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Temperature and age, individually and interactively, shape the size, weight, and body composition of adult female mosquitoes

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A R T I C L E I N F O

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A B S T R A C T

Most insects are poikilotherms and ectotherms, so their body temperature fluctuates and closely aligns with the temperature of their environment. The rise in global temperatures is affecting the physiology of insects by altering their ability to survive, reproduce, and transmit disease. Aging also impacts insect physiology because the body deteriorates via senescence as the insect ages. Although temperature and age both impact insect biology, these factors have historically been studied in isolation. So, it is unknown whether or how temperature and age interact to shape insect physiology. Here, we investigated the effects of warmer temperature (27 °C, 30 °C and 32 °C), aging (1, 5, 10, and 15 days post-eclosion), and their interaction on the size and body composition of the mosquito, *Anopheles gambiae*. We found that warmer temperatures result in slightly smaller adult mosquitoes, as measured by abdomen and tibia length. Aging alters both abdominal length and dry weight in a manner that correlates with the increase in energetic resources and tissue remodeling that occurs after metamorphosis and the senescence-based decline that ensues later. Moreover, the carbohydrate and lipid contents of adult mosquitoes are not meaningfully affected by temperature but are altered by aging: carbohydrate content increases with age whereas lipid content increases over the first few days of adulthood and then decreases. Protein content decreases with both rising temperature and aging, and the aging-associated decrease accelerates at warmer temperatures. Altogether, temperature and age, individually and to a lesser extent interactively, shape the size and composition of adult mosquitoes.

1. Introduction

Most insects are poikilothermic ectotherms, meaning that their internal body temperature fluctuates with, and closely matches, the temperature of their environment. Insect body temperature dictates the efficiency of many physiological processes and affects their ability to survive, reproduce, and transmit disease (Agyekum et al., 2021; Delatte et al., 2009; Ferguson and Adamo, 2023; Gloria-Soria et al., 2017; González-Tokman et al., 2020; Ma et al., 2021; Nosrat et al., 2021; Reinhold et al., 2018; Soh et al., 2018; Walsh et al., 2019). Current climate changes are increasing global temperatures, so insects are being subjected to temperatures that are higher than what they have become accustomed to (Couper et al., 2021; Deutsch et al., 2008; Ma et al., 2021). These warmer temperatures quicken development (Bayoh and Lindsay, 2003; Ciota et al., 2014; Du Plessis et al., 2020; Gilbert and Raworth, 1996; Huxley et al., 2021; Kirby and Lindsay, 2009; Reiskind and Zarrabi, 2012), increase the metabolic rate (González-Tokman et al., 2020; Huestis et al., 2012), and decrease longevity (Agyekum et al., 2022b; Agyekum et al., 2021; Aytekin et al., 2009; Christiansen-Jucht et al., 2014; Miquel et al., 1976; Mołoń et al., 2020). Furthermore, warmer temperatures alter the ability of insects to combat pathogens by strengthening some immune responses while weakening others, resulting in complex immune outcomes to infection (Adamo and Lovett, 2011; Ferguson and Adamo, 2023; Murdock et al., 2013; Murdock et al., 2012a; Murdock et al., 2012b; Wojda, 2017). Thus, most insects—including those that are pollinators, agricultural pests, or disease vectors—are being impacted by the global rise in temperature.

In addition to temperature, aging also impacts insect physiology. As insects age, they undergo senescence. Senescence is the irreversible deterioration of body condition with increased age and includes lower metabolic rates and a weakened immune response to infection (Boggs, 2009; King and Hillyer, 2013; League et al., 2017; Monaghan et al.,

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2008; Stanley, 2012). Senescence is also accompanied by a decrease in fertility and fecundity and aging-related mortality (Gaillard and Lemaître, 2020; Jehan et al., 2021; Levitis, 2011; Monaghan et al., 2008; Styer et al., 2007).

Individually, both temperature and age shape insect physiology. However, these factors have historically been studied in isolation. That is, we do not understand how temperature impacts the rate of senescence. Aging is often conceptualized as a function of time, which refers to chronological age. However, aging can also be conceptualized as a function of physiological efficiency and performance, which refers to physiological age. In homeotherms, chronological and physiological aging are usually coupled because body temperature is stable and constant, meaning that an organism's age in terms of time corresponds to how well the organism's body functions. However, because the physiology of most insects is influenced by body temperature, and their body temperature fluctuates with environmental temperature, the physiological age of an insect likely differs from its chronological age. We hypothesize that warmer environmental temperature accelerates the rate of senescence, thereby decoupling chronological and physiological age, and quickening the physiological aging of insects.

Here, we begin to test this hypothesis in the mosquito, *Anopheles gambiae*, which is a primary vector of human malaria. *A. gambiae* inhabits tropical and subtropical Africa, where warmer temperatures are forcing this species to adapt or alter its geographic range, leaving more people vulnerable to the pathogens that it transmits (Couper et al., 2021; Deutsch et al., 2008; Jia et al., 2017; Lindsay et al., 1998; Ngonghala et al., 2021). Warmer temperatures during development result in smaller larvae, pupae, and adults (Agyekum et al., 2022a, 2022b; Agyekum et al., 2021). These smaller mosquitoes ingest smaller blood meals, have decreased fecundity, and have shorter lifespans (Ameneshewa and Service, 1996; Briegel, 1990; Takken et al., 1998). Less clear is how adult aging affects mosquito size. The chitinous exoskeleton of a mosquito sclerotizes and becomes largely fixed following eclosion (Chapman et al., 2013; Klowden, 2013), so aging should not greatly alter mosquito size. Nevertheless, temperature and aging may affect the internal body composition of mosquitoes, and this would be reflected in their carbohydrate, lipid, and protein content. These parameters—size and body composition—are critical because they influence how well a mosquito can survive, mate, reproduce, and transmit disease (Briegel, 1990; Takken et al., 1998; Vanhandel, 1984).

Here, we tested how temperature (27 °C, 30 °C and 32 °C) and age (1, 5, 10 and 15 days post-eclosion), individually and interactively, alter the size and body composition of adult mosquitoes. We found that mosquitoes are slightly smaller when reared at warmer temperatures. Additionally, both abdominal length and dry weight change with aging in a manner that corresponds with the initial increase in energetic resources and tissue remodeling that occurs following metamorphosis and later with the decrease in resources that occur as the mosquito senesces. Moreover, carbohydrate and lipid contents are not meaningfully affected by temperature but change with age: carbohydrate content increases with age whereas lipid content first increases and then decreases. Finally, protein content decreases with both rising temperature and aging, and the aging-associated decrease accelerates at warmer temperatures. Taken altogether, we found that both temperature and age affect the size and composition of adult mosquitoes.

2. Materials and methods

2.1. Mosquito rearing and colony maintenance

A colony of *Anopheles gambiae* Giles *sensu stricto* (G3 strain; Diptera: Culicidae) was maintained in an environmental chamber at a constant temperature of 27 °C, 75% relative humidity, and a 12 h:12 h, light:dark cycle. Adult mosquitoes in this colony were fed defibrinated sheep blood (Hemostat Laboratories Inc., Dixon, CA) using a Hemotek PS5 artificial membrane feeding system (Hemotek Ltd., Blackburn, UK) that was heated to 37 °C. The eggs laid by these mosquitoes were collected and moved to one of three separate environmental chambers, each set to one of three constant temperatures: 27 °C, 30 °C, or 32 °C. Eggs were then hatched and reared to adulthood at each of these temperatures, and maintained there for the duration of the experiment. Humidity was kept constant at 75% humidity, with a 12 h:12 h, light:dark cycle. These temperatures represent warming temperatures experienced by this species in tropical Africa (Deutsch et al., 2008; Lindsay et al., 1998).

