

EFFECTS OF HIGH TEMPERATURE ON THE EGGS OF *Aedes Aegypti* (L.) AND SUBSEQUENT STAGES DEVELOPED THERE FROM

HUMAYUN REZA KHAN* AND FARJANA RAHMAN

Department of Zoology, University of Dhaka, Dhaka-1000, Bangladesh

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Abstract

The eggs laid by the emerged *Aedes aegypti* (L.) (Diptera: Culicidae) females were treated with high temperature at 35°C at different exposure periods, viz., half an hour, one, two, four, eight and 24 hours, separately along with their control. At different exposure periods at 35°C and control - the percentage of egg hatching ranged from 59 to 97; larval and pupal mortality ranged from zero to 12 and 3 to 14 per cent, respectively. Larval and pupal periods were 76 to 115 and 33 to 42 hours, respectively. Lengths of 2nd, 3rd, 4th instars were 3.615 to 4.518; 5.575 to 6.455 and 7.13 to 7.62 mm, respectively. Mean diameters of the head capsule of 1st, 2nd, 3rd and 4th instar larvae were 0.243 to 0.336, 0.387 to 0.476, 0.644 to 0.695 and 0.723 to 0.907 mm, respectively. Length of the cephalothorax of pupae was 1.49 to 1.907 mm. Body lengths of male and female adults were 2.9 to 3.00 and 3.33 to 3.51 mm, respectively.

Introduction

Aedes aegypti (L.) (Diptera: Culicidae) is a dangerous mosquito species. It is found in Dhaka city⁽¹⁻⁴⁾. It is suspected to transmit human diseases in the city⁽⁵⁾ and plays a role in the transmission of diseases like dengue, hemorrhagic fever, yellow fever⁽⁶⁾. The people of the city are threatened by this mosquito species. During March-August, 2000, Bangladesh witnessed a large-scale outbreak of dengue fever mainly in the urban areas of the country. *Aedes aegypti* has not been reported from the rural areas of Bangladesh⁽⁷⁾. One possible reason might be due to the fact that this mosquito species prefer artificial containers to natural containers, and its breeding containers are readily and largely available in the urban areas.

Aedes aegypti is exclusively container breeder. Its immature stages are found in containers with water and the most frequently encountered artificial and natural breeding site were: used tyres, discarded tins, plastic containers, abandoned car parts, brick holes, dead leaves on ground, coconut shell, flower vases, tree holes and rock pools^(8,9). Adult mosquitoes usually stay at bushes around houses, dark corners of room, storehouse, and back side of furniture. Most of them come out to feed at dawn and dusk.

*Author for correspondence: <hrkha@hotmail.com>.

Temperature has an impact upon insects in various ways. The extremes of temperature limit insect activities both in space and time. Their rates of metabolism and consequently those of growth, reproduction and general behaviour are largely controlled by temperature. The most important factors in the environment which influence the physiology of insects are temperature and humidity⁽¹⁰⁾. The higher temperature has a harmful influence on the development of insect⁽¹¹⁾. The optimum temperature for the insect species is generally taken to be 28°C; any increase above this appears to be increasingly unfavourable, and over 40°C it is quickly fatal and insects die from the effects of heat⁽¹²⁾. The effects of temperature on various stages of mosquito were studied by Hagstrum and Workman⁽¹³⁾, Nayar⁽¹⁴⁾, Dakshinamurty and Sharma⁽¹⁵⁾.

The present paper deals with the effects of high temperature on the eggs of *Ae. aegypti* and the stages developed there from after hatching. The following aspects were observed after the eggs were exposed to high temperature in different exposure durations: the hatching efficiency of the eggs, the mortality rates of the larvae and the pupae; the durations of the larval and pupal stages; morphometry of some external body parts; and the fecundity of the female adults emerged.

