Zoology and Ecology, 2023, Volume 33, Number 2
Online ISSN: 2165-8013
https://doi.org/10.35513/21658005.2023.2.9

DESICCATION TOLERANCE IN LARVAE OF *CULEX QUINQUEFASCIATUS* SAY, 1823 AND *ANOPHELES STEPHENSI* LISTON, 1901 (DIPTERA: CULICIDAE): IMPLICATIONS FOR MOSQUITO CONTROL

Susobhan Datta Mudi, Sampa Banerjee, Krishnasish Bhattacharya, Goutam Kumar Saha, Gautam Aditya*

Department of Zoology, University of Calcutta, 35, Ballygunge Circular Road, Kolkata-700019, India
*Corresponding author. Email: gautamaditya2001@gmail.com
Susobhan Datta Mudi: https://orcid.org/0000-0003-4466-4987
Sampa Banerjee: https://orcid.org/0000-0001-9861-338X
Krishnasish Bhattacharya: https://orcid.org/0009-0007-6729-7872
Goutam Kumar Saha: https://orcid.org/0000-0002-9754-5458
Gautam Aditya: https://orcid.org/0000-0001-9445-879X

Datta Mudi S., Banerjee S., Bhattacharya K., Saha G.K., Aditya G. 2023. Desiccation tolerance in larvae of *Culex quinquefasciatus* Say, 1823 and *Anopheles stephensi* Liston, 1901 (Diptera: Culicidae): implications for mosquito control. *Zoology and Ecology* 33(2), 179–192. https://doi.org/10.35513/21658005.2023.2.9

Article history
Received: 05 April 2023;
accepted 22 October 2023

Keywords:
Desiccation tolerance; *Anopheles stephensi*; *Culex quinquefasciatus*; temperature; mosquito control

Abstract. A laboratory assessment of desiccation tolerance in larval stages of mosquitoes *Anopheles stephensi* Liston, 1901 (Diptera: Culicidae) and *Culex quinquefasciatus* Say, 1823 (Diptera: Culicidae) was carried out using five temperature levels and three levels of exposure duration as explanatory variables. The increase in the duration of exposure to desiccation from 15 min to 60 min was noted to result in a substantial increase in water loss (in mg) in mosquito larvae. Similarly, the increase in temperature levels was observed to cause larval mortality and a substantial decrease in the emergence of adults. Both the temperature and the duration of exposure to desiccation were found to be influential factors in determining survival and subsequent emergence of adults. Desiccation is a primary factor in determining the survival of mosquito larvae, particularly in the conditions where habitat permanence is uncertain, e.g., in temporary pools and containers serving as mosquito larval habitats. The results of the present study provide a glimpse into the effects of desiccation at varied temperatures and exposure durations on the successful emergence of adults in mosquitoes *Cx. quinquefasciatus* and *An. stephensi*. Although primary, the obtained results show that desiccation can be a probable way of regulating the development of mosquito larvae in conditions where the permanence of the habitat for mosquito larvae is uncertain. Further studies on desiccation tolerance may be carried out using different mosquito species that are adapted to temporary pools and containers as sources for breeding.

INTRODUCTION

Mosquitoes (Diptera: Culicidae) are a major threat to the health of millions of people all over the world. Apart from being vectors of various diseases including malaria, filariasis, dengue, Japanese encephalitis, many mosquito species are considered as nuisance pests (Silver 2008; Becker et al. 2010). An estimated 219 million people are known to suffer from various mosquito-borne diseases, the majority of which are recorded in Asian, African, and South American continents (WHO 2017). Among the known vector mosquitoes, *Anopheles stephensi* is an established vector of malaria (Subbarao et al. 2019; WHO 2022; Whittaker et al. 2023; Tian 2023), and *Culex quinquefasciatus* is a major vector of filariasis (WHO 2013; Gopalakrishnan and Veer 2018), posing health risk to several million people globally.

Mosquitoes breed in a wide range of habitats including sewage drains, plastic containers, earthen pots, waste tires, as well as suitable phytotelmata (Silver 2008; Banerjee et al. 2010; Becker et al. 2010). In several habitats, variations in temperature and relative humidity cause water evaporation and increase the risk of desiccation stress in mosquitoes. Any environmental change that acts to reduce the fitness of an organism qualifies as stress (Koehn and Bayne 1989). Desiccation is one of the critical environmental stresses that an insect may face during its life cycle. The capacity of an organism to withstand an arid environment without the loss of viability can be considered as desiccation tolerance. Among mosquitoes, *Orthopodomyia signifera* is the one that is unable to tolerate drought but *Aedes triseriatus* can produce drought-resistant eggs and demonstrate speedy larval development (Bradshaw

and Holzapfel 1988). Desiccation may positively affect the oviposition habitat selection by mosquitoes during post-draught periods (Duchet et al. 2020). However, Benedict et al. (2010) noted significant differences in desiccation tolerance between *An. arabiensis* and *An. gambiae*, where, *An. arabiensis* had a much-reduced desiccation tolerance compared to that of *An. gambiae* (Benedict et al. 2010). For dealing with desiccation or overcoming it, insects use three recognized physiological mechanisms: (i) enhancement of water storage either in the form of metabolic water (molecules of water obtained directly from catabolism) or in that of bulk water (water molecules obtained from sources other than catabolism) or both, (ii) regulation of water loss through respiration and trans-cuticular transpiration, and (iii) development of tolerance to water loss (Gray and Bradley 2005; Archer et al. 2007).The lower cuticular permeability, lower water loss rates, and higher dry and wet masses are the adaptations that have all been shown to be associated with desiccation resistance in Drosophilids (Parsons 1970). In *Drosophila melanogaster,* lower water loss rates and increased bulk water contents were the two most important physiological mechanisms for desiccation resistance (Archer et al. 2007).

