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Gonotrophic cycle duration, fecundity and parity of *Anopheles gambiae* complex mosquitoes during an extended period of dry weather in a semi arid area in Baringo County, Kenya

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ABSTRACT
An entomological longitudinal survey was carried out over a 22 month period in two semi-arid villages in Baringo District in Kenya to study how adult malaria vectors survive under semi-arid conditions and during extended periods of dry weather.
Methods: Wild caught female mosquitoes were dissected to examine ovarian lobes for parity status and to determine number of gonotrophic cycles they had undergone. Duration of the first and second gonotrophic cycles were estimated using cage-reared F_1 females. Blood-fed females were kept individually in plastic vials and percent oviposition incidence recorded.
Results: Significantly fewer mosquitoes laid eggs during the dry than the wet season. The average duration of the first gonotrophic cycle in the wet season was 4.1 d after blood feeding, 1.1 d (36%) longer than the dry season (3.0 d). The average duration of the second gonotrophic cycle in the wet season was 2.9 d after second blood meal, 0.7 d (31.8%) longer than those in the dry season. Chi-square tests showed the gonotrophic cycle duration was significantly shorter during the dry than the wet season. Both gonotrophic cycle duration and physiological age varied significantly between wet and dry seasons.
Conclusion: These findings suggest the duration of gonotrophic cycle among *Anopheles gambiae* in dry lands with scarce breeding sites is shorter during the dry than wet season. Low fecundity rates during the dry season could be a sign of reduced reproductive activity. However lack of variation in seasonal mating frequency is a clear indication that oviposition and mating kinetics are influenced differently even under the same environmental conditions. It is likely that the results of this study will shed an understanding on spatial and temporal heterogeneities experienced in malaria transmission in semi-arid regions of the world where malaria and indeed mosquito-borne diseases are a public health menace.

Keywords: *Anopheles gambiae,* Gonotrophic cycle duration, fecundity, mating behavior.

1. Introduction
Semi-arid areas experience deficits of rain accompanied by high temperatures during dry seasons and extended periods of dry weather. It is observed that the abundance of the malaria vector species drops dramatically with the onset of the dry season, and this may depress the incidence of severe malaria [1, 2]. The onset of the rains, however, brings a rapid explosion in mosquito numbers and a concomitant increase in malaria [3, 4].

Data on the Anophelinae are not conclusive with respect to how each life stage contributes to long-term survival in semi-arid conditions and the rapid population rise that occurs following the onset of the rains. Contributions to long-term survival during the dry season and in semiarid zones could be made during the egg, larval, and/or adult (including pupal) stages. Adult stages make an important contribution to dry season/ semi-arid population dynamics. Small changes in the temperature of malaria vector resting sites in the relatively hot semi-arid areas may have a significant impact on the kinetics of mosquito blood digestion and consequently in their overall breeding ecology [5]. Subsequently, effects may be seen on population reproduction rates through changes in the duration of the gonotrophic cycle. Mosquito vectors require blood meals for egg development, and the rate of digestion of these blood meals is normally directly proportional to increase in temperature.

Increased frequency in egg-laying would require increased rates of feeding on human hosts resulting in enhanced vectorial capacity. Moreover, seasonal increases in ambient temperature may accelerate *Plasmodium* parasites maturation rates and consequently enhance the vectorial capacity.

It is hypothesized that during dry season mosquitoes go through reduced reproductive activities. Study observations are discussed in relation to their implications for malaria transmission in semi-arid complexes of the world that are currently experiencing malaria epidemics. It is likely that the results of this study will shed an understanding on spatial and temporal heterogeneities experienced in malaria transmission in these regions.

2. Methods

2.1 Study site

The study was based in Marigat division of Baringo district, Kenya. Marigat town is about 250 km north-west of the Capital City of Nairobi and is situated 0.45N, 36E and about 700 meters above the sea-level, most of which is rangelands (Figure 1). Temperatures in this zone are high [above 32 ^{o}C], with low average rainfall [500-600 mm]. Temporary habitats are scarce and whenever they are formed rarely last for over one week after the sporadic rain showers due to the exceedingly high temperatures and consequent evaporation rates. Permanent water sources are therefore the main drivers of mosquito breeding.

