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How much is needed to survive? Minimal nutritional levels for complete development of *Aedes aegypti* (Diptera: Culicidae)

Gilberto Dinis Cozzer[1,*], Thiago Sendeski Lara[1], Jacir Dal Magro[1], Daniel Albeny-Simões[2] and Renan de Souza Rezende[1]

[1] Communitarian University of Chapecó Region (Unochapecó) - Chapecó, Santa Catarina, Brazil.
[2] BioVectors Vector Control Solutions – Chapecó, Santa Catarina, Brazil.

[*] Corresponding author: pinocozzer.ps@unochapeco.edu.br

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ABSTRACT

How much do you need to survive? Minimal nutritional levels to complete the development on *Aedes aegypti* (Diptera: Culicidae)

Aedes aegypti mosquito larvae develop in various aquatic microhabitats, including water-holding tires, cups and bottles. These environments may vary in nutritional characteristics, an important factor for the development of larvae and resulting adult mosquitoes. Compromised larval nutrition can result in developmental failure or affect the growth and reproductive capacity of adults. Understanding these nutritional necessities can help optimize the laboratory rearing of mosquitoes. We tested the effects of sixteen (0, 0.0013, 0.0033, 0.0066, 0.0133, 0.02, 0.025, 0.03, 0.035, 0.04, 0.045, 0.05, 0.1, 0.15, 0.2, 2 g/L) different food concentration treatments of Spirulina Alcon® larval diet on larval survival and life history characteristics of *Ae. aegypti*. The experiment was conducted under controlled conditions, with a temperature of 27 ± 2ºC, relative humidity of 70–80 % and a photoperiod of 12:12 h. A total of 623 (43.26 %) of the initial 1440 *Ae. aegypti* individuals died during the experiment. Survival curves differed significantly among food concentration treatments (Chi-Square Test = 1271, df = 15, $p < 0.001$). The concentrations of 0.025 (60/66.66 %) and 0.03 g/L (67/74.45 %) had the lowest survival rates and 0.15 g/L (76/84.45 %) the highest. The concentrations of 0.025 and 0.03 g/L had the shortest larval development times (8.80; 8.86 days) and longevity (9.95; 8.70 days), but adult sizes were smallest for 0.025 (3.00 mm) and largest for 0.03 (3.15 mm). The concentration of 0.15 g/L had the longest larval development time (9.59 days) and longevity (12.41 days), with intermediate adult size (3.09 mm). Laboratory survival rates for *Ae. aegypti* are generally associated with high mortality on low-quality and low-quantity of resources. Nutritional stress was found to impair larval development, as well as adult size and longevity. Analyzing responses to different feeding regimes is important for understanding the main mechanisms involved in larval development and the requirements for optimizing mosquito rearing systems.

Key words: food limitation, larval development, mosquito development, nutrition, specific dietary regime

RESUMO

Quanto é necessário para sobreviver? Níveis nutricionais mínimos para o desenvolvimento completo do* Aedes aegypti *(Diptera: Culicidae)

Larvas do mosquito Aedes aegypti *se desenvolvem em vários microhabitats aquáticos, incluindo pneus, copos e garrafas que contêm água. Esses ambientes podem variar em termos de características nutricionais, fator importante para o desenvolvimento das larvas e dos mosquitos adultos resultantes. A nutrição comprometida das larvas pode resultar em falha no desenvolvimento ou afetar o crescimento e a capacidade reprodutiva dos adultos. A compreensão dessas necessidades nutricionais pode ajudar a otimizar a criação de mosquitos em laboratório. Testamos os efeitos de dezesseis (0, 0.0013, 0.0033, 0.0066, 0.0133, 0.02, 0.025, 0.03, 0.035, 0.04, 0.045, 0.05, 0.1, 0.15, 0.2, 2 g/L) tratamentos com diferentes concentrações de Spirulina Alcon® na dieta larval sobre a sobrevivência das larvas e as características da história de vida do* Ae. aegypti*. O experimento foi realizado em condições controladas, com temperatura de 27 ± 2ºC, umidade relativa de 70-80 % e fotoperíodo de 12:12 h. Um total de 623 (43,26 %) dos 1440 indivíduos iniciais de* Ae. aegypti *morreram durante o experimento. As curvas de sobrevi-*

vência diferiram significativamente entre os tratamentos de concentração de alimentos (Teste Qui-Quadrado = 1271, df = 15, p < *0.001). As concentrações de 0.025 (60/66.66 %) e 0.03 g/L (67/74.45 %) apresentaram as menores taxas de sobrevivência e 0.15 g/L (76/84.45 %) as maiores. As concentrações de 0.025 e 0.03 g/L apresentaram os menores tempos de desenvolvimento larval (8.80; 8.86 dias) e longevidade (9.95; 8.70 dias), mas os tamanhos dos adultos foram menores para 0.025 (3.00 mm) e maiores para 0.03 (3.15 mm). A concentração de 0.15 g/L apresentou o maior tempo de desenvolvimento larval (9.59 dias) e a maior longevidade (12.41 dias), com tamanho intermediário de adulto (3.09 mm). As taxas de sobrevivência do* Ae. aegypti *em laboratório geralmente estão associadas à alta mortalidade em recursos de baixa qualidade e baixa quantidade. Verificou-se que o estresse nutricional prejudica o desenvolvimento larval, bem como o tamanho e a longevidade dos adultos. A análise das respostas a diferentes regimes de alimentação é importante para compreender os principais mecanismos envolvidos no desenvolvimento das larvas e os requisitos para otimizar os sistemas de criação de mosquitos.*

