Biological traits of wild-caught populations of *Aedes aegypti* in dengue endemic and non-endemic regions of Kenya

Caroline Wanjiku[1,2], David. P. Tchouassi[1], Catherine L. Sole[2], Christian W.W. Pirk[2], and Baldwyn Torto[1,2]✉

[1]*Behavioural and Chemical Ecology Unit, International Centre of Insect Physiology and Ecology (icipe), Nairobi, Kenya, btorto@icipe.org*

[2]*Department of Zoology and Entomology, University of Pretoria, Hatfield 0028, Republic of South Africa*

Received 21 July 2020; Accepted 6 October 2020

ABSTRACT: Variation in vector traits can modulate local scale differences in pathogen transmission. Here, we compared seasonal variation in the wing length (proxy for body size) and energy reserves of adult wild-caught *Aedes aegypti* populations from a dengue endemic (Kilifi) and non-endemic (Isiolo) area of Kenya. Vector sampling in the dengue endemic site was conducted during the dry and wet seasons. In the non-endemic area, it was limited to the dry season which characterizes this ecology where sporadic or no rainfall is commonplace during the year. We found variation by site in the body size of both sexes, with an overall smaller size of *Ae. aegypti* populations collected from Isiolo than those from Kilifi. Our results show that although total carbohydrates and lipids levels were highest in both sexes during the dry season, they were two-fold higher in males than females. However, we found weak correlations between body size and energy reserves for both sexes, with body size being more sensitive in identifying differences at a population level. These results provide insights into the determinants of the vectoring potential of *Ae. aegypti* populations in dengue endemic and non-endemic ecologies in Kenya. ***Journal of Vector Ecology* 46 (1): 19-23. 2021.**

Keyword Index: Energy reserves, body size, *Aedes aegypti*, dengue, ecological adaptation.

INTRODUCTION

Studies on mosquito physiology can improve our understanding of vector behavior, ecological adaptations, and their capacity for disease transmission (Briegel 2003). Critical factors in the acquisition and spread of pathogens by disease vectors include their immune responses, biting frequency, dispersal, fecundity, and survival rates (Maciel-de-Freitas et al. 2007, Fragkoudis et al. 2009, Smith et al. 2012, Farjana and Tuno 2013). In laboratory studies, these factors are correlated to physiological parameters, such as wing length (proxy for body size) (Schneider et al. 2011). In *Aedes aegypti*, body size influences the life history of males, with larger-sized individuals shown to produce more seminal fluid and spermatozoa (Ponlawat and Laura 2007, Helinski and Harrington 2011) that in turn increases their reproductive success. Larger males are also reported to outlive their smaller counterparts in the wild (Maciel-de-Freitas et al. 2007). In females, size has been shown to influence reproductive potential, blood-feeding frequency, flight ability, and even susceptibility to pathogens, but often the relationships between size and these traits are not always consistent across the studies.

Briegel et al. (2001) observed that smaller females had a shorter life span and poor flight potential compared to their larger counterparts. In contrast, smaller females were found to survive equally well in both laboratory and field settings and dispersed over longer distances than their larger counterparts (Maciel-de-Freitas et al. 2007). Furthermore, while small females exhibited increased host-seeking behavior with consequent higher blood-feeding frequency (Farjana and Tuno 2013), it is the larger-sized individuals that had a higher chance of developing disseminated infections with viruses (Schneider et al. 2007). In *Anopheles gambiae*, a study comparing the reproductive potential of wild and laboratory-reared males found that body size and lipid reserves of wild males was greater than their laboratory counterparts (Huho et al. 2007). These findings clearly demonstrate that data on laboratory-reared insects should be interpreted with caution especially in making predictions about wild populations (Huho et al. 2007). Thus, there is a need for field-based studies to improve our knowledge of the significance of biological traits on the life history, population dynamics, and even pathogen vectoring potential of mosquito populations.

In Kenya, *Ae. aegypti* is widely distributed but recurrent dengue outbreaks occur mostly in the coastal part of the country (Lutomiah et al. 2016, Agha et al. 2019). Recent studies show that populations from the coastal region are the most likely to acquire and transmit Chikungunya and dengue 2 viruses (Chepkorir et al. 2014, Agha et al. 2017a). Vector competence of *Ae. aegypti* populations has been linked to environmental temperature (Chepkorir et al. 2014, Agha et al. 2017a). However, vector competence, and ultimately vector potential, is influenced by a variety of genetic and non-genetic factors, such as diet, gut microbial community, age, reproductive status, and body size (Lefèvre et al. 2013, Zirbel et al. 2018). We tested the hypothesis that there are variations in body size and energy reserves of local *Ae.*

aegypti populations and that the differences may impact on their vectorial capacity. To achieve this, we measured the wing length (proxy for body size) and energy reserves (total carbohydrates and lipids) and examined their seasonal variation in two *Ae. aegypti* populations collected from a dengue endemic and a non-endemic area of Kenya.

