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Effects of Elevated Temperatures on the Growth and Development of Adult *Anopheles gambiae (s.l.)* (Diptera: Culicidae) Mosquitoes

Thomas P. Agyekum,[1,7] John Arko-Mensah,[1] Paul K. Botwe,[1] Jonathan N. Hogarh,[2] Ibrahim Issah,[1] Duah Dwomoh,[3] Maxwell K. Billah,[4] Samuel K. Dadzie,[5] Thomas G. Robins,[6] and Julius N. Fobil[1]

[1]Department of Biological, Environmental and Occupational Health Sciences, School of Public Health, College of Health Sciences, University of Ghana, Accra, Ghana, [2]Department of Environmental Science, Kwame Nkrumah University of Science and Technology, Kumasi, Ghana, [3]Department of Biostatistics, School of Public Health, College of Health Sciences, University of Ghana, Legon, Ghana, [4]Department of Animal Biology and Conservation Science, University of Ghana, Accra, Ghana, [5]Parasitology Department, Noguchi Memorial Institute for Medical Research, University of Ghana, Accra, Ghana, [6]Department of Environmental Health Sciences, University of Michigan, Ann Arbor, MI, USA, and [7]Corresponding author, e-mail: thomaspagyekum@gmail.com

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Abstract

Higher temperatures expected in a future warmer climate could adversely affect the growth and development of mosquitoes. This study investigated the effects of elevated temperatures on longevity, gonotrophic cycle length, biting rate, fecundity, and body size of *Anopheles gambiae (s.l.)* (Diptera: Culicidae) mosquitoes. *Anopheles gambiae (s.l.)* eggs obtained from laboratory established colonies were reared under eight temperature regimes (25, 28, 30, 32, 34, 36, 38, and 40°C), and 80 ± 10% RH. All adults were allowed to feed on a 10% sugar solution soaked in cotton wool; however, some mosquitoes were provided blood meal using guinea pig. Longevity was estimated for both blood-fed and non-blood-fed mosquitoes and analyzed using the Kaplan–Meier survival analysis. One-way ANOVA was used to test the effect of temperature on gonotrophic cycle length, biting rate, and fecundity. Adult measurement data were log-transformed and analyzed using ordinary least square regression with robust standard errors. Increasing temperature significantly decreased the longevity of both blood-fed (Log-rank test; $X^2(4) = 904.15$, $P < 0.001$) and non-blood-fed (Log-rank test; $X^2(4) = 1163.60$, $P < 0.001$) mosquitoes. In addition, the fecundity of mosquitoes decreased significantly (ANOVA; $F(2,57) = 3.46$, $P = 0.038$) with an increase in temperature. Body size ($\beta = 0.14$, 95% CI, 0.16, 0.12, $P < 0.001$) and proboscis length ($\beta = 0.13$, 95% CI, 0.17, 0.09, $P < 0.001$) significantly decreased with increasing temperature from 25 to 34°C. Increased temperatures expected in a future warmer climate could cause some unexpected effects on mosquitoes by directly influencing population dynamics and malaria transmission.

Key words: *Anopheles*, body size, fecundity, longevity, temperature

Mosquitoes are important vectors that have gained significant public health attention due to their involvement in transmitting diseases that globally affect human health (Shahrudin et al. 2019). The mosquito's survival is of paramount interest as it affects the vector's ability to transmit diseases such as malaria, chikungunya, yellow fever, dengue, and West Nile virus (WNv) (Cailly et al. 2012, Caraballo and King 2014).

Mosquito abundance—a pivotal factor that influences mosquito-borne disease's resurgence (Wang et al. 2011) depends on the number of adults entering and exiting the population. The high abundance of mosquitoes is usually a prelude to an epidemic (Roiz et al. 2014). Both the human biting rate and mosquito abundance are significantly influenced by temperature (Lyons et al. 2013). Additionally, many multicellular organisms have distinct life stages that differ in

morphology, physiology, size, and other measurable characteristics. Conditions encountered at different habitats, seasonal environments, and microclimates could cause the mosquito to develop different physiological responses and sensitivities, affecting the organism's overall fitness (Kingsolver et al. 2011).

Global warming scenarios have prompted many studies focusing on the effects of elevated temperatures on both the morphology and the biology of various species (Aytekin et al. 2009). Temperature is regarded as the fundamental factor influencing mosquito development (Shahrudin et al. 2019). In addition, temperature affects key life history physiognomies such as fecundity, wing size, adult longevity, blood feeding behavior, gonotrophic cycle length, and biting rate (Davies et al. 2016, Shapiro et al. 2017, Ezeakacha and Yee 2019). These physiognomies can affect mosquito survival, parasite development, and influence transmission by affecting fecundity (Christiansen-Jucht et al. 2014). Temperature can also affect vector competence (Shapiro et al. 2017) and the mosquito's innate ability to reproduce and transmit pathogens (Buckner et al. 2016).