The present study into the combined effects of larval exposure to different temperatures and to desiccation for different durations was undertaken with a view to elucidate the regulation of larval development in mosquitoes. The possible mode of regulation of mosquitoes occurring in different larval habitats can be revealed by highlighting desiccation as a regulatory factor. Recent evaluation suggests that humidity is a crucial factor in shaping the population of mosquitoes, directly or jointly with temperature variations (Brown et al. 2023). Due to desiccation during egg or larval stages, the adult population of *Aedes* mosquitoes varies throughout their life history stages (Juliano et al. 2002; Schmidt et al. 2018). Due to the oviposition at different sites with a higher probability of drying up, mosquito larvae face uncertainty regarding habitat permanence and therefore tend to alter their developmental path. This may result in higher mortality rates than can be expected in natural conditions or delayed development time of the concerned mosquito species. In Kolkata, India, the mosquitoes *An. stephensi* and *Cx. quinquefasciatus* are common and breed in varied types of larval habitats with extreme levels of habitat permanence (Pramanik and Raut 2000, 2002; Banerjee et al. 2010). If sewage drains and septic tanks may provide wide access to nutrient-filled water for breeding, smaller habitats such as temporary pools and containers hold small amounts of water. Smaller habitats such as tree holes and containers are filled with limited amounts of water and are prone to drying with increasing temperature, which makes them poor-quality habitats. Mosquito larval development in habitats with uncertain permanence requires developmental pace adjustment, and successful emergence of adults appears to be more challenging. Therefore, in the present study, the loss of water content in mosquito larvae was assessed under exposure to dry conditions and high temperature to provide an explanation for the observed deviation in successful emergence of adult mosquitoes. The results of the study will help to understand the contribution of habitat permanence towards the developmental pace and successful emergence of adult mosquitoes, which is particularly relevant when considering smaller habitats as breeding sites of common mosquitoes in Kolkata and similar regions in India. The results will help to evaluate the potential of individual mosquito larvae to develop under conditions of uncertainty.

MATERIALS AND METHODS

Collection of mosquito larvae

The larvae of *Cx. quinquefasciatus* were collected from sewage drains located at Boral (22°44'59.3"N, 88°37'11.5"E), Kolkata, West Bengal, while *An. stephensi* larvae were collected from different freshwater containers in and around the Ballygunge Science College Campus (22°52'73.6"N, 88°36'28.5"E), Kolkata, West Bengal. All the larvae were collected following Robert et al. (2002) with the help of a plankton net (200 µm mesh size, rectangular in shape), using a circular plastic net (10 mm mesh size round in shape) or a glass dropper depending on the type of mosquito habitat. All the collected larvae were poured into a plastic bag (2 L) or in a 100 ml plastic sample container (Tarsons®, India) with a little amount of water and brought to the laboratory. In the laboratory, all the mosquito larvae were segregated with the help of a glass dropper using an appropriate identifying key (Nagpal et al. 2005; Rattanarithikul et al. 2005; Harbach 2007).

Experimental design

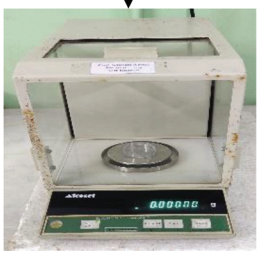
This experiment was conducted on all fully developed late fourth instar larvae. The desiccation experiment (Figure 1) was performed on larvae of each mosquito species at different temperature levels (15 ± 2°C, 20 ± 2°C, 25 ± 2°C, 35 ± 2°C were selected while the room temperature 30 ± 2°C served as control). Tissue paper in two states (completely dry and slightly moist; 0.05ml of water was added to make it moist), and three exposure durations (15, 30, and 60 min) were used as variables in the experiment. The exposure duration was chosen based

on the survival rates of the exposed mosquito larvae. A single mosquito larva was placed on a fully dry tissue paper and again on another dry piece of tissue paper until no water absorption was evident on the tissue paper. Next, each larva was placed in a pan balance (Afcoset®), and the weight to the nearest to 0.1 mg was recorded. Then, in one set of experiment, a larva was transferred to a single well of a six-well plate with a piece of dry tissue paper (2 × 2 cm in size; 15 gram per square meter (GSM) thick) laid on the bottom of each well. In another set of experiments, a larva was transferred to a piece of moist tissue paper (2 × 2 cm in size; 15 GSM thick and 0.05 ml of water) laid on the bottom of each well of a six-well plate. This arrangement prevented contact among the selected larvae for exposure to desiccation. Each six-well plate was then placed in an incubator set with one of the five different temperature conditions. With five different temperatures (15 ± 2°C, 20 ± 2°C, 25 ± 2°C, 35 ± 2°C, and 30 ± 2°C as room temperature), three different exposure durations (15, 30, and 60 min), and

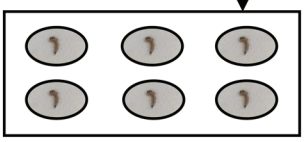
IV instar *Cx. quinquefasciatus* larvae

IV instar *An. stephensi* larvae

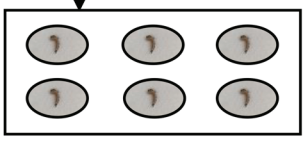
Transfer of larvae onto the tissue paper for water absorption



Larvae weighed in an Afcoset® pan balance nearest to 0.1 mg



Larva placed on a piece of dry tissue paper on a six-well plate



Larva placed on a moist tissue paper on a six-well plate

A total of 30 experimental combinations, involving exposure to five different temperatures (15±2, 20±2, 25±2, 35±2, and 30±2°C as room temperature) for three different exposure durations(15, 30, and 60min), using two different states of the tissue paper (completely dry and slightly moist) were considered for each mosquito species. A total of 120 individuals of mosquito larvae (instar IV) of the two mosquito species were considered for each combination.

After the time allotted for exposure, each larva was weighed in a pan balance again and transferred back to the water for rehydration. Then, all the larvae were checked individually for viability and the obtained data were recorded. Subsequently, the number of the emerged pupae in the samples that were co-exposed to each temperature and each desiccation duration was recorded. Lastly, the number of the emerged mosquito adults was recorded.

Figure 1. Schematic representation of the experimental protocol followed in the present study on desiccation tolerance in *Cx. quinquefasciatus* and *An. stephensi* under exposure to five different temperatures.

two different states of tissue paper (completely dry and slightly moist), a total of 30 experimental combinations for each mosquito species was considered in this present study. A total of 120 individual larvae for each of the two mosquito species were considered for each combination (120 × 5 temperature levels × 3 time intervals × 2 states = 3600 larvae for each mosquito species). The larvae were placed in the incubator on a piece of tissue paper without any cover to desiccate. After the time allowed for desiccation, the individual larva was weighed in a pan balance and its weight was recorded. After that it was transferred back to the water for rehydration in a plastic container (@120 larvae in 200 ml water). Later, all the larvae were individually checked for viability, and the data on survival were recorded. The surviving larvae were further reared with supply of yeast as food to pupae and later to the adult stage individuals. Data on the number of adults emerging was recorded.