MATERIALS AND METHODS

Study area

The study was conducted at two sites with differing dengue epidemiology: Kilifi County (endemic) and Isiolo County (non-endemic). In Kilifi, the study was conducted within and around the KEMRI-Wellcome Trust Research Program (KWTRP) facility and the Kilifi district hospital, both located in Kilifi town (3.63229° S, 39.8569° E). In Isiolo County, trapping was conducted within the Garbatullah District Hospital facility (0.4111° N, 38.5690° E) in Garbatullah town. Kilifi town is an urban settlement and located within Kilifi County which is part of the larger coastal region of Kenya. The area experiences annual temperatures ranging between 21° to 32° C and has two rainy seasons: long rains occurring between March/April to May/June, and the short rains between October and December (Owino et al. 2014). The months between July to October and December to March/April are generally dry, with temperatures ranging between 28° to 31° C. In 2017, there was a delayed onset of long rains in Kilifi and throughout the country resulting in an extended drought period. However, in 2018 the long rainy season extended to the end of July and was marked by heavy flooding. Unlike Kilifi, the ecology of Garbatullah located within Isiolo County consists predominantly of arid and semi-arid lands with annual temperatures ranging between 24° to 30° C. The area is usually dry for the greater part of the year with minimal rain that is erratic and it is common that some years may miss a rainy season. The rainfall is often accompanied by heavy storms and flash floods.

Design and mosquito trapping

In Kilifi, mosquito surveys were each conducted during the dry (July and August, 2017) and rainy seasons, (July and August, 2018). Trapping during each season was conducted for ten consecutive days. In Isiolo, only one mosquito survey during the dry period (March, 2018) was conducted for five consecutive days. Mosquitoes were sampled using BG-Sentinel traps baited with carbon dioxide (dry ice). Daily, ten traps (at least 50 m apart) were set up at dawn (06:00) and retrieved shortly before dusk (18:30). After retrieval, mosquitoes were killed by freezing, sorted, counted, and stored in cryo-vials in liquid nitrogen before shipment to the *icipe* laboratories in Nairobi.

Estimation of mosquito size

The right wing of each individual adult was carefully spread on a glass slide with a drop of ringer solution and excised at the root using a scapel. Wing length was measured from the joint at the root of the wing to the apex, including the fringe scales (Briegel 1990), using a Leica HD digital microscope equipped with the Q-capture Pro7 software (Biocompare) that has a digital micrometer. Body size was determined by calculating the cubic value of wing length as described by Briegel (1990). The distribution of wing length of males and females irrespective of site and season was determined. Based on the median value for each sex, the wing sizes were classified into two groups as follows: 1) large (> than median size) and 2) small (≤ median size).

Mosquito processing and energetics analysis

Since samples were to be analyzed for sugars, lipids, and DNA analysis (in a separate study), they were each processed following the methods of Nyasembe et al. (2018) but with the following modifications: 1) After removal of the wings, phosphate buffered saline (PBS) instead of 0.5% hypochlorite solution was used to rinse the individual mosquitoes (to remove any debris) before being transferred into sterile microcentrifuge tubes and crushed using individual sterile polypropylene pestles and 2) 100 µl of sterile 0.3M sodium acetate was added to 200 µl of absolute molecular grade ethanol and the samples incubated for 30 min at -20° C (first step of the DNA extraction). After incubation, the homogenates were centrifuged (Eppendorf 5417r) at 4° C for 10 min at 12,000 RPM and the resulting supernatant separated for total sugar and lipid analysis while the remaining homogenates were stored for molecular assay in a separate study.

The sugar and lipid fractions were processed using the hot anthrone and vanillin phosphoric acid solutions as described previously (Van Handel 1985a, 1985b). However, the latter test was modified as follows: instead of using a mixture of chloroform/methanol to extract lipids as described by Van Handel (1985a), we added 200 µl of chloroform directly to the supernatant obtained in the extraction step above. The mixture was then centrifuged (Eppendorf 5417r) for 1 min at 1,000 RPM, then 200 µl of sterile double distilled water was added and the sample centrifuged at 1,000 RPM for 1 min to activate phase separation. The non-aqueous phase (chloroform layer) was then separated and processed using the vanillin phosphoric method: the chloroform was evaporated on a heating block, 200 µl of sulphuric acid added to the tubes and heated for 10 min then allowed to cool and 1 ml of vanillin phosphoric acid reagent added. The purpose of using sulfuric acid is to convert unsaturated lipids to water soluble sulfonic acid derivatives which is what gives a red color after the addition of vanillin-phosphoric acid reagent (visual indicator for presence of lipids). The sugar fraction was processed using the hot anthrone method that entailed addition of 1 ml of anthrone reagent and heating the mixture for 17 min at 110° C.

Both total lipids and sugars were quantified in each sample using a microplate plate reader (Epoch, Biotek) using the Gen5 software (Biotek) on a 96 well plate. First, the absorbance values of blank wells were measured at a wavelength of 625 nm. Then, 200 µl of either sugar or lipid samples, as well as a set of standard solutions, were dispensed in triplicates in three adjacent wells and the absorbance values read. Lemon grass oil and glucose dissolved in 70% ethanol served as controls for lipids and sugars, respectively.

They were each run at different concentrations spanning the expected analyte concentration range. From these, calibration curves and linear equations (R^2 = 0.97; Y = 1E+06x−8E + 06) were generated to estimate the amounts of lipids and sugars in each sample. Samples with amounts below the limit of detection (LoD) (8.1µg/ml for sugars and 179 µg/ml for lipids) were excluded in the data analysis.

Quantities of carbohydrates and lipids detected in the samples were expressed per cubic value of mean wing length for each respective cohort to normalize them for body size as described by Briegel (1990).

Data analysis

We used a Chi-square test-of-independence to compare proportions of wing class sizes for both males and females at each site. A one-way analysis of variance, and welch t-test were also used to compare quantities of energy reserves (total carbohydrates and lipids) between and within sexes. Where significant differences were found, we used the Tukey HSD test for pair-wise comparison of means. We also used the Pearson correlation coefficient to assess the relationship between body size and energetic reserves. All the statistical tests were done at a 95% significance level using the R software (R Core Team 2020).