Palavras chave: *desenvolvimento larvar, desenvolvimento de mosquitos, limitação alimentar, nutrição, regime alimentar específico*

INTRODUCTION

Mosquitoes (Diptera: Culicidae) are the clinically most important group of insects due to the number of etiological agents they transmit and the magnitude of these diseases for the health of animals including humans. This is especially true for *Aedes (Stegomyia) aegypti* (Linnaeus, 1762) (Wilke et al., 2020), a species recorded on six continents (Murray et al., 2013) and one of the main threats to public health (Wilke et al., 2020) given its ability to transmit arboviruses such as urban yellow fever, dengue, Zika and chikungunya (Amraoui et al., 2018). The eggs of the species remain viable for long periods of time and hatch once submerged in water, thus increasing infestation during rainy and warm periods (Becker et al., 2010). During cold and/or dry periods, however, these organisms are able to temporally suspend their metabolism (even eggs) via the mechanism of diapause, which enables survival during harsh periods (Diniz et al., 2017; Garzón et al., 2020). After hatching, *Ae. aegypti* larvae can develop in a wide variety of aquatic habitats including water-holding cups, bottles, tires, trunk hollows, and bromeliad leaf axils (Albeny-Simões et al., 2014; Rezende et al., 2020). Larvae are able to consume the most varied types and amounts of food in these environments, such as microorganisms, plant and animal detritus, biofilm, and other organic matter (Rogers & Yee, 2019). However, several environmental factors can affect the survival rate and developmental success of larval *Ae. aegypti*, including temperature (De Majo et al., 2019), larval density (Chandrasegaran et al., 2018), microcosm microbiota (Rogers & Yee, 2019), and larval diet (Souza et al., 2019).

Several studies have shown that the resources available in the environment where mosquito larvae develop are strong determinants of adult characteristics (Levi et al., 2014; Galon et al., 2020; Schoor et al., 2020). The conditions typically found in container systems occupied by *Ae. aegypti* larvae are larval-food limited (Bellamy & Alto, 2018). Such containers usually receive random and irregular inputs of nutrients, including litter detritus, sediments, plant remains, and invertebrates (Daugherty et al., 2000; Rogers & Yee, 2019). Nutritional restrictions can negatively affect individual size, body reserves, feeding behavior, fecundity, longevity, and overall vector competence (Breaux et al., 2014; Vantaux et al., 2016). A lack of adequate nutrition during *Ae. aegypti* larval development causes developmental delay or failure (Schoor et al., 2020) and high larval and pupal mortality rates (Levi et al., 2014), resulting in smaller adults and reduced longevity (Vantaux et al., 2016).

Most of the nutrition assimilated during larval development is allocated for structural growth (Padmanabha et al., 2012), making these allocations crucial for developing insects as they must pass certain physiological checkpoints to progress through each developmental stage (Plaistow et al., 2004). *Aedes aegypti* mosquitoes have four larval developmental instars (Carvalho et al., 2023) and to successfully molt from the 4th instar into pupa, the larva must undergo a high transformation pe-

riod, during which juvenile hormone levels quickly disappear and ecdysone becomes predominant (Levi et al., 2014). Since metamorphosis requires a large amount of energy, proper larval growth and development are required for its normal occurrence (Becker et al., 2010), resulting in the emergence of larger adults with increased body mass and greater energy reserves, with effects on survival and longevity (Chandrasegaran et al., 2018). Complex life cycles with an aquatic and a terrestrial stage have different life histories (Costanzo et al., 2011). Premetamorphic stages (larvae aquatic stage) are committed to growth (Padmanabha et al., 2012), while postmetamorphic stages (adult terrestrial stage) are dedicated to reproduction and dispersal (Briegel et al., 2001).

Larval diet needs to provide a wide range of nutrients to avoid the risk of deficiencies that could affect either breeding productivity or adult fitness (Timmermann & Briegel, 1999). In laboratory tests, Singh & Brown (1957) found that mosquito larvae require sugar, nucleotides, polyunsaturated fatty acids, sterols, vitamins, and fourteen essential amino acids for proper development. Analysis of larval nutrition and the resulting impacts on growth, pupation, and emergence facilitates a deeper understanding of the requirements mediating developmental processes in immature mosquitoes (Schoor et al., 2020). However, diets for rearing mosquito larvae vary significantly among laboratories in terms of the type and amount of food used in rearing protocols (Levi et al., 2014; Bond et al., 2017). Examples of laboratory diets include fish food (Cozzer et al., 2022), turtle food (Andrade et al., 2017), liver powder (Chen et al., 2015), brewer's yeast and lactalbumin (Bellamy & Alto, 2018), and several varieties of rat, cat, and dog food (Bond et al., 2017; Pooraiioubi et al., 2018; Almadiy, 2020).