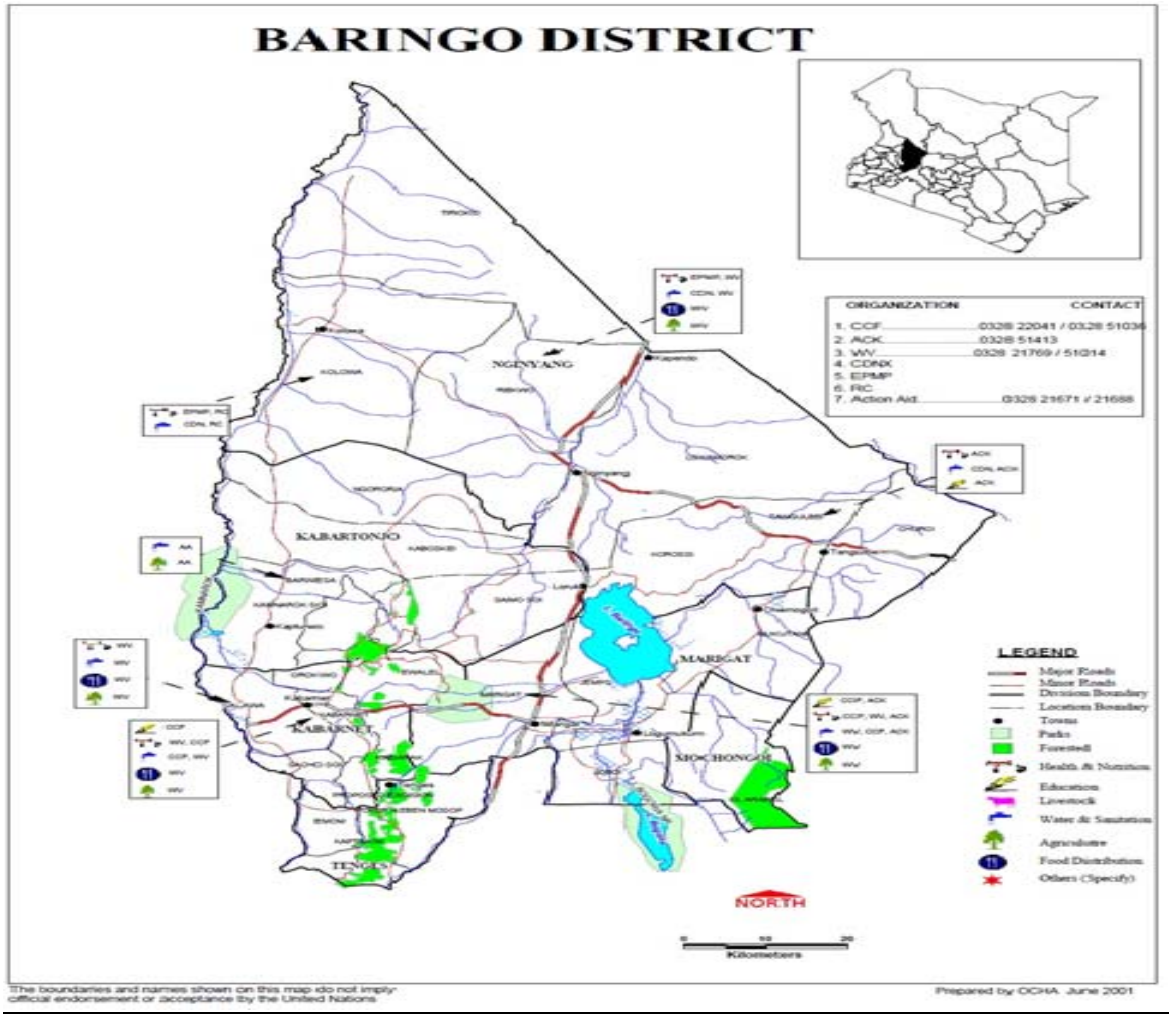

Fig 1: Map of Baringo District

2.2 Mosquito sampling

Ten houses were randomly selected from two villages; Kamarimar and Tirion. The selection of study villages was based on village location in relation to known breeding sites with the former being adjacent to the Loboi swamp, while the latter is located approximately 3 km away from the swamp. Longitudinal sampling of adult mosquitoes was done fortnightly in each of the ten fixed houses per village for 22 months from July 2008 to April year 2010, unless circumstances required that a nearby house be substituted. Pyrethrum spray collections (PSC) were done once weekly in the morning between 0700 and 1100 hours. CDC light traps (J.W. Hock Ltd, Gainesville, FL, U.S.A.) were operated between 1800 and 0600 hours in the main bedroom of each house every fortnight.

