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Effect of change in mean monthly temperature and pH on the larvae of *Aedes triseriatus* Say, 1823 (Diptera: Culicidae) from North 24 Parganas of West Bengal

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ABSTRACT: *Aedes triseriatus* Say, 1823 commonly called the Eastern Tree Hole mosquito is the vector of La Crosse virus. Its larval density is highest in spring – early summer. Environmental parameters such as temperature and pH affect the life cycle of mosquitoes. Temperature affects every stage of the life cycle of *Aedes* sp. Effect of changes in the mean monthly temperature (MMT) and pH of the larval habitat on the larval count of *A. triseriatus* in North 24 Parganas district of West Bengal, India studies found that the larval count varied significantly with MMT (p value = 0.003) but not with pH (p value = 0.445). The maximal larval count was obtained in the temperature range of 27°C and 36°C with the highest at 33°C. The pH range of 6.65 to 7.05 supported a high larval count with the maximum count obtained at a pH of 7.05. © 2017 Association for Advancement of Entomology

KEY WORDS: *Aedes triseriatus*, life cycle, larval count, mean monthly temperature, pH

INTRODUCTION

Mosquitoes are one of the significant vectors of parasites and pathogens which have a devastating impact on human beings (Gajanana *et al*., 1997). A large portion of the world's population is greatly affected by mosquito borne diseases which are prevalent in more than hundred countries, being mostly prevalent in the tropical ones. Mosquitoes serve as vectors of malaria, yellow fever, dengue fever, chikungunya fever, filariasis and encephalitis. *Aedes triseriatus* or *Ochlerotatus triseriatus* Say, 1823 (Diptera: Culicidae) commonly called the Eastern Tree Hole mosquito or "Tris" is an invasive mosquito species which has been reported for the first time in India. The mosquitoes are terrestrial and are commonly found in forest regions where the canopies can be as high as 27m (Obenauer *et al*., 2009). The range of flight is limited to around 200m (Turell *et al*., 2005). Tree holes, tyres, artificial containers (Borucki *et al*., 2002) etc. serve as perfect breeding sites for its larvae in the urban areas. *A. triseriatus* is a known vector of La Crosse virus in North America which is the most common cause of paediatric arboviral encephalitis in U.S.A with 42 to 172 cases reported annually (Borucki *et al*., 2002). Apart from this, studies have shown *A. triseriatus* to be a competent vector of West Nile virus experimentally (Styer *et al*., 2007) and of Venezuelan equine encephalitis (Davis *et al*., 1966), Eastern equine encephalitis, Western equine encephalitis, Dengue (type I), St Louis encephalitis virus and Yellow Fever virus under laboratory conditions (Freier and Grimstad, 1983).

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Temperature affects the developmental stages of the life cycle of *Aedes* sp. The length of developmental stages has been found to be inversely proportional to enhancement of temperature with the ambient temperature for completion of the life cycle ranging between 20°C and 36°C (Marinho *et al*., 2016). Mortality is higher in environments with high nutrient concentration at 35°C (Farjana *et al*., 2012). Mosquito larvae can survive in a wide range of pH, much greater than those tolerated by other aquatic animals. There is no evidence that pH limits the survival of larvae in nature (Clark *et al*., 2004). The reported pH values for larval habitats range from 3.3 to 8.1 for *Ochlerotatus taeniorhynchus*, 4.4–9.3 for *A. geniculatus*, 3.3–9.2 for *Psorophora confinnis*, and 4.4–9.3 for *Anopheles plumbeus*. *A. flavopictus* has been reared in pH ranging from 2–9 and *Armigeres subalbatus* in the pH range of 2–10 in the laboratory (Clark *et al*., 2004). Thus a study on the effect of change in temperature and pH on the larval counts of important genera of mosquitoes is of great interest at the moment in India from the point of view of development of effective vector-control programme.

The work embodied in this paper probes the effect of changes in the mean monthly temperature (MMT) and pH of the larval habitat on the larval count of *A. triseriatus* in the North 24 Parganas district of West Bengal during the period June, 2015 and June, 2017.