In this way, feed tolerance experiments are important for determining the optimal conditions for larval development and size and adult longevity in the laboratory, mainly because: (i) resource availability is a limiting factor for development, with effects on adult life history traits; and (ii) there is extensive variation in the diets used in rearing protocols for rearing mosquito larvae in terms of the types and amounts of food. These are crucial aspects in studies of mosquito larval biology since the impact of the amount of larval diet on survival rates and "fitness" parameters, such as immature development time, adult body size, and stored reserves, affect longevity (Cozzer et al., 2022), and can contribute to the elaboration of effective populational control strategies (Bellamy & Alto, 2018). As *Ae. aegypti* is strongly regulated by density-dependent effects and is not a good competitor, resources scarcity allows the use of compromised life expectancy as a key parameter in determining disease transmission risk (Alto et al., 2005). In addition, resource availability may improve procedures for rearing *Ae. aegypti* in the laboratory by promoting accelerated larval development, higher survival rates, and the production of a homogeneous adult population.

Considering that larval diet is an important factor in the mass rearing of *Ae. aegypti* (Bond et al., 2017), and based on the assumptions that (i) food limitation is common in artificial containers inhabited by immature forms of *Ae. aegypti* (Bellamy & Alto, 2018), (ii) larval diet must provide sufficient nutrients to reach a minimum size and allow development to proceed to pupation (Lounibos, 1979), and (iii) larval populations of *Ae. aegypti* are strongly influenced by density-dependent effects (Bellamy & Alto, 2018; Galon et al., 2020; Cozzer et al., 2022), we hypothesized that distinct amounts of resources will have differential effects on the survival and life history characteristics of *Ae. aegypti*. Therefore, we evaluated the effects of different food concentrations of larval diet on larval survival, growth, and development of *Ae. aegypti*. We predicted that: (i) limited resources will severely decrease larval survival and development, as well as adult size and longevity, of *Ae. aegypti*; and (ii) over-resourced environments will not result in differences in life history characteristics of *Ae. aegypti*.

MATERIALS AND METHODS

Experiment location

The experiment was conducted in the experimental room of the Laboratório de Entomologia Ecológica (LABENT-Eco) located in the Universidade Comunitária da Região de Chapecó (Unochapecó), city of Chapecó, state of Santa Cata-

rina, southern Brazil. It was carried out under controlled conditions with a temperature of 27 ± 2 ºC, relative humidity of 70–80 % and a photoperiod of 12:12 h. The *Ae. aegypti* larvae used in the experiment came from the LABENT-Eco mosquito colony. Genetic variability of the colony is maintained by annually collecting, identifying, and releasing wild strains of the *Ae. aegypti* mosquito, but there is no generational control. Mosquito eggs were hatched by immersing oviposition papers in trays (30 × 15 × 5 cm) containing 1.5 L of cistern water and allowing them to hatch for 24–48 hours. The presence/absence of diapause eggs was not observed/measured. Larval density ranged from 750 to 1250 larvae per tray.

Experimental treatments

Sixteen different concentration treatments (0, 0.0013, 0.0033, 0.0066, 0. 0133, 0.02, 0.025, 0.03, 0.035, 0.04, 0.045, 0.05, 0.1, 0.15, 0.2, 2 g/L) of Spirulina Alcon® fish feed were tested. Feed was weighed on an analytical balance (Bel Engineering SKU M - 0.0001g), added to a beaker with 1 L of water and diluted for three minutes in a magnetic stirrer. Experimental microcosms consisted of plastic cups holding 150 mL of nutrient solution, with six replicates for each concentration. Fifteen newly hatched *Ae. aegypti* larvae were added to each replicate (01 larva/10 mL) following Bellamy & Alto (2018). This density is based on the average number of *Ae. aegypti* larvae captured in larvae traps used in the field by Bellamy & Alto (2018). Evaporative water loss was compensated for by refilling experimental microcosm to the original volume once a day. Supplemental larval food was added to each treatment every three days with the entire system, except for the larvae, being substituted to ensure no resource depletion and to remove the toxic effect of nitrogenated excreta (Bellamy & Alto, 2018).

Experimental procedures

The number of live mosquito larvae in each replicate of every treatment was counted every 24 hours, which was subtracted from the larvae survival data recorded on the previous day. With the onset of pupation, pupae from each treatment/replicate were transferred to a 50 mL plastic vial containing 20 mL of water, which was inserted into an entomological trap (small Berlese funnel trap, Bioquip) to capture emerging adults. Pupae and adults were kept in the same biological room as the larvae (temperature 27 ± 2 ºC, relative humidity 70–80 %, photoperiod 12:12 h). Individuals emerging to adulthood were released into circular cages (10 cm diameter × 12 cm high), each identified by emergence date, treatment, and replicate. New cages were used for each day.

