Bangladesh J. Zool. **50**(2): 251-259, 2022 ISSN: 0304-9027
eISSN: 2408-8455

DIFFERENTIAL EFFECTS OF TEMPERATURE AND DIET ON THE BODY SIZE-FECUNDITY RELATIONSHIP OF *AEDES ALBOPICTUS* (DIPTERA: CULICIDAE)

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Abstract: To investigate whether *Aedes albopictus* females cease reproduction under adverse conditions, they were offered manipulated diets (high and low diets) at 26°C and 28°C. Both of the diets showed a profound effect on development time and body size. Lower temperature and high diet resulted in larger body size, and that further affected fecundity. There was an insignificant relationship between the number of eggs laid and body size excluding high diets at a lower temperature. As fecundity increased with the body size, *Ae. albopictus* laid more eggs at 28°C under a high diet. *Ae. albopictus* reserved more eggs at 28°C, however, body size and egg retention were significantly related under a lower diet at 26°C and 28°C. Our findings indicate that the difference in warm temperature and diet condition contributed to fecundity and the results might help in vector.

Key words: *Aedes albopictus*, temperature, body size, diet, fecundity.

INTRODUCTION

The Asian tiger mosquito, *Aedes albopictus* is documented as a common vector species in Bangladesh. Owing to the distribution of *Ae. albopictus* the world population is at risk from vector-borne diseases, including dengue, yellow fever, Chikungunya and Zika (Maurice *et al.* 2010, Weaver and Lecuit 2015). Such prevalence is a consequence of globalization and travel that led the vector species into communities despite struggles to suppress mosquitoes (CDC 2019, Dengue and Severe Dengue 2020). In disease transmission, there is a close epidemiological association between the behaviors of mosquitoes and the environment (Chandrasegaran *et al.* 2020). Temperature variation and diet are the most important environmental features that affect the body size and vectorial capacity of mosquitoes. It is important to identify the indices in a mosquito population's physiological response in different meteorological conditions that are better predictors of vectorial capacity. The vectorial capacity of females includes several parameters such as fecundity, both of which are

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 DOI: https://doi.org/10.3329/bjz.v50i2.62057

associated with body size (Takken *et al.* 2013, Christiansen-Jucht *et al.* 2015, Barreaux *et al.* 2018, Gutierrez *et al.* 2020). For example, small *Ae. aegypti* females were more susceptible to dengue virus infection than their larger counterparts (Alto *et al.* 2008). Fecundity affects the progeny of mosquitoes, and thus influences local mosquito abundance. However, few studies have investigated how these traits (fecundity) can be influenced by body size. The body size may be altered by temperature and food availability during the larval stage (Moller-Jacobs *et al.* 2014). For instance, high nutrients with lower temperature leads to large body size in adults, conversely, low diet linked with higher temperature raises smaller body size (Sibly and Atkinson 1994, Kingsolver and Huey 2008). Moreover, the impact of body size of *Ae. albopictus* on the fecundity has been little explored. A more recent study added temperature change could cause increases of body size in *Ae. albopictus* (Alam and Tuno 2020). Body size is linked with mosquito-borne disease and specifically, we studied how body size is associated with fecundity. To do so, we compare the impact of body size on the fecundity of *Ae. albopictus*. We set up cohorts with two temperatures and diets to produce different body sizes of mosquitoes. We recorded the number of eggs, and egg retention capacity of the various treatment groups. We sought to gain a better understanding of the future forecasts of disease and prepare the public health sector.

MATERIAL AND METHODS

Mosquito colony: Aedes albopictus were obtained from Dhaka city in adult and larval form in June 2021 to continue a laboratory colony. In the rearing incubator mosquitoes were reared at temperature 26°C with 70–90% RH and 14:10 h L:D. Human blood from the author's hand was offered to female mosquitoes to induce oviposition. In a plastic tray (25 cm width × 34 cm length × 6 cm height) the eggs were kept for further hatching. The first instar was fed with finely minced fish food (TetraMin, Spectrum Brands Japan Co. Ltd., Yokohama, Japan). The rearing colony was checked every day until pupation. Then the adults were kept in mosquito cages with 3% sucrose. The generations continued up to F3.

