mosquitoes collected was inadequate in our view to detect a reliable difference between host types (GLMM:

$P = 0.614$; Table 3). Moreover, the proportion of *An. coustani* (77%) caught in the calf-baited MET was significantly higher than that in the human-baited MET (GLMM: OR = 0.30, $P = 0.001$). The proportion of *An. arabiensis* caught in the METs was significantly higher in the cattle-baited traps in the early wet season and during the dry season ($\chi^2_{\text{Mar}} = 25.8$, $\chi^2_{\text{Apr}} = 19.7$, $\chi^2_{\text{Jul}} = 72.5$; $P < 0.0001$), while by the mid to late wet season there was no difference between the host-baited traps (Fig. 5).

Discussion

Information on the seasonal change in the abundance and host preference of primary and secondary malaria vectors, and how these contribute to malaria transmission, is required to strengthen and improve integrated vector management strategies in the context of the current state of residual transmission [10, 20, 21, 49, 50]. In this study, primary [23, 51, 52] and secondary [36, 53] malaria vectors exhibited similar, yet distinct, abundance and feeding patterns within the wet season, extending across the transition between the wet and dry seasons. The proportion of mosquitoes that fed on humans suggests that both primary and secondary vectors may differentially contribute to the risk of malaria transmission during the wet season, whereas only the

Table 2 The effect of host prevalence on primary and secondary malaria vector abundance

Household number	Host abundance (n)										Primary vector abundance										Secondary vector abundance									
	Humans		Bovine		Chickens		Sheep		Dogs		Cats		Goats		Total (n)	Mean	RR ^a	Lower ^a	Upper ^a	P-value ^a	Total (n)	Mean	RR ^a	Lower ^a	Upper ^a	P-value ^a				
5	18	30	18	17	6	1	0	0	6	1	0	0	0	336	3.73	1.00	-	-	-	152	1.69	1.00	-	-	-	N/A				
1	22	>200	>35	60	20	10	0	0	20	10	0	0	0	152	1.69	0.55	0.33	0.91	0.0195	54	0.60	0.40	0.16	0.96	0.0395					
2	15	>70	>50	0	0	3	0	0	0	3	0	0	0	169	1.88	0.51	0.31	0.84	0.0078	40	0.44	0.13	0.05	0.35	0.0001					
3	23	>58	>70	10	5	3	6	0	5	3	6	0	0	177	1.97	1.14	0.70	1.86	0.6045	64	0.71	0.34	0.14	0.84	0.0196					
4	14	10	>35	14	2	4	0	0	2	4	0	0	0	173	1.92	0.67	0.41	1.10	0.1127	33	0.37	0.15	0.06	0.41	0.0002					
6	5	0	20	0	0	0	0	0	0	0	0	0	0	119	1.32	0.31	0.19	0.52	0.0000	49	0.54	0.29	0.12	0.72	0.0074					
7	13	35	30	0	1	2	0	0	1	2	0	0	0	151	1.68	0.83	0.51	1.36	0.4561	74	0.82	0.60	0.25	1.45	0.2558					
8	22	20	25	0	3	0	0	0	3	0	0	0	0	66	0.73	0.26	0.15	0.45	0.0000	22	0.24	0.11	0.04	0.32	0.0000					
9	13	>20	>25	0	2	2	2	2	2	2	2	2	2	116	1.29	0.46	0.28	0.77	0.0031	33	0.37	0.16	0.06	0.42	0.0002					
10	18	9	>30	0	4	3	0	0	4	3	0	0	0	109	1.21	0.39	0.23	0.65	0.00	32	0.36	0.13	0.05	0.35	0.00					

GLMM Generalized linear mixed model, RR relative risk

^a Household number 5 was used as reference in the GLMM

Table 3 Blood meal sources identified from primary and secondary malaria vectors

<i>Anopheles</i> vector species	Blood meal sources										
	Bovine	Human	Dog	Goat	Sheep	Bovine/ Human	Bovine/Dog	Bovine/Goat	Human/Dog	Goat/ Dog	Unknown
<i>An. arabiensis</i> ^a	138	13	5	4	172	1	–	–	–	–	16
<i>An. funestus</i> ^a	5	13	–	–	–	1	–	–	–	–	–
<i>An. lessoni</i>	34	10	5	1	–	–	–	–	–	–	–
<i>An. rivolurum</i>	3	1	–	–	–	1	1	–	–	–	–
Unknown <i>An. funestus</i> sensu lato	13	1	1	1	–	–	–	–	–	–	–
<i>An. squamosus</i>	33	5	1	–	–	–	–	2	1	–	–
<i>An. coustani</i>	31	2	–	–	1	–	–	–	–	1	–
<i>An. pharoensis</i>	35	6	–	–	–	–	–	–	–	–	–
<i>An. ziemanni</i>	3	–	–	–	–	–	1	–	–	–	–
Total	295	51	12	6	173	3	2	2	1	1	16