Adult *Ae. aegypti* were provided with access to water, but not nutrition, ad libitum via daily renewed moistened cotton. Changes in adult survival under starvation conditions were measured to avoid the possibility that adult nutrition could mask the potential effects of larval stressors in the different treatments. Adults were examined daily, and dead mosquitoes were sexed, counted, and recorded. Dead adults were collected daily, organized in Eppendorf tubes by treatment/replicate and stored at -18 °C. Sexing and measurement of the left wing (ventral view) was performed for each dead adult individual as a measure of allometry (Gutiérrez et al., 2022). These measurements were made using a Zeiss Stemi 305 binocular stereoscopic microscope at 40× magnification. All measures were calibrated at the same magnification and the wing was placed in the center of the visual field to allow accurate size comparisons and reduce the risk of optical distortion. Larval development time (hatching to pupation in days), adult size, and adult survival (emergence to adulthood to death, expressed in days) were measured for each replicate of each experimental treatment. These data were measured separately for each individual in the sample.

Statistical analysis

Differences in larval development time, wing size and adult longevity (dependent variables) were evaluated for effects of treatment (food concentration), sex (males and females) and their interaction with Two-Way Factorial Generalized Linear Models (GLM). Gaussian error distribution was used for all three GLMs (link = identity, test = F; Crawley, 2007). All models were test-

ed for under or overdispersion by the hnp package and function (Moral et al., 2017). *Post hoc* orthogonal contrasts and model simplification were also used to assess differences in response variables (R vegan package). The response variables were ranked from the lowest to the highest and tested pairwise. A step-by-step simplification of the model was subsequently carried out by sequentially adding treatment values that did not affect the model and testing with the next variable in the sequence (Crawley, 2007). Tukey *post hoc* tests (R vegan package, lsmeans function) were also used to compare interactions between evaluated factors. The survival package was used to perform Kaplan-Meier survival analysis followed by statistical comparison of the survival curves using the Chi-square test. The median time to death was also calculated for each treatment using the Kaplan-Meier product-limit method with the log-rank test (Ferreira et al., 2010; Martins et al., 2017). All analyses were carried out with the program R, version 4.1.3 (R Development Core Team, 2014).

RESULTS

Survival

No *Ae. aegypti* survived in the concentration treatments of 0, 0.0013, 0.0033, 0.0066, 0.0133, 0.02 and 2 g/L. Only the concentration treatments of 0.025 (60/66.66 %), 0.03 (67/74.45 %), 0.035 (73/81.11 %), 0.04 (68/75.55 %), 0.045 (73/81.11 %), 0.05 (71/78.89 %), 0.1 (70/77.78 %), 0.15 (76/84.45 %) and 0.2 g/L (65/72.22 %) had survivors from the 90 initial individuals for each treatment (Fig. 1). A total of 817 (56.74 %) of 1440 initial *Ae. aegypti* individuals survived during the experiment. Survival curves differed among all treatments (Chi-square test = 1271, df = 15, $p < 0.001$) and were affected by food concentration.