Generating larger and smaller size mosquitoes: The body size yielding experiments were performed based on the following treatments: 26°C x low diet, 26°C x high diet, 28°C x low diet and 28°C x high diet. The dietary source (Tetramin®) was administered at the first to fourth instar: 0.05 and 0.1 mg/ larvae/day as a low diet, and 0.4 and 0.8 mg /larvae/day as a high diet (Farjana and Tuno 2012). In each experimental trial, groups of 200 (L1) larvae were counted and transferred in four different plastic trays (25 cm width × 34 cm length × 6 cm height). In all trials a similar larval number was used. The water was changed prior to the daily addition of diet in each tray. The larval development, molting and pupation were checked to compare the diet effects. Pupae were collected daily and transferred individually in plastic vials (3 cm diameter × 6 cm height) until their emergence into the adult. The adult males and females which emerged from each diet and temperature treatment were kept in mosquito cages (20 cm width × 20 cm height× 30 cm length) with 7% sucrose.

Developmental time, oviposition and retained eggs: The development time of emerging adults was recorded from each treatment. In order to measure oviposition, a total of 50 adult females were taken per diet and temperature treatment. From each treatment, females were housed with males for mating purposes. After mating, they offered a blood meal from the rat up to engorge. Then females were placed individually into a vial (3 cm diameter × 6 cm height) with a part of wet filter paper on the bottom and a cotton ball soaked with sugar solution on a mesh net covering the vial mouth. Each female was checked after five days and they were kept for more than two days for further inquiries. The number of reserved eggs in the ovaries was checked and oviposited females were dissected (Farjana and Tuno 2012). The number of laid eggs and retained eggs in the ovaries were counted. Females that laid no eggs (from not mating) were omitted from the analyses. To assess the body size variation of females, we measured the wing size of each female by using a micrometer.

Data analysis: Differences in development time and body size between temperature and diet treatments were analyzed by generalized linear models (GLM). To know the combined influence of temperature, diet and body size on fecundity; a generalized linear model (GLM) was used. The analysis of fecundity was done with the identity link function and the normal distribution error. For explanatory variables temperature, diet, body size and the interaction terms of these parameters were used. Based on the results of the analysis by GLM, the number of laid eggs and retained eggs were further compared with body size. Statistical analyses were examined in SPSS version 23 (SPSS Inc. IBM).

RESULTS AND DISCUSSION

Effects of temperature and diet on the adult development time and body size: Body size (wing length), number of laid eggs, retained eggs, and the total number of eggs in *Ae. albopictus* that were developed under different temperatures and diets conditions (Table 1). In this experiment, we determined how different temperature and diet affect the fitness traits of *Ae. albopictus*. According to GLM analysis both temperature and diet treatment had a substantial effect on the development time of adult *Ae. albopictus* (GLM: X^2 = 41.78, df = 1, *P* < 0.0001). However, higher temperatures and low diet levels profoundly increased the development time. Our result showed that temperature and diet strongly influence the developmental time that is concordance with the earlier studies (Farjana *et al.* 2012, Kivuyo *et al.* 2014). However, at low temperatures, mosquitoes potentially require increased developmental time due to taking longer to forage. Consequently, raising temperatures initiate shortened development time, which is advantageous for life history traits. Other studies have shown that larvae fed on low diet leads to late development in adults (Vantaux *et al.* 2016, Zeller and Koella 2016, Aznar *et al.* 2018). A lower temperature (26°C) and a high diet increased the body size of *Ae. albopictus* (2.81 ± 0.14 mm) and individual body size was reduced (2.31 ± 0.10mm) at higher temperature (28°C) and low diet treatment (Table 1). There was a noteworthy difference observed in body size under different temperatures and diet treatments. Body size is significantly affected by temperature and diet as well as their interaction terms in *Ae. albopictus* (*P*< 0.0001) (Fig. 1). Temperature and diet affected the mosquito body size of mosquito. Large size mosquitoes were produced in lower temperature (Mohammed and Chadee 2011, Dodson *et al.* 2012) and lower food supply reduced the body size (Zeller and Koella 2016).