MATERIALS AND METHODS

Mosquito larvae were collected from abandoned tyres, artificial containers and tree holes by immersing clean sampling bottles of 50ml capacity and brought to the Parasitology laboratory of the Department of Zoology, University of Kalyani, Kalyani,West Bengal, India for identification. A total of three samples were collected every month during the period June, 2015 and June, 2017.

Determination of mean monthly temperature (MMT) and pH: Temperatures were recorded using a thermometer on each day of the month. MMT (°C) was calculated as the average of the daily maximum temperatures of the month. Similarly, the average of the daily minimum temperatures of the month yielded the mean monthly minimum temperature. MMT was calculated as the average of the mean monthly maximum temperature and mean monthly minimum temperature. The pH of the water was measured during sample collection using a portable pH meter. The averages of the pH values measured each month during sampling have been used as the final pH values in this study.

Identification of larvae and determination of larval count: The mosquito larvae were identified by studying their body parts under the 10X objective of a phase contrast microscope (Olympus Corporation, Model : KH) following the work of Farajollahi and Price (Farajollahi and Price, 2013). The larval count per sample was ascertained.

Mean larval count (M), standard deviation (SD) and standard error of mean (SE) were calculated using the Graph Pad software (http://graphpad.com/quickcalcs/CImean1/).

Identification of adults: The larvae were reared at 26±2°C in a photoperiod of 12h light and 12h dark on a diet comprising of yeast extract and finely ground dog biscuits in the ratio 1:3 to obtain adults. The adults were identified following the adult pictorial key designed by the Crans and Reed of the Center for Vector Biology, School of Environmental and Biological Sciences, Rutgers, The State University of New Jersey, New Brunswick, NJ 08901-8536 (http://vectorbio.rutgers.edu/Adult_Pictorial_Key.pdf).

Determination of optimal MMT and pH for maximal larval count: The data (Table 1) was organized into six continuous temperature classes and four continuous pH classes. For determining the optimal MMT and pH for maximal larval count, larval counts were plotted against the temperatures and pH values corresponding to the class marks (mean of the upper class limit and lower class limit) of the respective classes (Table 2 and Table 3). The larval counts were expressed as the sum of mean larval count (M) of the class and standard error of mean (SE) (M±SE). The data set corresponding to MMT

= 21°C was omitted from the plots as the mean larval count was zero in this case.

Statistical analysis: A one-way ANOVA was performed to determine whether larval count significantly varied with temperature and pH. Test of homogeneity of variances was performed using the Levene's test. Data analysis was performed using the SPSS software (version 19).

RESULTS AND DISCUSSION

Identification of larvae and adults: The features of the larval body parts were compared with the specimen studied by Farajollahi and Price in 2013 for identification. The specimen under study is a larva of *A. triseriatus* based on the larval body parts (Table 4, Fig. 1). The adults were identified using the adult pictorial key designed by Crans and Reed (http://vectorbio.rutgers.edu/Adult Pictorial Key.pdf). The current specimens are adults of *A. triseriatus* (Fig. 2).

Determination of optimal MMT and pH for maximal larval count: The data obtained during the sampling period (Table 1) was organized into six continuous temperature classes and four continuous pH classes for statistical analysis using SPSS. For determining

Table 1. Showing the Mean Monthly Temperature (MMT) (°C), pH, mean larval count (M), standard deviation (SD) and standard error of mean (SE) as obtained during the period of sampling

Period	MMT (°C)	pH	M	SD	SE
Jun'2015	33.5	6.9	76.33	6.03	3.48
Jul'2015	31.5	7.7	80	5	2.89
Aug'2015	31.5	6.7	79.67	2.52	1.45
Sep'2015	32	7.17	85.67	4.04	2.33
Oct'2015	30.5	8	72.33	8.74	5.04
Nov'2015	29	7.1	71	3.61	2.08
Dec'2015	26	7.4	68.33	3.51	2.03
Jan'2016	25	8	60	11.14	6.43
Feb'2016	29.5	6.7	77.33	6.81	3.93
Mar'2016	32.5	8	94	10.39	6
Apr'2016	36	8	79	5.29	3.06
May'2016	34.5	7.5	80.67	7.51	4.33
Jun'2016	34	7.3	81.67	3.51	2.03
Jul'2016	32	7.7	77.33	6.81	3.93
Aug'2016	31	7	86.67	7.64	4.41
Sep'2016	31	7.8	81.33	4.04	2.33
Oct'2016	29.5	7.62	76.33	5.13	2.96
Nov'2016	26.5	6.9	75.67	4.04	2.33
Dec'2016	25.5	7.37	73.33	12.22	7.06
Jan'2017	20	6.8	52.67	4.62	2.67
Feb'2017	24	7.3	55	10.44	6.03
Mar'2017	27	6.7	88.33	12.58	7.26
Apr'2017	31	7	88.33	2.89	1.67
May'2017	32	7.1	93.33	11.55	6.67
June'2017	31	7.5	87.33	2.52	1.45

