Differential Effects of Larval and Adult Nutrition on Survival, Fecundity, and Size of the Yellow Fever Mosquito Aedes Aegypti

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Abstract

Background: The yellow fever mosquito, *Aedes aegypti*, is the principal vector of multiple infectious pathogens that can cause severe illness such as dengue fever, yellow fever and Zika. Their transmission potential for these arboviruses is largely shaped by their life history traits, such as their survival and fecundity. These life history traits depend on environmental conditions, such as larval and adult nutrition (e.g., nectar availability). Both these types of nutrition are known to affect the energetic reserves and life history traits of adults, but whether and how nutrition obtained during different stages have an interactive influence on mosquito life history traits remains largely unknown.

Results: Here, we experimentally manipulated both larval and adult diets to create four nutritional levels, that is, a high amount of larval food plus poor (weak concentration of sucrose) adult food: HL+PA, high larval plus good (normal sucrose concentration) adult food: HL+GA, low larval plus poor adult food: LL+PA and low larval plus good adult food: LL+GA. We then compared the size, survival and fecundity of mosquitoes reared from these nutritional regimes. We found that larval and adult nutrition affected mosquito size and survival, respectively, without interactions, while both larval and adult nutrition synergistically influenced mosquito fecundity. There was a positive relationship between mosquito size and fecundity. In addition, this positive relationship was not affected by nutrition.

Conclusions: These findings highlight how larval and adult nutrition differentially influence mosquito life history traits, suggesting that studies evaluating nutritional effects on vectorial capacity traits should account for environmental variation across life stages.

Background

The yellow fever mosquito (Diptera: Culicidae), *Aedes aegypti* (Linnaeus, 1762), is the principal vector of several arboviruses such as dengue, yellow fever, chikungunya and Zika, which continue to impose a heavy burden on public health globally [1–5]. Dengue virus (DENV), for example, is estimated to cause 390 million cases of human infection each year, 96 million of which have clinical manifestations[6]. These arboviruses have been re-emerging in many regions and expanding their ranges across the globe, partly due to urbanization and subsequent expansion of the distribution of *Ae. aegypti* [7]. Given their medical importance, the vectorial capacity of mosquitoes has been studied for decades [8, 9]. Yet understanding the variation in transmission potential between areas requires in-depth knowledge of vector traits that potentially influence vectorial capacity in a single locality. Mosquito life history traits, such as body size, survival and fecundity, are important correlates of vectorial capacity. For example, Alto et al [10] found that smaller-sized *Ae. aegypti* females were more susceptible to DENV infection and more likely to disseminate it than their larger counterparts. Longevity is a key competent for vectorial capacity as vectors must survive long enough to allow pathogens to replicate to a high level before the virus can be disseminated in subsequent bites [11]. Despite the importance of life history traits, however, relatively few studies have examined how these traits can be influenced by the joint environment experienced by mosquitoes across their developmental stages.
As an organism with a complex life cycle, mosquitoes experience highly distinct habitats from larval to adult stages and environmental factors may play a critical role in their fitness and performance. The environment experienced by larvae may affect adult phenotypes through so called “carry-over effects” [12, 13]. For example, larval competition, food quantity and temperature have been reported to affect adult survival, size, longevity and vector competence [14–18]. At the same time, the environment experienced by adults, such as food quality/availability, or air temperature and humidity, can also directly affect their life histories and vector competence [19, 20]. Nonetheless, how environmental factors in both larval and adult stages may interactively affect mosquito life histories remains largely unknown (but see [21] for the influence of both larval and adult nutrition on mosquito behavior).