Relationship of body with fecundity: Results of analysis for the fecundity indicated that females fed a diet (low and high) significantly (*P*<0.0001) related to the fecundity; however, temperature was not influencing the fecundity (Table 2). Surprisingly, fecundity was affected by temperature and body size interaction since the number of eggs laid by females did not differ significantly by temperature. Other reports, suggest that higher temperature reduced mosquito fecundity (Ezeakacha and Yee 2019). In addition, fecundity was significantly affected by the body size (GLM: x^2 = 5.04, *P*<0.0001) (Table 2). The temperature and diet pointedly impacted the fecundity (GLM: x^2 = 3.10, *P*<0.0001) (Table 2). Likewise, the association between temperature and body size (GLM: x^2 = 23.6, *P*<0.001) and diet and body size (GLM: x^2 = 1.05, *P*<0.001) on fecundity was significant (Table 2). Overall, the number of eggs was influenced by body size, temperature and diet (GLM: x^2 = 12.91, *P*<0.001) (Table 2). When mosquito species were exposed to the high diet as adults were laid more eggs than the lower diet. Notably, in our findings significant relationship was found between diet supply and fecundity (Yan *et al.* 2021). In addition, diet and body size significantly affected mosquito fecundity. We found that egg numbers also depend on diet, even controlling body size, similar to the findings of Yan *et al.* (2021). Regression analysis showed that the number of laid eggs and body size were not significantly (P>0.05) related in *Ae. albopictus* except at 26°C under high diet (Fig. 2). The same pattern was observed in *Ae. triseriatus*, with no link between size and fecundity when mosquitoes were raised at lower temperature, whether the temperature continued constant or fluctuate (Westby and Juliano 2015). This indicates that the size-fecundity relationship is robust to temperature and diet variation. We postulate that larval environments that promote proportionally greater lipid reserves reduce the relationship between fecundity on size (Briegel 1990, Briegel *et al.* 2002). However, the association between number of retained eggs and body size were also insignificant (Linear regression, P>0.05) (Figs. 3A and C). The regression results revealed that the ratio of females with retained eggs was influenced by body size at 28°C under low diet treatment (*P*< 0.00) (Fig. 3D). The highest number of eggs (68.09 ± 33.2) were laid at high temperature and high diet treatment and at low temperature and low diet treatment laid the lowest number of eggs (19.58 ± 20.45) (Table 1).

Table 1. Wing length (mm), number of laid eggs, retained eggs and total number of eggs (laid+ retained eggs) in *Aedes albopictus* under different temperature and diet treatment

Species	Temperature	Diet	Wing length (mm)		No. of laid eggs	No. of retained eggs	Total no. of eggs
	(°C)		(Mean±SD)	n[1]	(Mean± SD)	(Mean ± SD)	(Mean± SD)
Ae. Albopictus	26	High	2.81±0.14	49	58.59±40.66	12.4± 24.81	69.82±49.09
	26	Low	2.43±0.06	48	15.71±11.28	14.32±11.82	15.08±11.48
	28	High	2.78±0.09	47	68.09± 33.2	10.38±20.82	74.38±37.22
	28	Low	2.31±0.10	50	19.58±20.45	12.44±19.73	32.02±37.47

n[1]: Number of females observed for counting the number of eggs.

Table 2. GLM analysis on the fecundity under different temperature and diet treatment

Variable	Fecundity	
	Likelihood ratio x^2	*P*-value
Temperature	3.75	0.53
Diet	21.24	<0.0001
Body size	5.04	<0.0001
Temperature * body size	23.6	<0.0001
Diet * body size	1.05	<0.0001
Temperature* diet	3.10	<0.0001
Temperature*diet* body size	12.91	<0.0001

Temperature: 26°C and 28°C, diet: high and low, body size: wing length

To conclude, temperature and diet may have varied consequences on body size. Life history traits, including fecundity, are greatly affected by body size. *Ae. albopictus* was able to lay more eggs and retained a few eggs. Body size difference has a putative role to predict how species respond to the spread of infectious diseases. It has potentially essential ramifications for understanding the vectorial capacity that might be measured when tracking factors affecting mosquito Fig.1.