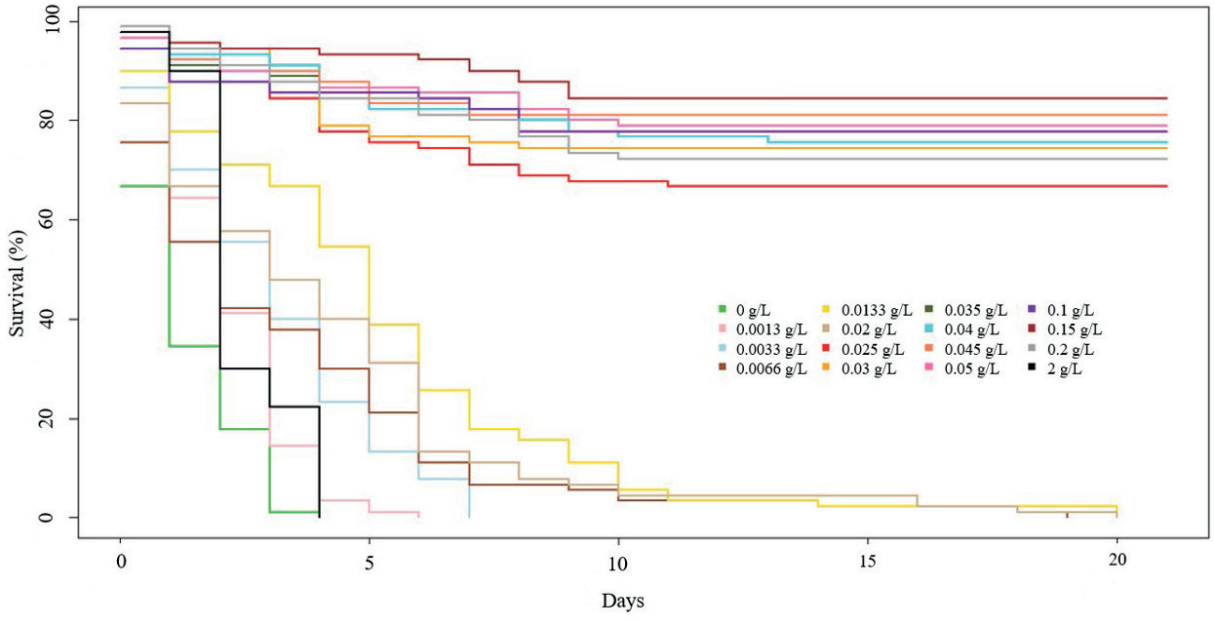

Figure 1. Kaplan–Meier Survival Analysis shows the percentage of larvae remaining for each concentration (0, 0.0013, 0.0033, 0.0066, 0.0133, 0.02, 0.025, 0.03, 0.035, 0.04, 0.045, 0.05, 0.1, 0.15, 0.2, 2 g/L) of the diet for twenty-one days, until they were all dead or reached the pupation. The log-rank paired comparison of all diets resulted in significant differences between pupation rates ($p < 0.001$) for all comparisons. *A Análise de Sobrevivência Kaplan-Meier mostra a porcentagem de larvas restantes para cada concentração (0, 0.0013, 0.0033, 0.0066, 0.0133, 0.02, 0.025, 0.03, 0.035, 0.04, 0.045, 0.05, 0.1, 0.15, 0.2, 2 g/L) da dieta durante vinte e um dias, até que todas estivessem mortas ou chegassem à pupação. A comparação logaritmizada pareada de todas as dietas resultou em diferenças significativas entre as taxas de pupação (*$p < 0.001$*) para todas as comparações.*

Table 1. Generalized Linear Models (GLM) between treatments (0.025, 0.03, 0.035, 0.04, 0.045, 0.05, 0.1, 0.15, 0.2 g/L), sexes (Males and Females) and the interaction between these factors for Larval Development Time (A), Adult Longevity (B) and Wing Size (C). In addition, orthogonal contrast analyses for treatments and sexes; Degrees of freedom (Df), Deviation (Deviation), Residual Degrees of Freedom (Df Res.), Residual Deviance (Dev) and Values of F and *p* (Pr > F). *Modelos Lineares Generalizados (GLM) entre tratamentos (0.025, 0.03, 0.035, 0.04, 0.045, 0.05, 0.1, 0.15, 0.2 g/L), sexos (Machos e Fêmeas) e a interação entre estes fatores para o Tempo de Desenvolvimento Larvar (A), Longevidade Adulta (B) e Tamanho da Asa (C). Além disso, análises de contraste ortogonal para tratamentos e sexos; Graus de liberdade (Df), Desvio (Desvio), Graus de liberdade residuais (Df Res.), Desvio residual (Dev) e Valores de F e* p *(Pr > F).*

	Df	Deviation	Df Res.	Dev	F	Pr(>F)	Contrast Analyzes
A. Larval Development Time							
WITHOUT EFFECT			593	504.96			
Treatments	8	84.021	585	420.94	15.586	**< 0.001**	0.025 = 0.03 = 0.04 = 0.045 < 0.035 < 0.1 < 0.05 < 0.15 < 0.2
Sexes	1	24.875	584	396.06	36.915	**< 0.001**	Males < Females
Treatments:Sexes	8	7.929	576	388.13	1.471	0.165	Table MS1
B. Adult Longevity							
WITHOUT EFFECT			593	8161.2			
Treatments	8	990.48	585	7170.7	10.364	**< 0.001**	0.025 = 0.03 = 0.035 = 0.045 < 0.04 < 0.05 < 0.2 < 0.15 < 0.1
Sexes	1	73.89	584	7096.9	6.185	**0.013**	Females < Males
Treatments: Sexes	8	215.86	576	6881.0	2.259	**0.022**	Table MS2
C. Wing Size							
WITHOUT EFFECT			593	129.763			
Treatments	8	1.736	585	128.026	2.698	**0.006**	0.025 = 0.04 = 0.045 = 0.1 < 0.2 < 0.15 < 0.035 < 0.05 < 0.03
Sexes	1	81.505	584	46.521	1013.373	**< 0.001**	Males < Females
Treatments: Sexes	8	0.194	576	46.327	0.302	0.965	Table MS3

Larval development time

There were significant effects for treatment (GLM; $F_{(8, 585)} = 15.58$, $p < 0.001$), sex (GLM; $F_{(1, 584)} = 36.91$, $p < 0.001$) and their interaction (GLM; $F_{(8, 576)} = 1.47$, $p = 0.165$) on larval development time (Table 1A). The treatments that differed significantly from each other in pairwise tests were: (i) 0.045 and 0.05 g/L for females (8.765 ± 0.688; 9.657 ± 1.145) and males (8.525 ± 0.921; 9.179 ± 0.876); (ii) 0.1 and 0.15 g/L for males (8.976 ± 0.820; 9.475 ± 0.741); and (iii) 0.025 g/L between males and females (8.393 ± 1.048; 9.296 ± 1.047) (Table S1, Supplementary information, available at https://www.limnetica.net/en/limnetica). Larval development time was shortest in 0.025 and 0.03 g/L (8.80 and 8.86 days, respectively) and longest in 0.15 g/L (9.59 days).

