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Short Communication

The Assessment of Morphometric Variation of *Aedes aegypti* Larvae with the Seasonal Environment Temperature Inversion in Selected Areas of Lahore, Pakistan

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Aedes aegypti, Dengue vector, *Fourth-instar larvae*, Larval body length, Seasonal temperature

Abstract | Temperature is one of the critical abiotic environmental factors that can influence biological and physiological processes, including mobility, development, and reproduction in poikilotherms. Due to the medical importance of *Aedes aegypti* as a vector of several medically important pathogens, evaluating the body length variation of *Aedes aegypti* larvae with the changing seasonal temperature is important. The study was conducted to observe the difference in body size and different body structures of *Ae. aegypti* larvae in two seasons, i.e., southwest monsoon (June through September) and retreating monsoon (October and November). The fourth instar larvae were collected from areas of district Lahore. The collected larvae were preserved in formalin and transported to the laboratory of the Department of Environmental Science and Engineering at the Government College University Faisalabad for further analysis. The larval morphological measurements were carried out using a stereomicroscope, which included changes in head length and width, thoracic length and width, abdominal length and width, and total length of the larva. Every month, the fourth instar larvae (n=36) were investigated for body size measurement. The results showed that low temperatures of breeding water significantly increase ($P \leq 0.05$) the body size, head, thorax and abdomen of larvae. The results convinced that temperature inversion affects the immature development stages of *Ae. aegypti*. This study concluded that, *Ae. aegypti* larvae's body size depends upon seasonal temperature inversion in the breeding water. These findings can help in predicting the variation in the development rate of *Ae. aegypti* larvae under different seasonal temperatures.

Novelty Statement | The determines the body length variation of *Aedes aegypti* larvae with the changing seasonal temperature and evaluates how breeding water temperature affects its development.

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Introduction

Mosquitoes are an important group of insects because they spread pathogens that cause disease in human

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and animal (Giesen *et al.*, 2020). The primary vector of various human arboviral diseases, including dengue, Zika, and chikungunya fever, is a mosquito of the genus *Aedes,* namely *Aedes aegypti* (Rocklov and Dubrow, 2020).

As a poikilotherm, the internal temperature of insects, including mosquitoes, fluctuates and rely on the temperature of the nearby environment (Reinhold *et al.* 2018). Temperature changes affect physiology, behaviour, ecology, and insect survival (Wimalasiri-Yapa *et al.* 2021). Because of seasonal thermal fluctuations, insect development faces the risks, such as desiccation, metabolic changes, and loss of mobility (Caminade *et al.*, 2019).

The development of insects primarily depends on temperature and can be delayed or accelerated by altering the temperature (Beck-Johnson *et al.*, 2013). The *Aedes* mosquitoes have 4 life stages: egg, larva, pupa and adult. The entire life cycle, from an egg to an adult, takes approximately 8-10 days. In *Ae. aegypti*, there is a direct association between the mosquito's immature development stage and the temperature (Farjana *et al.*, 2012). The development rate improved linearly with temperatures (22°C to 28°C) (Farjana *et al.*, 2012; Barreaux *et al.*, 2018; Sasmita *et al.*, 2019). It is critical to follow on for about ten days the advancement from the egg to the adult stages (Bayoh and Lindsay, 2003) and Christiansen (2015) described that no adults appeared at temperatures below 18°C or above 34°C after pupation, but at 35°C, all larvae died before emergence (Christiansen-Jucht *et al.*, 2015).

Since mosquitoes are also poikilotherms, almost all biological activity is affected by ambient environmental conditions, such as humidity and temperature (Wimalasiri-Yapa *et al.*, 2021). Given the increasing phenomenon of climate change, it is important to understand how mosquitoes respond to changes in body characteristics and critical environmental parameters needed to predict survival rates. The present study assessed the changes in body sizes of *Ae. aegypti* larvae in two different seasons of the year, i.e., Southwest Monsoon (June through September) and Retreating Monsoon (October and November), to better understand the fluctuation in the development rate of *Ae. Aegypti* larvae in different environmental temperatures.