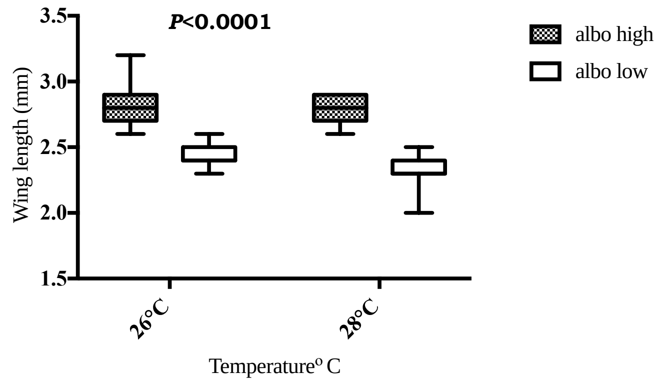

Fig 1. Differences in body size (wing length) of *Aedes albopictus* between treatment levels. Temperature: 26°C and 28°C, diet: high and low.

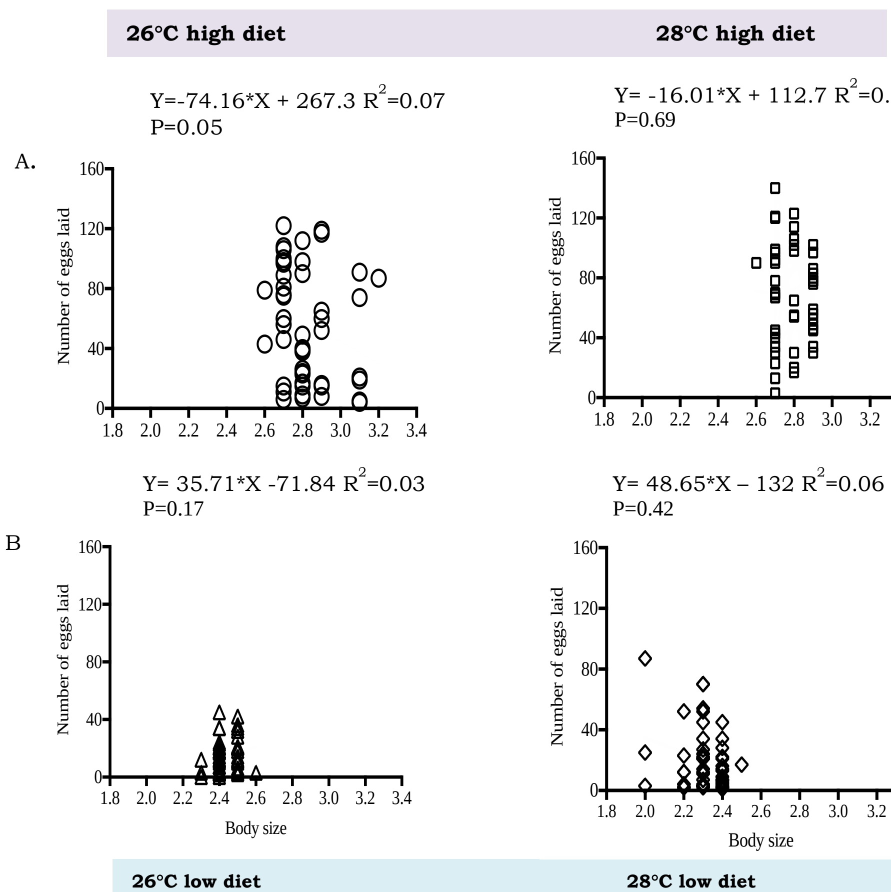

Fig. 2. Regression relationship between body size (wing length/mm) and fecundity (number of eggs laid) by *Aedes albopictus* (circle indicates 26°C high diet, triangle indicates 26°C low diet, square indicates 28°C high diet, and diamond indicates 28°C low diet).