How temperature affects adult *Anopheles gambiae (s.l.)* mosquito growth and development is essential, especially in forecasting malaria control in a future warmer climate. For example, a shorter gonotrophic cycle length and increased biting rates may lead to the production of more generations because the female mosquito will come into contact more often with their hosts. This results in higher mosquito densities and more population genetic variations as mosquitoes have increased potential to acclimatize to different environments (Bellone and Failloux 2020). Furthermore, an organism's size affects almost all aspects of its physiology, performance, morphology, and fitness (Kingsolver and Huey 2008). For instance, an adult mosquito's body size can affect many bionomic factors, including spatial dispersal, fecundity, and host attack rate. The immune responses of *An. gambiae (s.l.)* to *Plasmodium* infections are influenced by the adult mosquito's body size (Gleiser et al. 2000).

Despite the plethora of information linking temperature to mosquito life history traits, relatively few studies have assessed the effect of temperature on the growth and development of adult *An. gambiae (s.l.)* mosquitoes. For instance, there is a data gap on the effects of temperature on the biting rate and gonotrophic cycle length of *An. gambiae (s.l.)* mosquitoes (Agyekum et al. 2021). In Ghana and many African countries, *An. gambiae (s.l.)* mosquito is one of the most predominant and important malaria vectors (Baffour-Awuah et al. 2016, Riveron et al. 2016). An understanding of the population dynamics of *An. gambiae (s.l.)* mosquitoes and their association with temperature is vital to studying the epidemiology of mosquito-borne diseases (Shaman and Day 2007) in the light of a future warmer climate. Therefore, this study investigated the effects of elevated temperatures on the growth and development of *An. gambiae (s.l.)* mosquitoes by explicitly assessing the effects of temperature on longevity, gonotrophic cycle length, biting rate, fecundity, and body size.

Methods

Experimental Design

Mosquitoes were reared in climate incubators (RTOP-1000D, Zhejiang, China) at African Regional Postgraduate Program in Insect Science (ARPPIS), University of Ghana. Ghana has relatively high ambient temperature with an average annual temperature ranging between 25 and 30°C (Asante and Amuakwa-Mensah 2015). Hence, for experimental purposes, three temperatures were initially selected to coincide with this range: 25, 28, and 30°C. Additionally, to evaluate the effects of elevated temperatures on immature mosquitoes, a 2°C increment at interval from 30°C was added to the selected temperatures until 40°C maximum temperature was reached. The incubators were set at 80 ± 10% relative humidity and programmed to have a photoperiod of 12:12 (light:dark) h (Agyekum et al. 2022). A HOBO MX1102 CO_2 logger (Onset Computer Corp., Cape Cod, MA) was placed in each incubator to monitor the daily temperature and relative humidity (Shapiro et al. 2017). Data were downloaded daily between 10:00 a.m. and 11:00 a.m. to ensure that temperature and humidity remained stable throughout the experiment (Supp Table S1 [online only]).

Mosquito Colony Maintenance

This study used *Anopheles gambiae (s.l.)* mosquitoes (Tiassalé strains). This strain [a mixture of *An. gambiae (s.s.)* and *An. coluzzii*] is a resistant strain initially originated from Tiassalé, Cote d'Ivoire (Edi et al. 2012) and maintained in the Vestergaard—Noguchi Memorial Institute for Medical Research Vector Labs (VNVL) insectary since 2010 (Chabi et al. 2019). The eggs on filter paper were obtained from the insectary of Vestergaard—Noguchi Memorial Institute for Medical Research Vector Labs (VNVL). Mosquito eggs (about 400 per group) were placed in round plastic bowls (28 cm top diameter, 18 cm bottom diameter, 9 cm height) with 1 liter of de-chlorinated tap water and 1.5 ml of yeast to induce hatching (Farnesi et al. 2017). Upon hatching, larvae were fed daily on 10 mg of TetraFin goldfish flakes (Tetra Werke, Melle, Germany). All adults were allowed to feed on a 10% sugar solution soaked in cotton wool. However, some adults to be used to estimate fecundity and longevity of blood-fed mosquitoes were given a chance to feed on a guinea pig for 20 min on day 4. Cotton pads containing sugar were changed daily to prevent fermentation and drying out, especially at higher temperatures. The positions of the cages were rotated daily to control the effects of position within the chamber. Ethical approval concerning the use of guinea pigs for blood-feeding was sought from the University of Ghana Institutional Animal Care and Use Committee (UG-IACUC) with permit number UG-IACUC 001/20-21.

Molecular Identification of *An. gambiae (s.l.)* Mosquitoes (Tiassalé Strain)

To confirm the composition of *An. gambiae (s.l.)*, polymerase chain reaction (PCR) procedures were performed on the parent mosquitoes. One hundred adult female mosquitoes (3–5 d old non-blood-fed) were randomly selected for molecular analysis. DNA was extracted from the sampled mosquitoes using the Cetyl Trimethyl Ammonium Bromide (CTAB) extraction method following the procedures described by Mouhamadou et al. (2019).