Eggs were hatched in water and larvae were fed koi fish food and Baker's yeast daily. Similar larval density was maintained in each temperature condition. Pupae were collected every 24 h and placed in 2.4-liter plastic containers with a fine mesh marquisette top. After eclosion, adults were fed 10% sucrose solution *ad libitum*. Sucrose was selected as the dietary source because it is the carbohydrate that is preferred by mosquitoes (Ignell et al., 2010). Experiments were conducted on adult female mosquitoes at 1, 5, 10, and 15 days post-eclosion that had been maintained at each temperature (Fig. 1).

2.2. Body size and weight measurements

Body size was determined by measuring the length of the abdomen, hind tibia, and wing, with minor modification (Brown et al., 2018). Briefly, individual mosquitoes were pinned through the dorsal side of the thorax on Sylgard 184 silicone elastomer (Dow Corning, Midland, MI) that had been polymerized in 60 × 15 mm Petri dishes. The right wing and right hind leg were removed with forceps and placed next to the pinned body, flat on the dish. Each body part was imaged under brightfield illumination using a 4x objective on a Nikon Eclipse Ni-E compound microscope connected to a Nikon Digital Sight DS-Qil monochrome digital camera and Advanced Research NIS Elements Software (Nikon, Tokyo, Japan). The length of each feature was measured three independent times using the NIS Elements length analysis tool, and the average of the three values was recorded (Fig. 2). The length of the abdomen was measured from the posterior of the thoracic postnotum to the end of the eighth abdominal segment, excluding the cercus. The length of the tibia was measured from the end of the femur to the beginning of the tarsus, joint to joint. The length of the wing was measured from the axillary incision (located near the proximal region of the ambient costa) to the junction between the ambient costa and the radius 3, excluding the fringe (Chintapalli and Hillyer, 2016). Three independent trials were conducted for each temperature and age combination, with each trial composed of 10–12 mosquitoes per combination.

Body size was also determined by measuring the dry weight of mosquito whole bodies. Briefly, 10–15 adult female mosquitoes from each temperature-age combination were pooled inside a pre-weighed 1.5 mL tube and then frozen for 1–2 h. Tubes were subsequently moved into a desiccator filled with Drierite (Xenia, OH) and dried for ≥48 h in a light vacuum. The tubes were weighed again, and the weight of an individual mosquito was calculated by subtracting the initial weight of a tube from the final weight of that same tube and dividing the value by the number of mosquitoes that had been placed inside the tube. Weighing of tubes was performed using a Denver Instrument SI-114 analytical balance (Bohemia, NY), accurate to 0.0001 g. Three independent trials were conducted for each temperature and age combination, with each trial composed of 3 biological samples.

2.3. Carbohydrate measurements

Total carbohydrate content was determined using the colorimetric phenol–sulfuric acid method (Ahmed, 2013; Ellison et al., 2015; Nielsen, 2017), with minor modifications. Between 3 and 5 whole mosquitoes were homogenized in 200 μL of distilled water. The homogenate was centrifuged at 15,000 rcf for 1 min at 4 °C, 10 μL of supernatant was transferred to a 1.5 mL tube, and 390 μL of distilled water was added. Then, the sample was mixed with 10 μL of 80% aqueous phenol (Thermo

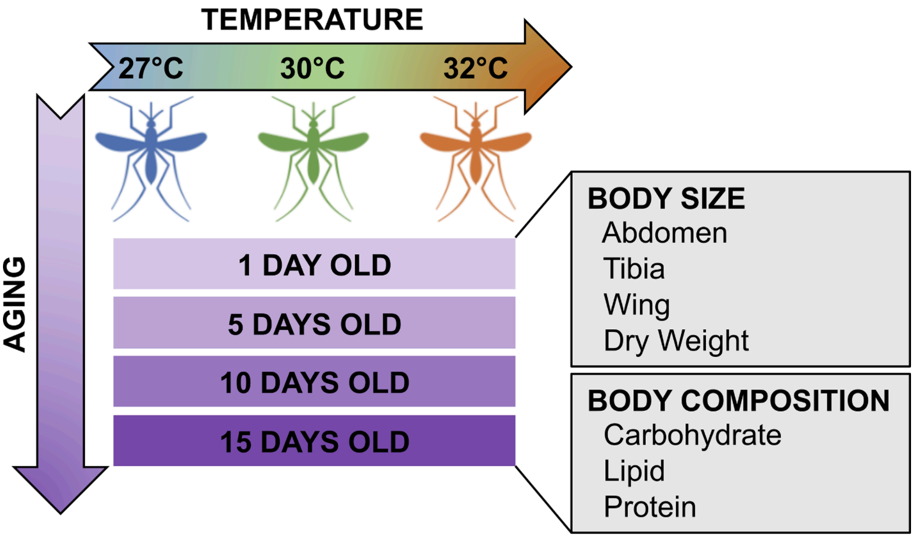

Fig. 1. Experimental overview for the determination of the effects of temperature and age on body size and body composition. Mosquitoes were reared and maintained at 3 constant temperatures (27 °C, 30 °C, or 32 °C) and size and body composition were measured at 1, 5, 10, and 15 days post-eclosion.

Fisher Scientific, Waltham, MA, USA), and 1 mL of concentrated sulfuric acid (Thermo Fisher Scientific) was added. The solution was incubated for 10 min at room temperature, vortexed, and the absorbance was read at 490 nm using an Eppendorf Biophotometer Plus (Eppendorf AG, Hamburg, Germany). The absorbance value was compared to a standard calibration curve that was generated from a 10 mg/mL D-glucose (Thermo Fisher Scientific) solution that was diluted from 50 μg/mL to 10 μg/mL. Between 4 and 5 independent trials were conducted for each temperature-age combination, with each trial composed of three biological samples. Each biological sample was split and analyzed in technical duplicate, and the average of the two technical duplicates was recorded. Using each absorbance value, the standard curve, and the number of mosquitoes in a sample, the total carbohydrate content per mosquito was calculated.

2.4. Lipid measurements

Total lipid content was determined using the colorimetric sulfo-phospho-vanillin method (Cheng et al., 2011; Ellison et al., 2015; Van-handel, 1985). Between 3 and 5 mosquitoes were homogenized in 200 μL of 2:1 chloroform: methanol, v/v. The homogenate was centrifuged at 15,000 rcf for 1 min at 4 °C, and 8 μL of supernatant was transferred to a 1.5 mL tube and heated to 90 °C for 10 min to evaporate the solvent. A volume of 100 μL of concentrated sulfuric acid was added, and the tube was incubated at 90 °C for 20 min. Once the sample cooled to room temperature, 50 μL of vanillin-sulfuric acid reagent (0.5 mg vanillin per 1 mL 17% phosphoric acid; Thermo Fisher Scientific) was added. The sample was vortexed, and after 5 min of color development, the absorbance was read at 550 nm using an Eppendorf Biophotometer Plus. The absorbance value was compared to a standard calibration curve that was generated from a solution of canola oil prepared in 2:1 chloroform: methanol, v/v, at 15 mg/mL that was then diluted from 120 μg/mL to 7.5 μg/mL. Between 4 and 5 independent trials were conducted for each temperature-age group combination, with each trial composed of three biological samples. Each biological sample was split and analyzed in technical duplicate, and the average of the two technical duplicates was recorded. Using each absorbance value, the standard curve, and the number of mosquitoes in a sample, the total lipid content per mosquito was calculated.

2.5. Protein measurements

Total protein content was determined using the Bradford assay following the manufacturer's guidelines of the Coomassie Protein Assay Kit (Thermo Fisher Scientific), with minor modifications (Bradford, 1976; Ellison et al., 2015). Briefly, between 3 and 5 mosquitoes were homogenized in 200 μL of distilled water. The homogenate was centrifuged at 15,000 rcf for 1 min at 4 °C, and 15 μL of supernatant was transferred to a 2 mL tube and mixed with 750 μL of Coomassie Reagent. The sample was incubated for 10 min at room temperature for color development, and the absorbance was measured at 595 nm using an Eppendorf Biophotometer Plus. The absorbance value was compared to a bovine serum albumin (BSA) standard calibration curve that was serially diluted from 2000 μg/mL to 25 μg/mL. Between 4 and 5 independent trials were conducted for each temperature-age group combination, with each trial composed of three biological samples. Each biological sample was split and analyzed in technical duplicate, and the average of the two technical duplicates was recorded. Using each absorbance value, the standard curve, and the number of mosquitoes in a sample, the total protein content per mosquito was calculated. Because the first step for carbohydrate and protein determination is the same, aliquots from the initial homogenates were used for both carbohydrate and protein measurements.