Materials and Methods

Larval collection, selection and identification

A total of 216 (n= 36/month) *Ae. aegypti* larvae were collected during the southwest monsoon (June through September) and retreating monsoon (October and November). The larvae were collected from selected areas of Wahga town and Allama Iqbal town of the district Lahore. The collected samples were then transported to the Department of Environmental Science and Engineering at the Government College University Faisalabad for further investigations. The larval samples were preserved in 70% formalin according to (Khan *et al.*, 2018) method. The collected larvae were identified to species level with the aid of a microscope by (Rueda, 2004) identification key. For the control, larvae were reared at laboratory maintained optimum temperature and relative humidity i.e., 28±1°C and 80%±5, respectively.

Morphometric characterization of larvae

After mounting with Hoyer's medium for morphometric parameters assessment, the dead larvae were scrutinized. The head length, head width, thoracic length, thoracic width, abdominal length, abdominal width, and total length of larvae were determined with the aid of a stereomicroscope (BOECO BST-606, Germany) and method described by (Gunathilaka *et al.*, 2019; Sutiningsih *et al.*, 2019).

Statistical analysis

The statistical determination of the length and width of variations in body segments of the larva was analyzed with Student's t-test using Prism v.7 (GraphPad Software, San Diego, CA, USA). The P-value ($p<0.05$) was considered significant.

Results

The results showed that seasonal thermal fluctuations affected the total body size, head length and width, abdominal length and width, and thoracic length and width of *Ae. aegypti* larvae (Figure 1). It was found that the average head length (0.51±0.01mm) during southwest monsoon significantly increased ($P \leq 0.01$) (0.62 ±0.02mm) during the retreating monsoon season (Figure 1a). Similarly, the average head width in southwest monsoon was 0.54±0.01mm, which significantly increased ($P \leq 0.05$) in retreating monsoon (0.63±0.02mm) (Figure 1b). The abdominal length in southwest monsoon (3.57 ±0.07mm) got significantly increased ($P \leq 0.05$) in retreating monsoon (3.91±0.09mm) (Figure 1c). At the same time the abdominal width in southwest monsoon (0.68±0.02mm) was also significantly increased ($P \leq 0.05$) in retreating monsoon (0.74 ±0.02mm) (Figure 1d). Furthermore, the thoracic length significantly increased ($P \leq 0.05$) during retreating monsoon from 0.83±0.01mm in southwest monsoon to 0.90±0.03mm (Figure 1e). The average thoracic width during southwest monsoon was recorded at 0.95±0.02mm which also significantly increased ($P \leq 0.05$) in retreating monsoon (1.07±0.04mm) (Figure 1f). The overall size of *Ae. aegypti* larvae in Southwest Monsoon was recorded at 4.91±0.05mm that increased highly significantly ($P \leq 0.001$) in retreating monsoon (5.43±0.20mm) (Figure 1g). While the average overall size of the larvae under laboratory temperature were recorded as 4.343±0.40 mm. The temperature variation during Southwest Monsoon and Retreating Monsoon are shown in (Figure 2).

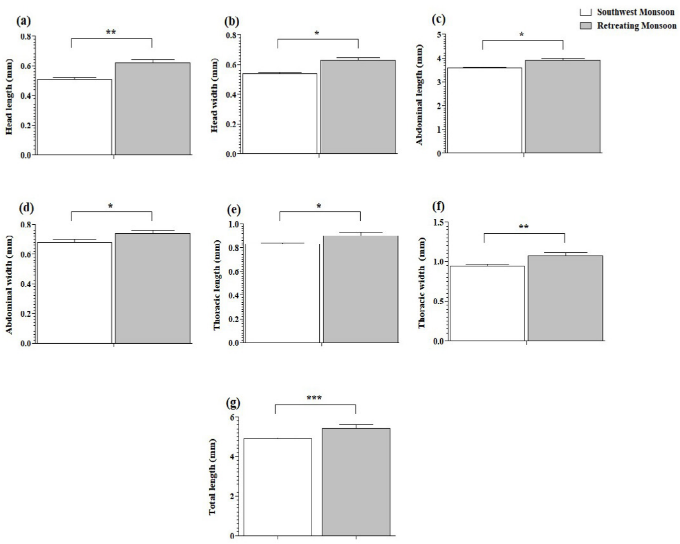
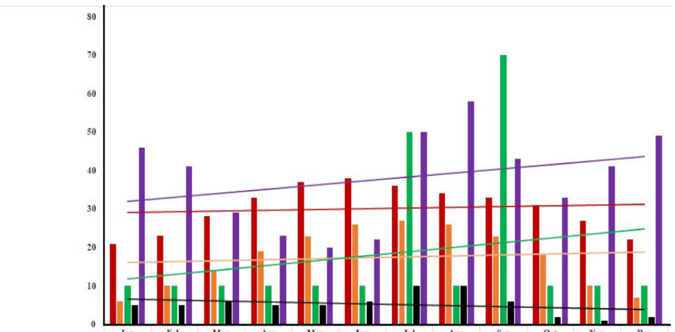


