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First report of *Aedes japonicus japonicus* Theobald (Diptera: Culicidae) from India with special reference to the effect of temperature and relative humidity on its larvae

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ABSTRACT: *Aedes japonicus japonicus* Theobald, 1901 an invasive mosquito species is a competent vector of West Nile virus, La Crosse virus and Japanese Encephalitis virus. Environmental parameters such as temperature and relative humidity affect the life cycle of mosquitoes. The length of the developmental stages has been found to vary inversely with an increase in temperature and relative humidity. The effects of habitat and weather parameters on this mosquito are not well documented. Therefore investigations were carried out to identify the larvae and adults of *Ae. japonicus japonicus* and probe the effect of temperature and relative humidity on its larvae. We found by regression analysis that the weather parameters (temperature and relative humidity) and the larval count were positively correlated. Subsequently a one way ANOVA proved that the larval count varied significantly with these two parameters. The maximal larval count was obtained in the temperature range of 25.5 and 37.5°C with the highest at 28.5°C. The relative humidity range of 51.5 to 81.5% supported a high larval count with the maximum count being obtained at 72.5%.

KEY WORDS: Invasive mosquito, life cycle, larval count, weather parameters

INTRODUCTION

Mosquito borne diseases affect a large portion of the world's population being mostly prevalent in the tropical countries. Mosquitoes serve as vectors of many diseases like malaria, yellow fever, dengue fever, chikungunya fever, filariasis etc. *Aedes japonicus japonicus* Theobald, 1901 is enlisted as one among the top hundred invasive mosquitoes. Though the mosquito is endemic to Korea, Japan, Taiwan, Russia and southern China but, it is now also being reported to be found in parts of Europe, New Zealand, Canada and U.S.A. The larvae are slender and appear brownish-yellow or darker in color with a long siphon (Kampen *et al*., 2012). They are found in a variety of natural and artificial aquatic habitats like rock pools, tyres, bird baths, tree holes etc with varying sunlight, elevation and detrital content (Andreadis *et al*., 2001 and Lorenz *et al*., 2013). Adults are relatively large with golden scales on the scutum and are found in forested areas (Andreadis *et al*., 2001). They are active during the daytime and crepuscular hours with the females feeding preferentially on mammals (Turell *et al*., 2005). *Ae. japonicus japonicus* is a known vector of West Nile virus in U.S.A (Andreadis *et al*., 2001;

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Turell *et al*., 2005). Apart from this, laboratory studies have shown it to be a competent carrier of Japanese encephalitis virus (Takashima and Rosen 1989) and La Crosse virus (Sardelis *et al*., 2002). The mosquito is also a moderately effective vector of Saint Louis encephalitis virus (Sardelis *et al*., 2003), Eastern equine encephalitis virus (Sardelis *et al*., 2002), Chikungunya virus, Dengue virus (Schaffner *et al*., 2011) and Rift Valley fever virus (Turell *et al*., 2013).

Temperature and relative humidity affect the stages of the life cycle of *Aedes* sp. The length of these stages has been found to be inversely proportional to increment of these weather parameters. The ambient temperatures range between 20°C and 36°C for successfull completion of its life cycle (Marinho *et al*., 2016). At 35°C mortality is higher in environments with high nutrient concentration (Farjana *et al*., 2012). Intensity of the temperature effect is influenced by relative humidity. High relative humidity supports survival of female mosquitoes and is responsible for higher egg production (fecundity) (Costa de Almeida *et al*., 2010). Females of *Aedes* sp. have significantly higher oviposition rates at 84% relative humidity than those at 34% relative humidity (Canyon *et al*., 1999). Hatching rates of the eggs of *Aedes* sp. is directly proportional to relative humidity values (Costa de Almeida *et al*., 2010). Fecundity, oviposition rates and hatching rates determine the larval counts in the habitat. Thus a study on the effect of change in temperature and relative humidity on the larval counts of important genera of mosquitoes will contribute towards the understanding of their population dynamics and help in developing effective vector-control programmes. The work embodied in this paper probes the effect of change in the temperature and relative humidity on the larval count of *Ae. japonicus japonicus* during the period August, 2015 and August, 2017.

MATERIALS AND METHODS

Collection of larvae: Mosquito larvae were collected as per the protocol (Chakraborti and Bandyopadhyay, 2017) from Hoogly district of West Bengal, India. The Hoogly district in West Bengal, India spans between the coordinates; 22.8963° N, 88.2461° E covering an area of 3149 sq. Km. Larvae were brought for identification to the Parasitology laboratory at the Department of Zoology in the University of Kalyani, Kalyani,West Bengal, India. A total of seven samples were collected every month during the period of study between August, 2015 and August, 2017.