2.6. Statistical analyses

In this study, a biological trial is an independent experiment that uses mosquitoes from an independent egg batch whereas a technical duplicate is an experimental resampling of a biological trial. For experiments with technical duplicates, the values were averaged, and the average was used for plotting and statistical analyses. Statistical analyses were conducted on GraphPad Prism, version 9 (GraphPad Software, San Diego, CA). First, each dataset was assessed for outliers by the ROUT outlier test, and it was determined that no outliers were present; thus, all the data collected were used in the analyses. Second, the normality of each dataset was assessed by the Shapiro-Wilk test, and all datasets were found to conform to a normal distribution. Third, the data were analyzed by two-way ANOVA with temperature and age as the variables, followed by Tukey's post-hoc tests. Results of two-way ANOVA, including Tukey's tests for main effects, are presented in Appendix A: Tables S1-S7. All data are presented in Appendix B: Data.

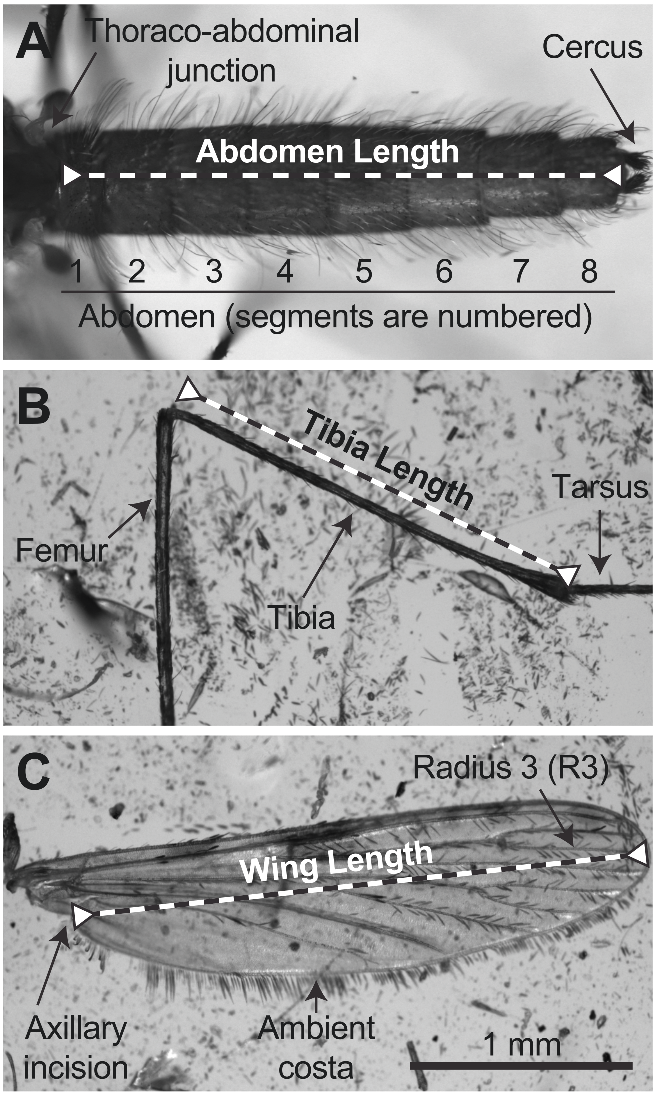

Fig. 2. Method for measuring the length of the abdomen, tibia, and wing. (A) The length of the abdomen was measured from the posterior of the thoracic postnotum to the end of the eighth abdominal segment, excluding the cercus. (B) The length of the tibia was measured from the end of the femur to the beginning of the tarsus, joint to joint. (C) The length of the wing was measured from the axillary incision to the junction between the ambient costa and the radius 3, excluding the fringe.

3. Results

3.1. Mosquitoes have slightly smaller abdomens and tibias when the temperature is warmer, but wing length is unchanged

By measuring the lengths of the abdomen, tibia, and wing, we examined how warmer temperatures during development and adulthood, irrespective of age, alter the body size of the adult mosquito. We measured multiple body parameters to capture the full effects of temperature on mosquito body size because the size of a single feature, such as a mosquito's wing, may not scale in a linear manner with the size of another feature, such as the tibia or abdomen (Brown et al., 2018).

We found that both the abdomen and tibia are smaller in mosquitoes reared in warmer temperatures (Fig. 3A-D). Specifically, the abdomen of mosquitoes reared at 27 °C averaged 2.53 mm, which is 4.0% larger than the abdomen of mosquitoes reared at either 30 °C or 32 °C (Fig. 3A). The tibia of mosquitoes reared at 27 °C averaged 1.80 mm, which is 4.0% larger than the tibia of mosquitoes reared at 30 °C or 32 °C (Fig. 3C). However, changes in temperature did not influence the length of the wing (Fig. 3E-F). The average wing of mosquitoes reared at 27 °C measured 2.62 mm in length, which is almost identical to the wing of mosquitoes reared at 30 °C or 32 °C (Fig. 3E). Overall, warming temperature results in mosquitoes with slightly smaller abdomens and legs, but similarly sized wings.

3.2. The length of the mosquito abdomen marginally changes with aging, but the tibia and wing remain unchanged

We next tested whether aging, irrespective of temperature, alters the body size of the adult mosquito. We found that the length of the abdomen changes with age (Fig. 4A-B), but this change does not scale in a linear manner with aging. Instead, the mean abdomen length increased by 2.8%—from 2.43 mm to 2.50 mm—during the first 5 days of adulthood and then decreased by 2.3% over the subsequent 10 days (Fig. 4A). We infer that the abdomen lengthens initially during adulthood as tissue remodeling following metamorphosis is completed and nutritional reserves are restored, and then shortens due to deterioration as the mosquito senesces.

Unlike for the abdomen, the tibia was almost identical across the 4 ages assayed, ranging in length from an average of 1.75 mm at 1-day post-eclosion to 1.77 mm at 15 days post-eclosion (Fig. 4C-D). Similar to the tibia, the average wing of 1-day-old mosquitoes (2.58 mm in length) was nearly identical to the wing of mosquitoes that were 5, 10, and 15 days old (Fig. 4E-F). In summary, the length of the abdomen slightly changes with aging, but the length of the tibia and wing do not.

3.3. Wing and tibia length are weakly shaped by the interaction between temperature and age

Having determined how temperature and age independently impact body size, we next assessed how the effects of temperature are modified by aging, and vice versa. We found that temperature and age do not interact to shape abdominal length (Figs. 3B, 4B). However, the interaction between temperature and age shapes the length of both the tibia (Figs. 3D, 4D) and the wing (Figs. 3F, 4F). This interaction is subtle and is most clearly seen in comparisons made (i) across all temperatures at a single age and (ii) across all ages at a single temperature. To illustrate the first type of comparison, the temperature-based change in the length of the tibia and wing was most pronounced at 10 days of age relative to the other ages investigated (Fig. 3D, F). To illustrate the second type of comparison, the age-based change in the length of the wing was most pronounced at 27 °C relative to the other temperatures investigated (Fig. 4F). Regardless, we found that the interaction between temperature and age contributes to <4% of the total variation for tibia length and <6% of the total variation for wing length (Tables S2-S3). So, although under specific conditions this interaction alters body size, we conclude that this interaction is not biologically meaningful.

3.4. Mosquito dry weight is primarily dependent on adult age

We expanded our analysis of body size by investigating how temperature and age—individually and interactively—alter the dry weight of the adult mosquito. Previous work suggests that body length is directly correlated with the dry weight of the mosquito (Price et al., 2015; Reiskind and Zarrabi, 2012; Takken et al., 1998). Therefore, we hypothesized that dry weight decreases as temperature increases because mosquitoes reared at warmer temperatures are smaller, but that aging would have a non-linear effect on dry weight because of our earlier findings on abdominal length.