Ethical approval

Approval for the study was obtained from the Scientific and Ethics Review Unit of the Kenya Medical Research Institute (KEMRI-SERU) (Protocol number: SERU 2787). After explaining the objective of the study, verbal consent was also sought from local authorities to set up mosquito traps in the respective study sites.

RESULTS

Wing length

We found that wing lengths of both males and females followed a normal distribution with median length of 2 mm for males and 2.5 mm for females (Figure 1, Table 1). When wing lengths were classified as either large or small, we found that the cohort collected during the dry season from Kilifi comprised a significant proportion of large males and almost equal proportions of large and small females (males: $\chi^2 = 11.56$; df =1, $P < 0.05$); females: $\chi^2 = 2.56$ df = 1, $P > 0.05$) (Figure 2, Table 1). In the wet season, the proportions of large and small-winged males were almost equal while a significantly higher proportion (1.5 times more) of females were small-winged (males: $\chi^2 = 1.44$, df = 1, $P > 0.05$; females: $\chi^2 = 4$, df = 1, $P < 0.05$) (Figure 2, Table 1). However, in Isiolo, both males and females consisted of small-winged individuals (1.6 and 1.7 times, respectively, more than their large counterparts)

Table 1. Summary of wing lengths of wild-caught male and female *Aedes aegypti* from the dry and wet seasons in Kilifi and the dry season in Isiolo. No. = number analyzed; SEM = standard error of the mean; row values denoted by different letters are significantly different at the 95% level of significance.

				Wing length class distribution (%)		
	Site (season)	Range (mm) (No.)	Mean wing length (mm) ± SEM	Large	Small	*Chi-square statistic (df =1)*
Females	Kilifi (dry season)	1.33 - 3.80 (360)	2.55 ± 0.01	58[a]	42[a]	$\chi^2 = 2.56$; $P = 0.11$
	Kilifi (wet season)	1.89 – 3.26 (100)	2.49 ± 0.03	40[a]	60[b]	$\chi^2 = 4$; $P = 0.05$
	Isiolo (dry season)	1.19 – 3.18 (132)	2.38 ± 0.02	36[a]	64[b]	$\chi^2 = 7.84$; $P = 0.01$
Males	Kilifi (dry season)	1.01 - 2.7 (133)	2.06 ± 0.02	67[a]	33[b]	$\chi^2 = 11.56$; $P = 0.001$
	Kilifi (wet season)	1.12 – 2.55 (100)	1.93 ± 0.02	44[a]	56[a]	$\chi^2 = 1.44$; $P = 0.23$
	Isiolo (dry season)	1.21 – 2.5 (133)	1.87 ± 0.02	38[a]	62[b]	$\chi^2 = 5.76$; $P = 0.02$

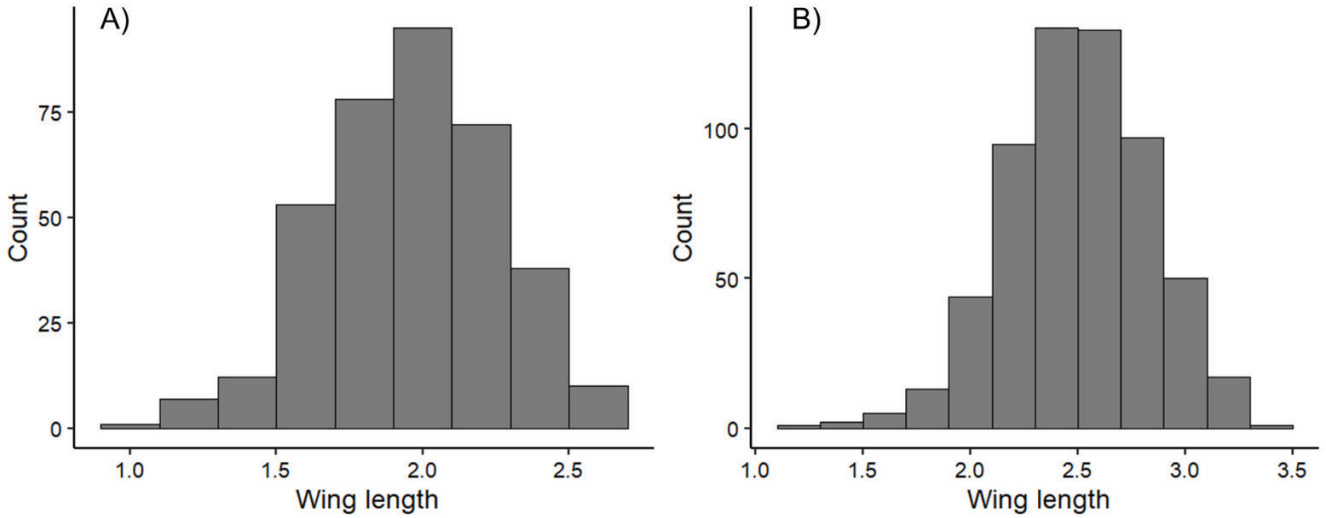

Figure 1. Distribution of wing lengths (mm) of *Aedes aegypti* A) males and B) females collected during the dry and wet seasons in Kilifi and the dry season in Isiolo.

(males: $\chi^2 = 5.76$, df = 1, $P < 0.05$; females: $\chi^2 = 7.84$, df = 1, $P < 0.05$) (Figure 2, Table 1).