Determination of temperature and relative humidity: The temperature (°C) and relative humidity (%) values were recorded on days of sample collection using a portable thermometer hygrometer every month. The averages of these temperature and relative humidity values were used for the study.

Identification of larvae and determination of larval count: Identification of the mosquito larvae was performed by studying their body parts under the 10X objective of a phase contrast microscope (Olympus Corporation, Model: KH). The work of Farajollahi and Price (Farajollahi and Price, 2013) was followed for identification. Larval counts per sample were determined. The temperature (°C), relative humidity (%), mean larval count (M), standard deviation (SD) and standard error of mean (SE) was determined on a monthly basis throughout the period of study (Table 1). The Graph Pad software (http://graphpad.com/quickcalcs/CImean1/) was used to calculate mean larval count (M), standard deviation (SD) and standard error of mean (SE) throughout.

Identification of adults: The larvae were reared at 27±2°C, 75% relative humidity in a photoperiod of 12h light and 12h dark. They were fed a diet comprising of yeast extract and finely ground dog biscuits in the ratio 1:3 to obtain adults. The adult pictorial keys by William W. Stanuszek of the Saginaw County Mosquito Abatement Commission (Stanuszek, 2013) were followed for identifying the adults.

Determination of optimal temperature and relative humidity for maximal larval count:

Ungrouped data was organized into three continuous temperature classes and seven continuous relative

Table 1. Temperature (°C), relative humidity (%), mean larval count (M), standard deviation (SD) and standard error of mean (SE) as obtained during the period of sampling (M, SD and SE were calculated using Graph Pad software)

Sampling period	Temperature (°C)	Relative humidity (%)	Meanlarval count	Standard deviation	Standard error of mean
Aug'2015	31	75	94.86	15.83	5.98
Sep'2015	32	68	100.14	12.48	4.72
Oct'2015	31.5	63	95.71	10.84	4.10
Nov'2015	28	52	91.29	9.55	3.61
Dec'2015	25	43	47	4.51	1.70
Jan'2016	26.2	41	50	7.92	2.99
Feb'2016	28.4	50	63.71	8.26	3.12
Mar'2016	31.8	51	80.43	9.8	3.7
Apr'2016	35	50	61.43	8.77	3.32
May'2016	35	58	56	9.73	3.68
Jun'2016	34.2	62	69	7.85	2.97
July'2016	32	68	94.57	11.31	4.28
Aug'2016	30.8	76	95.57	11	4.16
Sep'2016	31.2	81	94.14	11.39	4.31
Oct'2016	28.7	71	94.86	10.87	4.11
Nov'2016	26	56	78.57	10.89	4.12
Dec'2016	24.5	45	52	8.33	3.15
Jan'2017	20.1	40	40.14	7.13	2.69
Feb'2017	23	41	53.29	5.19	1.96
Mar'2017	28	57	74.86	9.56	3.61
Apr'2017	32	65	83.57	7	2.64
May'2017	33	70	84.14	7.93	3
Jun'2017	32.3	78	82.43	12.49	4.72
July'2017	31.2	69	94.29	14.29	5.40
Aug'2017	32	72	96.71	13.06	4.94

humidity classes. For determining the optimum temperature and relative humidity which yield maximal larval count, larval counts (M±SE) (values corresponding to the different classes of temperature and relative humidity) were plotted against the temperature and relative humidity values corresponding to the class marks (mean of the upper class limit and lower class limit) of the respective classes.

Regression analysis was done using the Graph Pad software (http://graphpad.com/quickcalcs/linear1/) to probe any correlation between the dependent (larval count) and independent (temperature and relative humidity) variables. A one-way ANOVA was performed to ascertain that the variation of larval count with temperature and relative humidity was significant. 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 larval specimen was slender and dark brownish-yellow in appearance with a big siphon and the details of the larval body parts was identified under the 10X objective of a phase contrast microscope (Table 2, Fig. 1). It was identified as a larva of *Ae. japonicus japonicus* by following the pictorial keys of Farajollahi and Price (Farajollahi and Price, 2013). The adult specimens were dark in appearance with golden scales on scutum. They were identified as adults of *Ae. japonicus japonicus* (Fig. 2) by following the adult pictorial keys of William W. Stanuszek (Stanuszek, 2013).

Determination of optimal temperature and relative humidity for maximal larval count:

A high larval count (M±SE) was obtained in the temperature range of 25.5 to 37.5°C with the maximal larval count being obtained at 28.5°C. The larval count (M±SE) was high in the relative humidity range of 51.5 to 81.5% with the maximum number of larvae surviving at 72.5% (Fig. 4).