We found that both temperature and age alter dry weight (Fig. 5). Specifically, temperature and age accounted for 4.2% and 46.8% of the total variation in dry weight, respectively, which indicates that dry weight is primarily shaped by age (Tables S4). When considering temperature, mosquitoes reared at 30 °C were the lightest (Fig. 5A). These

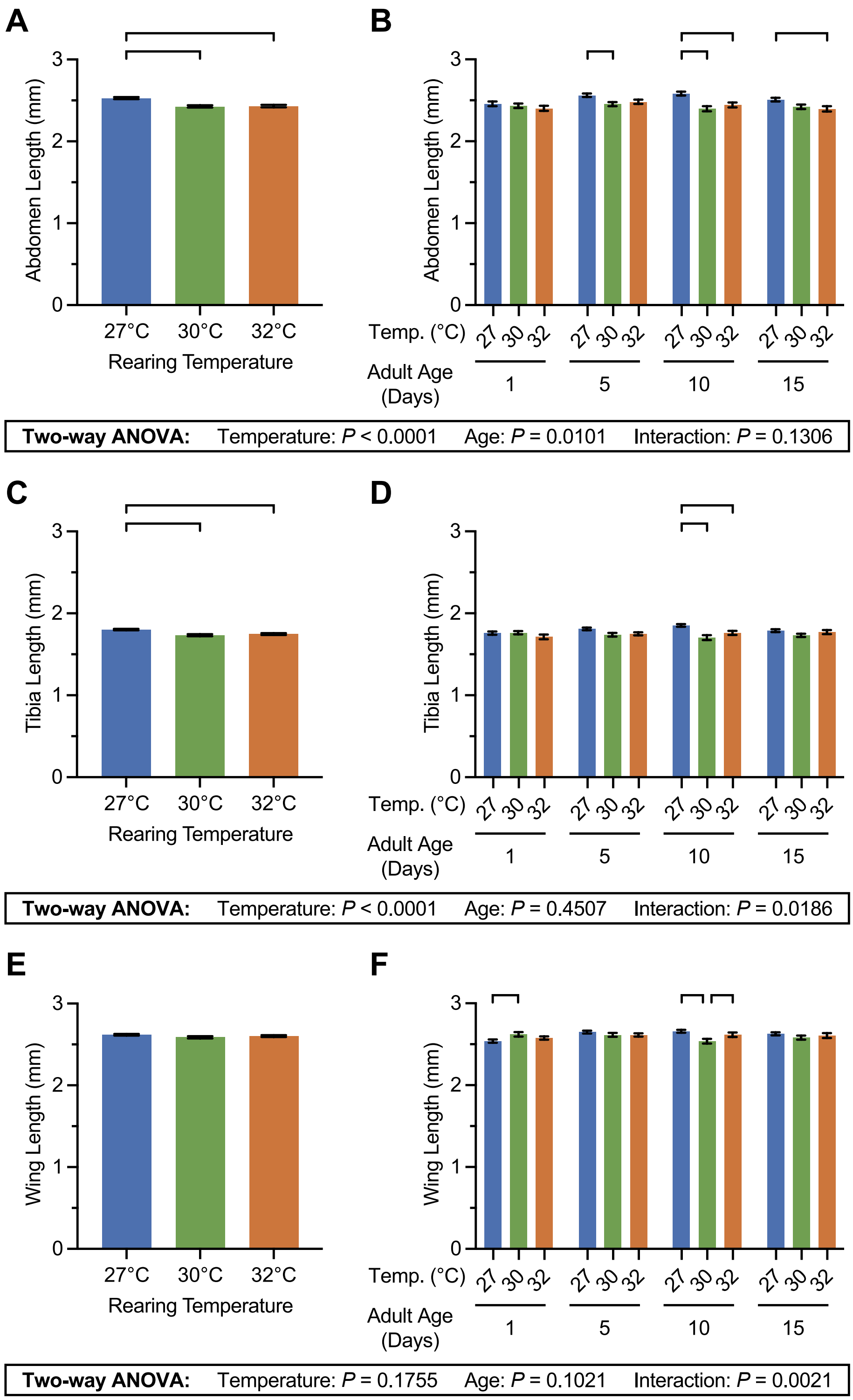

Fig. 3. At warmer temperatures, the abdomen and tibia are smaller, but wing length is unchanged. (A-B) Length of the abdomen. The abdomen is smaller when the rearing temperature is warmer. (C-D) Length of the tibia. The tibia is smaller when the rearing temperature is warmer. (E-F) Length of the wing. Wing size is unaltered by rearing temperature. Columns mark the mean and whiskers denote the standard error of the mean (S.E.M.). Data were analyzed by a two-way ANOVA followed by a Tukey's post-hoc test. Brackets denote Tukey's $P < 0.05$. Panels A, C and E aggregate the data by temperature irrespective of age. The same measurements are plotted in Figs. 3 and 4 but grouped or arranged differently.

mosquitoes averaged 0.3098 mg, which is 6.7% less than those reared at 32 °C and 4.4% less than those at 27 °C. When considering aging, we found that mosquito dry weight resembles a parabolic curve (Fig. 5C). One-day-old adults were the lightest, weighing an average of 0.2796 mg, and by 5 days post-eclosion, the weight increased by 29.4% to 0.3619 mg. By 10 days post-eclosion, weight decreased by 11.6% to 0.3200 mg and remained unchanged through day 15. Similar to the aging-related changes we observed in abdominal length, a potential explanation for this age effect is that newly eclosed mosquitoes are lighter because of the cost of metamorphosis; then, mosquitoes increase in weight as they feed and restore energy reserves prior to a senescence-based decline.

When we investigated whether temperature altered the age-related changes in dry weight, these two factors did not interact (Fig. 5B, D). In summary, aging—and not temperature or the interaction between temperature and age—is the primary factor driving the differences in mosquito dry weight.