2.3 Identification of *Anopheles* mosquitoes and determination of parity status

All mosquitoes from each collection were preserved in labeled vials containing anhydrous calcium sulphate for purposes of identification. Adults were identified using morphological criteria [6, 7, 8] and PCR was used to identify members of the *Anopheles gambiae* complex according to the protocols of Scott and others [9]. The ovary tracheation method of Detinova [10] was used to determine parity. Multiparous groups were distinguished using the protocols of Polovodova [11, 12] as modified by Hoc [13]. Ovary development was classified according to Christophers [14] stages, as modified by Clements and Boocock [15].

2.4 Estimation of gonotrophic cycle duration

Two hundred 4-d-old virgin F1 female *Anopheles gambiae* mosquitoes were placed into a 30 by 30 by 30 cm^3 cage and blood fed from a rabbit for 30 min under standardized laboratory conditions. Mosquitoes that failed to feed were aspirated out of the cage. An equal number of virgin *Anopheles gambiae* male mosquitoes were introduced into the cages to allow females to mate for 24 h. Cages were suspended from the laboratory roof at a distance of 2 m above the ground in the laboratory using greased suspension twines to block ants from reaching the cages. Each of the 100 female blood-fed mosquitoes was transferred into an individual oviposition cup after 24 hours. The oviposition cups, 10 cm in width and 12 cm in height, each contained a piece of filter paper on a wet cotton wool pad to provide an oviposition substrate, and the cups were placed on a table inside the laboratory. Grease was applied on table legs to prevent ants from accessing the oviposition cups. The number of eggs oviposited per female mosquito during the first gonotrophic cycle was counted under a dissection microscope and recorded. Females that oviposited were given a second blood meal to determine the duration of the second gonotrophic cycle but no egg counts were done. The studies were carried out during the dry season in September 2009 and repeated during the rainy season in the month of February 2010.

2.5 Climate Data Collection

HOBO data loggers (Onset Computer Corporation, Bourne, MA) were placed inside the laboratory where the gonotrophic cycle length was measured, to record temperature and relative humidity. The data loggers were suspended from the roof, 2 m above the ground. Outdoor temperature was recorded by placing three HOBO data loggers in standard meteorological boxes, 2 m above the ground for the same time period described above.

2.6 Data Analysis

Monthly average temperatures were calculated from the daily record of minimum or maximum temperature. Daily mean temperature was computed as the arithmetic mean of the 24 hourly temperature records of a day, and mean monthly temperature as the average of daily mean temperatures. Mean gonotrophic cycle duration is operationally defined as the average number of days that gravid mosquitoes took to oviposit after taking a blood meal. The number of eggs laid in each season and the proportion of mosquitoes that laid eggs were compared using the chi-square test.

3. Results

The average monthly maximum outdoor temperature during the study period was 28.40 and 23.65 °C in the dry and wet seasons respectively. However, the average minimum temperature (12.5 °C) in the dry season was 4.91 °C lower than that in the wet season (7.59 °C) (t=5.98, df=16, p=0.001; Fig. 1). On the other hand mean monthly outdoor temperature was 26.32 °C during the dry season and 24.82 °C during the wet season. The average monthly temperature during the dry season was 1.5 °C higher than that of the dry season (t= 2.59, df=16, P=0.0075), and the average monthly maximum temperature was 4.75 °C higher (t = 8.85, df=16, p<0.001).

3.1 Species composition and abundance

Four Anopheles species were collected in the two study sites during the 22-month period. These included *An. gambiae s.l.* (66.8%), *An. funestus* (17.9%), *An. pharoensis* (14.5%) and *An. coustani* (0.8%). *Anopheles gambiae s.l.* and *An. coustani*, respectively, were the most and the least abundant species in both study sites. *Anopheles pharoensis* was the second most abundant species in Kamarimar, while An. funestus was the second most abundant species in Tirion. For all species, light trap collections were more productive than PSC. rDNA PCR analysis of 500 *An. gambiae s.l* samples revealed all turned out to be as *An. Arabiensis.*

3.2 Seasonal variation in gonotrophic Cycle Duration

During the dry season, significantly fewer mosquitoes (38.2%) laid eggs than those in the wet season (61.8%) (t=8.85, df=1, p<0.05 Table 1). The average duration of the first gonotrophic cycle in the wet season was 4.1 d after blood feeding, 1.1 d (36%) longer than those in the dry season (3.0 d) (z=11.1 , P <0.001; Table 1). The average duration of the second gonotrophic cycle in the wet season was 2.9 d after second blood meal, 0.7 d (31.8%) longer than those in the dry season (2.2 d) (Z=7.1, P<0.001; Table 1). Chi-square tests showed that the duration of the gonotrophic cycle was significantly different between the wet and dry seasons (X^2 =96.68, df=2, p<0.001).