Adult longevity and size

There were significant effects for treatment (GLM; $F_{(8, 585)} = 10.36$, $p < 0.001$), sex (GLM; $F_{(1, 584)} = 6.18$, $p = 0.013$) and their interaction (GLM; $F_{(8, 576)} = 2.26$, $p = 0.022$) on adult longevity (Table 1B). Only the treatments of 0.05 and 0.1 g/L (for males) differed significantly from each other in pairwise tests (11.105 ± 1.479; 13.414 ± 5.407) (Table S2, Supplementary information, available at https://www.limnetica.net/en/limnetica). Adult survival was shortest in 0.025 and 0.03 g/L (9.95 and 8.70 days, respectively) and longest in 0.15 g/L (12.41 days).

There were significant effects for treatment (GLM; $F_{(8, 585)} = 2.70$, $p = 0.006$) and sex (GLM; $F_{(1, 584)} = 1013.37$, $p < 0.001$), but not for their interaction (GLM; $F_{(8, 576)} = 0.30$, $p = 0.965$), on wing size (Table 1C). Treatments that differed

significantly from each other in pairwise tests were: (i) 0.045 and 0.05 g/L for females (3.394 ± 0.230; 3.517 ± 0.248) and males (2.590 ± 0.246; 2.780 ± 0.185); (ii) 0.1 and 0.15 g/L for males (2.679 ± 0.387; 2.765 ± 0.267); and (iii) 0.025 g/L between males and females (2.637 ± 0.351; 3.367 ± 0.298) (Table S3, Supplementary information, available at https://www.limnetica.net/en/limnetica). Adult size was smallest in 0.025 g/L (3.00 mm) and largest in 0.03 g/L (3.15 mm), with the treatment 0.15 g/L having individuals of intermediate size (3.09 mm).

DISCUSSION

Survival

Survival of *Ae. aegypti* was positively affected by increasing resource concentration, as observed in other studies (Levi et al., 2014). The resources provided reduced mortality rates and positively affected larval development. There was also low mortality in the concentration treatments of 0.025, 0.03, 0.035, 0.04, 0.045, 0.05, 0.1, 0.15 and 0.2 g/L (overall survival rate of 76.9 %). Survival rates of *Ae. aegypti* in laboratory experiments are generally associated with high mortality on low-quality resources at low-quantities (Schoor et al., 2020). However, due to low differences in food concentrations of the treatments used, the larvae survival rates for concentrations in which individuals achieved pupation did not differ very much. As diet plays an important role in immature insect development, nutritional limitation compromises *Ae. aegypti* survival (Bellamy & Alto, 2018; Schoor et al., 2020). The environments of larval *Ae. aegypti* receive random inputs of resources that are typically composed of a mixture of leaf and animal detritus, whereas optimal *Ae. aegypti* development has been found to rely on high nitrogen content (Schoor et al., 2020). Spirulina Alcon® is rich in protein, which is one of the several nutrients found in natural *Ae. aegypti* habitats where carbohydrates are available from plant-based materials, supplemented with other nutrients like protein from animal detritus and cohabiting bacteria (Andrade et al., 2017). In cases of low protein levels, it can be supplanted later in life via vertebrate blood meals typically required for egg development by anautogenous female mosquitoes, such as *Ae. aegypti* (Becker et al., 2010).

No living *Ae. aegypti* larvae were found in treatments with concentrations lower than 0.02 g/L and in the 2 g/L concentration. Lack of resources due to very low concentrations leads to starvation mortality (Chandrasegaran et al., 2018). Mosquito larvae perform gas exchange by exposure of the respiratory siphon to the atmosphere (Becker et al., 2010). Compounds that do not dilute properly or form a film on the water surface after saturation of the medium (2 g/L very high concentration) can impede gas exchange and cause larval death by asphyxiation (Torres et al., 2014). First instar larvae are more predisposed to these events due their smaller size and incapacity to break through such film (Torres et al., 2014). Mass rearing of *Ae. aegypti* mosquitoes requires a balanced diet that favors high survival and uniform, short larval development (Bond et al., 2017). Because larvae eat almost continuously and grow very fast under optimal developmental conditions (Schoor et al., 2020), energy reserves are synthesized and accumulated for use in metamorphosis and to provide lipids and glycogen for the adult stage (Bond et al., 2017). Therefore, the nutritional dependencies of larval mosquitoes could be an important target for the development of efficient mosquito rearing techniques and alternative larval control measures (Bond et al., 2017).