Species-specific primers targeting the rDNA gene [intergenic spacer (IGS)] were first used to identify species of the *An. gambiae* complex using an established protocol (Scott et al. 1993). Subsequently, SINE PCR was used for the identification of *An. coluzzii* and *An. gambiae (s.s.)* using the primers, F6.1a (5′-TCGCCTTAGACCTTGCGTTA-3′) and R6.1b (5′-CGCTTCAAGAATTCGAGATAC-3′), respectively (Santolamazza et al. 2008). The PCR reactions were performed in a 25 µl reaction, which contained 0.4 µM of each primer. The other reagents included 2× GoTaq Green Master Mix (Promega, Madison, WI) 12.5 µl, and 4 µl of DNA template extracted from individual mosquitoes. The volume was adjusted with DNase-free water (6.5 µl). The amplifications were done in a thermocycler (Alpha Cycler, UK) and programmed as follows: 94°C for 5 min followed by 35 cycles of 94°C for 30 s, 59°C for 30 s, and 72°C for 1 min. At the end of amplification, the mixture was subjected to a final extension at 72°C for 10 min. The PCR products were allowed to migrate on 2% agarose gels stained with gel red. The species expected band profile

was 249 bp for *An. gambiae (s.s.)*, 479 bp for *An. coluzzii*, and 249, 479 bp for hybrid (*An. gambiae* M and S forms) after visualization with a BioDoc-it imaging system (UVP, Upland, CA).

Longevity of Adults

The longevity of mosquitoes was measured as the time of adult emergence to the time of death, which was assessed daily between 9:00 and 10:00 a.m. One hundred (50 males and 50 females) newly emerged mosquitoes were assigned to each temperature regime to monitor daily mortality and longevity. Any mosquito that had lost its wings and could no longer fly was considered moribund and recorded dead (Agyekum 2017). Dead mosquitoes were removed, observed, identified for sex allocation, and enumerated. This experiment was repeated three times under all temperature regimes, and longevity was estimated for blood-fed and non-blood-fed mosquitoes. Taking blood meal is a realistic behavior of female mosquitoes. In addition, one of the important factors in malaria transmission is how long a mosquito can live after a potential infectious blood meal, hence, the need to study longevity of blood-fed mosquitoes (Barreaux et al. 2018). Adult *An. gambiae (s.l.)* mosquitoes kept at 25 and 28°C were blood-fed three times (days 4, 8, and 14); those kept at 30°C were also blood-fed twice (days 4 and 8). Mosquitoes kept at 32 and 34°C were blood-fed only once (day 4) because of high mortalities that resulted in fewer females for which reason the mosquitoes could not be fed 3 times as those reared under 25 and 28°C temperature regimes.

Estimation of Length of Gonotrophic Cycle, Biting Rate, and Fecundity

One hundred (3–5 days old) adult mosquitoes (50 males and 50 females) were placed in a cage to estimate the gonotrophic cycle length. The mosquitoes were blood-fed on two (2) occasions. The number of days taken for mosquitoes to lay eggs after the first and second feeding with blood meal was recorded, and from the mean value, the length of the gonotrophic cycle was calculated. When the females failed to lay a second batch, the number of days to lay the first batch was considered to be the length of the gonotrophic cycle. The biting rate of *An. gambiae (s.l.)* mosquitoes was estimated as the reciprocal of the mean gonotrophic cycle for each temperature regime (Shapiro et al. 2017). These experiments were repeated five times for all colonies reared under the different temperature regimes.

In estimating fecundity, first day after the blood meal, twenty (20) fully engorged mosquitoes were randomly selected from each temperature regime and transferred individually into oviposition cups with 10% sugar solution. In each of the cup, five males were added to ensure that females were mated. Whatman filter paper on wet cotton wool was placed in each oviposition cup, provided substrate, and observed for egg-laying (a measure of fecundity). The filter paper was removed daily and checked for eggs, and the number of eggs on the filter paper under each temperature regime was enumerated. Fecundity was estimated from only the first batch of eggs laid by the mosquitoes, which, according to Costa et al. (2010), is a prognosis of total fecundity.

Body Weight, Size, and Proboscis Length

Fifty (50) of the four (4) d old adults (25 males and 25 females) that were not fed with a blood meal were randomly selected for each temperature regime, freeze-killed, and body weights were measured using an XS104 ultra-micro balance (Mettler Toledo Inc., Columbus, Ohio). Mosquitoes were weighed three times, and their averages were used as the body weight of the mosquito. After weighing, the same mosquitoes were used for body size and proboscis length measurements. Mosquito wing length has been used as a proxy for body size (Barreaux et al. 2018, Phasomkusolsil et al. 2018). Each mosquito's proboscis and wings were dissected, mounted on a slide, and their images captured at a magnification of 20×, using a Leica Stereomicroscope fitted with an inbuilt camera (Leica EZ4 HD, Leica Microsystems Limited, Switzerland), and with the capability of taking linear measurements. The Leica Application Software, version 3.4.0 (Leica Microsystems Limited, Switzerland), was used to measure proboscis length from the labial basal setae to the labella apex. Wings were also measured from the tip to the alula's distal end (excluding the fringe) (Barreaux et al. 2016a). The mean length of the two wings was used in the analyses; however, only one was used when a wing was missing.