Nutrition is one of the environmental factors that affects all mosquito life history traits as it fuels mosquito development, growth and performance. During the larval stage, microorganisms and particulate organic detritus are major nutritional resources and their abundance is readily affected by environmental changes, such as rainfall and predators of larvae [22]. As a container breeder, larval populations of *Ae. aegypti* can be regulated by nutritional stress derived from food limitation in the aquatic habitat [23]. After emergence, adult *Ae. aegypti* start foraging for food from terrestrial habitats nearby. Most mosquito species need to feed on both plant sugar and vertebrate blood as nutritional resources for energy supply and egg production. Previous studies suggested that female *Ae. aegypti* rarely feed on sugar [24] and that feeding on human blood alone may provide them with a fitness advantage [25, 26]. However, sugar-feeding by female *Ae. aegypti* may not be as unusual as thought previously, as support for frequent sugar-feeding in certain environments has been reported [27, 28], and this propensity has been used to design attractive toxic sugar baits for *Ae. aegypti* control [29, 30]. Like larvae, adult mosquitoes may also be influenced by nutritional stress derived from changes in food quality (e.g., sugar concentration[31]). Both larval and adult nutritional stress has been shown to associate with adult survival, reproduction, and growth [32–34]. However, little is known about whether larval nutritional stress will interact with adult nutritional stress to influence adult life history traits.

Here we experimentally examined the potential interactive effects of larval (quantity) and adult (sucrose concentration) nutrition on survival and fecundity of adult female *Ae. aegypti*. To do that, we set up cohorts with two amounts of food during larval stages and two concentrations of sucrose solution during the adult stage and compared mosquito life history traits between different treatments.

**Methods**

**Mosquito rearing and treatments**

All mosquitoes were cultured using the F19 generation of an *Ae. aegypti* colony established from eggs collected in Key West, FL. Eggs were hatched overnight in an enamel pan (35 × 25 × 6 cm) filled with 500 mL of deionized (DI) water and 2 g of brain heart infusion (Difco Laboratories, Detroit, USA). To minimize the potential effect of variation in larval density on mosquito fitness and performance [35], first-instar larvae were randomly counted and 100 of them were placed in each enamel pan filled with 500 mL
of DI water. The larvae were reared under two nutritional regimes, following Telang et al.\cite{36}: a well-nourished treatment where 100 mg of rabbit chow: lactalbumin: yeast (1:1:1) diet (Sigma-Aldrich, St. Louis, USA) was provided on days 2, 4, 5 and 6 post hatching, representing high larval nutrition (hereafter HL); while a malnourished treatment where 100 mg of the same diet was provided only on days 2 and 6 post hatching, representing low larval nutrition (hereafter LL). Eclosed adults from each larval nutrition treatment were maintained in paperboard cages (20.5 cm height × 18.5 cm diameter) and randomly assigned to one of two nutritional regimes with different food quality: a well-nourished treatment with *ad libitum* access to 10% sucrose solution, representing good adult nutrition (hereafter GA); or a malnourished treatment with *ad libitum* access to 1% sucrose solution, representing poor adult nutrition (hereafter PA). Hence, there are four nutritional treatments in total (Fig. 1; Additional file 1). After keeping males and females together for 3–5 days to allow for mating, mosquitoes were cold-anesthetized at 4 °C and sexed on chilled Petri dishes using a stereomicroscope (Stemi DV4, Carl Zeiss AG, Jena, Germany). Females were retained in smaller paperboard cages (12 cm height × 11 cm diameter) with *ad libitum* access to the same adult nutritional treatments as above. Larvae and adult mosquitoes were kept in incubators (I-36VL, Geneva Scientific LLC, Fontana, USA) at 27 (± 1) °C and 75 (± 5)% relative humidity (RH) under a 12:12 h Light (L): Dark (D) photoperiod throughout the experiments.