Larval development time

Interactions between factors, such as resource availability and intraspecific density/competition, influence *Ae. aegypti* larval development time (Chandrasegaran et al., 2018). Males and females responded differently to the same treatments for larval development time. Resource quantity affects both growth and competition (Steinwascher, 2020), but given the protandry and sexual dimorphism of the species one would expect different effects for the sexes (Kleckner et al., 1995; Chandrasegaran et al., 2018). Male and female mosquito larvae compete for the same food resources, with competition being greater while males are in the system (Kleckner et al., 1995). Because males are smaller and emerge earlier as adults

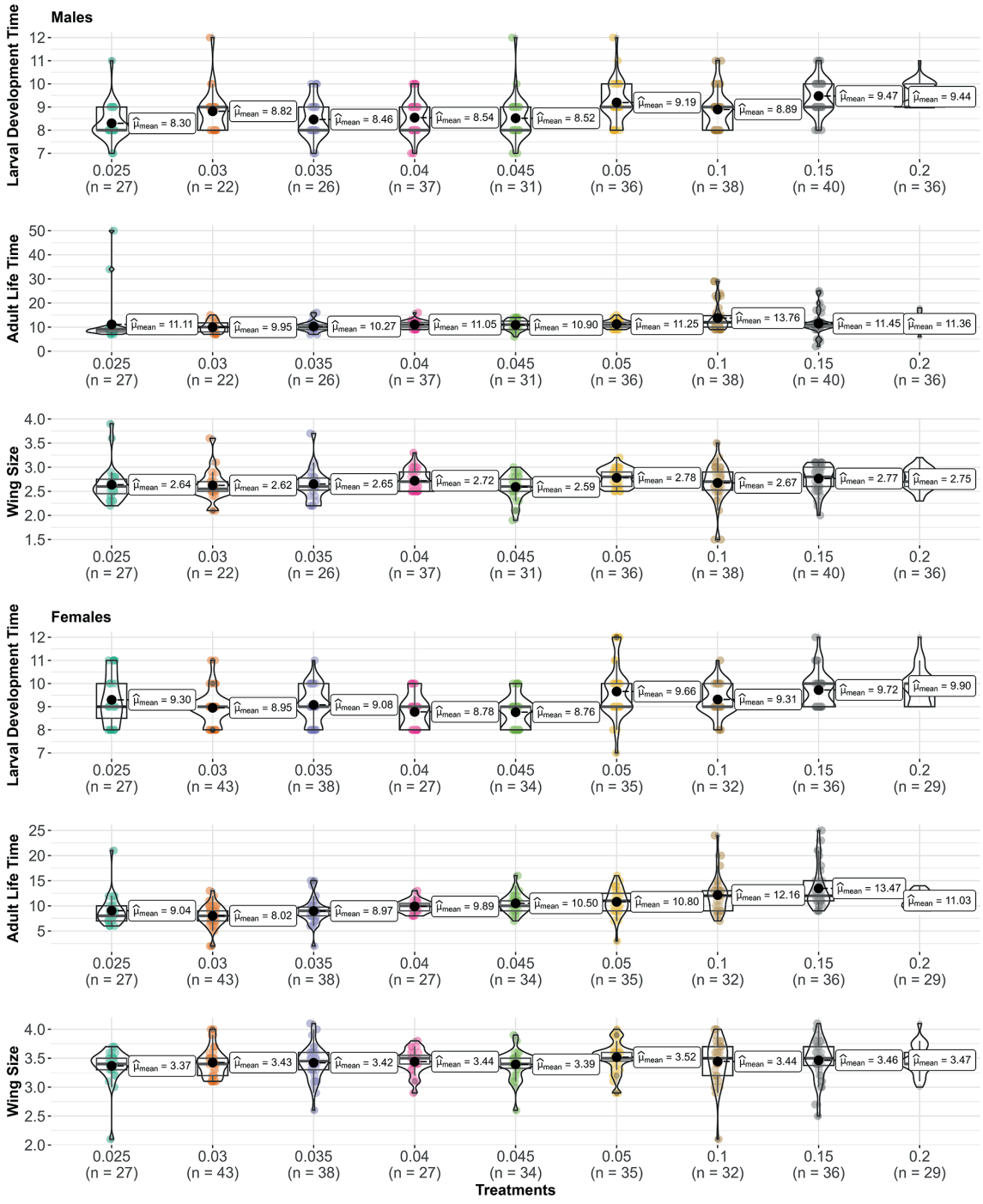

Figure 2. Responses in larval development time (in days), adult longevity (in days), and wing size (in mm) of different sexes (males and females) to different treatments (0.025, 0.03, 0.035, 0.04, 0.045, 0.05, 0.1, 0.15, 0.2 g/L). The violin boxes represent the quartiles; the black circles in the horizontal represent the average; the horizontal-bold line represents the median; and the vertical line represents the upper and lower limits. *Respostas no tempo de desenvolvimento larval (em dias), longevidade adulta (em dias), e tamanho da asa (em mm) dos diferentes sexos (machos e fêmeas) a diferentes tratamentos (0.025, 0.03, 0.035, 0.04, 0.045, 0.05, 0.1, 0.15, 0.2 g/L). Os violinos representam os quartis; os círculos pretos na horizontal representam a média; a linha horizontal em negrito representa a mediana; e a linha vertical representa os limites superior e inferior.*

to mature sexually (Kleckner et al., 1995), their departure from the system releases females from the negative pressure of the density-dependent effect, improving their development (Fig. 2) (Steinwascher, 2020).