Regression analysis established a positive correlation between the dependent (larval count) and independent (temperature and relative humidity) variables (Fig. 3). Regression analysis showed that the larval count significantly varied with change in temperature and relative humidity with the regression equation, r^2 and p values for larval count versus temperature being $y = 2.705x-3.242$, $r^2 = 0.2985$, $p = 0.0047$ and that for larval count versus relative humidity being $y = 1.255x+1.777$, $r^2 = 0.7076$, $p<0.0001$ respectively.

The one way ANOVA proved that the larval count varied significantly with the temperature (ºC) and relative humidity (%) (p value < 0.05). Levene's test of homogeneity of variances for both temperature and relative humidity signified that the variances among the different classes of temperature and relative humidity were homogeneous. The p values for the Levene's test for temperature and relative humidity were 0.205 and 0.064 respectively.

The study was conducted on the effects of the two environmental parameters namely, temperature and relative humidity on the larval count of *Ae. japonicus japonicus*. The Hoogly district in West Bengal, India experiences a tropical wet and dry climate. The temperature (values corresponding to

Table 2. Comparing the larval body parts of the specimen to the one studied by Farajollahi and Price

Larval body parts of *Aedes japonicus japonicus*	Body parts as described by Farajollahi and Price	Remarks: Present or Undetected in the test specimen
Head hair	Straight line arrangement	Present
Upper head hair 5-C	Multiple	Present
Lower head hair 6-C	Multiple	Present
Preantennal 7-C	Multiple	Present
Pecten teeth	Distally detached	Undetected
Comb scales	Within pecten patch	Present
Anal saddle	Heavily spiculated	Present
Siphonal tuft 1-S	Multiple	Present
Lateral hair 1-X	On saddle, single	Present
Anal papillae	Equal and tapering	Present