Data analysis

Differences in the body weight of the treated larvae were noted and considered as response variables against the duration of exposure to desiccation and temperature levels as explanatory variables (treatment factor). A simple linear regression was constructed to link the differences in the weight loss with the temperature and duration of exposure, for both *Cx. quinquefasciatus* and *An. stephensi* mosquitoes (Zar 1999). Further, a three-way factorial ANOVA was applied for the data on the weight loss against different treatments involving moist or dry substrate, temperature, and the duration of exposure to desiccation in *XL*STAT (Addinsoft 2010). A total of 900 observations on the changes in the body weight against duration of exposure to desiccation and temperature treatments for each of the mosquito species were analyzed.

RESULTS

In this experiment, larvae of both mosquito species, i.e., *An. stephensi* and *Cx. quinquefasciatus,* were found to be capable of tolerating lower temperature and shorter exposure duration. Under dry conditions, larval weight loss was also noted to be lower but as the temperature and duration of exposure increased, larval weight loss increased and the survivability decreased. As for *Cx. quinquefasciatus* on dry tissue paper, the maximum survivability of larvae (114 individuals) was recorded at 20°C and exposure duration of 15 min, the highest pupation rate (60 individuals), and adult emergence (41 individuals) being recorded at 25°C and 15 min exposure duration (Table 1a). Not a single larva was found alive beyond 35°C and 15 min exposure duration (Table 1a). The survivability and pupation rate of *Cx. quinquefasciatus* larvae on moist tissue paper were the highest (118 and 66 individuals, respectively) at 20°C and 15 min exposure duration, but adult emergence was maximum (56 individuals) at 25°C and 15 min exposure duration (Table 1a). Beyond 35°C and 30 min exposure duration, none of the larvae managed to survive (Table 1a). Larval survivability and adult emergence reached peak

Table 1. Data on survival, pupation rate, and adult emergence of 120 instar IV larvae of *Cx. quinquefasciatus* and *An. stephensi* mosquitoes exposed to five different temperatures (15 ± 2°C, 20 ± 2°C, 25 ± 2°C, 35 ± 2°C, 30 ± 2°C as room temperature) for three different exposure durations (15 min, 30 min, 60 min) using two states of tissue paper (Dry and Moist).

State of tissue paper	Temp (°C)	15°C			20°C			25°C			30°C			35°C		
	Exp. Time (min)	15 min	30 min	60 min	15 min	30 min	60 min	15 min	30 min	60 min	15 min	30 min	60 min	15 min	30 min	60 min
a. *Cx. quinquefasciatus*																
Dry	Larva	102	107	82	114	102	94	102	93	73	97	81	26	40	0	0
	pupa	47	41	34	53	50	41	60	45	32	45	37	11	13	0	0
	adult	32	28	24	39	28	20	41	27	17	22	15	6	3	0	0
Moist	Larva	114	106	99	118	110	100	110	99	82	103	97	40	52	31	0
	pupa	60	52	41	66	57	51	65	50	39	54	44	26	31	9	0
	adult	49	41	32	41	35	32	56	32	21	35	23	13	15	4	0
b. *An. stephensi*																
Dry	Larva	87	79	70	98	83	75	77	64	41	69	55	10	17	0	0
	pupa	41	35	31	42	48	37	32	23	15	22	16	2	10	0	0
	adult	19	18	13	25	12	8	14	16	9	8	7	0	4	0	0
Moist	Larva	106	101	90	117	111	104	91	85	63	81	72	27	39	11	0
	pupa	55	46	39	56	52	44	38	26	18	37	27	11	18	1	0
	adult	26	20	17	23	24	13	30	19	11	24	16	4	7	0	0