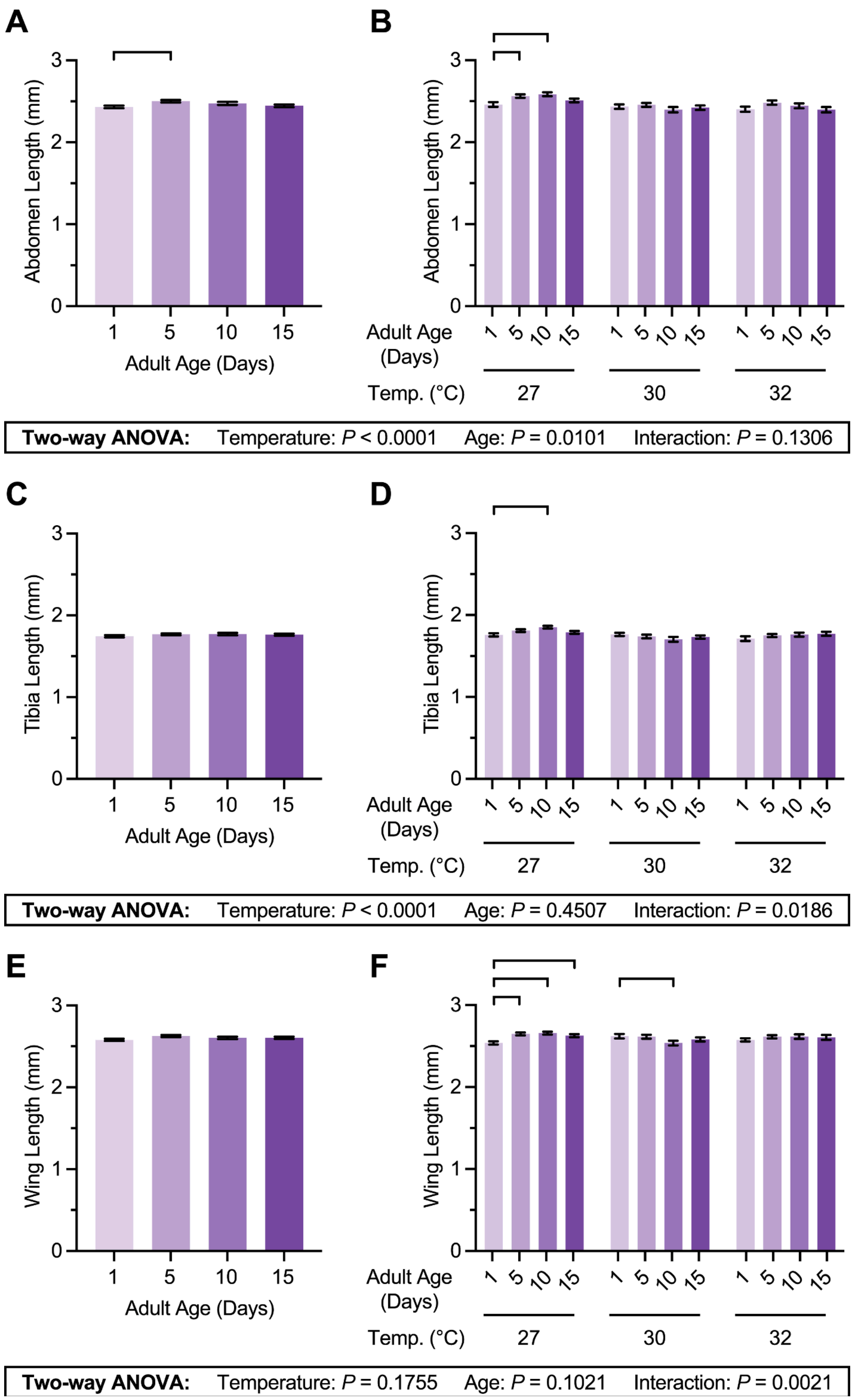

Fig. 4. During aging, the size of the abdomen changes, but the tibia and wing are unchanged. (A-B) Length of the abdomen. Abdomen length first increases and then decreases with aging. (C-D) Length of the tibia. Tibia length is unaltered by aging. (E-F) Length of the wing. Wing length is unaltered by aging. Columns mark the mean and whiskers denote the S.E.M. Data were analyzed by a two-way ANOVA followed by a Tukey's post-hoc test. Brackets denote Tukey's $P < 0.05$. Panels A, C and E aggregate the data by age irrespective of temperature. The same measurements are plotted in Figs. 3 and 4 but grouped or arranged differently.

3.5. Aging is the primary driver of changes in carbohydrate content in a mosquito

We next examined how warmer temperature, older age, and their interaction shape the carbohydrate, lipid, and protein content in mosquitoes because these are the structural building blocks of the body and the mosquito's primary sources of energy. We began assessing body condition by measuring how warming temperature shapes carbohydrate content. We found that temperature alters the carbohydrate content of adult mosquitoes, but the carbohydrate content does not track linearly with warming temperatures (Fig. 6A-B). Instead, mosquitoes reared at 30 °C have the lowest carbohydrate content, averaging 47.7 μg, which is 16% less than mosquitoes reared at 32 °C and 9% less than mosquitoes reared at 27 °C (Fig. 6A). We do not have a strong explanation for this finding, but it is possible that our observation is due to the natural variation in carbohydrate intake across mosquitoes, which results from differences in feeding frequency (adults were fed sugar *ad libitum*).

We then measured how age shapes carbohydrate content and found that carbohydrate content increases with aging (Fig. 6C). Specifically, carbohydrate content increased by 40% from 1 to 5 days of age,

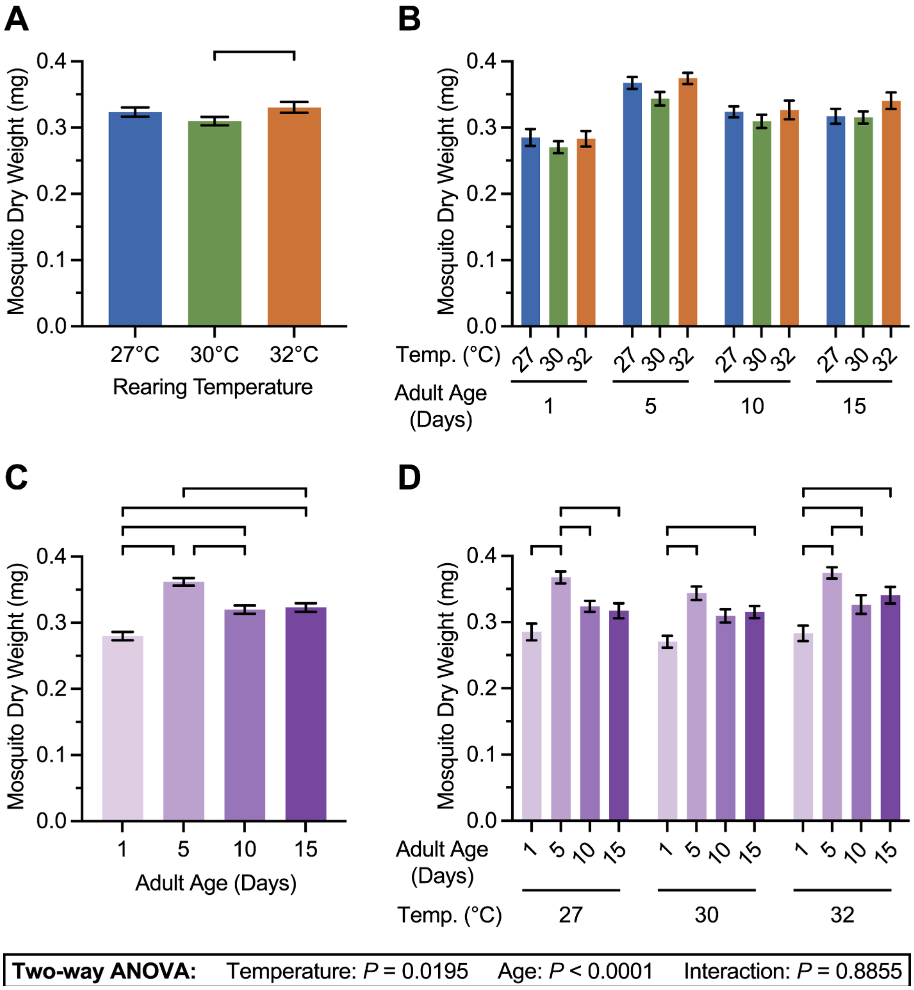

Fig. 5. Dry weight is primarily shaped by aging. (A-B) Effect of temperature on dry weight. As the temperature warms, mosquito weight is unchanged. (C-D) Effect of age on dry weight. Dry weight changes in a parabolic manner as a mosquito ages. Columns mark the mean and whiskers denote the S.E.M. Data were analyzed by a two-way ANOVA followed by a Tukey's post-hoc test. Brackets denote Tukey's $P < 0.05$. Panel A aggregates the data by temperature irrespective of age, and panel C aggregates the data by age irrespective of temperature. The measurements in panels A and B are re-grouped or re-arranged and plotted again in panels C and D.

remained constant from 5 to 10 days of age, and increased by 17% from 10 to 15 days of age. On average, mosquitoes increased their carbohydrate content from 39 µg to over 60 µg during their adult lifetime. This increase matches the sucrose-only diet we provided these adults, which allowed them to continuously feed to replenish and build carbohydrate reserves as they aged.

Finally, we investigated whether temperature alters the age-associated change in carbohydrate content. Temperature and age did not interact to shape carbohydrate content (Fig. 5B, D), and therefore, we conclude that aging—and not temperature or the interaction between temperature and age—is the primary factor driving changes in the carbohydrate content of mosquitoes.