Table 2. Showing the temperature classes, class mark, larval count, mean larval count (M), standard deviation (SD) and standard error of mean (SE)

Temperature classes (°C)	Class mark (°C)	Larval count	Mean larval count (M)	Standard Deviation (SD)	Standard Error of Mean (SE)
19.5 – 22.5	21	52.67±2.67	0	0	0
22.5 – 25.5	24	55±6.03 60±6.43	57.5	3.54	2.5
25.5 – 28.5	27	88.33±7.266 8.33±2.03 75.67±2.33 73.33±7.06	76.415	8.512	4.256
28.5 – 31.5	30	88.33±1.67 87.33±1.45 72.33±5.04 71±2.08 77.33±3.93 86.67±4.41 81.33±2.33 76.33±2.96	80.081	6.863	2.426
31.5 – 34.5	33	93.33±6.67 76.33±3.48 80±2.89 79.67±1.45 85.67±2.33 94±6 81.67±2.03 77.33±3.93	83.5	6.879	2.432
34.5 – 37.5	36	79±3.06 80.67±4.33	79.835	1.18	0.835

the optimal MMT and pH for maximal larval count, larval counts (mean larval count of the class (M) ± standard error of mean (SE)) were plotted against the temperatures and pH values corresponding to the class marks (mean of the upper class limit and lower class limit) of the respective classes (Table 2 and Table 3). MMT ranging between 27°C and 36°C showed a high larval count (M±SE) with the highest larval count (M±SE) at 33°C (Fig. 3). The larval count (M±SE) was high in a pH ranging between 6.65 and 7.05 with the maximum number of larvae surviving in the environment whose pH was neutral i.e. 7.05 (Fig. 3).

Levene's test of homogeneity of variances for both temperature and pH signified that the variances among the different classes of temperature and pH were homogeneous. The p values for the Levene's test for temperature and pH were 0.293 and 0.85 respectively. It became evident from one way ANOVA that the larval count varied significantly with the MMT (p value = 0.003) but, not with pH (p value = 0.445).

The study probed the effect of the two environmental parameters namely, mean monthly temperature and pH on the larval count of *A. triseriatus* collected from North 24 Parganas district of West Bengal, India. The district of North 24 Parganas in West Bengal, India covers an area of 4094 km^2 and spans between the coordinates; 22.6168°N and 88.4029°E and has a tropical wet and dry climate. The MMT (values corresponding to the class marks of temperature classes) in North