Statistical Methods

The assumptions of normality and homogeneity of variances were assessed using Shapiro–Wilk and Bartlett's tests, respectively, in Stata version 15.1 (StataCorp LLC, TX). One-way analysis of variance (ANOVA) was used to explore the relationship between temperature and gonotrophic cycle length, biting rate, and fecundity. In cases where the overall model showed statistically significant differences, a post hoc analysis using the Tukey test was further used to identify where the differences existed. In addition, adult weight, body size (wing length), and proboscis length were log-transformed and analyzed using ordinary least square regression analysis with robust standard errors. Sensitivity analysis was further conducted using quantile and robust regression methods to determine how the regression coefficients and their respective standard errors change with respect to using different statistical models to assess the effects of temperature on adult weight, body size (wing length), and proboscis length.

With regard to adult longevity, a non-parametric (Kaplan–Meier) survival analysis was performed to determine the effects of temperature and blood meal on the longevity of adult *An. gambiae (s.l.)* mosquitoes. In addition, log-rank test and Cox proportional hazards models were used to test the null hypothesis that adult longevity did not change across the different rearing temperatures. The log-rank test compared the overall longevity trend for the five temperature regimes and blood meal effect on longevity, whereas Cox proportional-hazards model was used for two-sample comparisons at one temperature against the longevity at the baseline temperature (25°C) (Christiansen-Jucht et al. 2014). Median longevity with 95% confidence intervals was estimated for each of the temperature regimes.

Continuous variables with normal distribution were presented as mean (standard deviation [SD]), and non-normally distributed variables were reported as median (interquartile range [IQR]). In all statistical computations, a *P*-value of less than 0.05 was considered significant. P_{all} values represent *P* values for both blood-fed and non-blood-fed mosquitoes.

Results

Composition of *An. gambiae (s.l.)* (Tiassalé Strain) Mosquitoes

Molecular analysis of mosquitoes was conducted to identify the composition of *An. gambiae (s.l.)* mosquitoes used in the study. The results showed that *An. gambiae (s.l.)* samples used in this study consisted of two species: *An. gambiae (s.s.)* (26.53%) and *An. coluzzii* (23.47%). A hybrid of the two species constituted 50.00% (Table 1).

Longevity of Adults

The effects of temperature on the longevity of adult mosquitoes were assessed at different temperature regimes: 25, 28, 30, 32, 34,

36, 38 and 40°C. Eggs incubated at 40°C failed to hatch even after seven (7) d, and those that hatched at 38°C died before pupation. Although adult mosquitoes emerged at 36°C, they died within 24 h. Therefore, longevity was estimated for only mosquitoes reared at 25, 28, 30, 32, and 34°C. The results showed that the median adult longevity decreased with increasing temperature and differed between blood-fed and non-blood-fed mosquitoes. The difference in the median longevity of non-blood-fed and blood-fed mosquitoes was eight (8) d at 25°C and two (2) d at 34°C, respectively (Table 2).

Among blood-fed mosquitoes, Kaplan–Meier plots showed that longevity decreased with increasing temperature from 25°C (22 d) to 28°C (19 d), 30°C (14 d), 32°C (9 d), and 34°C (5 d) (Fig. 1A). A similar trend was observed in the longevity of non-blood-fed mosquitoes, although non-blood-fed mosquitoes lived longer than blood-fed mosquitoes (Fig. 1B). The longevity of mosquitoes significantly decreased with increasing temperature [Log-rank test: $X^2(4) = 904.15$, $P < 0.001$ for blood-fed group, $X^2(4) = 1163.60$, $P < 0.001$; non-blood-fed group] (Supp Table S2 [online only]).

Cox proportional hazard model was further used for two-sample comparisons at each of the temperature regimes (28, 30, 32, and 34°C) against the longevity at the baseline temperature (25°C). Compared to the baseline temperature (25°C), the results showed that adult longevity of blood-fed and non-blood-fed *An. gambiae (s.l.)* mosquitoes significantly decreased when temperature was increased to 28°C (HR = 2.89, 95% CI: 2.43, 3.44, $P < 0.001$ for non-blood-fed group; HR = 1.26, 95% CI: 1.07, 1.49, $P = 0.005$ for blood-fed group), 30°C (HR = 6.86, 95% CI: 5.62, 8.37, $P < 0.001$ for non-blood-fed group; HR = 2.71, 95% CI: 2.28, 3.23, $P < 0.001$ for blood-fed group), 32°C (HR = 16.46, 95% CI: 13.28, 20.40, $P < 0.001$ for non-blood-fed group; HR = 5.27, 95% CI: 4.37, 6.36, $P < 0.001$ for blood-fed group), and 34°C (HR = 22.65, 95% CI: 18.15, 28.26, $P < 0.001$ for non-blood-fed group; HR = 11.16, 95% CI; 9.11, 13.67, $P < 0.001$ for blood-fed group). In addition, blood meal significantly [log-rank test; $X^2(1) = 217.63$, $P < 0.001$] reduced the longevity of adult mosquitoes in all the temperature regimes (Supp Table S2 [online only]).