3.6. *Aging is the primary driver of changes in lipid content in a mosquito*

In the midgut, ingested carbohydrates are converted into lipids, which are then transported and stored in the fat body or ovaries (Arrese and Soulages, 2010; Gondim et al., 2018; Vanhandel, 1984). We first examined the effects of temperature and found that temperature alters the quantity of lipids in the mosquito, but the lipid content does not track linearly with warming temperature (Fig. 7A-B). Mosquitoes reared at 30 °C had the lowest quantity of lipids, averaging 80.73 µg, which is 17.5% less than mosquitoes at 32 °C and 11.0% less than mosquitoes at 27 °C (Fig. 7A). These results match what was observed for carbohydrates, and because of the link between carbohydrate and lipid metabolism, we propose that this is due to stochastic differences in carbohydrate ingestion.

We then considered the effects of age and found that as a mosquito ages, the lipid content resembles a parabolic curve (Fig. 7C-D), which is similar to the findings on dry weight. On average, lipid content increased by 154%, from 45 µg to 114 µg, during the first 5 days of adulthood, remained constant from 5 to 10 days of age, and decreased by 30.2% from 10 to 15 days of age (Fig. 7C). We propose that the increase in lipid content during the first 5 days of adulthood is due to the restoration of energy reserves after metamorphosis and in anticipation of reproduction, and the decrease in lipid content that ensues later is due to senescence.

Finally, we investigated whether temperature and age interact to shape lipid content and found that temperature alters the age-related change in lipid content, and vice versa (Fig. 7B, D). For all temperatures, lipid content showed an aging-dependent parabolic change, but this change was more attenuated at 30 °C (Fig. 7D). At 30 °C, lipid content increased by 130% from 1 to 5 days post-eclosion, and then marginally decreased by 3.3% from 5 to 10 days post-eclosion and by 5% from 10 to 15 days post-eclosion. At 27 °C, however, lipid content increased by 189% from 1 to 5 days post-eclosion, increased further by 15% from 5 to 10 days post-eclosion, and then decreased by 35.8% from 10 to 15 days post-eclosion. At 32 °C, lipid content increased by 154% from 1 to 5 days post-eclosion, remained constant from 5 to 10 days post-eclosion, and then decreased by 46.3% from 10 to 15 days post-eclosion. Although this interaction was significant, it accounted for <5% of the variation across samples (Tables S5). Thus, we conclude that aging—and not temperature or the interaction between the temperature and age—is the primary factor driving changes in lipid content.

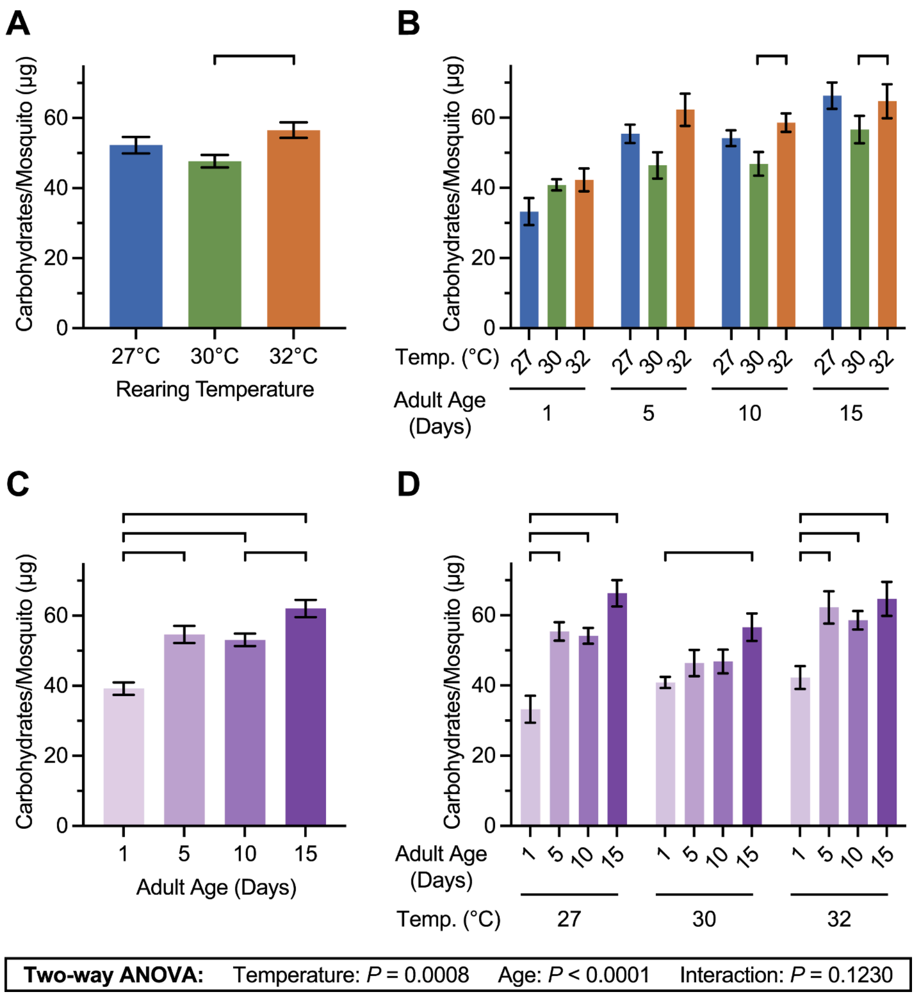

Fig. 6. Carbohydrate content is primarily shaped by aging. (A-B) Effect of temperature on carbohydrate content. Carbohydrate content did not track linearly with temperature. (C-D) Effect of age on carbohydrate content. Carbohydrate content increases with aging. Columns mark the mean and whiskers denote the S.E.M. Data were analyzed by a two-way ANOVA followed by a Tukey's post-hoc test. Brackets denote Tukey's $P < 0.05$. Panel A aggregates the data by temperature irrespective of age, and panel C aggregates the data by age irrespective of temperature. The measurements in panels A and B are re-grouped or re-arranged and plotted again in panels C and D.

3.7. Warmer temperatures accelerate the aging-associated decrease in protein content

Finally, we assessed how protein content changes with warming temperature and aging. We found that as the temperature warms, the quantity of protein decreases (Fig. 8A-B). Specifically, protein content at 27 °C averaged 31.3 µg and decreased by 12.0% and 15.7% as the temperature warmed to 30 °C and 32 °C, respectively (Fig. 8A). This decrease in protein content is likely due to an accelerated metabolic rate at warmer temperatures while under a diet that lacks proteins. Another possible minor contributor is that mosquitoes at warmer temperatures are slightly smaller.

We then considered the effects of age and found that protein content decreases with aging (Fig. 8C-D), with each 5-day increase in age resulting in a significant decrease in protein content (Fig. 8C). On average, protein content decreased by 32.5% within the first 5 days post-eclosion, by 16.1% from 5 to 10 days post-eclosion, and by 11.4% from 10 to 15 days post-eclosion. This is a 50% decrease in the total protein content, from 41 µg to 20.5 µg, as the mosquito ages. Therefore, protein stores are consumed as mosquitoes senesce, and presumably continue to decline unless replenished by a blood meal.

Finally, we investigated whether temperature and age interact to shape protein content and found that temperature accelerates the age-associated decline in protein content (Fig. 8B, D), with younger mosquitoes at a warmer temperature exhibiting features of older mosquitoes at a cooler temperature. For example, protein content in 10-day-old mosquitoes at 27 °C was similar to that of 5-day-old mosquitoes at 30 °C or 32 °C (Fig. 8B). This suggests that physiological aging is accelerated in warmer temperatures, resulting in a faster depletion of protein stores as the temperature warms.

4. Discussion

Although many independent factors, biotic or abiotic, shape the life history of an insect, so too do the interactions between these factors. Here, we investigated how temperature, age, and their interaction shape the size and body composition of the mosquito, *A. gambiae*. We discovered that both temperature and age, individually and in some cases interactively, affect the length and weight of a mosquito, as well as their carbohydrate, lipid, and protein content.