**Bioassays and life history traits**

Six-to-eight day old female mosquitoes were provided with access to bovine blood (Hemostat Laboratories, Dixon, USA) for 45 min via Hemotek Membrane Feeding Systems (PS6, Hemotek Ltd, Blackburn, UK). Prior to the blood-feeding assay, these mosquitoes had been starved for 24 h by depriving them of sucrose solutions. From 24 – 12 h prior to the blood-feeding assay a cotton roll soaked with DI water was provided to mosquitoes. Engorged mosquitoes were separated from unfed mosquitoes on chilled petri dishes after cold-anesthesia at 4 °C for 10 min. Fifty engorged mosquitoes were randomly selected from each of the 4 treatments and placed individually in a small paperboard cage (5.5 cm height × 9 cm diameter) for life history assays. In each cage, a germination paper was placed surrounding the inner wall and kept wet daily from day 2 to 7 post blood-feeding to allow for egg laying. All caged individuals were provided with *ad libitum* access to either 1% or 10% sucrose solution daily until death (see Fig. 1). Mortality of mosquitoes was checked daily. Here, the survival of a mosquito was recorded as the number of days from blood-feeding to death (hereafter post blood-feeding longevity). Immediately after the death of a mosquito, all the eggs inside a cage (including germination paper and all inner surface of the cage) were counted using the stereomicroscope. The fecundity of a mosquito was recorded as the total eggs counted in a cage. Dead mosquitoes were removed and stored at -80 °C for wing length measurement, which was used as a proxy of body size. Wing length was measured as the distance from the axial incision to the apical margin excluding the fringe of scales \cite{33}. The measurement of wing length was conducted using an inverted microscope (IX51, Olympus, Japan) and Olympus cellSens Entry 2.3 software.

**Statistical analyses**
Pairwise t-tests with Bonferroni correction were conducted to detect whether there were significant differences in post blood-feeding longevity, body size and fecundity between any two of the nutritional treatments. The normality of these three variables was examined in normal quantile plots. Generalized linear models (GLMs) were also fitted to examine whether there was an interactive effect of larval and adult treatments on post blood-feeding longevity and fecundity, respectively. To further assess the effects of larval and adult nutritional stress on daily survival of mosquitoes, a survival analysis was performed using the packages survival [37] and survminer [38] with Kaplan-Meier Method and Log-Rank Test. We also explored if there is any trade-off between life history traits by performing linear regression between each pair of traits. To test whether there was a significant difference in the slopes of linear regressions, analysis of covariance (ANCOVA) was performed between treatments using the packages car [39]. Statistical analyses were carried out in R software v. 3.6.3 [40].

Results

The mean wing length of mosquitoes from treatment of low larval plus poor adult nutrition (LL + PA), high larval plus poor adult nutrition (HL + PA), low larval plus good adult nutrition (LL + GA) and high larval plus good adult nutrition (HL + GA) was 2.48 ± 0.02 SE mm, 2.78 ± 0.01 SE mm, 2.50 ± 0.02 SE mm and 2.75 ± 0.01 SE mm, respectively (Fig. 2). There was a significant difference between low and high larval treatments regardless of adult treatments (pairwise t test, each P < 0.001, Fig. 2), indicating that larval food quantity significantly affected adult size. As wing length is fixed in adults, no difference was found between adult treatments when larval treatments were the same (pairwise t test, each P > 0.05, Fig. 2).

The mean fecundity of mosquitoes from treatment of LL + PA, HL + PA, LL + GA and HL + GA was 35.80 ± 4.06 SE, 74.59 ± 3.56 SE, 50.02 ± 2.90 SE and 87.82 ± 3.30 SE, respectively (Fig. 3). There was a significant difference between any two of the four treatments (pairwise t test, each P < 0.05, Fig. 3), indicating that both larval and adult treatment, as well as food quantity and quality can affect mosquito egg-laying. In addition, egg number positively increased with nutrition combination from the worst (LL + PA) to the best (HL + GA) combination, indicating that larval and adult food may synergistically influence mosquito fecundity. However, no effect of the interaction between larval and adult treatments on fecundity was detected (estimate ± SE = 0.99 ± 6.97, t = 0.14, p = 0.89).