Values in table are the number of blood-fed mosquitoes per *Anopheles* species and blood meal source

^a Primary vectors

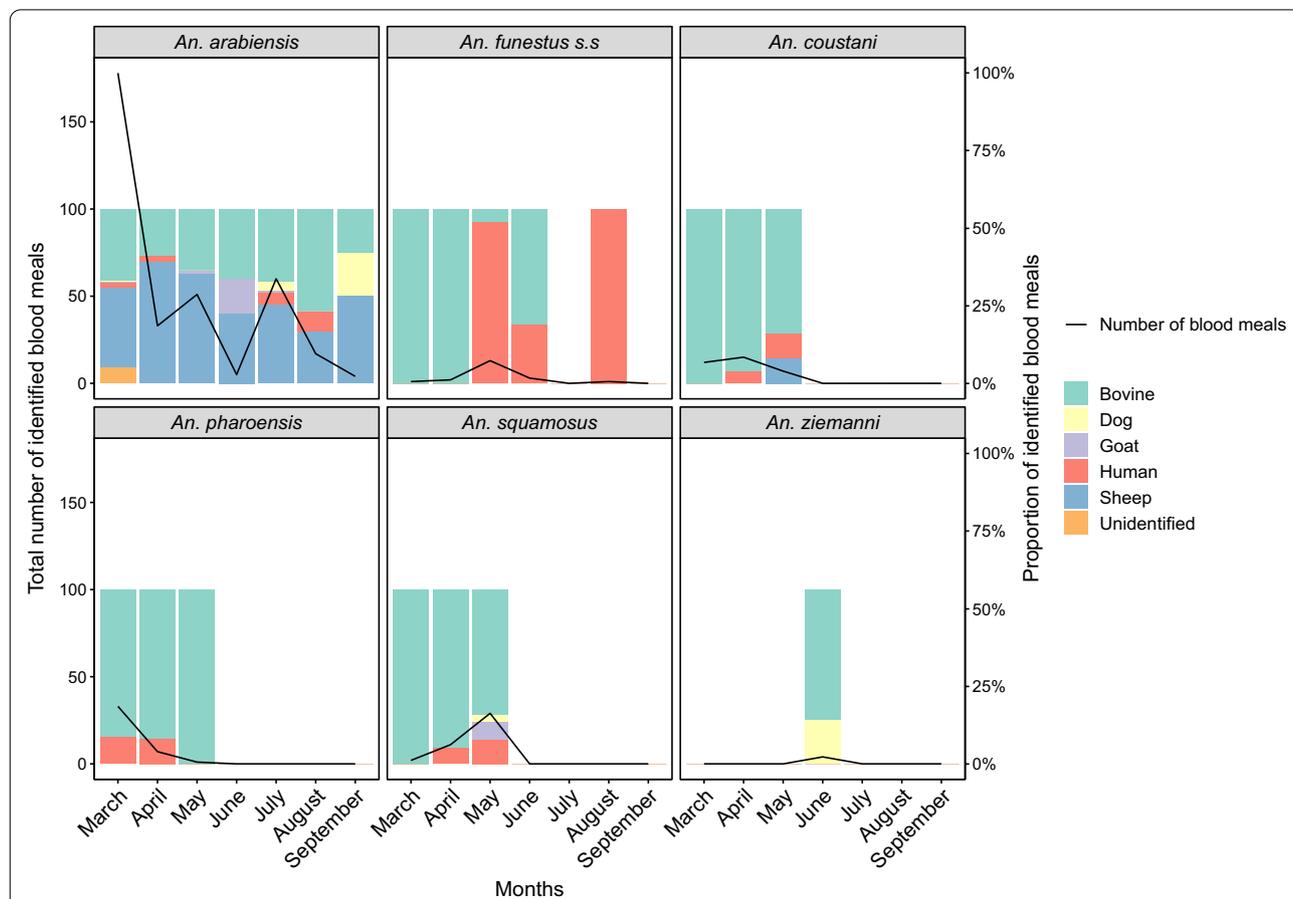
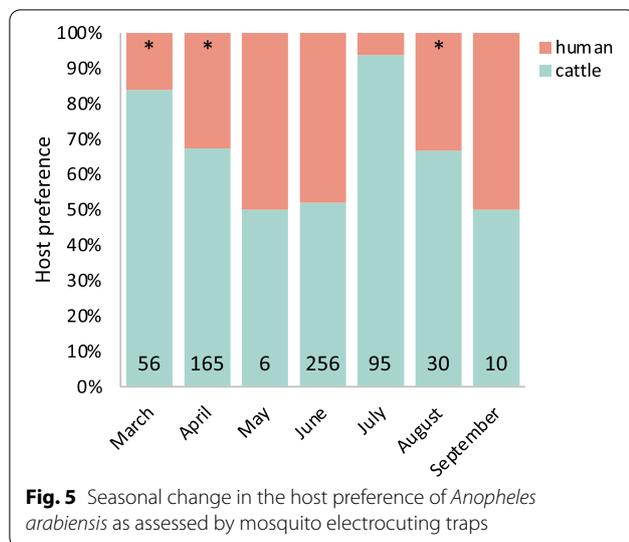