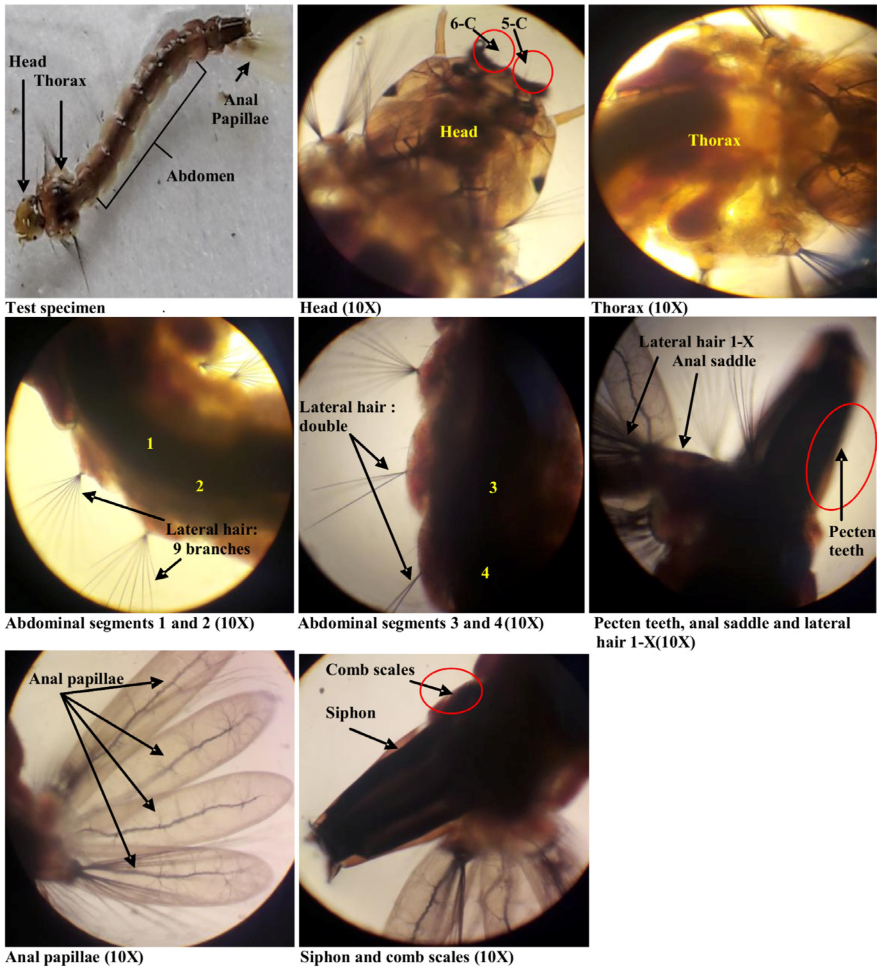
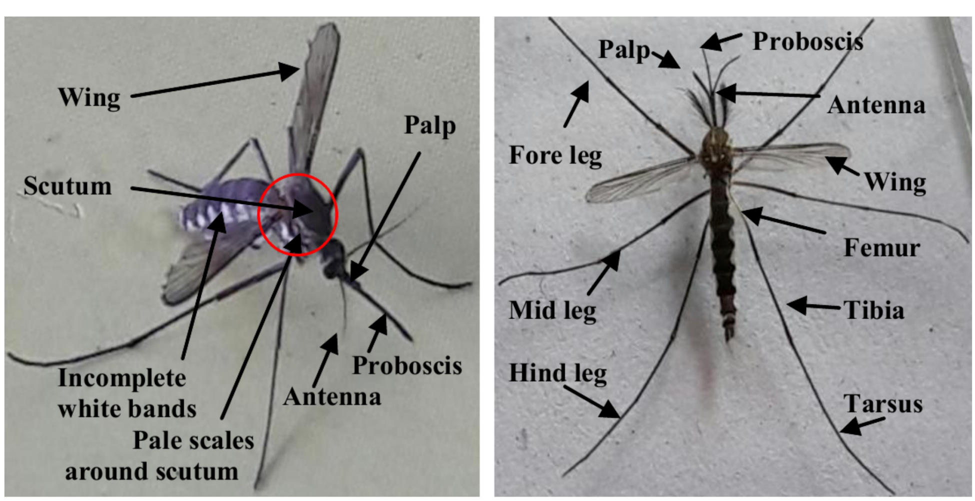
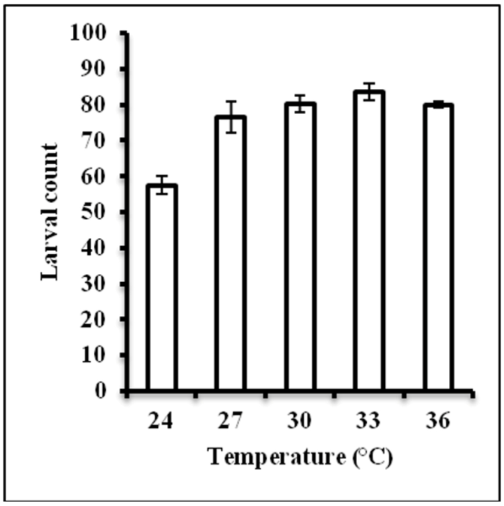


