Bangladesh J. Zool. ***51***(3): 361-369, 2023 - Scientific Note
ISSN: 0304-9027
eISSN: 2408-8455

TEMPERATURE EFFECTS ON THE REPRODUCTIVE PERFORMANCE OF *AEDES AEGYPTI* (DIPTERA: CULICIDAE) MOSQUITOES IN DHAKA CITY, BANGLADESH

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In 2022, Bangladesh experienced unprecedented weather patterns during the monsoon season, characterized by warm temperatures and reduced rainfall. This inconstancy from typical climate conditions in July and August has been observed over the past three decades, demonstrating a concerning trend of rising temperatures and decreased precipitation during the monsoon period. To explore the potential effect of these climate extremes on the dengue vector mosquito, *Aedes aegypti*, this study was conducted in the regions of Keranigonj, Jatrabari, and Uttara in Dhaka city. The study focused on collecting adult mosquitoes, larvae, and pupae to assess their survival fitness. Upon maturing the collected larvae and pupae into adults, we examined their reproductive ability. The findings revealed that during the first gonotrophic cycle, the fecundity and hatchability rate were significantly low, indicating that the initial reproductive capacity of wild caught mosquitoes was severely affected by the extreme climate conditions. However, as the study progressed, the adult mosquitoes exhibited enhanced reproductive capacity in subsequent gonotrophic cycles, reflecting the direct impact of climate extremes on the reproductive capacity. The study highlights the vulnerability of *Ae. aegypti* mosquitoes to climate change and emphasizes the importance of proactive measures to mitigate the impact of extreme weather events on mosquito populations. By understanding and addressing the consequences of climate change on mosquito populations, communities can better protect themselves from associated health risks and work for sustainable solutions in an ever-changing world.

Global climate change will have a critical effect on all forms of life. The environmental changes potentially lead to the extinction of some species, nevertheless, mosquitoes take advantage due to climate variations. Mosquito-borne diseases including malaria, yellow fever, dengue, chikungunya virus, West Nile virus, and zika are significantly influenced by climatic change (Rocklöv and

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 DOI: https://doi.org/10.3329/bjz.v51i3.72069

Dubrow 2020). These diseases are threatening for the world's population that increases a serious global health challenge (Franklions *et al.* 2019). Presently, *Ae. aegypti* has achieved establishment in 167 countries (Wilkerson *et al.* 2020), with a gradual expansion of 1.5% per decade in conducive environmental conditions. It is expected that this trend will accelerate over the course of the next century (Iwamura *et al.* 2020).

Weather events have a significant impact on the species distribution (Khormi and Kumar 2014; Thai and Anders 2011). Temperature impacts on vector behavior, survival, reproductive rates, and the development of the pathogens (Bellone and Failloux 2020). Likewise, precipitation influences vectors to transmit diseases in aquatic stages, and humidity play crucial role transmit diseases by vectors without such stages (Chowdhury *et al.* 2018). The relationship between meteorological factors and disease incidence patterns, with climate variables explaining the geographic spread of most vector-borne diseases (Bouzid *et al.* 2014). Overall, the effects of climate change can be challenging to determine, especially when vectors are protected from weather or when pathogens have extended development periods in nature. Predictive models indicate that dengue transmission is influenced by climate change and other factors, with evidence supporting its increasing prevalence globally (Tran *et al.* 2020). It is crucial to comprehend the impact of severe weather events on the reproductive dynamics of vectors such as *Ae. aegypti*. Currently, there is no effective vaccine, and interventions targeting the vector have limited evidence of effectiveness (Gubler 2011). This understanding is essential for effectively managing vector populations and controlling the transmission of diseases. Through the examination of the reproductive behaviors of these vectors in response to severe weather conditions, we can formulate focused strategies aimed at mitigating their influence and ensuring the protection of public health.

In Dhaka, the capital city of Bangladesh, dengue outbreaks have been a recurring problem, and the primary vector responsible for its transmission is the *Ae. aegypti* mosquito (Rahman *et al.* 2022), where an average annual temperature is 29.7°C, with winter temperatures occasionally dropping below 19.6°C (Haque *et al.* 2023). With an annual rainfall of 1854 mm, which provides favorable habitat for the proliferation and reproduction of *Ae. aegypti* mosquitoes (Haque *et al.* 2023). Several studies demonstrated that *Ae. aegypti* is particularly sensitive to temperature fluctuations, with higher temperatures leading to shorter developmental periods and smaller adult sizes (Delatte *et al.* 2009; Farjana and Tuno 2012; Reinhold *et al.* 2018). In recent years, Bangladesh has faced repeated dengue outbreaks during the monsoon season, particularly in 2019 and 2021. However, in 2022, an unusual phenomenon occurred, as the monsoon season was exceptionally dry, resulting in significantly reduced rainfall. Paradoxically, this dry monsoon was followed by a dengue outbreak starting in September.

To investigate this unusual situation, we conducted a study where we collected wild populations of *Ae. aegypti* mosquitoes in July and August and examined their reproductive capacities. By sampling mosquitoes during this period, we aimed to understand how the mosquitoes responded to the abnormal weather conditions and how it affected their ability to reproduce. This study sought to shed light on the potential factors contributing to the dengue outbreak in the context of a dry monsoon.