Materials and Methods

The larvae of *Ae. aegypti* were collected from the earthen pots and aquaria placed in the Zoological garden, Curzon Hall campus, University of Dhaka. The larvae were collected with the help of a large dropper. The collected larvae were kept in a clean beaker and brought to the laboratory. They were then washed gently in tap water for several times to clean those from impurities. The larvae were then transferred to another beaker containing clean water. Ground glucose biscuits were provided the larvae as their food. The collected larvae were placed on slides within a few drops of water, examined under a compound microscope and identified following service⁽¹⁶⁾. The rearing of the mosquito and heat-experiment was carried out at an ambient environment (temperature $28 \pm 4^\circ\text{C}$ and 70 - 80% RH) of the Entomological laboratory in the Department of Zoology, University of Dhaka.

The collected larvae were kept in a plastic bowl containing tap water and provided with ground biscuits as their foods. The larvae were transferred from one bowl to another, whenever necessary, with the help of a large dropper. The water was changed and ground biscuits were provided daily. Similar procedure was followed to rear the subsequent 2nd, 3rd and 4th instar larvae.

The 4th instar larvae moulted into pupae, which is a non-feeding stage in the life cycle of mosquito. The pupae were separated daily from the larval bowl with the use of a dropper and kept them in a plastic bowl which was previously filled with tap water. Since pupae do not take any food⁽¹⁷⁾, no food was, therefore, supplied to them. The plastic

bowl with the pupae was kept in a mosquito rearing cage for the emergence of adult mosquito.

After emergence inside the cage, the adults were provided with 10% glucose solution daily as their food. The glucose solution was soaked in a cotton wad and placed it on a Petri dish, which was then kept inside the cage. The male mosquito only takes glucose solution as food throughout their life time. For the first two or three days of emergence, the females were also fed with the glucose solution.

Blood feeding is required for the nourishment and maturation of the eggs of the mosquito. From the 3rd day after emergence, the adult female mosquitoes were allowed to feed on blood meal from a pigeon. The feather was removed from the breast region of the pigeon and kept in a tight small iron cage which could easily be placed inside the rearing cage. The blood feeding was initiated on the 4th day after adult emergence and continued as long as the females were alive. After taking the blood meal, the abdomen of the females became large and reddish.

After taking blood meal, the females mated with the males. A plastic bowl containing tap water was taken and a filter paper strip was placed around the rim of the water in order that half of the paper was emerged in it. The bowl was then placed inside the rearing cage for the females to oviposit. The eggs were laid singly on the filter paper, abundantly a little bit above the water edge; sometimes the females laid eggs on the water surface singly. The number of eggs laid per female was recorded. The eggs containing filter paper were removed and left at room environment for five - seven days for drying. A drought condition is required for the maturation and development of the eggs of *Ae. aegypti*⁽¹⁷⁾.

After desiccation, the eggs were dipped into tap water in a plastic bowl. When flooded with water, the eggs were hatched within a few minutes. The larvae were then reared again following the above procedure to obtain a continuous supply of adequate larvae for the experiment.

Only the healthy eggs were selected for temperature treatment. The eggs of *Ae. aegypti* in a group of 50 in each of three replicates were used for this experiment. The eggs were exposed to 35°C in an incubator (SLI-600L, Japan) for half an hour, one, two, four, eight and 24 hours, separately. A control treatment of three replicates was also taken in which the eggs were not treated with temperature.

Six Petri dishes with filter paper had been kept in an incubator at 35°C for 24 hours before the heat-treatment began. Three strips of filter paper with 50 eggs of *Ae. aegypti* each, were then placed on each Petri dish. For each of the above mentioned exposure treatment, one Petri dish was taken out of the incubator and kept it at room temperature and humidity for five minute for cooling. The three strips with eggs were then dipped into the tap water contained in three plastic bowls in an ambient laboratory condition. The eggs were immersed in the water for 24 hours. The number of first instar larvae

hatched in 24 hours in the water. The same procedure was followed for all exposure periods, i.e. half an hour, one, two, four, eight and 24 hours, separately. The data were recorded on the following aspects: Hatching efficiency of the temperature treated eggs; Larval mortality; Pupal mortality; Larval duration; Pupal duration; Measurements of larval length and its head capsule; Measurements of pupal length and its cephalothorax; Size of male and female adults emerged and fecundity.