Fig. 1 Showing the body parts of the larval specimen under study



Female mosquito **Male mosquito**

Fig. 2 Showing the body parts of the adult specimens under study



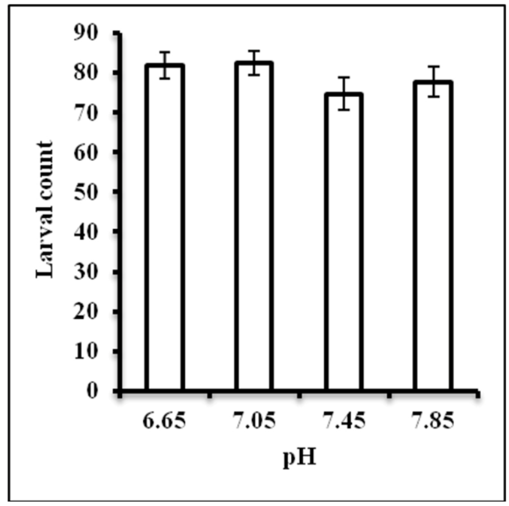


Fig. 3 Showing the plots of larval count versus MMT (°C) and pH

Table 3. Showing the pH classes, class mark, larval count, mean larval count (M), standard deviation (SD) and standard error of mean (SE)

pH classes	Class mark	Larval count	M	SD	SE
6.45 - 6.85	6.65	8.33±7.26 79.67±1.45 77.33±3.93	81.776	5.794	3.345
6.85 – 7.25	7.05	88.33±1.67 93.33±6.67 76.33±3.48 85.67±2.33 71±2.08 86.67±4.41 75.67±2.33	82.428	8.12	3.069
7.25 – 7.65	7.45	55±6.03 87.33±1.45 68.33±2.03 80.67±4.33 81.67±2.03 76.33±2.96 73.33±7.06	74.665	10.621	4.014
7.65 – 8.05	7.85	80±2.89 72.33±5.04 60±6.43 94±67 9±3.06 77.33±3.93 81.33±2.33	77.712	10.231	3.867

Table 4. Comparing the larval body parts of the specimen under study to the one studied by Farajollahi and Price, 2013

Larval body parts of *Aedes triseriatus*	Body parts as described by Farajollahi and Price	Remarks : Present or Undetected in the test specimen
Head hair	Has a box arrangement	Present
Upper head hair 5-C	Single	Present
Lower head hair 6-C	Double/triple	Present
Preantennal 7-C	Multiple	Undetected
Pecten teeth	Evenly placed	Present
Comb scales	Beyond pecten, partly double row.	Present
Anal saddle	Smooth	Present
Siphonal tuft 1-S	Double	Undetected
Lateral hair 1-X	On saddle, multiple	Present
Anal papillae	Unequal and tapering	Present

24 Parganas district of West Bengal, India ranges between 21°C and 36°C which is more or less similar to that of near by areas within the state where the average monthly temperature ranges between 19°C and 30°C (Khan *et al*., 2017). We found that the larval count varies significantly with the MMT (p value = 0.003) within temperature range of 24°C to 36°C with the maximum number of larvae surviving at 33°C. However, the larval count decreased above 33°C which may be due to suppressed embryonic development. The time taken for completing the life cycle and temperature are inversely related (Beserra *et al*., 2009). Above the optimal temperature, rate of development remains steady and may decrease slightly until the temperature reaches an upper limit of around 38°C to 42°C (Eisen *et al.,* 2014) which corroborates the results. Mosquito larvae can tolerate a wide range of pH from 2-10, much greater than those tolerated by other aquatic animals (Clark *et al*., 2004). However, there is no concrete evidence suggesting that pH limits the survival of mosquito larvae in nature. Tolerating sudden changes in pH suggests that major rearrangements pertaining to transporter expression are not required when faced with either a highly acidic or alkaline environment. The ability to withstand rapid changes in pH may be attributed to presence of separate mechanisms for acid and base secretion in larvae, rather than an adaptation providing the capacity to tolerate the sudden changes in pH. This is true in case of our findings wherein the larval count of *A. triseriatus* did not vary significantly with pH (p value = 0.445) although maximum larvae survived at a pH of 7.05.

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