In July and August of 2022, *Aedes aegypti* mosquitoes were collected from several locations within Dhaka city, namely Uttara (23° 52' N, 90° 22' E), Jatrabari (23° 71'N, 90° 43' E), and Keranigonj (23° 69' N, 90° 37' E). The larvae of third and fourth instar and pupae were collected from various breeding habitats, such as discarded containers and flower vases, in the areas of Keranigonj and Jatrabari. This was accomplished by utilizing pipettes. Furthermore, we employed the leg catch method (Ndenga *et al.* 2017) to gather adult females from the Uttara region. The larvae, pupae, and adult female specimens were carefully collected and subsequently transferred to a temporary cooler box. They were then transported to the Entomology laboratory for further examination and analysis.

Larvae and pupae were carefully collected and subsequently transferred into designated rearing cups. Throughout the rearing process, the specimens were nourished using fish food, specifically TetraMin (Germany). Newly emerged males and females were retained for the purpose of copulation and were provided with a 3% sucrose solution. The identification of female *Ae. aegypti* was confirmed based on morphological characteristics. Adult female individuals were administered blood meals in order to sustain their gonotrophic cycles.

Adults collected from Uttara were allowed to blood feed until engorged. Similarly, in the case of the Jatrabari and Uttara population, female mosquitoes obtained a blood meal after their emergence. To facilitate oviposition, we place 200 mL plastic cups containing dechlorinated water and paper strips within each of the breeding cages. The number of eggs produced per individual was counted using contrast microscopy. Following a period of seven days, the surviving female underwent an additional blood meal to initiate the second gonotrophic cycle. The eggs were collected, and the total number was counted. During the course of fourteen days, the surviving female specimens were once again subjected to blood feeding for the purpose of initiating their third gonotrophic cycle. To measure the hatchability rate, the previously counted eggs were collected and kept in a wet petri dish. After a few days, the number of larvae and no hatched eggs was counted with the aid of a microscope. The hatching rate for each gonotrophic cycle was

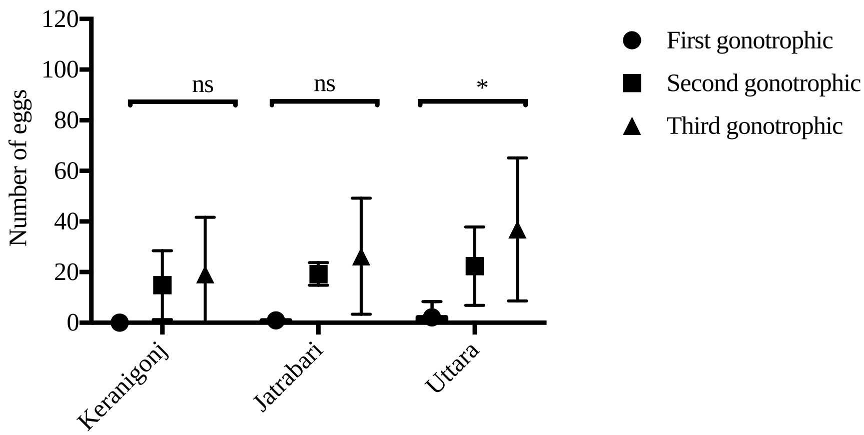

Fig. 1. Number of eggs laid by *Aedes aegypti* in different gonotrophic cycle in three sites.

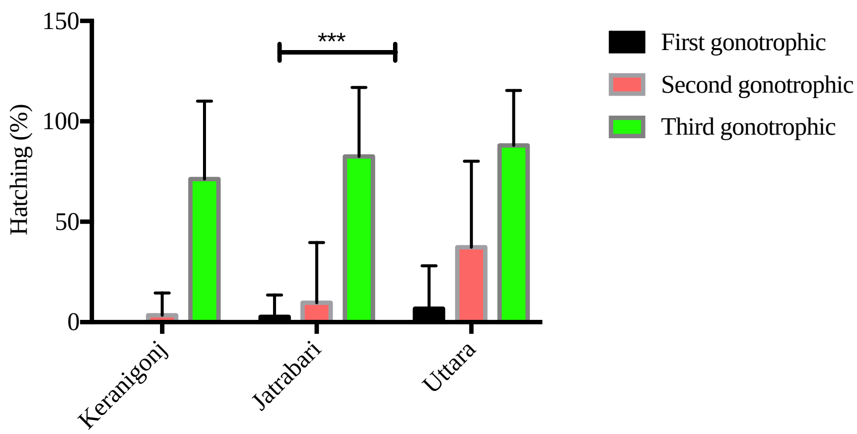

Fig. 2. Hatching rate of *Aedes aegypti* in different gonotrophic cycle in three sites.

calculated as the number of egg hatchings/number of total eggs (hatched and un-hatched) observed. The numbers of larvae hatching from the laid eggs were recorded as a measure of fertility.