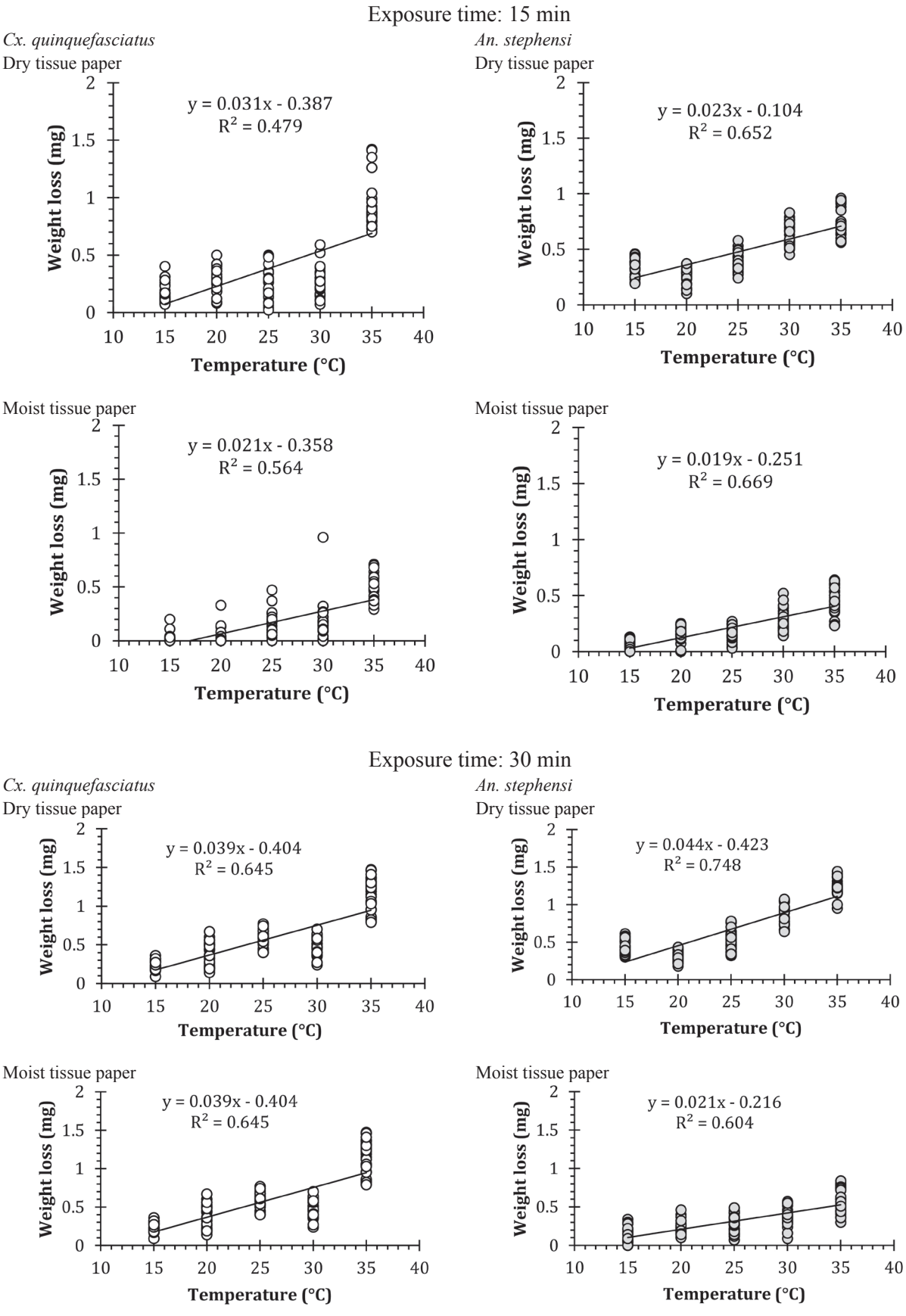


Figure 2. Water loss (in mg), measured as a difference in the body weight of an individual instar IV larva (> 12 hours of age) before and after exposure to the desiccation condition, shown as a function of temperature. In all instances, water loss was observed in at least 120 individual mosquito larvae of *Cx. quinquefasciatus* and *An. stephensi* exposed to a particular temperature for a particular exposure duration (varying between 15 min and 60 min).

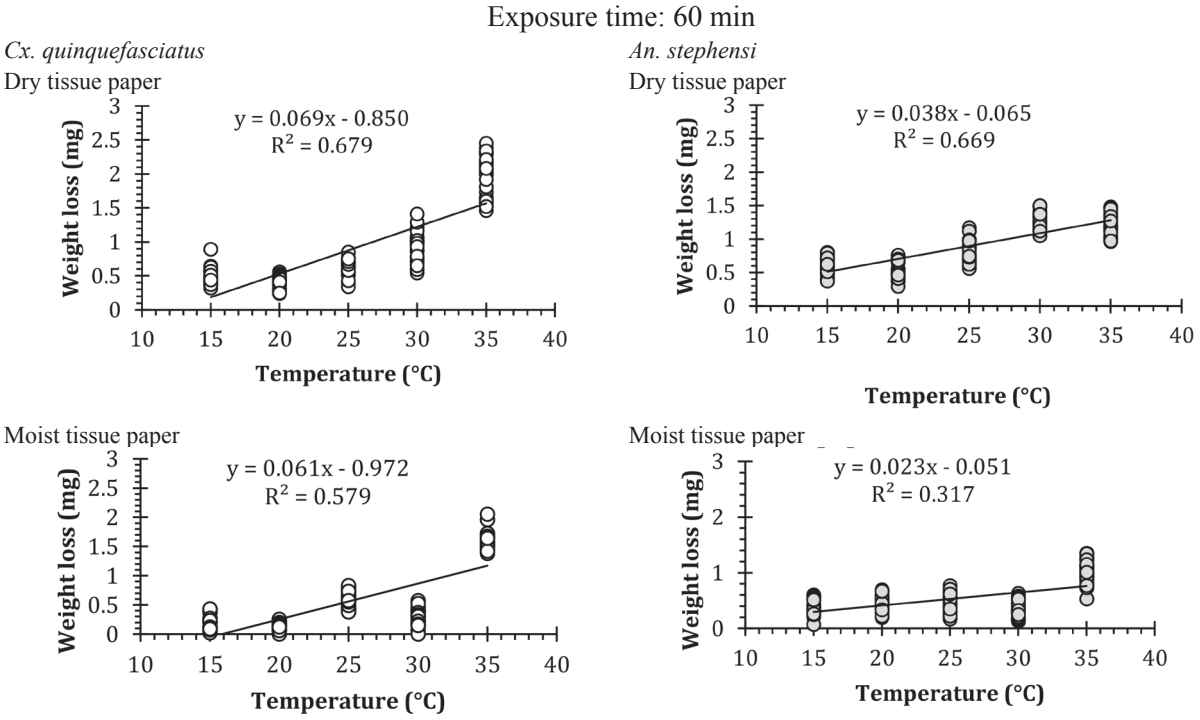


Figure 2 (continued). Water loss (in mg), measured as a difference in the body weight of an individual instar IV larva (> 12 hours of age) before and after exposure to the desiccation condition, shown as a function of temperature. In all instances, water loss was observed in at least 120 individual mosquito larvae of *Cx. quinquefasciatus* and *An. stephensi* exposed to a particular temperature for a particular exposure duration (varying between 15 min and 60 min).

Table 2. The results of ANOVA and the *post hoc* Tukey test on the weight loss (mg) in instar IV *Cx. quinquefasciatus* larvae using the state of tissue paper, exposure duration, and temperature as explanatory variables. The values in bold indicate significance at $p < 0.05$ level. The data represent the observations under two different post-exposure conditions – dry and moist tissue paper, five different temperatures, 15 ± 2, 20 ± 2, 25 ± 2, 35 ± 2, and 30 ± 2 °C as room temperature for control, and three different exposure durations, 15, 30, and 60 min amounting to 900 observations in all for a particular mosquito species.

(a) The ANOVA table

Source of variation	Sum of squares	DF	Mean squares	F
State	13.567	1	13.567	**297.641**
Temperature	121.384	4	30.346	**665.753**
Time of exposure	29.61	2	14.805	**324.808**
Error	40.659	892	0.046	
Total	205.22	899		

(b) The *post hoc* Tukey test for the duration of exposure and temperature levels

	Contrast	Difference	Standardized difference	Critical value
State of tissue	Dry *vs* moist	0.246	**17.252**	1.963
Time of exposure (minutes)	60 *vs* 15	0.442	**25.352**	2.348
	60 *vs* 30	0.261	**14.951**	2.348
	30 *vs* 15	0.181	**10.400**	2.348
Temperature (˚C)	35 *vs* 15	1.005	**44.680**	2.733
	35 *vs* 20	0.970	**43.119**	2.733
	35 *vs* 30	0.812	**36.089**	2.733
	35 *vs* 25	0.779	**34.613**	2.733
	25 *vs* 15	0.227	**10.067**	2.733
	25 *vs* 20	0.191	**8.507**	2.733
	25 *vs* 30	0.033	1.476	2.733
	30 *vs* 15	0.193	**8.591**	2.733
	30 *vs* 20	0.158	**7.031**	2.733
	20 *vs* 15	0.035	1.560	2.733

values (98 and 25 individuals, respectively) at 20°C and 15 min exposure duration, but pupation rate was the highest (48 individuals) at 20°C and 30 min exposure duration for *An. stephensi* on dry tissue paper (Table 1b). None of the larvae survived at 35°C after 15 and 30 min long exposure (Table 1b). Like *Cx. quinquefasciatus*, *An. stephensi* on moist tissue showed the highest larval survivability and pupation rate (117 and 56 individuals, respectively) at 20°C and 15 min exposure duration, but the highest adult emergence (30 individuals) of this species was recorded at 25°C and 15 min exposure duration (Table 1b). There were no alive larvae found at 35°C and after 60 min long exposure (Table 1b).