A. gambiae larvae, pupae, and adults are smaller and lighter when reared at a warmer temperature (Agyekum et al., 2022a, 2022b; Agyekum et al., 2021). This decrease in size correlates with the warming-based acceleration of larval development, which offers less time for feeding, growing, and the storing of nutrients prior to pupation and eclosion (Aytekin et al., 2009; Bayoh and Lindsay, 2003; Kirby and Lindsay, 2009; Lang, 1963; Lyons et al., 2013). For example, *A. gambiae* adults that were reared at 34 °C have a smaller proboscis and wings than those reared at 25 °C (Agyekum et al., 2022b). Additionally, the wings of *Aedes aegypti* reared at 35 °C are smaller than those of mosquitoes reared at 24 °C (Mohammed and Chadee, 2011). Here, we investigated how warming temperature shapes the size of the adult mosquito. Wing length is commonly used as the sole parameter to approximate mosquito body size (Ameneshewa and Service, 1996; Huxley et al., 2021; Mohammed and Chadee, 2011). However, because an earlier study found that a treatment may not equally shape the size of all body features (Brown

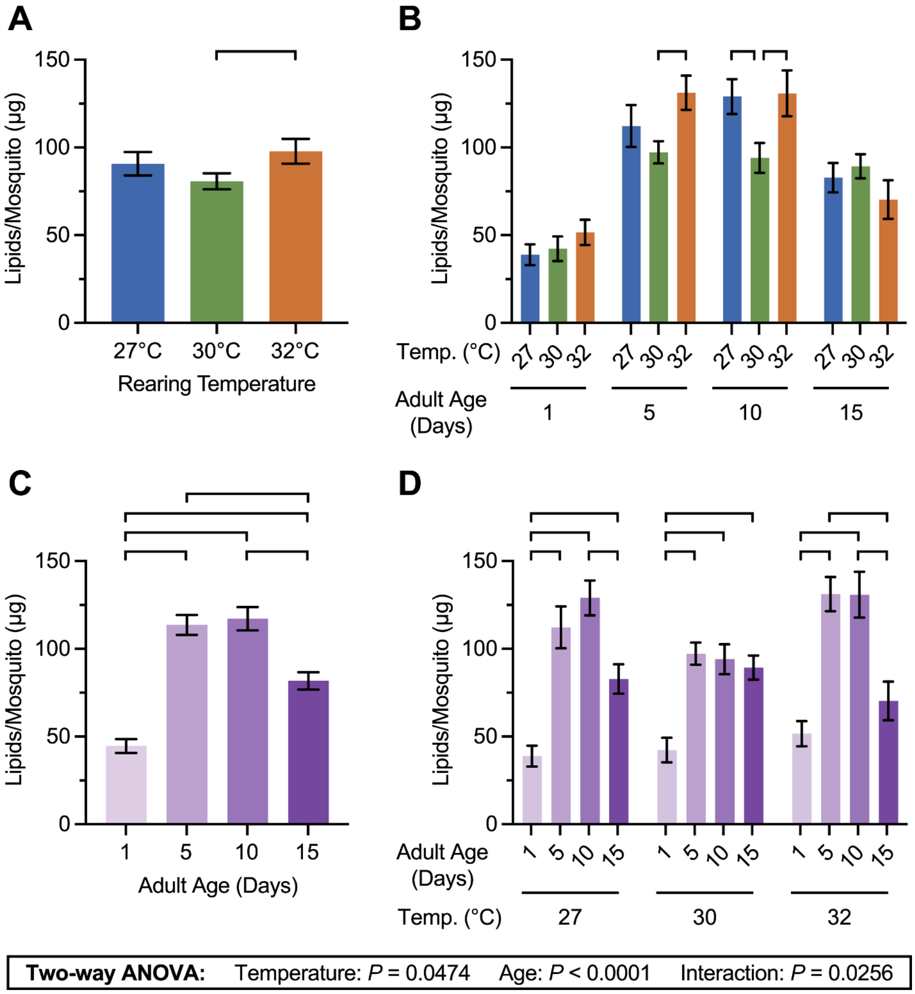

Fig. 7. Lipid content is primarily shaped by aging. (A-B) Effect of temperature on lipid content. Lipid content did not track linearly with temperature. (C-D) Effect of age on lipid content. Lipid content changes in a parabolic manner as a mosquito ages. Columns mark the mean and whiskers denote the S.E.M. Data were analyzed by a two-way ANOVA followed by a Tukey's post-hoc test. Brackets denote Tukey's $P < 0.05$. Panel A aggregates the data by temperature irrespective of age, and panel C aggregates the data by age irrespective of temperature. The measurements in panels A and B are re-grouped or re-arranged and plotted again in panels C and D.

et al., 2018), we took a multi-pronged approach that measured the length of three features: the abdomen, hind tibia, and wing. We found that the abdomen and tibia are slightly smaller when the developmental temperature is increased from 27 °C to 32 °C. Unexpectedly, wing length remained unchanged as the temperature warmed, which contradicts what had been reported previously (Agyekum et al., 2022b; Huxley et al., 2021; Mohammed and Chadee, 2011). There are several plausible explanations to this apparent contradiction on wing size. Mainly, previous reports on changes in wing size examined a wider range of temperatures (Agyekum et al., 2022b; Huxley et al., 2021; Mohammed and Chadee, 2011), so our temperature range may have been too narrow to identify a meaningful change. Regardless, the data on the length of the abdomen and tibia confirm that as the developmental temperature warms, the resultant adults are smaller. Moreover, our findings further establish that studies assessing mosquito size should take a multi-dimensional approach instead of relying on the size of a single feature.

Adult mosquitoes are encased in a hard, largely inflexible exoskeleton made of chitin and protein that, once sclerotized following eclosion, cannot grow (Chapman et al., 2013; Klowden, 2013). Therefore, although body condition deteriorates as the insect senesces, we predicted that aging would not affect body size. Instead, we found that the length of the abdomen changes with aging, while the hind tibia and wing are unaffected. This change of abdominal size was unexpected, but in retrospect, makes sense. The abdomen, although constrained by sclerotization, expands to accommodate large blood or sugar meals. This is easily seen when the width of the abdomen dramatically increases following feeding, which is mechanically accomplished by the unfolding of the pleuron (Chapman et al., 2013; Jones, 1978). Here, we infer that the age-associated increase in abdominal length during the first five days of adulthood results from a combination of pleuron unfolding and the pulling of the articular membranes that link the tergites and the sternites. From a physiological perspective, we hypothesize that this change is likely a consequence of tissue remodeling and nutrient reserve restoration after metamorphosis, including the expansion of fat body attached to the integument (Arrese and Soulages, 2010). Then, the abdomen shortens during the subsequent days because of senescence.

As another method for assessing mosquito size, we measured dry weight. Dry weight is non-linear and removes any influence of water that was ingested prior to sample collection. We predicted that dry weight would correlate with our linear measurements, such that smaller-bodied mosquitoes—those reared at warmer temperatures—would be the lightest. However, we found that temperature only had a minor influence on dry weight. Instead, age was the primary factor driving changes in dry weight. These age-associated changes resemble a parabola that matched the age-related changes in abdominal length: increase over the first few days of adulthood followed by a decrease. Similar to what we infer for abdominal length, these age-associated changes may be due to tissue remodeling and nutrient reserve restoration after metamorphosis, followed by the expenditure of energy stores (Briegel, 1990; Huestis et al., 2012; Rivera-Perez et al., 2017).