Table 1: Duration of the first and second gonotrophic cycles of *A. gambiae* mosquitoes in dry and wet seasons.

			First gonotrophic cycle			Second gonotrophic cycle		
Season	**Mean indoor Temp**	**Mean outdoor Temp**	**Proportion of mosquitoes that laid eggs (%)**	**Mean duration (d)**	**Mean fecundity**	**Proportion of mosquitoes that laid eggs (%)**	**Mean duration (d)**	**Mean fecundity**
Dry	28.22± 1.1	26.32 ± 0.33	43	4.52± 0.12	70.65± 0.74	8.7	3.0± 0.2	69.02± 0.1
Wet	27.12± 1.2	24.82 ± 0.33	64	6.14± 0.27	54.24± 1.02	6.2	4.1 ± 0.3	50.36± 0.2

Field collected F1 female mosquitoes that had blood fed and mated were used. Standard deviation for mean indoor and mean outdoor temperatures, gonotrophic cycle duration, and fecundity is shown.

Table 2: Parity status of for *A. gambiae* mosquitoes.

Month/year	Dilatation number and total with each				
	1	2	3	4	5
July 2008	345 (3.24)	210 (7.39)	0(0.00)	0(0.00)	0(0.00)
August2008	120 (1.13)	80 (2.82)	4(2.03)	4(13.79)	0(0.00)
September 2008	76 (0.71)	30(1.06)	0(0.00)	0(0.00)	0(0.00)
October 2008	0 (0.00)	0(0.00)	0(0.00)	0(0.00)	0(0.00)
November 2008	2100 (19.74)	520(18.31)	0(0.00)	0(0.00)	0(0.00)
December 2008	520 (4.89)	140(4.93)	0(0.00)	0(0.00)	0(0.00)
January 2009	123 (1.16)	27(0.95)	0(0.00)	5(17.24)	0(0.00)
February2009	356 (3.35)	80(2.82)	0(0.00)	7(24.14)	0(0.00)
March2009	21 (0.20)	30(1.06)	2(1.02)	1(3.45)	1(9.09)
April2009	400 (3.76)	271(9.54)	0(0.00)	2(6.90)	0(0.00)
May2009	121 (1.14)	50(1.76)	0(0.00)	1(3.45)	0(0.00)
June2009	152 (1.43)	5(0.18)	0(0.00)	5(17.24)	6(54.55)
July2009	68 (0.64)	13(0.46)	6(3.05)	1(3.45)	2(18.18)
August2009	43 (0.40)	21(0.74)	10(5.08)	0(0.00)	1(9.09)
September2009	62 (0.58)	50(1.76)	0(0.00)	0(0.00)	1(9.09)
October 2009	1170 (11.00)	200(7.04)	0(0.00)	0(0.00)	0(0.00)
November 2009	450 (4.23)	57(2.01)	43(21.83)	0(0.00)	0(0.00)
December 2009	230 (2.16)	76(2.68)	4(2.03)	0(0.00)	0(0.00)
January 2010	940 (8.84)	250(8.80)	19(9.64)	0(0.00)	0(0.00)
February 2010	301 (2.83)	348(12.25)	45(22.84)	3(10.34)	0(0.00)
March 2010	1800 (16.92)	379(13.35)	64(32.49)	0(0.00)	0(0.00)
April 2010	1240 (11.66)	300(10.56)	0(0.00)	0(0.00)	0(0.00)
Total/mean	10638	2840	197	29	11

3.3 Seasonal trends in parity

Parity varied over time, ranging between 88% and 100%. Four peaks in parity were recognized in November 2008, June 2009 and in March and April 2010 (Figure 2). Parous females were categorized into different multiparous groups as shown in table 2. A majority of the mosquitoes were parous-1, suggesting a largely young vector population. During the dry season, a majority of females were parous-1 at 75.5% (n=3916) followed by parous-2 at 22 % (n=1107). No parous-5 mosquitoes were encountered during the dry seasons sample collections for dissection, but three parous-4 females were collected. Overall no mosquitoes were found that had undergone more than five gonotrophic cycles in either season throughout the study period, raising questions on vector longevity in this area, a subject that requires further investigation.