Total carbohydrate content

In both Kilifi and Isiolo, the levels of total carbohydrates of males were two-fold higher than those of females at both sites (Kilifi(dry), t = 4.74, df = 133.39, $P < 0.0001$; Kilifi(wet), t = 3.54, df = 21.78, $P < 0.05$; and Isiolo (dry), t = 3.524, df = 141.39, $P < 0.05$). However, the quantities varied between the sites for both sexes (males, df = 2, F = 9.289, $P < 0.05$; females, df = 2, F = 79.38, $P < 0.0001$) (Figure 3). Irrespective of season, total carbohydrate content of males did not vary significantly between Isiolo and Kilifi (Isiolo (dry): Kilifi (dry), $P > 0.05$; Isiolo (dry): Kilifi(wet), $P > 0.05$). However, males from the dry season in Kilifi had two-fold higher ($P < 0.05$) amounts than their wet season counterparts (Figure 3). Similarly, the total carbohydrate content of females collected from the dry season in Kilifi was not significantly different from those collected from Isiolo ($P > 0.05$) (Figure 3). However, the amounts were three and 2.7 times, respectively, higher than their counterparts from the wet season in Kilifi (Kilifi (dry): Kilifi (wet), $P < 0.0001$; Isiolo (dry): Kilifi (wet), $P < 0.05$) (Figure 3).

Total lipid content

Males had significantly higher levels of lipids than females (two- and three-fold higher in Kilifi and Isiolo, respectively) (Kilifi(dry), t = 10.957, df = 270.12, $P < 0.0001$; Kilifi (wet), t = 7.386, df = 20.124, $P < 0.0001$, and Isiolo (dry), t = 14.804, df = 176.29, $P < 0.0001$). Similar to total carbohydrates, the quantities of lipids also varied across sites for both males (df = 2, F = 20.87, $P < 0.0001$), and females (df = 2, F = 15.86, $P <$

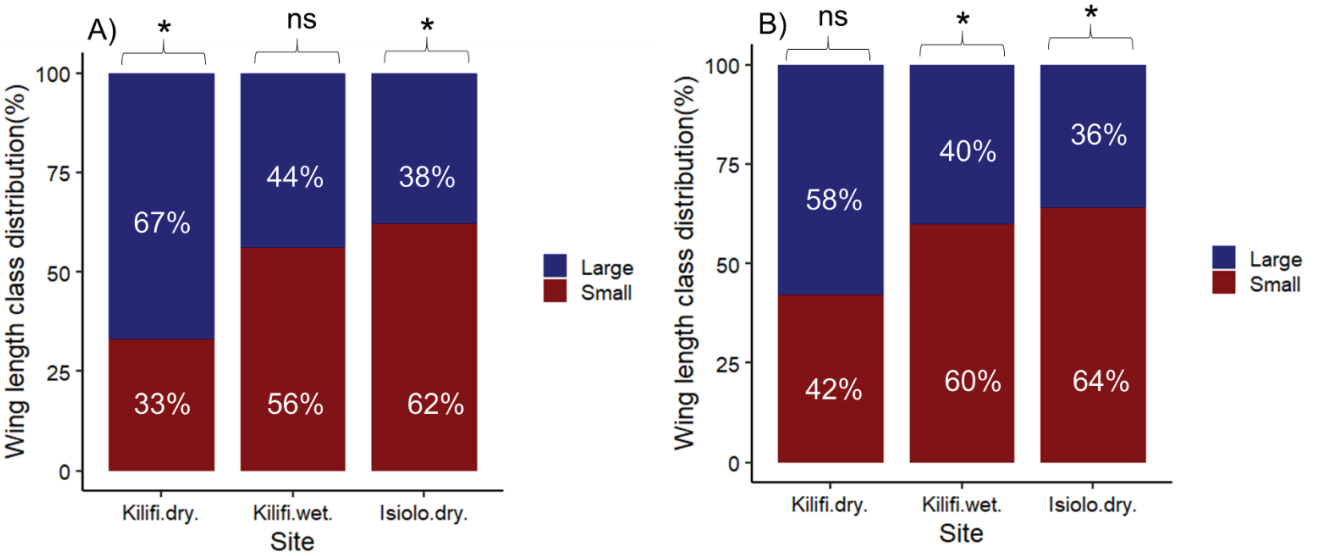

Figure 2. Proportions of wing length classes (large and small) of A) male and B) female *Aedes aegypti* caught during the dry and wet seasons in Kilifi and the dry season in Isiolo; bars with * and ns indicate statistical significance and non-significance, respectively at 95% level of significance.

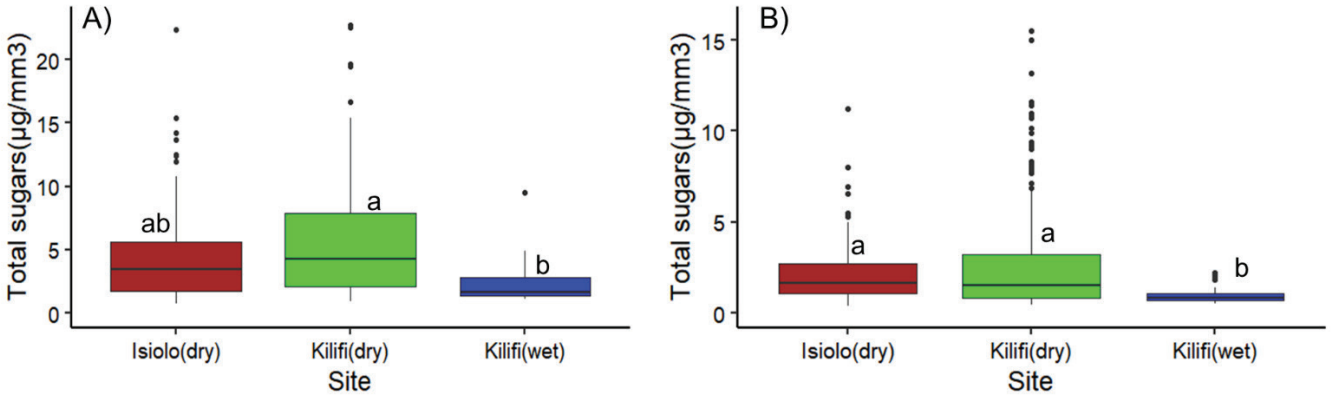

Figure 3. Proportions of wing length classes (large and small) of A) male and B) female *Aedes aegypti* caught during the dry and wet seasons in Kilifi and the dry season in Isiolo; bars with * and ns indicate statistical significance and non-significance, respectively at 95% level of significance.