Size and weight measurements only detected small changes, and they do not capture the changes in body composition that occur as an insect deteriorates at different temperatures or over time. So, we investigated how carbohydrate, lipid, and protein content changes with temperature and aging as a measure of body condition. We hypothesized that carbohydrate, lipid, and protein content would (i) be lower at warmer

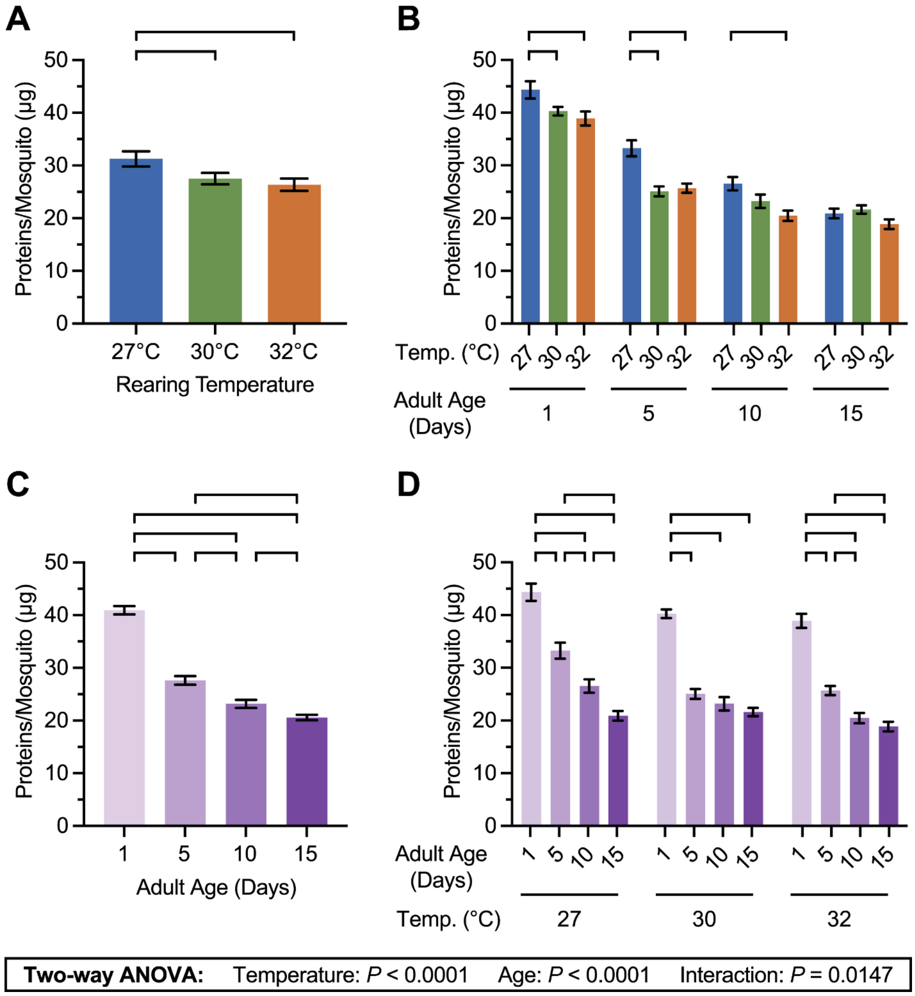

Fig. 8. The age-associated decline in protein content accelerates in warmer temperatures. (A-B) Effect of temperature on protein content. As the temperature warms, protein content decreases. (C-D) Effect of age on protein content. Protein content decreases with aging. Altogether, warmer temperature accelerates the age-dependent decline in protein content. Columns mark the mean and whiskers denote the S.E.M. Data were analyzed by a two-way ANOVA followed by a Tukey's post-hoc test. Brackets denote Tukey's $P < 0.05$. Panel A aggregates the data by temperature irrespective of age, and panel C aggregates the data by age irrespective of temperature. The measurements in panels A and B are re-grouped or re-arranged and plotted again in panels C and D.

temperatures due to an accelerated metabolic rate (González-Tokman et al., 2020; Huestis et al., 2012), (ii) decrease with aging due to deterioration of the body condition (Boggs, 2009; Monaghan et al., 2008), and (iii) decrease faster in warmer temperatures due to an accelerated rate of senescence. We found that age, and not temperature or their interaction, played a primary role in shaping the carbohydrate and lipid content of an adult mosquito. Specifically, at 1-day post-eclosion, carbohydrate and lipid content were low, which we postulate is because of the energetic cost of metamorphosis (Arrese and Soulages, 2010; Vanhandel, 1984). During the next 5 days, carbohydrate and lipid contents increase, which is a consequence of replenishing energy stores by feeding on sucrose *ad libitum*. Carbohydrate and lipid content then remain constant over the next 5 days, perhaps because of the combination of carbohydrate consumption, carbohydrate usage, carbohydrate conversion to lipids, lipid storage, and lipid usage (Briegel, 1990; Gondim et al., 2018). However, after 10 days of age, carbohydrate content continues to increase while lipid content decreases. We postulate that this shift is a consequence of senescence. Overall, these findings highlight the interdependency of carbohydrate and lipid metabolism and how it changes with aging.

The typical diet of adult female mosquitoes consists of both blood and sugar. Blood is high in both proteins and lipids, and in *A. gambiae* this food source is required for oocyte maturation and egg development (Gondim et al., 2018). However, in our study, mosquitoes were maintained on a carbohydrate-only diet, and carbohydrates are not directly used for protein synthesis. We found that protein content decreases as the temperature warms from 27 °C to 32 °C. At these warmer temperatures, mosquitoes are also smaller. Therefore, the decrease in protein content could be in part due to a decrease in body size, although the magnitude of the decrease in protein content was considerably larger than the magnitude of the decrease in size. A more likely explanation is that the decrease in protein content is a consequence of an increase in the metabolic rate. The metabolic rate accelerates when the temperature is warmer (González-Tokman et al., 2020; Huestis et al., 2012), and we hypothesize that this increases the rate in which proteins and amino acids within the mosquito are broken down.

Protein content also dramatically decreases with aging. Proteins are not part of our experimental mosquitoes' diet, and therefore, all proteins and amino acids in our mosquitoes were carried over from the larval life stage. We hypothesize that the decrease in protein content that we observed with age is a consequence of senescence and the lack of available new proteins in their diet.

The findings in this study show the intragenerational effects of warmer temperature on size and body composition. That is, this study examined mosquitoes that were reared and maintained at different temperatures, but whose eggs were all laid by mosquitoes maintained at 27 °C. Hence, the inference is that parental provisioning to the eggs is similar for all mosquitoes, and that the changes observed are due to the temperature experienced within a generation. An intriguing question for future investigation is whether these intragenerational changes are similar or different to what occurs when the temperature has also been manipulated for the earlier generations.

Both temperature and age affect mosquito life history, but whether these two factors interact to influence size and body condition remained unknown. We detected a statistically significant interaction between temperature and age for shaping the length of both the tibia and the wing, but these interactions are not biologically meaningful; the magnitude of the changes are very small. We also detected a statistically significant interaction between temperature and age for shaping lipid content, but a small difference was only detected between all ages at a single temperature. Where a statistically significant and meaningful interaction was detected was in the age-dependent decline in protein content, which significantly accelerated as the temperature warmed. Altogether, this study uncovered that temperature and age, individually and to a lesser extent interactively, shape the size and body composition of the adult mosquito. Furthermore, this study demonstrates the importance of measuring multiple factors when assessing the effects of a treatment on the size and body condition of an insect.

CRediT authorship contribution statement

Jordyn S. Barr: Conceptualization, Methodology, Investigation, Formal analysis, Writing – original draft, Writing – review & editing, Visualization. **Tania Y. Estevez-Lao:** Methodology, Investigation, Formal analysis. **Marina Khalif:** Investigation, Formal analysis, Writing – review & editing. **Saksham Saksena:** Investigation, Formal analysis, Writing – review & editing. **Sagnik Yarlagadda:** Investigation. **Ommay Farah:** Investigation. **Yasmine Shivere:** Investigation. **Julián F. Hillyer:** Conceptualization, Methodology, Formal analysis, Writing – original draft, Writing – review & editing, Visualization, Supervision, Funding acquisition.

Declaration of Competing Interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Julian F. Hillyer reports financial support was provided by National Science Foundation. Julian Hillyer is in the Editorial Board of Journal of Insect Physiology.

Data availability

All data are available in Appendix B: Data.

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Data availability

All data are available in Appendix B: Data.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jinsphys.2023.104525.

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