Our study demonstrates that higher temperature decreased the reproductive capacity of *Ae. aegypti*. Based on the data presented in Table 1 and Fig. 1, it can be observed that there was a minimal or negligible number of eggs laid during the initial gonotrophic cycle in the field collection. It is important to note that none of the eggs were hatched from larvae that were captured in their natural habitat. The observed hatching rate during the initial gonotrophic cycle was

Table 1. Number of eggs and hatching rate in first, second and third gonotrophic cycle on July or August in 2022 of field collected *Aedes aegypti*.

Sampling location	Collected mosquito	First gonotrophic cycle				Second gonotrophic cycle				Third gonotrophic cycle			
		N female	N Egg	Hatching rate	x^2 test	N female	N Egg	Hatching rate	x^2 test	N female	N Egg	Hatching rate	x^2 test
Keranigonj	Larva	22	0	0		21	312	3.4	A	21	402	71.2	A
Jatrabari	Larva and pupa	32	29	2.7	A	31	598	9.6	B	30	788	82.5	B
Uttara	Adult	34	74	6.7	B	32	760	37.3	C	32	1254	88.1	B

relatively low. During the initial gonotrophic cycle, an observed hatching rate ranging between 2.7% and 6.7% was documented. The unincubated eggs displayed an unusual void, and it was fascinating to note that eggs laid by wild-caught mosquitoes were devoid of contents. Under laboratory conditions, female *Ae. aegypti* mosquitoes have been observed to produce between 27 and 80 eggs and hatching rate ranging from 80% to 90% (Sultana *et al.* 2023). The impact of temperature fluctuations and dietary factors on *Aedes species* exhibited variability (Sultana *et al.* 2022). During the second gonotrophic cycle, a larger number of eggs were deposited; however, it was noted that the hatching rate was comparatively low. A significant discrepancy in hatchability was observed during the second gonotrophic cycle, as evidenced by the Chi-square test results ($P < 0.05$) (Fig. 2). The adult subjects in the laboratory were kept at a controlled temperature of 25 ± 1°C for a period of 23 days or longer. The data presented in Table 1 and Figure 2 indicate a notable increase in both the number of eggs and the hatching rate observed during the third gonotrophic cycle. Following that, a thorough assessment was conducted on all the eggs that had not undergone incubation. The examination was performed using a stereomicroscope, which facilitated the observation of their unique appearance that closely resembled empty eggshells.

The present study entailed the examination of unoccupied eggs, which potentially play a role in the conservation of ovum under adverse environmental conditions. Sultana *et al.* (2022) investigated the egg retention ability of *Aedes albopictus*. The findings indicated that smaller females exhibited a higher inclination to retain a greater number of embryos compared to larger females. Small females, naturally nutritionally deprived and they need to disperse from their breeding sites. So, they will need larger energy reserves for future dispersal and may retain eggs as energy stocks. However, the egg absorption occurs in mosquito (Magnarelli 1983). Significantly reduced hatching rates were observed during both the initial and subsequent gonotrophic cycles. The potential impact of high temperatures and limited rainfall on the hatching process cannot be overlooked. Alam and Tuno (2019) conducted a study on *Ae. albopictus* and documented a scenario that bears resemblance to the one delineated in this discourse. In the year 2022, Bangladesh has encountered a weather pattern that deviates from the norm.

The weather report presents monthly averages for temperature and precipitation for the year 2022, with particular emphasis on July and August as the months characterized by the lowest levels of precipitation and the highest temperatures (BMD 2022). During the months of July and August in the year 2022, reproductive habitats experienced a notable decline in water levels and a scarcity of larvae and pupae. There exists a hypothesis suggesting that a rise in water temperature may impede the growth and maturation of larvae and pupae. The potential for hindered egg-laying exists due to climatic conditions, characterized by reduced precipitation and elevated temperatures, experienced during these particular seasons. Between the months of May and December, there was a noticeable decline in the quantity of embryos during the months of July and August. However, this particular data is currently not being exhibited. The average maximum temperature for the month of July was 33.7 °C, indicating a noteworthy rise of 2.6 °C in comparison to the average maximum temperature observed during the preceding three decades. Furthermore, the mean monthly precipitation in the month of July exhibited a reduction of 57.6% in comparison to the average monthly precipitation. This reduction amounted to 211 millimeters. The year 2022 witnessed a rise in the prevalence of dengue cases, characterized by a discernible upward trajectory, particularly during the period spanning from August to December. The occurrence of dengue epidemics in Bangladesh is significantly threatened by climate change. Hence, the task of forecasting the reaction of *Ae. aegypti* to forthcoming climatic fluctuations is exceedingly challenging.

The research revealed that the initial reproductive capacity of wild-caught mosquitoes was significantly affected by the warm temperatures and reduced rainfall during the monsoon season. However, the mosquitoes demonstrated an enhanced reproductive capacity in subsequent cycles. This highlights the vulnerability of *Ae. aegypti* mosquitoes to climate change and underscores the need for proactive measures to mitigate the impact on mosquito populations. Understanding and addressing these consequences is crucial for protecting communities from associated health risks and promoting sustainable solutions in a changing world.

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(Manuscript received on 23 September; 2023 revised on 15 November 2023)