In both mosquito species (Figs 2 and 3), the larval body weight loss significantly increased with the increase in temperature and exposure duration. *Cx. quinquefasciatus,* on both dry and moist tissue paper, showed the maximum weight loss [1.944 ± 0.057 mg (mean ± SE)] at 35°C and (1.622 ± 0.028 mg) after 60 min long exposure (Figure 4a). For *An. stephensi,* the maximum weight loss was 1.213 ± 0.031 mg and 1.020 ± 0.034 mg, respectively, for both states of tissue paper, i.e., completely dry, and slightly moist (Figure 4b).

The results of ANOVA indicate that the state of tissue (dry and moist), temperature, and exposure duration have a significant effect ($p < 0.05$) on larval weight loss

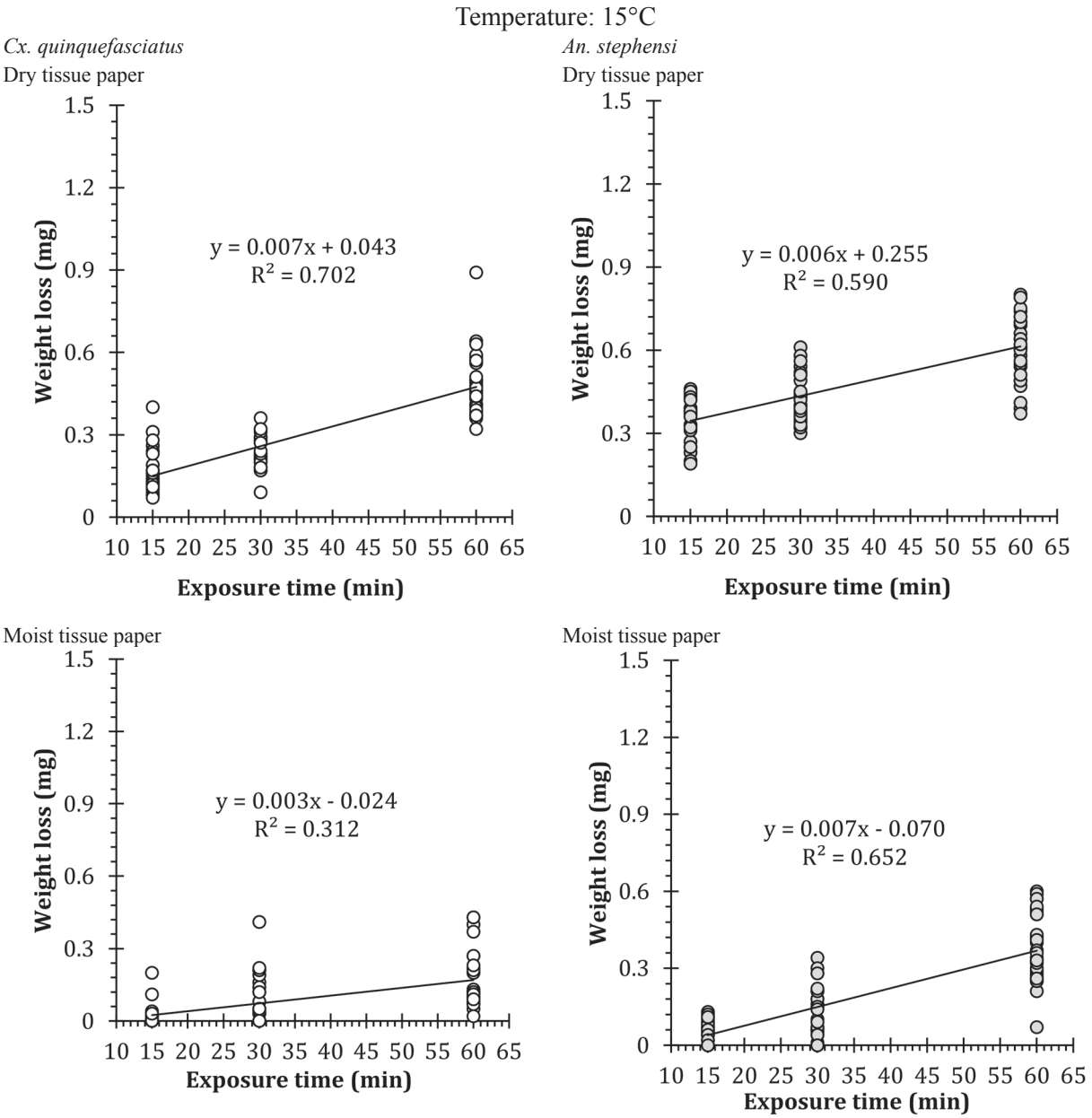


Figure 3. Water loss (in mg), measured as a difference in the body weight of an individual instar IV larva (> 12 hours of age) before and after exposure to the desiccation condition, shown as a function of exposure duration. In all instances, water loss was observed in 120 individual larvae of the two mosquito species, i.e., *Cx. quinquefasciatus* and *An. stephensi*, which were exposed to a particular temperature (varying between 15°C and 35°C) for a particular exposure duration.