Fig. 4 Seasonal change in the proportion of blood meals from various hosts. Blood meals were determined by enzyme-linked immunosorbent assay from bovine, dog, goat, and human hosts



primary vectors appear to contribute to the risk of transmission during the dry season. These findings provide additional information on the ecology of the primary and secondary vectors within the region, which needs to be taken into account when planning future local control efforts.

The seasonal variation in the abundance of both primary and secondary vectors driven by the availability of rainfall reflects what has been observed in other parts of the Kilombero valley, throughout Tanzania and in other regions of SSA [49, 53–60]. Of the primary vectors, *An. arabiensis*, the only member of the *An. gambiae* s.l. complex found in the Kilombero valley [51, 61], was consistently more abundant than *An. funestus* s.s. across the wet and dry seasons, as previously reported in this valley [58, 61] and other regions of SSA [59, 62–64]. The seasonal pattern in abundance of the main secondary vector, *An. coustani*, reflected that of *An. arabiensis* and *An. funestus*, with the peak of abundance following the height of the wet season and extending into the dry season [56]. While the population of the less abundant secondary vectors increased much earlier in the wet season, and reached the peak of abundance at a similar time as the primary vectors and *An. coustani*, these mosquito populations did not persist into the dry season. The observed change in vector abundance across seasons is likely explained by the variation in access to, and choice of, breeding habitat, and to a lesser extent the ability of the vector species to make use of the available blood meals. During the wet season, the overall numbers of anophelines collected outdoors doubled compared to those collected indoors, which is likely a result of the contribution of the secondary vector populations, which feed outside, predominantly on bovine blood.

Both the primary vectors, along with *An. coustani*, exhibit higher flexibility in their choice of breeding habitats, showing a tolerance to small stagnant pools of water [65–70], whereas the breeding sites of the other secondary vectors are closely linked to rainfall, large pools and flood plains [71, 72]. The rise in the secondary vector populations for *An. pharoensis* and *An. squamosus* in the early wet season (March and April) suggests that these species tolerate the heavy rain associated with the flushing off of, and the mechanical damage to, mosquito eggs and larvae. The sharp decline in the secondary vectors *An. pharoensis* and *An. squamosus* in July corresponded with the drying out of the valley, leading to mass decrease in large pools as breeding sites, while small, stagnant pools were maintained into the dry season as breeding sites for *An. coustani* and the primary vectors. The ability of the primary vectors to make use of smaller, human-made and/or stagnant bodies of water as breeding sites enables them to maintain their population numbers into the dry season, thereby suggesting that this is an important factor regulating malaria transmission during this period [65, 69, 70, 73–75].

Host blood meal prevalence varied across the seasons in the primary vectors, which was most evident in *An. arabiensis* and further supported by direct assessment of host preference. While a seasonal variation in the host blood meals in the secondary vectors was indicated, further analysis is needed for confirmation due to the low abundance of these vectors. The overall feeding patterns observed in this study are consistent with those previously described for the primary and secondary vector species in this study, in regions with similar host availability [76–78]. Blood meal analysis, as a proxy for host preference, is confounded by host availability [27, 36]; therefore, we used a direct measure of host preference, the METs, to provide supporting evidence of the seasonal shift in host choice in *An. arabiensis* [79]. These data showed a shift from a preference for cattle in the early wet season to opportunistic feeding late in the wet season and then a return to the preference for cattle in the transition to the dry season. A plausible explanation for the change in host preference may be related to the need to maximize the lifetime reproductive success [80] as the habitat conditions for *An. arabiensis* worsen in the dry season [73, 75]. Alternatively, this seasonal shift in host preference cannot be specifically stated, and it may simply reflect an effect of experience of a previous blood meal by the malaria vectors [81]. The ongoing discussion of host preference in *An. arabiensis* may be obscured by this apparent seasonality, leading some studies to conclude that this primary vector is zoophilic, while others argue for it being opportunistic [36, 82]. Further studies are required to elucidate how this apparent

change in host preference across seasons affects malaria transmission.