Populations of *Ae. aegypti* larvae subjected to nutritional stress have been associated with dysfunctions in survival, reproduction, and longevity of adults (Yan et al., 2020). Larval development time was shortest in the 0.025 and 0.03 g/L treatments (8.80 and 8.86 days, respectively) and among the longest in 0.15 g/L (9.59 days). This partially corroborates our predictions that (i) limited resources will severely compromise larval survival and development; and (ii) enhanced resources will positively affect larval survival and development. Although larval survival was differentially affected by resource availability, larval development time did not behave as predicted. According to Chandrasegaran et al. (2018), higher resource availability results in lower mortality rates and reduced larval development time (Levi et al., 2014). Treatments at the lowest concentrations with surviving individuals also had the shortest larval development times. On the other hand, the treatment of 0.15 g/L had among the longest larval development times, which does not corroborate the findings of Chandrasegaran et al., (2018). In contrast, the larval stage of *Ae. aegypti* at average temperatures of 25 °C, without competition and with resources in adequate amounts, is 8.42 days (Yan et al., 2020), with a range of 7.9–9.0 days (Becker et al., 2010). The treatments of 0.025 and 0.03 g/L were within this expected range, but the treatment of 0.15 g/L was in excess.

Adult size and longevity

Nutritional stress during the larval stage influences adult size and longevity of mosquitoes (Levi et al., 2014; Chandrasegaran et al., 2018). The existing difference in adult size between males and females of *Ae. aegypti* is due to sexual dimorphism, with females always being larger than males (Kleckner et al., 1995). In addition, females grow larger and faster than males under the same feeding conditions (Steinwascher, 2020). The limited resources present in the 0.025 g/L treatment of the present study severely compromised adult size (3.00 mm). As a result of resource scarcity, the individuals that advance from 4th instar to pupa emerged as adults of reduced body size (Telang et al., 2007; Chandrasegaran et al., 2018). Therefore, the investment of resources obtained in the larval stage was sufficient to build up reserves to metamorphose rather than to contribute to structural growth (Telang et al., 2007). The higher resource availability at 0.03 g/L allowed for greater investment in structural growth (3.15 mm), even if it implies having no remaining reserves after metamorphosis, thereby affecting longevity (Telang et al., 2007; Chandrasegaran et al., 2018). The enhanced features of the 0.15 g/L treatment did not result in a large physical size after metamorphosis (3.09 mm), but instead in individuals of intermediate size (Telang et al., 2007; Chandrasegaran et al., 2018).

Mosquitoes spend energy constantly (basal metabolic rate) and, in periods without food, live on the reserves accumulated in periods of food abundance (Arrese & Soulages, 2010). Thus, the longevity of adults is directly related to the amount of nutrients found, consumed, and stored (Briegel et al., 2001; Arrese & Soulages, 2010), and the body size of the individual (Reiskind & Lounibos, 2009). The treatments of 0.025 and 0.03 g/L had the shortest adult longevity (9.95 and 8.70 days). The reduced size of the individuals in the 0.025 g/L treatment allowed a greater longevity than those of the 0.03 g/L treatment, since their basal metabolic rate was also lower due to their smaller size (Reiskind & Lounibos, 2009; Arrese & Soulages, 2010). The treatment of 0.03g/L reached a larger size than did the treatment of 0.025g/L, the latter thus having greater energy demands, exhausting energy reserves earlier and resulting in shorter longevity. These results differ from those found by Chandrasegaran et al., (2018), in which wing length was strongly and positively related to adult longevity, and the risk of death decreased with increasing wing length. The 0.15 g/L treatment had the greatest longevity (12.41 days). With enhanced resources, much of what was consumed and assimilated was directed toward building energy reserves at the expense of structural growth, ensuring greater adult longevity.

CONCLUSIONS

Diet plays a very important role in the growth and development of all organisms and can vary significantly based on the life history of the species (Schoor et al., 2020). This is especially true for *Aedes aegypti*, which has evolved to occupy niches with potential nutritional limitations, such as human habitations (Bellamy & Alto, 2018). Environments occupied by mosquito larvae receive sporadic and random inputs of basal nutrients and adults may not find energy sources (Bellamy & Alto, 2018; Schoor et al., 2020). Mosquito larvae exposed to reduced nutrient levels in these locations experience retarded larval development that compromises size and longevity, even more so if adults cannot find resources as well (Chandrasegaran et al., 2018). Changes in mosquito life history traits can shift the effectiveness of control efforts and alter the results of ecological surveys of habitats and vectorial capacity (Bellamy & Alto, 2018; Chandrasegaran et al., 2018). Shortened adult longevity reduces the number of mosquitoes that survive the intrinsic incubation period of pathogens, influencing the transmission rate of diseases. Individuals of *Ae. aegypti* that develop under a better feeding regime exhibited more preferential life history traits, with greater survival rates and better energy stores to invest in reproduction, which is desired in rearing systems. Understanding feeding requirements, especially the optimum, is important for practical purposes, such as improving experimental replicability and advancing efficient mass rearing practices. In addition, such understanding can help to define vector control strategies, as it can result in increased efforts to control population sizes and reduce cases of disease in human populations.

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