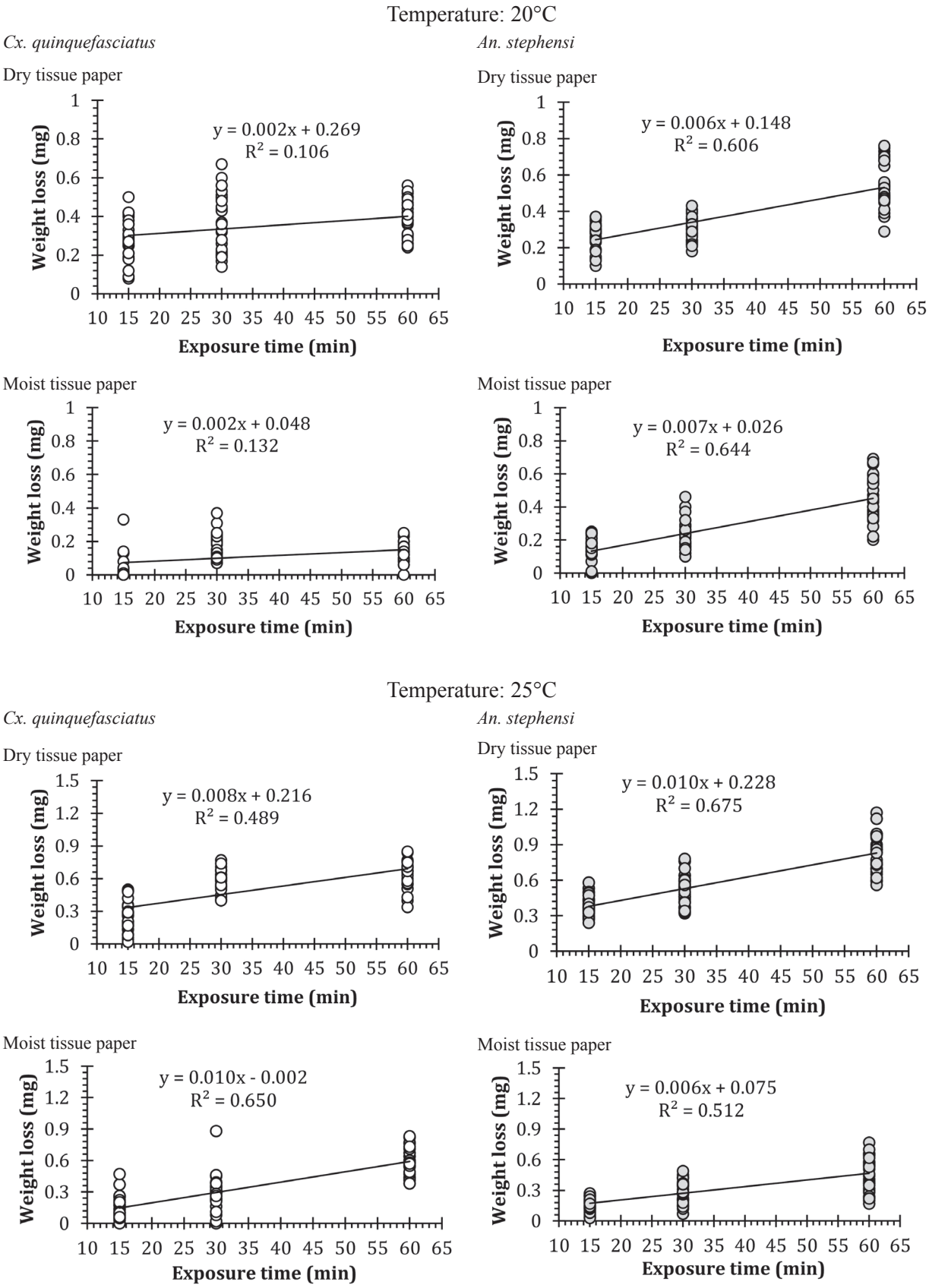


Figure 3 (continued). Water loss (in mg), measured as a difference in the body weight of an individual instar IV larva (> 12 hours of age) before and after exposure to the desiccation condition, shown as a function of exposure duration. In all instances, water loss was observed in 120 individual larvae of the two mosquito species, i.e., *Cx. quinquefasciatus* and *An. stephensi*, which were exposed to a particular temperature (varying between 15°C and 35°C) for a particular exposure duration.

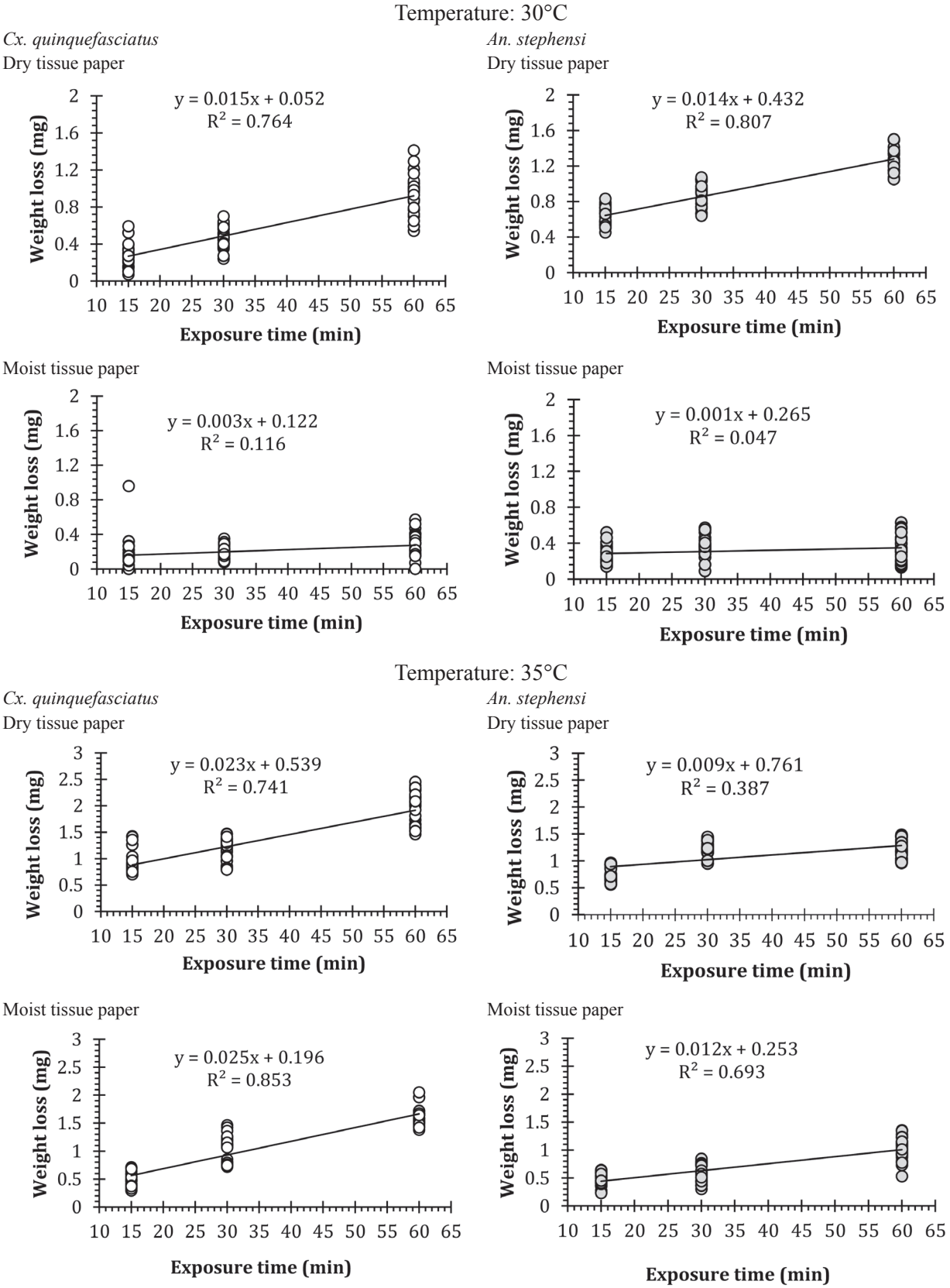
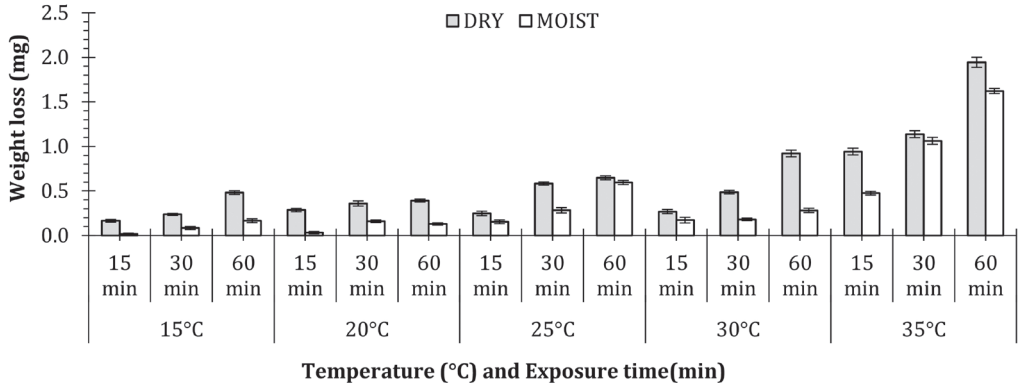