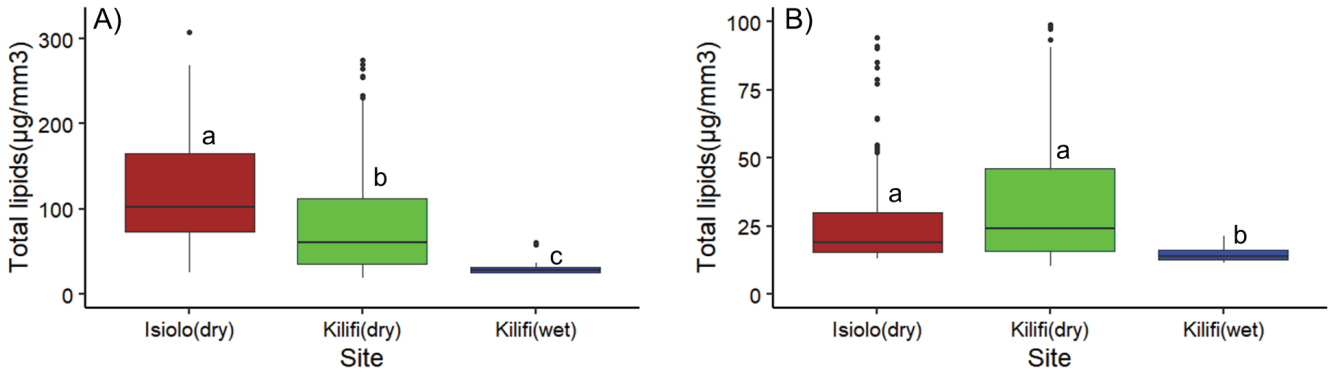

Figure 4. Quantities of total lipids detected in A) male and B) female *Aedes aegypti* caught during the dry and wet seasons in Kilifi and the dry season in Isiolo; quantities are expressed in micrograms per cubic value of the wing length (µg/mm3); boxes with different letters indicate significant differences at 95% level of significance.

0.0001) (Figure 4). The lipid content of males collected from the dry season in Isiolo was 1.3 and 3.6 times more than that of males from the dry and wet season in Kilifi, respectively (Isiolo (dry): Kilifi (dry), $P < 0.0001$; Isiolo (dry): Kilifi (wet), $P < 0.0001$) (Figure 4). Also, males collected during the dry season in Kilifi had significantly higher levels of lipids (2.6 times higher) than those collected from the same site during the wet season (Kilifi (dry): Kilifi (wet), $P < 0.05$) (Figure 4). In contrast, the total lipid content of females collected from Kilifi during the dry season was not significantly different from those sampled from Isiolo ($P > 0.05$) (Figure 4). However, females from both the dry seasons in Kilifi and Isiolo also had significantly more (two-fold higher) lipids than females collected from the wet season in Kilifi (Kilifi (dry): Kilifi (wet), $P < 0.0001$; Isiolo (dry): Kilifi (wet), $P < 0.0001$) (Figure 4).

Relationship between body size and energy reserves

There was overall weak correlation between male body size and total carbohydrates (Kilifi (dry), $R^2 = 0.004$; Isiolo (dry), $R^2 < 0.001$, Kilifi (wet), $R^2 = 0.002$) or lipids (Kilifi (dry), $R^2 = 0.02$; Isiolo (dry), $R^2 = 0.01$, Kilifi (wet), $R^2 = 0.03$). Similarly, correlations between female body size and total carbohydrate (Kilifi (dry), $R^2 = 0.02$; Isiolo (dry), $R^2 = 0.003$, Kilifi (wet), $R^2 = 0.04$) and lipids (Kilifi (dry), $R^2 = 0.03$; Isiolo (dry), $R^2 = 0.002$, Kilifi (wet), $R^2 = 0.02$) were also weak.

DISCUSSION

In this study, we examined seasonal variations in wing length (proxy for body size) and energy reserves of *Ae. aegypti* populations from a dengue endemic (Kilifi) and a non-endemic (Isiolo) site in Kenya. We found variation in wing lengths of males and females by site and season. The results are consistent with previous findings on wild *Ae. aegypti* populations from Thailand, Puerto Rico, Mexico, and south Texas (Scott et al. 2000, Strickman and Kittayapong 2003, Walsh et al. 2011, Olson et al. 2020). When classified as either small or large, we found that the distribution of wing lengths varied by sex, site, and season. In both sexes, the highest proportions of large and small-winged individuals were detected in the dry season in Kilifi and Isiolo, respectively. We also noted with interest the increase in the proportion of small-winged males and females during the wet season in Kilifi. Wing length is determined during larval development and is associated with the prevailing conditions at the breeding site (Briegel 1990, Maciel-De-Freitas et al. 2007). Thus, the cumulative smaller size of *Ae. aegypti* found for populations from Isiolo compared to Kilifi was an important revelation of our study. *Aedes aegypti* is known to breed in diverse containers (e.g., discarded tires, metal/plastic drums) and natural larval sites (e.g., tree holes, flower vases) which are abundant in coastal Kenya (Agha et al. 2017b). These sites