The mean post blood-feeding longevity of mosquitoes from LL + PA, HL + PA, LL + GA, and HL + GA was 20.58 ± 2.62 SE d, 20.57 ± 2.25 SE d, 35.00 ± 2.02 SE d and 31.63 ± 2.24 SE d, respectively (Fig. 4). There was a significant difference between poor and good adult treatments regardless of larval treatments (pairwise t test, each P < 0.001, Fig. 4), indicating that adult food quality significantly affected adult longevity. No difference was found between larval treatments when adult treatments were the same (pairwise t test, each P > 0.05, Fig. 4), indicating that larval food quantity did not affect adult survival. Survival curves also showed that there is significant difference between HL + GA and HL + PA (z = 2.39, p = 0.017; Fig. 5a) and between LL + GA and LL + PA (z = 2.24, p = 0.025; Fig. 5a). Cox Proportional Hazards Model including all four treatments and wing length as covariates indicated that poor adult nutrition increased the death risk of mosquitoes, and smaller-size mosquitoes tended to have a reduced death risk.
The effect of the interaction between larval and adult treatments on survival was not significant (estimate ± SE = 3.36 ± 4.59, t = 0.73, p = 0.47).

Linear regression showed that there was a positive correlation between wing length and fecundity (t = 10.98, p < 0.001, R² = 0.38). ANCOVA further showed that the positive relationship between wing length and fecundity did not change across different treatments (slope = 69.81, adjusted R² = 0.46; Fig. 6). In addition, there was a significant effect of the treatment on the fecundity after controlling for the effect of the wing length (F₄,₁₉₅ = 43.22, p < 0.001; Fig. 6). There was also a significantly positive relationship between mosquito fecundity and survival (t = 2.34, p = 0.02, R² = 0.03). No significant relationship was found between wing length and survival.

**Discussion**

In this study we tested the effects of larval and adult nutrition on several key life history traits of adult female *Ae. aegypti* mosquito. We found that while adult size is influenced by larval nutrition, and survival is influenced only by adult nutrition, fecundity is influenced by both larval and adult nutrition.

We found that larval nutrition affects mosquito wing length, a proxy for body size, consistent with previous studies [36, 41, 42]. Mosquito body size has been suggested to be an important life history trait of mosquitoes because of its close connection to or correlation with other traits that influence fitness and susceptibility to infection and dissemination [10]. Here, however, the effect of body size was only noticeable on fecundity.

Mosquitoes feeding on 10% sucrose solution showed significantly higher survival than their counterparts feeding on 1% sucrose solution, regardless of the level of larval nutrition. Similarly, Briegel et al [43] found that higher sucrose concentrations (0.5%-50%) extend the survival time of *Ae. aegypti*, probably because higher concentrations allow for greater increases in energy reserves. Larval nutrition, in our case, had no influence on adult survivorship. A negative effect of increased larval nutrition on adult *Ae. aegypti* longevity have been reported by previous studies [34, 42]. However, larval competition for nutrition can also reduce adult *Ae. aegypti* longevity under certain conditions (e.g., under stress related to low humidity) [16]. Opposite effects of larval nutrition on adult longevity have thus been reported, and the discrepancy could be caused by the methodological differences among studies, such as larval food quantity and quality as well as larval density. It is also possible that effects of larval nutrition on longevity are only expressed when mosquitoes are placed in stressful conditions, though the current study suggests that low adult nutrition at least does not induce that outcome.

We found that both larval and adult nutrition significantly affected mosquito fecundity. In particular, mosquitoes generated from good larval food and 10% percent sugar solution laid more eggs than their counterparts, respectively. This is in accordance with Vantaux et al [42] who found adult *Ae. aegypti* reared with low larval food produce significantly fewer eggs than their counterparts. While a number of studies focused on identifying the effects of blood meal quantity and source on mosquito fecundity (e.g.
relatively few have examined the effects of carbohydrates on egg production. Energy reserves can be a more decisive factor for fecundity than protein, for example, Mostowy and Foster [44] found that egg number of *Ae. aegypti* does not correspond to blood meal size but instead closely associated with the level of energetic reserves at the time of blood-feeding. Recent sugar meals, resulting in a full crop, however, can reduce blood meal intake and fecundity. In our study, where mosquitoes were starved for one day before blood feeding, crops would have been empty, and the effect on fecundity did not appear to depend on the nutritional reserves obtained during the larval stages, suggesting rather an additive effect of reserves on fecundity.