Figure 3 (continued). Water loss (in mg), measured as a difference in the body weight of an individual instar IV larva (> 12 hours of age) before and after exposure to the desiccation condition, shown as a function of exposure duration. In all instances, water loss was observed in 120 individual larvae of the two mosquito species, i.e., *Cx. quinquefasciatus* and *An. stephensi*, which were exposed to a particular temperature (varying between 15°C and 35°C) for a particular exposure duration.

(a) *Cx. quinquefasciatus*



(b) *An. stephensi*

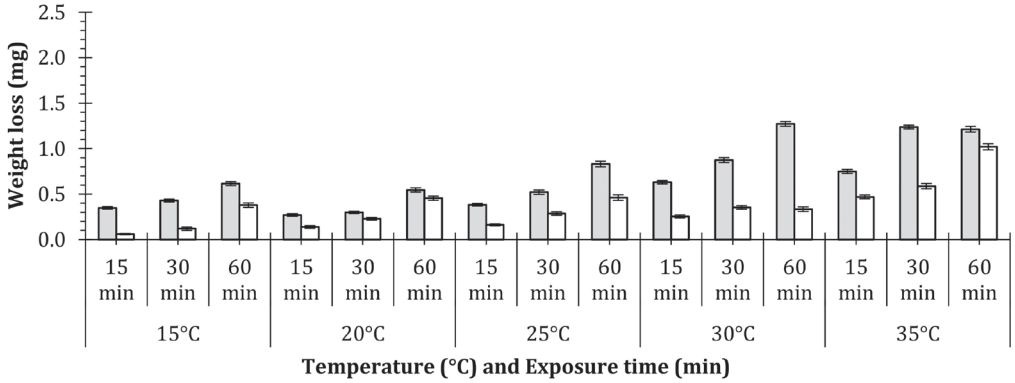


Figure 4. Mean (±SE) values of larval weight loss in two mosquito species: (a) *Cx. quinquefasciatus*, and (b) *An. stephensi* under exposure to five different temperatures (15 ± 2°C, 20 ± 2°C, 25 ± 2°C, 35 ± 2°C, 30 ± 2°C as room temperature), for three different durations (15 min, 30 min, 60 min) using two states (Dry and Moist) of tissue paper.

Table 3. The results of ANOVA and the *post hoc* Tukey (HSD) test on the weight loss (mg) in the instar IV *An. stephensi* larvae using the state of tissue paper, duration of exposure, and temperature as explanatory variables. The values in bold indicate significance at the $p < 0.05$ level. The data represent the observations under two different post-exposure conditions – dry and moist tissue paper, five different temperatures, 15 ± 2, 20 ± 2, 25 ± 2, 35 ± 2, and 30 ± 2 °C as room temperature for control, and three different exposure durations, 15, 30, and 60 min amounting to 900 observations in all for a particular mosquito species. In all instances, the instar IV larva was considered for the experiment.

(a) The ANOVA table

Source of variation	Sum of squares	DF	Mean squares	F
State	24.095	1	24.095	**865.425**
Temperature	39.991	4	9.998	**359.090**
Time of exposure	20.337	2	10.169	**365.233**
Error	24.835	892	0.028	
Total	109.258	899		

(b) The *post hoc* Tukey test for the duration of exposure and temperature levels

	Contrast	Difference	Standardized difference	Critical value
State of tissue	Dry *vs* moist	0.327	**29.418**	1.963
Time of exposure (minutes)	60 *vs* 15	0.366	**26.855**	2.348
	60 *vs* 30	0.219	**16.067**	2.348
	30 *vs* 15	0.147	**10.787**	2.348
Temperature (˚C)	35 *vs* 20	0.556	**31.634**	2.733
	35 *vs* 15	0.554	**31.526**	2.733
	35 *vs* 30	0.438	**24.925**	2.733
	35 *vs* 30	0.260	**14.770**	2.733
	30 *vs* 20	0.297	**16.864**	2.733
	30 *vs* 15	0.295	**16.757**	2.733
	30 *vs* 30	0.179	**10.155**	2.733
	30 *vs* 20	0.118	**6.709**	2.733
	30 *vs* 15	0.116	**6.602**	2.733
	15 *vs* 20	0.002	0.107	2.733

in both mosquito species. In the case of *Cx. quinquefasciatus,* temperature proved to exert a significant effect ($F = 665.75$, df = 4, 892, $p < 0.0001$) on larval weight loss (Table 2). The trend remained the same for both states of tissue paper and duration of exposure. The results of the *post hoc* Tukey test (Table 2) showed that all factors have a significant effect on larval weight loss except temperatures between 15°C and 20°C, and between 25°C and 30°C. As for *An. stephensi,* the state of tissue paper, i.e., dry and moist, had a significant effect ($F = 865.42$, df = 1, 892; $p < 0.0001$) on larval weight loss (Table 3). Temperature and exposure duration also followed the same trend. The results of the *post hoc* Tukey test (Table 3) showed that all parameters have a significant effect on larval weight loss except temperatures between 15°C and 20°C.