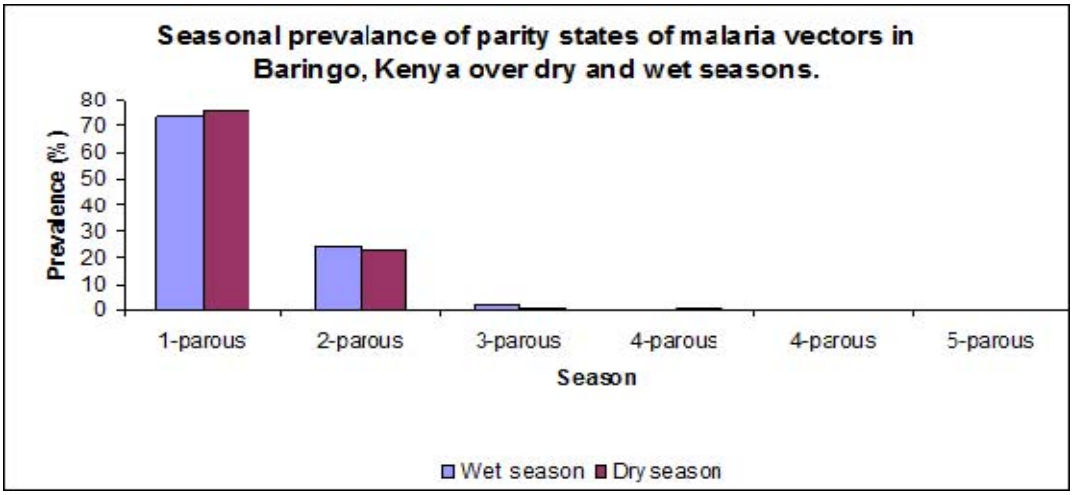

Fig 2: Seasonal prevalence of multiparous groups of *Anopheles gambiae*

3.4 Seasonal patterns of follicular development

A majority of follicles were in Christopher Stage I during the wet season at 57.08% (n=3051) as shown in table 3. Some 24.43% were in Stage II (n=1306%), 13.4 % (n=680) in stage III, 2.81% (n=250) in stage IV while stage V had the least number of females at 2.28% (n=255). During the dry season, however, Christopher stage II dominated at 56.68% (n=2742). Stage V mosquitoes were more prevalent during the dry than wet season collections by more than double at 5.27% (n=255). There was a reduction in the number of females with follicles in stage I to 18.83% (n=911). Stage III and IV follicle prevalence doubled during the dry season at 5.17% (n=250) and 5.27% (n=255) respectively.

Table 3: Follicular stages of *A. gambiae* mosquitoes

Month/year	Christopher stages of follicular developement				
	I	II	III	IV	V
July 2008	200 (3.46)	310(5.55)	3(0.18)	40(8.51)	2(0.37)
August2008	56(0.97)	132(2.36)	2(0.12)	2(0.43)	16(2.99)
September 2008	6(0.10)	60(1.07)	20(1.22)	5(1.06)	15(2.80)
October 2008	0(0.00)	0(0.00)	0(0.00)	0(0.00)	0(0.00)
November 2008	1500(25.95)	820(14.67)	150(9.15)	30(6.38)	120(22.43)
December 2008	280(4.84)	300(5.37)	57(3.48)	3(0.64)	20(3.74)
January 2009	30(0.52)	101(1.81)	10(0.61)	3(0.64)	11(2.06)
February2009	56(0.97)	272(4.87)	43(2.62)	32(6.81)	40(7.48)
March2009	8(0.14)	29(0.52)	4(0.24)	3(0.64)	11(2.06)
April2009	187(3.23)	348(6.23)	58(3.54)	4(0.85)	76(14.21)
May2009	18(0.31)	143(2.56)	4(0.24)	2(0.43)	5(0.93)
June2009	38(0.66)	84(1.50)	23(1.40)	18(3.83)	5(0.93)
July2009	27(0.47)	62(1.11)	1(0.06)	0(0.00)	0(0.00)
August2009	12(0.21)	48(0.86)	3(0.18)	6(1.28)	6(1.12)
September2009	28(0.48)	65(1.16)	3(0.18)	8(1.70)	10(1.87)
October 2009	148(2.56)	833(14.91)	315(19.22)	70(14.89)	4(0.75)
November 2009	39(0.67)	420(7.52)	36(2.20)	37(7.87)	18(3.36)
December 2009	132(2.28)	115(2.06)	10(0.61)	50(10.64)	3(0.56)
January 2010	297(5.14)	565(10.11)	194(11.84)	97(20.64)	56(10.47)
February 2010	320 (5.54)	246(4.40)	7(0.43)	7(1.49)	117(21.87)
March 2010	1700(29.41)	214(3.83)	276(16.84)	53(11.28)	0(0.00)
April 2010	699(12.09)	421(7.53)	420(25.63)	0(0.00)	0(0.00)
Total/mean	5781	5588	1639	470	535