Table 1. Composition of *An. gambiae (s.l.)* mosquitoes

Anopheles gambiae (s.l.)	Frequency (*N*)	Percentage (%)
An. gambiae(s.s.) (S form)	26	26.53
An. coluzzii (M form)	23	23.47
M/S hybrids	49	50.00
Total	98	100.00

Table 2. Median longevity of adult *An. gambiae (s.l.)* mosquitoes reared at different temperatures

	Median longevity (days) (95% CI)	
Temperature regime (°C)	Blood-fed	Non-blood-fed
25	10 (6, 15)	18 (15, 22)
28	10 (5, 14)	12 (8, 16)
30	6 (3, 10)	9 (6, 12)
32	4 (2, 6)	6 (3, 8)
34	2 (2, 4)	4 (2, 7)
Total	5 (3, 10)	9 (5, 14)

95% CI means 95% Confidence interval.

The longevity of female and male mosquitoes was also compared to determine if differences existed. Overall, the longevity of female mosquitoes was significantly higher [log-rank test; $X^2(9) = 925.98$, $P < 0.001$ for blood-fed group; $X^2(9) = 1198.52$, $P < 0.001$ for non-blood-fed group] than that of the male mosquitoes irrespective of whether the mosquitoes were blood-fed or not (Fig. 1C and D; Supp Table S3 [online only]). However, log-rank test showed no statistically significant difference in the longevity of male and female mosquitoes reared at 25°C [$X^2(1) = 3.36$, $P = 0.067$] and 32°C [$X^2(1) = 1.20$, $P = 0.274$] in the blood-fed group (Supp Table S3 [online only]).

Length of the Gonotrophic Cycle, Biting Rate, and Fecundity of *An. gambiae (s.l.)* Mosquitoes

The length of gonotrophic cycle, biting rate, and fecundity of mosquitoes were estimated at only three temperature regimes, 25, 28, and 30°C because eggs kept at 40°C failed to hatch, and larvae were unable to develop at 38°C. In addition, adult mosquitoes that emerged at 36°C died within the first day and adult mosquitoes that emerged from larvae at either 32 or 34°C did not lay eggs.

The results showed that the gonotrophic cycle length was similar at 25 and 28°C (both had 3.20 d) but reduced at 30°C (2.90 d) (Table 3). There was no statistically significant difference [one-way ANOVA: $F(2,12) = 1.00$, $P = 0.397$] in the gonotrophic cycle length among the various temperature regimes. With biting rate, increasing temperature from 25 to 30°C increased the biting rate from 0.31 (±0.03) to 0.35 (±0.03) d^{-1} (Table 3). However, one-way ANOVA showed no statistically significant difference [$F(2,12) = 0.83$, $P = 0.460$] in biting rates among the various temperature regimes. In addition, the average number of eggs laid per female mosquito ranged from 75.68 at 25°C to 55.43 at 30°C (Table 3). The mean fecundity significantly decreased [one-way ANOVA: $F(2,57) = 3.46$, $P = 0.038$) with increasing temperature. Further statistical test using Tukey post hoc test revealed a significant difference in fecundity only between 25 and 30°C ($P = 0.027$) (Supp Table S4 [online only]).

Measurement of Body Weight, Size, and Proboscis Length of Adult Mosquitoes

The body weight, size, and proboscis length of adult mosquitoes were estimated at 25, 28, 30, 32, and 34°C because eggs kept at 40°C failed to hatch. Larvae were unable to develop at 38°C and adult mosquitoes that emerged at 36°C died within the first day. The results showed that body weight, size, and proboscis length of adult mosquitoes decreased with increasing temperature. Adult weight decreased as temperature increased from 1.10 (IQR, 0.30) mg at 25°C to 0.90 (IQR, 0.40) mg at 32 and 34°C (Table 4). Further analysis using ordinary least square regression with robust standard errors showed that a change in temperature from 25 to 34°C resulted in a statistically significant decrease in adult weight by 0.13 (95% CI: 0.24, 0.01, $P = 0.031$; Table 5).

With regard to body size of mosquitoes, it decreased with increasing rearing temperature from 25°C [3.03 (IQR, 0.26) mm] to 34°C [2.62 (IQR, 0.15) mm] (Table 4). Compared to 25°C, body size significantly decreased with increasing temperature by 0.06 (95% CI: 0.08, 0.03, $P < 0.001$) at 28°C, 0.07 (95% CI: 0.10, 0.05, $P < 0.001$) at 30°C, 0.10 (95% CI: 0.12, 0.08, $P < 0.001$) at 32°C, and 0.14 (95% CI: 0.16, 0.12, $P < 0.001$) at 34°C (Table 5). Similarly, proboscis length decreased with increasing temperature from 2.18 (IQR, 0.32) mm at 25°C to 1.84 (IQR, 0.36) mm at 34°C (Table 4). Generally, proboscis length significantly decreased with an increase in temperature from the baseline (25°C) to 30, 32, and 34°C