DISCUSSION

In mosquitoes and similar insects with short life cycles, diverse life history stages, and adaptation to two distinct environments, the larval development time is a crucial factor for the successful life history accomplishment (Honěk 1993; Danks 2006). Variations in the time of larval development can cause alterations in the expression of life history traits and, therefore, impact fitness. The faster life cycle is linked with the completion of larval development at a faster pace but with an optimal and adequate body size to retain the functions indispensable for survival, reproduction, and reproductive success. In general, a shorter larval development implies a small-sized adult that would render an individual low in reproductive success (Renshaw et al. 1994), while a longer larval development would mean exposure to larger risks against the known and unknown adverse factors in the concerned larval habitats (Romero and Srivastava 2010). Thus, a successful completion of the life cycle includes a trade-off between the larval development and the growth pattern (Romero and Srivastava 2010). This may be reflected in the variations of life-history traits that are linked with reproductive success and fitness of mosquitoes (Agnew et al. 2000; Silver 2008; Mohan et al. 2017; Banerjee et al. 2017a, b). However, a prerequisite for the successful completion of the life cycle and reproductive success is habitat permanence. During development in freshwater larval habitats, mosquitoes grow at a faster pace due to undisturbed conditions of the habitat. Habitat permanence provides a cue for the pace of larval development and overall growth, thereby contributing, as a factor, to the maintenance of the mosquito population status (Merritt et al. 1992). In many instances, the growth of mosquitoes is affected by the daily per capita availability of nutrients. Nonetheless, the water loss in mosquito larvae is a deciding factor in determining their growth and development (Benedict et al. 2010). Although desiccation effects may vary among species and individuals within a species as well, the prolonged waterless condition of larval habitats forces mosquitoes to develop tolerance to water loss. In the present instance of the two different mosquito species examined, *Cx. quinquefasciatus* exhibited tolerance to a great variety of conditions, while *An. stephensi* preferred fresh and clean water for breeding. Although the effects of desiccation may vary between the two species (as reflected in regression equations, Figs 2 and 3), the larval stages of both mosquito species proved to be capable of withstanding dry conditions for a period of at least 3 days (Beier et al. 1990; Minakawa et al. 2001). The tolerance of the eggs of *Ae. aegypti* and *Ae. albopictus* to desiccation influenced competitive interactions and the dominance of one species (Juliano et al. 2002). Such effects manifest themselves in adult stages of mosquitoes as well, where the interactions between temperature and humidity determine the longevity of adult *Ae. aegypti* and *Ae. albopictus* mosquitoes (Schmidt et al. 2018).

The presence of adequate water in the body ensures the homeostatic condition in developing larvae. The progress of larval development is smooth and sequential if larvae are in an adequate physiological condition. The availability of sufficient water amount within the body serves as a prerequisite for the normal progression of development in mosquito larvae. In situations where water loss is common, larval development may slow down or reverse the pattern and pace to a large extent. As observed in the present instance, the loss of water caused larval development to change its course. The pattern in both *An. stephensi* and *Cx. quinquefasciatus* was similar but differing in the extent of response. The proportion of mosquito larvae reaching adulthood remained considerably low, proving that water content in the body is a significant contributor to the development. Desiccation influences the path and outcome of development in many arthropods, snails, and annelids (Bradley et al. 1999; Aboagye-Antwi and Tripet 2010; Glasheen et al. 2017; Kalinda and Chimbari 2022). Desiccation-induced water loss leads to delayed development and eventual death of individuals. The ambient temperature and evaporation are the two factors that control the desiccation impact on invertebrates such as insects, snails, and annelids (Sota and Mogi 1992; Asami 1993; Juliano et al. 2002). With an increase in temperature, the desiccation rate changes, increasing the number of individuals prone to high water loss. Water loss triggers various physiological processes eventually leading to death. At higher temperatures, the effect of desiccation was found to be considerably higher with a cascading effect on the physiology and mortality of the species. Similarly, the duration of exposure to desiccation appears to be a factor determining the loss of water and its effects on the physiological system that impairs normal development of mosquitoes. Long expo-

sure to dry conditions in combination with high ambient temperature was found to induce similar trends towards reduced survivorship and high mortality in *An. stephensi* and *Cx. quinquefasciatus*. Although further studies are required to prove the expedience of using desiccation for mosquito regulation purposes, the temperature- and exposure duration-dependent variations in the survivorship of *An. stephensi* and *Cx. quinquefasciatus* mosquitoes determined in this study were prominent. In the case of the fall armyworm (noctuid moth) *Spodoptera frugiperda*, the body water content and the water loss rate increased with the age across larval instar stages. However, the desiccation pre-treatment was not found to influence temperature tolerance and fecundity (Keosentse et al. 2022). Larvae of both *Cx. quinquefasciatus* and *An. stephensi* exhibited a similar pattern of desiccation tolerance under varying temperature and exposure duration to dry condition. In field conditions, particularly, in small larval habitats such as phytotelmata and puddles, water content is low and prone to fast drying. Under such a scenario, mosquito larvae may face intermittent habitat drying and increased risk of mortality. If desiccation tolerance is a determining factor in successful completion of the life cycle, the choice of habitat for oviposition may remain critical. Further studies into the effects of temperature and desiccation tolerance may provide additional insights into mosquito population dynamics.

ACKNOWLEDGEMENT

We thank Prof. Bimalendu B. Nath, Director, MIE-Savitribai Phule Pune University, Institute of Higher Education, Doha, Qatar, and another anonymous reviewer for the comments on the earlier version of the manuscript. We thank Dr. Denis Copilas-Ciocianu, Editor, for his constructive comments on the earlier version of the manuscript. We gratefully acknowledge the English language correction and enhancement of this manuscript by Ms. Laima Monkienė. We are grateful to the Head, Department of Zoology, University of Calcutta for the facilities provided in carrying out this experimental study. SDM acknowledges UGC [Ref. No.:685/ (CSIR-UGC NET JUNE 2018), Roll-341095, dated 15/04/2019] for the financial support. SB acknowledges Department of Science and Technology, Government of India, under DST-INSPIRE fellowship (sanction number: DST/INSPIRE Fellowship/2013/645, dated 17.12.13).

Conflict of Interest: As authors of this article, we declare no conflict of interest.

Author's contribution: Conceptualized and compiled by GA and GKS; Field collections and laboratory experiment by SDM, KB and SB; data analysis GA, SDM and SB; Draft compilation SDM and SB and final compilation GA and SDM.

Data availability: The data pertaining to the experiments and results of this experimental study can be made available upon authentic and reasonable request.

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