4. Discussion

Female age composition plays a key role in malaria transmission dynamics. Female parity status is a key index of both vector competence and vector longevity. The more multiparious a female vector is, the older the female is likely to be. Older females also have higher exposure rates to malaria parasites considering the number of pre-oviposition encounters they may have had with humans while questing for blood meals. They therefore have a higher probability of being infected with malaria parasites.

The vector population in Baringo had high proportions of parous females, a key pointer to continuous small-scale breeding of malaria vector species. This reproductive behavior is propped up by the almost year round guarantee of breeding site availability provided by the permanent swamps in the study area. The high proportions of parous females may also suggest mortality rates are low among emerged females. Abundance of such a population of vector in a population may result in high malaria transmission rates. It is interesting that a majority females captured in Baring were young (parous-1 and parous-2), making these age-groups epidemiologically the most important in relation to malaria transmission in the area. This can help explain the extremely low sporozoite rates reported elsewhere [16] among females realized in this study since such young females have most likely just emerged and are unlikely to be infected by sporozoites.

Results on follicular stages show high proportions of post teneral mosquitoes. Such wild caught females have ovaries that are not yet developed and are yet to take their first blood meal and would usually require at least one carbohydrate meal. Almost all females with follicles in stage II were nullipars. Most of the mosquito biting populations during the dry season were in stage II. This is an indication that gonotrophic concordance prevails for *Anopheles gambiae* in Baringo.

Fecundity rates were lower during the dry than the wet season. This could have been due to unfavorable climatic conditions, improper feeding or some fertilization factors. These observations suggest malaria incidence in semi-arid areas may increase based on seasonal temperature increase alone and not necessarily due to increased vector density [18, 19, 20].

We have demonstrated in this investigation that the duration of their first and second gonotrophic cycle by 1.1 days and 0.7 days shorter respectively during the dry than the wet season. Reduction in gonotrophic cycle duration is likely to increase malaria incidence due to increased egg laying and biting frequencies especially among old sporozoite-laden females [21, 10, 22, 23]. These cycles are especially sensitive to changes in environmental temperature [24, 25], being reduced with increments in temperature. It was observed that the average duration of the first gonotrophic cycle was longer than that of second cycle one, regardless of season. Wild caught fed females used in the study consisted of both nulliparous and parous individuals as is usually found in natural populations. The possible explanation for shorter duration observed in the second gonotrophic cycle may be that most of the wild-caught mosquitoes were parous. Parous females have been known to take a shorter time before oviposition than nullipars because in parous females the ovaries are usually already in the middle or late Christopher's stage II of ovarian development [26, 27, 2829, 30, 31].

5. Conclusions

These findings suggest the duration of gonotrophic cycle among *Anopheles gambiae* in dry lands with scarce breeding sites is shorter during the dry than wet season. That is, higher ambient temperatures during the dry season leads to faster

blood meal digestion and thus shorter gonotrophic cycle duration and higher biting frequency by the mosquitoes. The rates of feeding on humans by malaria vectors are an important determinant of vectorial capacity. Low fecundity rates during the dry season could be a sign of reduced reproductive activity. However lack of variation in seasonal mating frequency is a clear indicator that oviposition and mating kinetics are influenced differently even under the same environmental conditions. It is likely that the results of this study will provide crucial information on planning and timing of anti-vector measures in semi-arid settings.

6. Competing interests

The author(s) declare that they have no competing interests.

7. Author contributions

Albert O. Mala conducted the field studies, analyzed the data and wrote the manuscript. Elizabeth K. Mitaki analysed the data and helped in manuscript preparation. Josephat I. Shililu, John I. Githure, Joseph K. Njagi, and Charles M. Mbogo provided scientific guidance in data collection, analysis and manuscript preparation and planning, and implementation of day-to-day field and laboratory activities. Lucy W. Irungu provided overall supervision of the study and preparation of manuscript. All authors actively contributed to the interpretation of the findings and development of the final manuscript and approved the final manuscript.

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