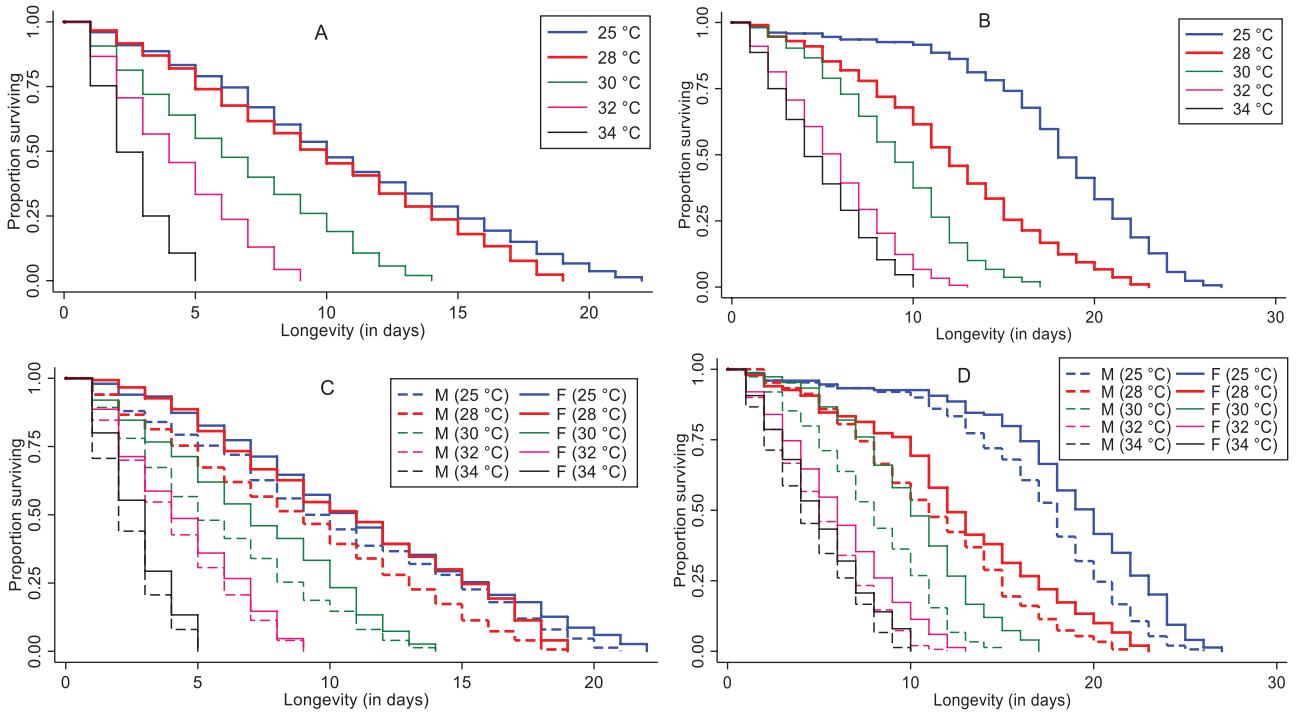

Fig. 1. Longevity of adult *An. gambiae (s.l.)* mosquitoes reared under different temperature regimes. (A) Longevity of blood-fed mosquitoes; (B) longevity of non-blood-fed mosquitoes; (C) longevity of male and female mosquitoes in the blood-fed group; (D) longevity of male and female mosquitoes in the non-blood-fed group. The 25°C temperature (blue) was set as the baseline against which longevity at different temperature was compared; 28°C (red); 30°C (green); 32°C (pink); 34°C (black); M and F represent Male and Female, respectively (See online version for color figure).

Table 3. Mean gonotrophic cycle length, biting rate, and fecundity of *An. gambiae (s.l.)* mosquitoes reared at different temperature regimes

Temperature regime (°C)	Gonotrophic cycle length (in days) Mean (±SD)	Biting rate (day-1) Mean (±SD)	Fecundity Mean (±SD)
25	3.20 (±0.27)	0.31 (±0.03)	75.68 (±21.69)
28	3.20 (±0.57)	0.32 (±0.06)	65.57 (±28.61)
30	2.90 (±0.22)	0.35 (±0.03)	55.43 (±22.06)
32	–	–	–
34	–	–	–

SD means Standard Deviation; mosquitoes at 32 and 34 did not lay eggs.

Table 4. *An. gambiae (s.l.)* weight, size, and proboscis length at different temperature regimes

Temperature regime (°C)	Adult weight (mg) median (IQR)	Adult size (mm) median (IQR)	Proboscis length (mm) median (IQR)
25	1.10 (0.30)	3.03 (0.26)	2.18 (0.32)
28	1.00 (0.40)	2.85 (0.17)	2.09 (0.37)
30	0.95 (0.50)	2.79 (0.19)	2.03 (0.29)
32	0.90 (0.40)	2.73 (0.15)	1.92 (0.39)
34	0.90 (0.40)	2.62 (0.15)	1.84 (0.36)

IQR, Interquartile range.

by 0.05 (95% CI: 0.09, 0.01, $P = 0.011$), 0.11 (95% CI: 0.15, 0.06, $P < 0.001$), and 0.13 (95% CI: 0.17, 0.09, $P < 0.001$), respectively. Sensitivity analysis using quantile and robust regressions showed consistent coefficients with the ordinary least square (OLS) regression (Table 5).