vary in their organic matter composition, including plankton, fungi, algae, bacteria, and other microorganisms on which larvae generally feed. Fewer larval sites from containers are not unexpected in Isiolo, which is a much drier ecology most of the year than Kilifi that has both wet and dry seasons. Thus, the observed differences between the sites may partly be due to variation in the quality and quantity of food available to larvae in their habitats. The reduction in mosquito size during the wet season in Kilifi was also interesting but not unexpected. Compared to the dry season, the rainy season in Kilifi is associated with increased vector density (Agha et al. 2017c). High larval densities within a breeding site results in competition for nutrients negatively affecting larval development and resulting in emergence of small-sized adults (Briegel et al. 2001, Mitchell-Foster et al. 2012). Thus, the variation in mosquito sizes between the wet and dry season in Kilifi is likely a reflection of changes occurring in the larval environment across the two seasons.

Seasonal influence in carbohydrate and lipid contents of the mosquito was evident and varied between the sites and was higher in males than in females. Mosquitoes obtain carbohydrates, and by extension lipids, largely from floral and extra floral nectar (Nyasembe et al. 2012, Nyasembe et al. 2018). In tropical environments such as our study sites, the flowering phenology of many plants is linked to rainfall (Kushwaha et al. 2011). It was therefore intriguing that independent of mosquito sex, both energy reserves were significantly higher in the dry than in the wet season. Due to limitations in natural sugar sources during dry periods, mosquitoes may opportunistically feed on seedpods and less nutritious stems of green flowerless plants (Müller and Schlein 2005, Junnila et al. 2010). Scarcity of these resources during the dry season may become limiting factors for mosquito survival, which may cause them to evolve adaptive strategies through physiological adjustments. When faced with harsh environmental conditions, some mosquito species undergo a period of dormancy either through diapause or quiescence (Diniz et al. 2017). The latter ensures that eggs survive dry conditions, a trait well known in *Ae. aegypti* (Diniz et al. 2017). This mosquito species also undergoes embryonic quiescence allowing the 1st instar larvae to remain inside the eggs until a time when an appropriate hatching stimulus is available (Perez and Noriega 2013). Both processes require significant investment in maternal energy reserves (Perez and Noriega 2013, Farnesi et al. 2015). Males may also store up enough energy reserves during dry periods to allow for longer range dispersal in search of females for mating or plant sources for sugars. Reproductive behaviors in males are energetically demanding. In the field, *Anopheles gambiae* males are reported to use up between 50% and 60% of their total sugars for swarming (Maïga et al. 2014). A combination of physiological needs for survival, especially during the dry season, may account for the observed seasonal differences.

Other factors that may have accounted for the higher levels of energy reserves in the dry than in the wet season may be the age of the mosquitoes. It is possible that the populations sampled during the dry season were older and therefore had accumulated more energy reserves. Further studies to correlate seasonality with age structure and energy reserves are warranted. Higher levels of energy reserves could also have resulted from increased sugar uptake or feeding on highly concentrated nectars. Temperature and moisture stress are two very important environmental factors that influence mosquito physiology (Huestis et al. 2011, Holmes and Benoit 2019). In fact, as little as a one-degree increment in environmental temperature can result in up to a 6% increase in metabolic rate (Huestis et al. 2011). Thus, during the dry season, increased uptake of plant sugar would mitigate dehydration and result in the accumulation of high levels of energy reserves that would produce increased metabolic rates. Also, the access to highly concentrated nectars during the dry season attributing to low relative humidity remains a possibility. Our data show that males had higher energy reserves than females at both sites and seasons, despite being smaller in size than females. Previously, Briegel (1990) observed that females with the same amount of lipids as males are often much larger than the males owing to their inherent adaptation that prevents them from accumulating lipids and leaving space for blood meals and the development of eggs. We also noted from our findings that body size did not correlate with the quantity of energy reserves. Similar lack of a clear relationship between these fitness traits has been previously demonstrated in field, rather than in laboratory studies (Huho et al. 2007). Thus, the outcome of fitness estimates may be more biologically complex in the natural setting (Charlwood 2003).

Overall, our data have shed light on variations in both mosquito body size and energy reserves that can influence mosquito survival, an important component of vectorial capacity. Thus, there is reason to believe that the observed trait variations between the different *Ae. aegypti* populations may impact on the vectoring potential between the two ecological sites. However, more work is needed to provide further insights on the ecologic dimensions of vector adaption and the pathogens they transmit. We conclude that, indeed, there are variations in biological traits, between the *Ae. aegypti* populations from Kilifi and Isiolo. However, between energy reserves and body size, the latter is the more sensitive parameter in unravelling differences at a population level. More studies are needed to link these physiological parameters to important phenotypes, such as their roles in pathogen transmission between the two different ecologies.