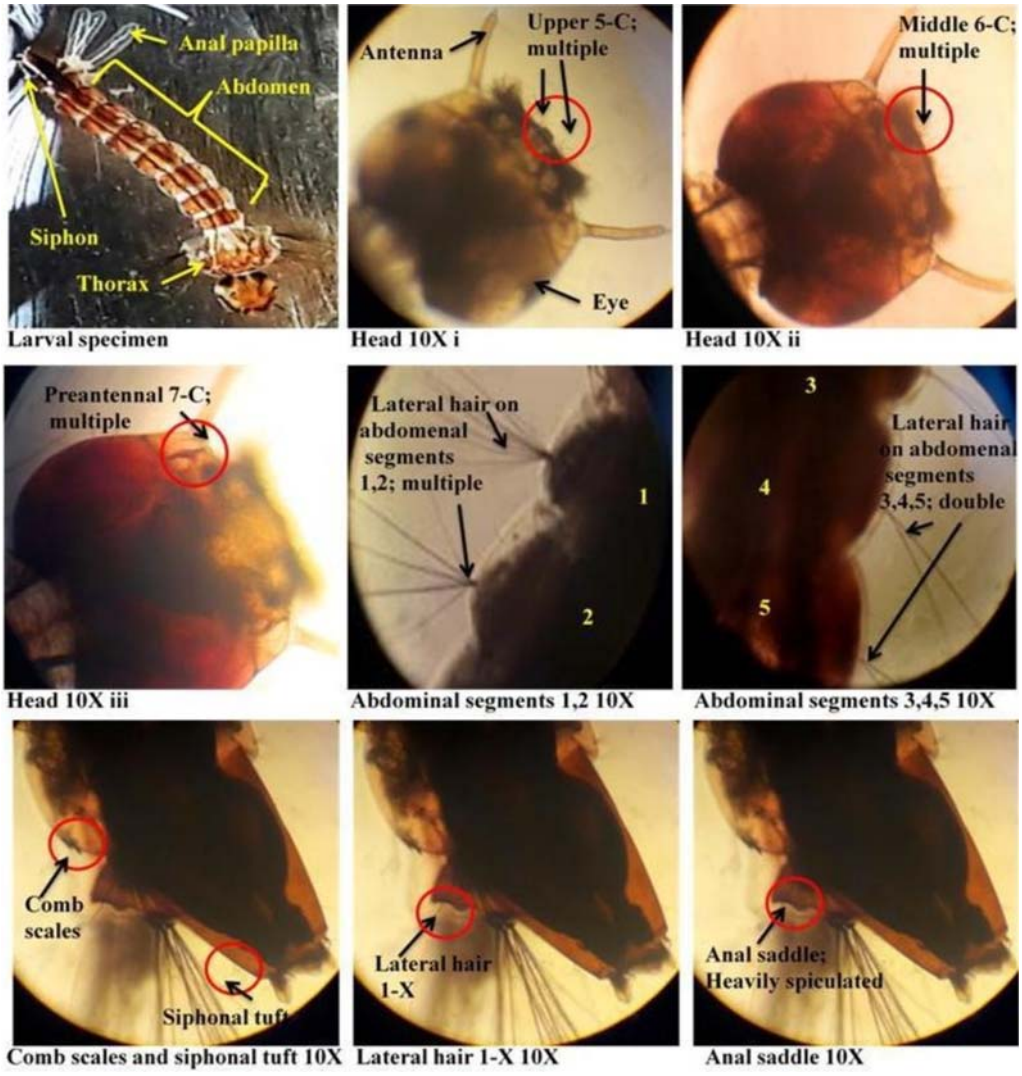

Fig. 1. Features of the body parts of the larva as seen under the 10X objective of a phase contrast microscope

the class marks of temperature classes) in this district ranges between 22.5°C and 34.5°C which is approximately close to that of the average monthly temperatures varying between 19°C and 30°C (Khan *et al*., 2017) in the neighbouring regions. It has been established that the time taken for life cycle completion and temperature are related inversely (Beserra *et al.,* 2009). Although the larval count varied significantly with temperature ($p<0.05$) but a decrease in the larval count was observed above 28.5°C as evident from the study. This may have been due to suppressed development of the embryo. Rise in temperature above the optimum temperature does not decrease the rate of development to a large extent. The rate of development may decrease slightly until the temperature reaches an upper limit of around 38°C to 42°C (Eisen *et al*., 2014). High humidity along with optimum temperatures promotes female survival, fecundity and hatching rates (percentage of larvae produced from total eggs (% of larvae ± SE). The oviposition time is affected by temperature irrespective of relative humidity (Canyon *et al*., 1999; Costa de Almeida *et al*., 2010). A study conducted on *Ae. aegypti* mosquito (Costa de Almeida *et al*., 2010) showed that the females survived for 11 days at 25ºC, 80% relative humidity producing 99.08 ± 3.56 eggs (M±SE) and only for