Discussion

Longevity of Mosquitoes Decreases With Increasing Temperature

Longevity of mosquitoes decreased with increasing temperature. This observation could be attributed to teneral reserves acquired from the immature stages in progressing to adulthood. Teneral reserves determine the amount of energy accessible for most adult life traits such as longevity, body size, vitellogenesis, and flight (Ukubuiwe et al. 2019). Larger mosquitoes (those that emerge at low temperatures) usually have more reserves and are likely to survive longer than smaller mosquitoes (those that emerge at high temperatures; Barreaux et al. 2018). Another possible explanation for the reduced longevity at high temperatures is the fast depletion of energy reserves because of the prolonged periods of high metabolic rates (Storey and Storey 2004). Higher temperatures may accelerate the reaction rate of various metabolic processes that affect the growth and development of the mosquito. The increased reaction rate could amplify the damage caused by the by-products of metabolism, such as reactive oxygen species (ROS) (Keil et al. 2015), leading to the death of the mosquito. Adult longevity influences the number of gonotrophic cycles and may limit disease transmission (Menge et al. 2005). These results support those of previous studies that have reported a decrease in longevity of *An. coluzzii* (Faiman et al. 2017) and *A. aegypti* (Marinho et al. 2016) with increasing temperatures.

The study also assessed the effect of blood meal on longevity of mosquitoes and found that mosquitoes fed on blood meal in addition to sugar solution had shorter longevity than those not fed with blood (these were fed on sugar only) in all the temperature regimes. The decreased longevity among blood-fed mosquitoes may be attributed to the intensity of oviposition, which requires much energy and is likely to decrease longevity (Marinho et al. 2016). A blood meal

Table 5. Relationship between temperature and adult *An. gambiae (s.l.)* weight, body size, and proboscis length

			Sensitivity analysis	
		OLS regression with robust standard errors	Quantile regression	Robust regression
Outcome	Temperature regime (°C)	β [95% CI]	β [95% CI]	β [95% CI]
Adult weight	25	Ref	Ref	Ref
	28	−0.05 [−0.15, 0.05]	−0.10 [−0.24, 0.05]	−0.04 [−0.16, 0.07]
	30	−0.06 [−0.16, 0.03]	−0.10 [−0.24, 0.05]	−0.08 [−0.20, 0.03]
	32	−0.09 [−0.19, 0.01]	−0.20 [−0.35, −0.05]**	−0.10 [−0.21, 0.01]
	34	−0.13 [−0.24, −0.01]*	−0.20 [−0.35, −0.05]**	−0.08 [−0.19, 0.03]
Adult size	25	Ref	Ref	Ref
	28	−0.06 [−0.08, −0.03]***	−0.06 [−0.09, −0.04] ***	−0.06 [−0.08, −0.04] ***
	30	−0.07 [−0.10, −0.05] ***	−0.08 [−0.10, −0.05] ***	−0.07 [−0.09, −0.05] ***
	32	−0.10 [−0.12, −0.08] ***	−0.10 [−0.13, −0.08] ***	−0.10 [−0.12, −0.08] ***
	34	−0.14 [−0.16, −0.12] ***	−0.14 [−0.17, −0.12] ***	−0.14 [−0.16, −0.12] ***
Proboscis length	25	Ref	Ref	Ref
	28	−0.04 [−0.08, 0.00]	−0.03 [−0.11, 0.05]	−0.04 [−0.09, 0.00]
	30	−0.05 [−0.09, −0.01]*	−0.05 [−0.13, 0.02]	−0.05 [−0.10, −0.01]*
	32	−0.11 [−0.15, −0.06] ***	−0.10 [−0.18, −0.03] **	−0.11 [−0.15, −0.07] ***
	34	−0.13 [−0.17, −0.09] ***	−0.14 [−0.22, −0.06] ***	−0.13 [−0.17, −0.09] ***

Adult weight, adult size, and proboscis length were log-transformed; OLS means Ordinary Least Square; single asterisk (*) represents significant difference at $P < 0.05$; double asterisk (**) means $P < 0.01$; triple asterisk (***) means $P < 0.001$; Ref means Reference; β means regression coefficients, 95% CI means 95% Confidence interval; *P*-values were generated using OLS with robust standard errors.

provides the female mosquitoes with the protein needed to synthesize yolk and develop their eggs (Nikbakhtzadeh et al. 2016). In a future warmer temperature, it is possible that adult mosquitoes may not be able to survive longer to reproduce and transmit diseases. This could help in the eradication of the vector and the disease it transmits.