Acknowledgments

We thank Alvin Karanja, Hilary Kirwa, and Onesmus Mwaura (Behavioral and Chemical Ecology Unit – BCEU (*icipe*, Nairobi)), for technical support in the laboratory. We also thank Martha Muturi and Dr. Joseph Mwangangi (KEMRI-Wellcome Trust Research Program), Ali Roba, and Godana Wachil (Garbatullah District Hospital) for logistical and technical support during field work in Kilifi and Garbatullah- Isiolo. This study was partly supported by the project, Combatting Arthropod Pests for better Health, Food and Climate Resilience (Project number: RAF-3058 KEN-18/0005) funded by Norwegian Agency for Development

Cooperation (Norad). We also gratefully acknowledge the financial support for this research by the following organizations and agencies: UK's Foreign, Commonwealth & Development Office (FCDO), the Swedish International Development Cooperation Agency (SIDA), the Swiss Agency for Development and Cooperation (SDC), the Federal Democratic Republic of Ethiopia, and the Government of the Republic of Kenya. CW was supported by the German Academic Exchange service (DAAD) in-region post-graduate program (Award number: 91636618). The views expressed herein do not necessarily reflect the official opinion of the donors. The authors declare that there are neither conflicts of interest nor competing financial interests associated with this work.

REFERENCES CITED

Agha, S.B., D.P. Tchouassi, M. J. Turell, A.D.S Bastos, and R. Sang. 2019. Entomological assessment of dengue virus transmission risk in three urban areas of Kenya. PLoS Negl. Trop. Dis. 13: 1–14.

Agha, S.B., E. Chepkorir, F. Mulwa, C. Togoi, S. Arum, M.M. Guarido, P. Ambala, B. Chelangat, J. Lutomiah, D.P. Tchouassi, M.J. Turell, and R. Sang. 2017a. Vector competence of populations of *Aedes aegypti* from three distinct cities in Kenya for chikungunya virus. PLoS Negl. Trop. Dis. 11: 1–11.

Agha, S.B., D.P. Tchouassi, A.D.S. Bastos, and R. Sang. 2017b. Assessment of risk of dengue and yellow fever virus transmission in three major Kenyan cities based on Stegomyia indices. PLoS Negl. Trop. Dis. 11: e0005858.

Agha, S. B., D.P. Tchouassi, A.D.S. Bastos, and R. Sang. 2017c. Dengue and yellow fever virus vectors: Seasonal abundance, diversity and resting preferences in three Kenyan cities. Parasit. Vectors 10: 1–10.

Briegel, H. 1990. Metabolic relationship between female body size, reserves, and fecundity of *Aedes aegypti*. J. Insect Physiol. 36: 165–172.

Briegel, H. 2003. Physiological bases of mosquito ecology. J. Vector Ecol. 28: 1–11.

Briegel, H., I. Knüsel, and S.E. Timmermann. 2001. *Aedes aegypti*: size, reserves, survival, and flight potential. J. Vector Ecol. 1: 21–31.

Charlwood, J.D. 2003. May the force be with you: Measuring mosquito fitness in the field. In: W. Takken and T.W. Scott (eds.), *Ecological Aspects for Application of Genetically Modified Mosquitoes.* Kluwer Publisher, Wageningen, The Netherlands. pp. 47–62.

Chepkorir, E., J. Lutomiah, J. Mutisya, F. Mulwa, B. Orindi, Z. Ng'ang'a, and R. Sang. 2014. The vector competence of *Aedes aegypti* mosquito populations from Kilifi and Nairobi for dengue-2 virus and the influence of temperature. Parasit. Vectors 7: 435.

Diniz, D.F.A., C.M.R. de Albuquerque, L.O Olivia, M.A.V. de Melo-santos, and C.F.J Ayres. 2017. Diapause and quiescence: dormancy mechanisms that contribute to the geographical expansion of mosquitoes and their evolutionary success. Parasit. Vectors 10: 1–13.

Farjana, T. and N. Tuno. 2013. Multiple blood feeding and host-seeking behavior in *Aedes aegypti* and *Aedes albopictus* (diptera: Culicidae). J. Med. Entomol. 50: 838–846.

Farnesi, L.C., R.F.S. Menna-barreto, A.J Martins, D. Valle, and G.L. Rezende. 2015. Physical features and chitin content of eggs from the mosquito vectors *Aedes aegypti*, *Anopheles aquasalis* and *Culex quinquefasciatus*: Connection with distinct levels of resistance to desiccation. J. Insect Physiol. 83: 43–52.

Fragkoudis, R., G. Attarzadeh-Yazdi, A.A. Nash, J.K. Fazakerley, and A. Kohl. 2009. Advances in dissecting mosquito innate immune responses to arbovirus infection. J. Gen. Virol. 90: 2061–2072.

Helinski, M.E. and L.C. Harrington. 2011. Male mating history and body size influence female fecundity and longevity of the dengue vector *Aedes aegypti*. J. Med. Entomol. 48: 202–211.

Holmes, C.J. and J.B. Benoit. 2019. Biological adaptations associated with dehydration in mosquitoes. Insects 10: 375.

Huestis, D.L., A.S. Yaro, A.I. Traoré, A. Adamou, Y. Kassogué, M. Diallo, S. Timbiné, A. Dao, and T. Lehmann. 2011. Variation in metabolic rate of *Anopheles gambiae* and *A. arabiensis* in a Sahelian village. J. Exp. Biol. 214: 2345–2353.

Huho, B.J., K.R. Ng'habi, G.F. Killeen, G. Nkwengulila, B.G.J. Knols, and H.M. Ferguson. 2007. Nature beats nurture: A case study of the physiological fitness of free-living and laboratory-reared male *Anopheles gambiae s.l.* J. Exp. Biol. 210: 2939–2947.

Junnila, A., G.C. Müller, and Y. Schlein. 2010. Species identification of plant tissues from the gut of *An. sergentii* by DNA analysis. Acta Trop. 115: 227–233.

Kushwaha, C.P., S.K. Tripathi, B.D. Tripathi, and K.P. Singh. 2011. Patterns of tree phenological diversity in dry tropics. Acta Ecol. Sin. 31: 179–185.