We did not detect any trade-offs between mosquito survival, size and fecundity, even within those treatments with limited access to both larval and adult nutrition. Trades-offs between life history traits of organisms have often been observed as a result of limited resource that is needed for growth, development and performance. In this context, organisms have to make a choice for allocating limited resource for growth, development or performance. Here, we found a positive relationship between wing length and fecundity, indicating that larger-size mosquitoes could lay more eggs, and egg numbers also depended on both larval and adult nutrition even after controlling for mosquito size. we also found a positive relationship between fecundity and survival, indicating that long-lived mosquitoes could also lay more eggs. Future work could explore whether other traits (e.g., related to immunity) do provide evidence of a trade-off in relation to mosquito nutrition.

**Conclusions**

In conclusion, mosquito larval and adult nutrition may have differential effects on mosquito life history traits. While larval food quantity and adult food quality influence mosquito size and survival respectively, both quantity and quality jointly affect mosquito fecundity. This has potentially important ramifications for our understanding of population dynamics of mosquitoes, in that the adult environment should be considered when tracking larval nutrition or competition and fecundity feedbacks.

**Declarations**

**Ethics approval and consent to participate**

Not applicable.

**Consent for publication**

Not applicable.

**Availability of data and materials**
All data generated or analysed during this study are included in this published article and its supplementary information files.

**Competing interests**

The authors declare that they have no competing interests.

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**Authors’ contributions**

JY and CMS conceived and designed the study. JY and RK carried out the experiments. JY and CMS performed the statistical analyses. JY drafted the first manuscript and all authors contributed to interpretation of the data, read and approved the final manuscript.

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**Figures**

**Figure 1**

The schematic diagram of experimental design. High or low larval nutrition represents an access to larval food on days 2, 4, 5, and 6 post hatching or on days 2 and 6 post hatching; Good or poor adult nutrition
represents an ad libitum access to 10% or 1% sucrose solution daily; Females were allowed to mate and take a blood meal before the start of the survival assay.

**Figure 2**

Wing length of Aedes aegypti from four treatments. HL+GA: high larval plus good adult nutrition (N=49), HL+PA: high larval plus poor adult nutrition (N=51), LL+GA: low larval plus good adult nutrition (N=50) and LL+PA: low larval and poor adult nutrition (N=50); The line within each box indicates the median and the edges of each box the first (Q1) and third (Q3) quartiles; the whiskers extend over 1.5 times the interquartile range; different letters indicate statistically significant treatments (each P<0.001).
Figure 3

Fecundity of Aedes aegypti from four treatments. HL+GA: high larval plus good adult nutrition (N=49), HL+PA: high larval plus poor adult nutrition (N=51), LL+GA: low larval plus good adult nutrition (N=50) and LL+PA: low larval and poor adult nutrition (N=50). The line within each box indicates the median and the edges of each box the first (Q1) and third (Q3) quartiles; the whiskers extend over 1.5 times the interquartile range; different letters indicate statistically significant treatments (each P<0.05).
Figure 4

Survival of Aedes aegypti from four treatments. HL+GA: high larval plus good adult nutrition (N=49), HL+PA: high larval plus poor adult nutrition (N=51), LL+GA: low larval plus good adult nutrition (N=50) and LL+PA: low larval and poor adult nutrition (N=50); The line within each box indicates the median and the edges of each box the first (Q1) and third (Q3) quartiles; The whiskers extend over 1.5 times the interquartile range; Different letters indicate statistically significant treatments (each P<0.001).
Figure 5

Effects of larval and adult nutrition on mosquito survival. (a) Survival curves for mosquitoes from four treatments; (b) Hazard ratios for mosquitoes from four treatments and different body size (wing length).
Figure 6

Relationship between mosquito wing length and fecundity. HL+GA: high larval plus good adult nutrition (N=49), HL+PA: high larval plus poor adult nutrition (N=51), LL+GA: low larval plus good adult nutrition (N=50) and LL+PA: low larval and poor adult nutrition (N=50).

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