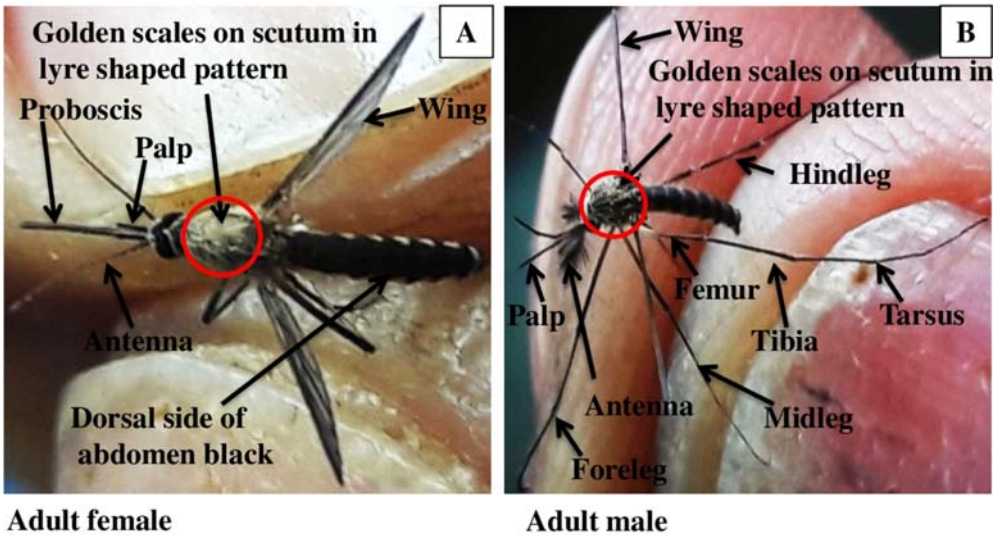

Fig. 2. Features of the body parts of the adult specimens as observed

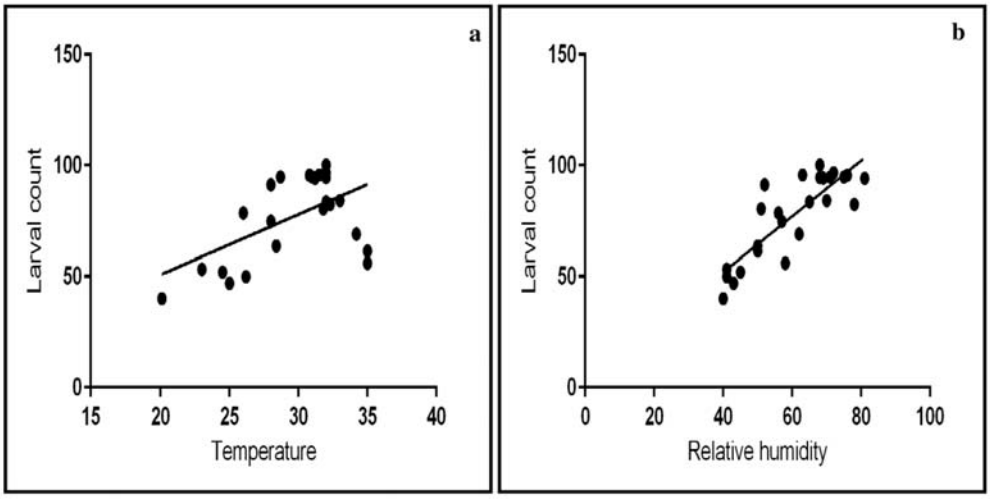

Fig. 3. Regression analysis between the dependent (larval count) and independent (temperature and relative humidity) variables

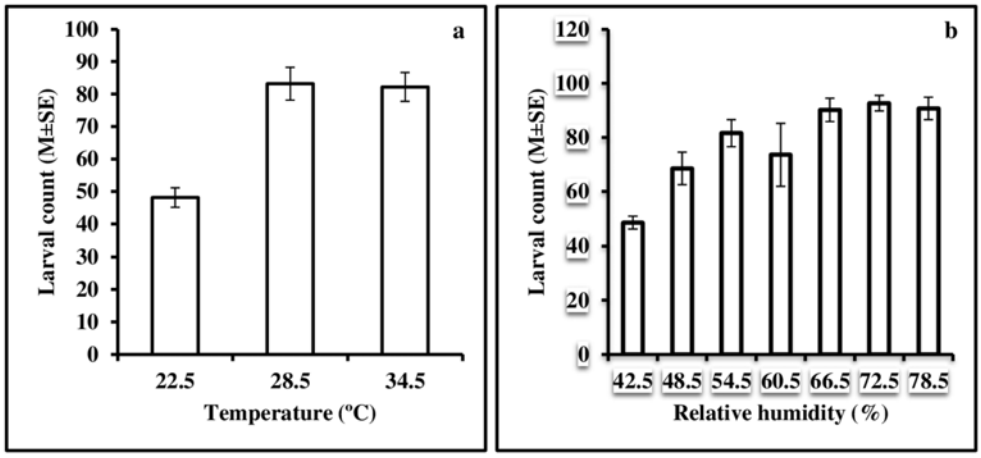

Fig. 4. Larval count (M ± SE) versus temperature (ºC) and relative humidity (RH) (%)

8 days at 25ºC, 60% relative humidity producing 85.99 ± 3.16 eggs (M±SE). Females survived for 7 days at 30ºC under both the conditions of relative humidity producing 75.75 ± 5.03 eggs (M±SE) at 80% relative humidity and 82.89 ± 3.33 eggs (M±SE) at 60% relative humidity. At 35ºC females survived for 5 days under both the humidity conditions although 20.9% females survived at 80% relative humidity and 12% survived at 60% relative humidity. The number of eggs (M±SE) produced were 59.62 ± 3.41 at 35ºC, 80% relative humidity and 54.53 ± 4.81 at 35ºC, 60% relative humidity. The percentage of larvae obtained from eggs (hatching rate) at 60% humidity reduced slowly with an increase in temperature. At 60% relative humidity eggs subjected to 25ºC produced about 10 and 20% more larvae than eggs at 30 and 35°C. At 80% relative humidity, hatching rates remained similar at 25 and 30 ºC i.e. 58.88 ± 4.87% and 70.67 ± 5.56% with a significant reduction at 35 ºC i.e. 43.08 ± 5.89 suggesting that optimum temperature and high relative humidity promotes higher female survival, fecundity and hatching rates. Oviposition time was 8 days at 25ºC, 6 days at 30ºC and 5 days at 35ºC irrespective of relative humidity. These findings corroborate our results i.e. the larval count varies significantly with the temperature and relative humidity (p value < 0.05) within temperature range of 22.5 to 34.5°C (class marks of temperature classes) and relative humidity range of 54.5 to 78.5% (class marks of relative humidity classes). The maximum number of larvae survived at 28.5°C and 72.5% relative humidity.

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