Lefèvre, T., A. Vantaux, K.R. Dabiré, K. Mouline, and A. Cohuet. 2013. Non-genetic determinants of mosquito competence for malaria parasites. PLoS Pathog. 9.

Lutomiah, J., R. Barrera, A. Makio, J. Mutisya, H. Koka, S. Owaka, E. Koskei, A. Nyunja, F. Eyase, R. Coldren, and R. Sang. 2016. Dengue outbreak in Mombasa City, Kenya, 2013–2014: Entomological investigations. PLoS Negl. Trop. Dis. 10: 2013–2014.

Maciel-De-Freitas, R., C.T. Codeço, and R. Lourenço-De-Oliveira. 2007. Body size-associated survival and dispersal rates of *Aedes aegypti* in Rio de Janeiro. Med. Vet. Entomol. 21: 284–292.

Maïga, H., A. Niang, S.P. Sawadogo, R.K. Dabiré, R.S. Lees, J.R. Gilles, F. Tripet, and A. Diabaté. 2014. Role of nutritional reserves and body size in *Anopheles gambiae* males mating success. Acta Trop. 132: S102-S107.

Mitchell-Foster, K., B.O. Ma, S. Warsame-Ali, C. Logan, M.E. Rau, and C. Lowenberger. 2012. The influence of larval density, food stress, and parasitism on the bionomics of the dengue vector *Aedes aegypti* (Diptera: Culicidae): Implications for integrated vector management. J. Vector

Ecol. 37: 221–229.

Müller, G. and Y. Schlein. 2005. Plant tissues: The frugal diet of mosquitoes in adverse conditions. Med. Vet. Entomol. 19: 413– 422.

Nyasembe, V.O., D.P. Tchouassi, C.W.W. Pirk, C.L. Sole, and B. Torto. 2018. Host plant forensics and olfactory-based detection in Afro-tropical mosquito disease vectors. PLoS Negl. Trop. Dis. 12: 1–21.

Nyasembe, V.O., P.E.A. Teal, W.R. Mukabana, J.H. Tumlinson, and B. Torto. 2012. Behavioural response of the malaria vector *Anopheles gambiae* to host plant volatiles and synthetic blends. Parasit. Vectors 5: 234.

Olson, M.F., S. Garcia-Luna, J.G. Juarez, E. Martin, L.C. Harrington, M.D. Eubanks, I.E. Badillo-Vargas, and G.L. Hamer. 2020. Sugar feeding patterns for *Aedes aegypti* and *Culex quinquefasciatus* (Diptera:Culicidae) mosquitoes in south Texas. J. Med. Entomol. 57: 1–9.

Owino, E.A., R. Sang, C.L. Sole, C. Pirk, C. Mbogo, and B. Torto. 2014. Field evaluation of natural human odours and the biogent-synthetic lure in trapping *Aedes aegypti*, vector of dengue and chikungunya viruses in Kenya. Parasit. Vectors 7: 451.

Perez, M.H. and F.G. Noriega. 2013. *Aedes aegypti* 1st instar quiescence affects larval fitness and metal tolerance. J. Insect Physiol. 58: 824–829.

Ponlawat, A. and H.C. Laura. 2007. Age and body size influence male sperm capacity of the dengue vector *Aedes aegypti* (Diptera:Culicidae). J. Med. Entomol. 44: 422–426.

Schneider, J.R., D.D Chadee, A. Mori, J. Romero-Severson, and D.W. Severson. 2011. Heritability and adaptive phenotypic plasticity of adult body size in the mosquito *Aedes aegypti* with implications for dengue vector competence. Infect. Genet. Evol. 11: 11-16.

Schneider, J.R., A. Mori, J. Romero-Severson, D.D. Chadee, and D.W. Severson. 2007. Investigations of dengue-2 susceptibility and body size among *Aedes aegypti* populations. Med. Vet. Entomol. 21: 370–376.

Scott, T.W., A.C. Morrison, L.H. Lorenz, G.G. Clark, D. Strickman, P. Kittayapong, H. Zhou, and J.D. Edman. 2000. Longitudinal studies of *Aedes aegypti* (Diptera: Culicidae) in Thailand and Puerto Rico: population dynamics. J. Med. Entomol. 37: 77–88.

Smith, D.L., K.E. Battle, S.I. Hay, C.M. Barker, T.W. Scott, and F.E. McKenzie. 2012. Ross, Macdonald, and a theory for the dynamics and control of mosquito-transmitted pathogens. PLoS Pathog. 8: e1002588.

Strickman, D. and D. Kittayapong. 2003. Dengue and its vectors in Thailand: calculated transmission risk from total pupal counts of *Aedes aegypti* and association of wing-length measurements with aspects of the larval habitat. Am. J. Trop. Med. Hyg. 68: 209–217.

Van Handel, E. 1985a. Rapid determination of total lipids in mosquitoes. J. Am. Mosq. Contr. Assoc. 1: 302–304.

Van Handel, E. 1985b. Rapid determination of glycogen and sugars in mosquitoes. J. Am. Mosq. Contr. Assoc. 1: 299–301.

Walsh, R. K., L. Facchinelli, J.M. Ramsey, J.G. Bond, and F. Gould. 2011. Assessing the impact of density dependence in field populations of *Aedes aegypti*. J. Vector Ecol. 36: 300–307.

Zirbel, K., B. Eastmond, and B.W. Alto. 2018. Parental and offspring larval diets interact to influence life-history traits and infection with dengue virus in *Aedes aegypti*. R. Soc. Open Sci. doi.org/10.1098/rsos.180539.