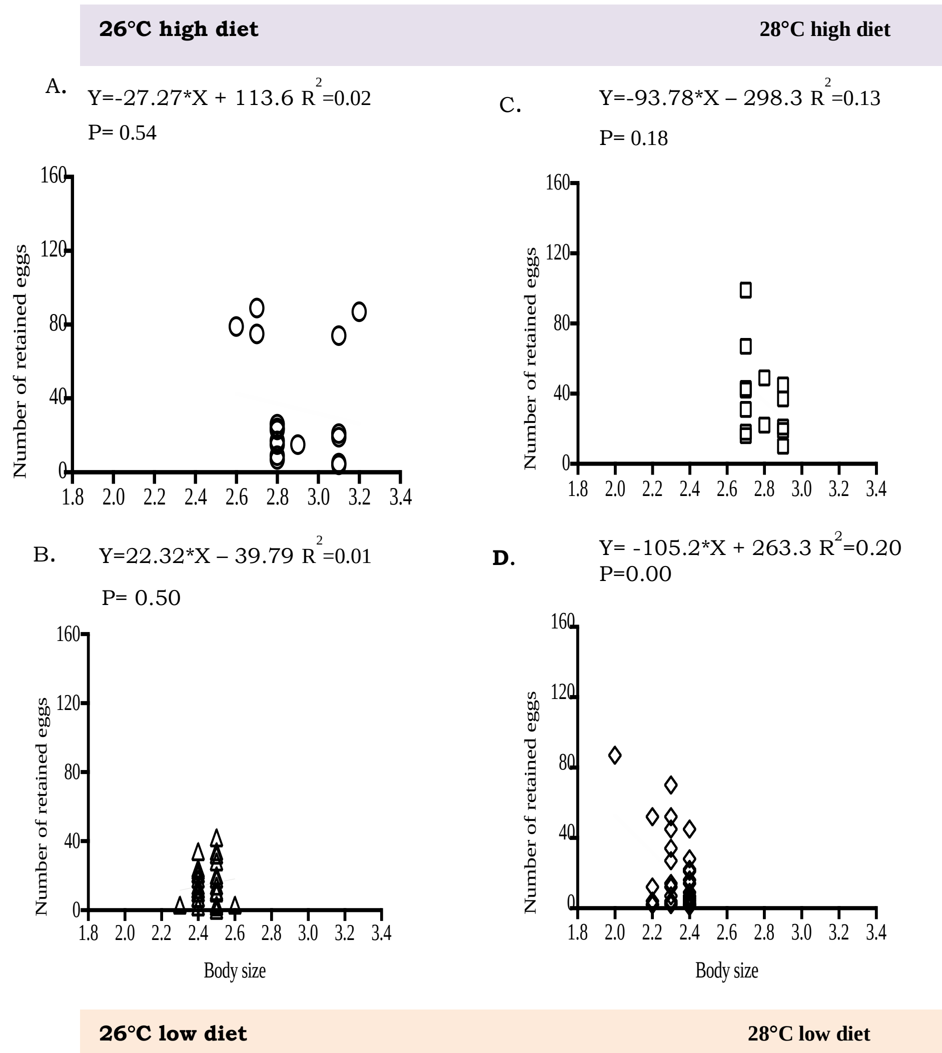

Fig. 3. Regression relationship between body size (wing length/mm) and fecundity (number of retained eggs) by *Aedes albopictus* (circle indicates 26°C high diet, triangle indicates 26°C low diet, square indicates 28°C high diet, and diamond indicates 28°C low diet).

fitness. The fitness variation affects disease transmission, thus making it a challenge in vector control. Thus, it might be useful in public health to gain more resilient and reliable forecasts of mosquito population aiming for better vector control.

Acknowledgements: We are grateful to the students and the field attendants of the Department of Zoology, Jagannath University. This work was partly conducted at icddr,b, Surya Kanta Kala-azar Research Centre, Mymensingh.

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(Manuscript received on 15 August 2022 revised on 26 August, 2022)