Figure 1: Body features of *Ae. aegypti* larvae (a) head length, (b) head width (c) abdominal length (d) abdominal width (e) thoracic length (f) thoracic width (g) total length. All data are expressed as means ± standard deviation (SD). The significance represented as ns P > 0.05, *P ≤ 0.05, **P ≤ 0.01, ***P ≤ 0.001 in Southwest Monsoon and Retreating Monsoon.



	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
High Temperature (°C)	21	23	28	33	37	38	36	34	33	31	27	22
Low Temperature (°C)	6	10	14	19	23	26	27	26	23	18	10	7
Rainfall (mm)	10	10	10	10	10	10	50	10	70	10	10	10
Rainy Days	5	5	6	5	5	6	10	10	6	2	1	2
Relative Humidity (%)	46	41	29	23	20	22	50	58	43	33	41	49

Figure 2: Variation in different climatic factors (minimum and maximum monthly temperatures, average rainfall and days per month, and average relative humidity) in Lahore.

Discussion

Changing environmental temperatures affect the development stages of mosquitoes and have a significant impact on their population dynamics (Couret and Benedict, 2014). The temperatures between 16°C-34°C are suitable for *Aedes aegypti* development, and at water temperatures below 8°C, the larvae become immobile and die within a few weeks (Cristophers, 1960). In Pakistan, dengue control field staff identify *Aedes* mosquitoes, on the bases of larval and siphon tube size. So, the information about the size is important to minimize the chances of wrong identification of the dengue vectors at the initial stage. Therefore, we conducted this study to observe the difference in body size and different body structures of *Ae. aegypti* larvae in two seasons, i.e., southwest monsoon (June through September) and retreating monsoon (October and November). To our knowledge, this is the first study to assess the morphometric variation of *Aedes aegypti* larvae with the seasonal environment temperature inversion in selected areas of Lahore, Pakistan.

Recent studies also showed that at lower temperatures, the survival of larvae is correlated with food availability in water and intraspecific density (Couret and Benedict, 2014; Couret *et al*., 2014). *Aedes aegypti* larvae with sufficient nutrient supply at cool ambient temperatures (15°C) can remain at a particular age for several months (Andrew and Bar, 2013; Brady *et al*., 2014; Foster and Walker, 2019).

It has been established that a temperature between 31°C-32°C is optimal for larvae to complete their development, and mortality threshold temperature ranges between 14°C and 38°C (Bar-Zeev, 1957, 1958). The survival of mosquitoes during the development stages depends on the regional temperature and their tolerance to cold and heat (Teng and Apperson, 2000).

Previously the pattern of the development time and size of the *A. aegypti* and *A. albopictus* mosquito is affected by changes in the temperature of the surrounding environment when it exceeds the lower critical development threshold (Yang *et al*., 2009). The findings of (Couret *et al*., 2014) showed that changes in the temperature could alter the stage and size of development starting from the larval stage. In a study conducted by (Farjana *et al*., 2012) the rate of development slowed and size increased as temperature decreased.

Conclusion

The study concluded that changes in meteorological conditions during the development of *Aedes aegypti* have a significant effect on the larval size and low temperatures of breeding water significantly increase (P≤0.05) the body size, head, thorax and abdomen of larvae. Climate change affects the immature stages of larval development. The findings might help in improving *Aedes aegypti* control measures and monitoring strategies by better understanding variation in larval development rates under different environmental temperatures. However, the study is limited in scope due to the smaller sample sizes and was carried out in two towns of one district only i.e., district Lahore. We suggest further studies with larger sample size and different settings across the dengue-endemic areas of the country.

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Conflict of interest

The authors have declared no conflict of interest.

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