Gonotrophic Cycle Length and Biting Rate of Mosquitoes Are Unaffected by Increasing Temperature

The associations between gonotrophic cycle length and biting rate of *An. gambiae (s.l.)* mosquitoes did not statistically change with increasing temperature. Previous studies have reported a significant decrease in the gonotrophic cycle length of *Anopheles* mosquitoes with increasing temperature (Paaijmans et al. 2013, Christiansen-Jucht et al. 2015, Shapiro et al. 2017). Also, Shapiro et al. (2017) found that biting rate significantly increased with increasing temperature. It is entirely possible that the difference in the findings between this study and previous studies may be attributable to the type of blood meal used to feed mosquitoes. In the studies above, mosquitoes were allowed to feed on human blood, compared to the above where mosquitoes were fed on guinea pigs. It has been reported that host blood source as food for mosquitoes could affect the fecundity and gonotrophic cycle length of mosquitoes (Shehata 2018). In this regard, high temperatures was found to speed up digestion of blood meals and reduce the gonotrophic cycle length (Afrane et al. 2006). Increased biting rate and reduced gonotrophic cycle length of *An. gambiae (s.l.)* mosquitoes could suggest that mosquitoes may feed more frequently, increasing egg-laying frequency and increasing their ability to transmit diseases (Afrane et al. 2012, Mala et al. 2014).

Fecundity of Mosquitoes Decreases With Increasing Temperature

Fecundity, which is the number of eggs laid per female mosquito, of mosquitoes decreased with increasing temperature. However, mosquitoes kept at 32 and 34°C failed to lay eggs. The reason for the decreased fecundity is not clear but it may have something to do with the size of mosquitoes as higher temperatures result in smaller adult mosquitoes. Temperature affects mosquito body size, which consequently influences mosquito's first meal choice. Smaller mosquitoes are more likely to take sugar meals than blood meals (Barreaux et al. 2018). Another possible reason for the inability of mosquitoes to lay eggs at 32 and 34°C could be that small mosquitoes (those that emerged at higher temperatures) were pre-gravid; that is, mosquitoes need at least two blood meals to complete the first gonotrophic cycle (Lyimo and Takken 1993) and such mosquitoes are not likely to live long enough. The findings are in line with those of previous studies (Aytekin et al. 2009, Phasomkusolsil et al. 2011, Christiansen-Jucht et al. 2015, Ezeakacha and Yee 2019) that have reported a decrease in mosquito fecundity with increasing temperature.

According to Christiansen-Jucht et al. (2015), when higher temperature affects mosquitoes fecundity, it also decreases the probability of mosquitoes to lay eggs. At high temperatures (32 and 34°C), the few mosquitoes that took blood meal even died within 2 days' post-feeding. It is possible that eggs may be present in the ovaries, just that mosquitoes did not live long enough to lay eggs (Ezeakacha and Yee 2019). However, this study did not dissect ovaries of dead mosquitoes to check insemination and egg development. These findings suggest that in a future warmer temperature, it is possible that the number of potential vectors may decrease (Charlwood and Bragança 2012) because of reduced fecundity of mosquitoes.

Body Size and Proboscis Length of Mosquitoes Decrease With Increasing Temperature

The effects of temperature on adult size and proboscis length could be attributed to the conditions experienced during larval development. Larval rearing temperature plays a significant role in shaping the overall size of adult mosquitoes (Ezeakacha and Yee 2019). The reduced body size of mosquitoes may have negative effects on fitness components such as reproduction, competition, and stress tolerance (Kingsolver and Huey 2008, Chidawanyika and Terblanche 2011).

The results are in agreement with previous studies (Aytekin et al. 2009, Phasomkusolsil et al. 2011, Charlwood and Bragança 2012, Christiansen-Jucht et al. 2015, Barreaux et al. 2016b, Barreaux et al. 2018) that reported reduced body size of *Anopheles* mosquitoes with increasing temperature. This current study did not investigate how temperature affected the ability of mosquitoes to insert proboscis into the host. It will be interesting for further studies to consider whether mosquitoes with the short proboscis (those kept at high temperatures) can reach the blood vessels of its host and acquire blood meal for egg development.

Conclusion

The findings of this study demonstrated that temperature significantly affected key growth and development characteristics of adult *An. gambiae (s.l.)* mosquitoes. At higher temperatures, the longevity of both blood-fed and non-blood-fed mosquitoes decreased significantly. In addition, gonotrophic cycle length, fecundity, body weight, size, and proboscis length decreased with increasing temperatures. Increased temperatures expected in a future warmer climate could likely cause some unexpected effects on mosquitoes by directly influencing population dynamics and malaria transmission. It is important to further investigate if the effects of temperature on proboscis length could influence the blood-feeding behaviour of female *An. gambiae (s.l.)* mosquitoes. We only considered the effects of elevated temperatures on the mosquito vector. It would also be interesting to evaluate the influence of high temperatures on the malaria parasite dynamics in mosquitoes. An increase or a decrease in the time a mosquito becomes infectious is key to malaria transmission.

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Authors' Contributions

T.P.A. conceived the study design, conducted all experiment, and drafted the manuscript. I.I. assisted during the experiment and D.D. helped in the data analysis. J.A.-M., P.K.B, J.N.H., M.K.B., S.K.D., T.R., and J.N.F. participated in the study design and critically reviewed important intellectual content. T.G.R. and J.N.F. acquired the funding for this study. All authors read and approved the final manuscript.

Supplementary Data

Supplementary data are available at *Journal of Medical Entomology* online.

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