Households with higher numbers of cattle and sheep had higher anopheline activity and a higher number of human-fed malaria vectors, suggesting that the abundance of non-human hosts, particularly cattle, in the study area may increase human-feeding rate, as previously reported [83–85]. In situations in which livestock are kept close to humans, the presence of animals may increase the risk of human-biting simply through the cattle attracting mosquitoes to the general proximity [85–87]. In contrast, the presence of livestock at households has also been shown to reduce human-feeding rates [77, 88, 89]. These contradictory observations may be attributed to variations in host preference and abundance of the dominant vector species, as well as in the distance of livestock from households [90]. This result emphasizes the need for further area-wide studies that incorporate direct measures of host preference, human-biting rates and host availability prior to implementing zooprophylaxis as a control strategy [91, 92].

Of the other non-human hosts available, dogs and goats were represented more often in the blood meals of *An. arabiensis* and several secondary vectors towards the end of the wet season and into the dry season. *Anopheles arabiensis* and the secondary vectors appeared to feed opportunistically throughout their activity periods based on blood meal analysis. The opportunistic behavior for the secondary vectors reflects what was previously observed in other regions of Tanzania [18], but contradicts findings from Zambia [93]. The determination of the degree of host-driven versus opportunistic behavior within populations and species may be easily misidentified when using blood meal analysis and host census data [94], and may be exacerbated in mosquito species which are in low abundance. In contrast to the seemingly opportunistic behaviors demonstrated by the other anophelines in this study, *An. funestus* demonstrated a potential switch in host choice from cattle to human [76, 95, 96], culminating at the peak of the wet season, thereby emphasizing the increasing importance of this species in malaria transmission in the region [10, 97]. During the dry season, there was increased availability of human blood meal due to a reduced use of bed nets compared to the wet season.

All malaria vectors collected in this study were negative for malaria parasites, emphasizing the decreasing malaria transmission in the region, most notably from 14% in 2011 to 11% in 2017 [30, 31]. Furthermore, other recent studies have indicated the lack of sporozoite-positive malaria vectors in the area [29]. This decline in malaria prevalence is likely due to urbanization, improved house construction and use of LLINs in conjunction with the

ongoing livestock-keeping lifestyle in the study area [23, 29, 88, 98, 99]. While the circumsporozoite protein ELISA used in these studies may underestimate the prevalence of malaria parasites, the mosquitoes identified are assuredly infectious, as compared to the more sensitive PCR approaches, which can contain the sporozoites from anywhere in the body [100].

Conclusions

This study provides updated seasonal information on the abundance, blood meal sources and host preference of the primary and secondary vectors in the Kilombero valley. *Anopheles arabiensis* and *An. coustani* were identified as the most abundant primary and secondary vectors, respectively. *Anopheles arabiensis* clearly demonstrated seasonality in host preference, while blood meal analysis only indicated this for *An. funestus* s.s. and the secondary vectors. While no sporozoite positive vectors were identified, the demonstrated seasonal changes in human biting are likely to contribute to low prevalence of malaria in the area. Further studies are required to assess the role of seasonal shifts in host preference in malaria transmission, and to increase our understanding of the ecology of *An. funestus* species complex.

Abbreviations

CDC: US Centers for Disease Control and Prevention; GLMM: Generalized linear mixed model; IRS: Indoor residual spraying; LLIN: Long-lasting insecticidal nets; MET: Mosquito electrocuting trap; SSA: Sub-Saharan Africa.

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Author contributions

RI, SRH, NJG and LLM conceived and designed the study. GCK, MH and SM collected the field samples. GCK analyzed the data and coordinated the laboratory analyses. GCK wrote the initial draft of the manuscript. RI, SRH, NJG and LLM critically revised the manuscript. All authors have read and approved the final version of the manuscript.

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Availability of data and materials

The dataset used and/or analyzed, as well as the materials collected, during the current study are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate

Written and signed consent was obtained from each household head in order to enroll his/her household in the study and access their private land.

All volunteers were provided with consent forms, translated into the local language (Kiswahili), to fill out before the study began. Ethical approval for the study was obtained from the Institutional Review Board of the Ifakara Health Institute (Ref. IHI/IRB/EXT/No: 23—2019) and the Medical Research Coordination Committee at the National Institute for Medical Research (NIMR) (Ref. NIMR/HQ/R.8a/Vol.IX/3085). The research team conducted meetings with local government administration officers and village leaders before the study was initiated in Sagamanga. In these meetings, the objectives of the study, methods and duration were discussed, and the staff of the study was introduced to the public.

Consent for publication

Permission to publish this work was also obtained from NIMR (Ref: NIMR/HQ/P.12 VOL 423 XXXIII/12).

Competing interests

The authors declare that they have no competing interests.

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