The data were reported as arithmetic mean \pm standard deviation (Sd). ANOVA was applied on the data to assess the treatment effect. When F-values indicated significant difference, DMRT was employed to discern specific difference among the treatments. All the statistical analyses were done on a computer using statistical software package SPSS. The corrected mortality was done by using Abbot's⁽¹⁸⁾ formula whenever necessary. The formula was $[(Po - Pc)/(100 - Pc)] \times 100$, where Po = Per cent mortality observed and Pc = Per cent mortality in control.

Results and Discussion

The eggs of *Ae. aegypti* hatched showed significant difference ($F = 7.358$, $p < 0.05$) among different exposure periods and control (Table 1). The high percentage of hatching occurred in control (96.66) and the lowest in 24 hour heat exposure (59.34). A trend of gradual reduction of egg hatching was observed from control to 24 hour of exposure treatment. These results indicate that the temperature at 35°C with different exposures had an impact on the egg hatching of the mosquito species. In the present findings, egg mortality increased gradually with increasing exposure periods at high temperature (35°C) which is in conformity with the observations that greater temperatures resulted in greater egg mortality⁽¹⁹⁾. About 28% of the egg mortality of *Ae. aegypti* was reported at 35°C⁽²⁰⁾.

The mortality of the larvae and pupae of *Ae. aegypti* is shown in Tables 2 and 3, respectively. Larval mortality in control was corrected by following Abbot's formula. In the present findings high temperature and different exposures showed insignificant effects [$F = 1.15173$, $p > 0.05$ in case of larvae (Table 2) and $F = 0.649446$, $p > 0.05$ in case of pupae (Table 3)] on larval and pupal mortalities.

Temperature has, however, some effects on the larval and pupal mortality of *Ae. aegypti* and the sensitiveness of the larvae of the mosquito increased with later instars and pupa, the second instar congregating between 23 and 32°C whilst later stages and pupa did so between 28 and 32°C⁽²¹⁾.

Table 1. Hatching efficiency of the eggs of *Ae. aegypti* treated at 35°C in different exposure periods and control in an ambient condition of the laboratory (at 28 ± 4°C and 80% RH).

Exposure period per (hrs)	No. of eggs replicate	No. of replicate	No. of larvae hatched (mean ± SD)	No. of larvae hatched replicate (%)	F-value
Control	50	3	48.33 ± 1.15 ^a	96.66	
0.5	50	3	44.00 ± 2.645 ^{ab}	88.00	
1	50	3	40.33 ± 3.511 ^b	80.66	
2	50	3	41.67 ± 2.516 ^{ab}	83.34	7.3588*
4	50	3	38.33 ± 4.618 ^{bc}	76.66	
8	50	3	32.67 ± 4.509 ^{ab}	65.34	
24	50	3	29.67 ± 7.023 ^d	59.34	

*Significant at 5% level (F = 7.3588, p < 0.05); same letters in the column showing insignificant difference at 5% level in DMRT.

Table 2. Mortality of the larvae of *Ae. aegypti* hatched from the eggs treated at 35°C in different exposure periods and control in an ambient condition of the laboratory (at 28 ± 4°C and 80% RH).

Exposure period (hrs)	Total no. of larvae hatched	Total no. of larvae died	Number of larvae died (mean±SD) (%)	Mean larval mortality	Corrected mortality (%)	F value
Control	145	8	2.66 ± 2.081	5.22	0.0	
0.5	132	22	7.33 ± 3.214	14.66	9.855	
1	121	21	7.00 ± 3.00	14.00	9.158	
2	125	25	8.33 ± 4.163	16.66	11.967	1.15173
4	115	30	10.00 ± 4.582	20.00	15.495	
8	98	21	7.00 ± 5.291	14.00	9.158	
24	89	18	6.00 ± 1.732	12.00	7.045	

Insignificant at 5% level (F = 1.151732, p > 0.05); Larval mortality was corrected by following Abbott's formula.

The duration of larval and pupal periods as a result of high temperature at 35°C for various exposure periods and control was observed significantly (F = 64.44, p < 0.05 in case of larvae and F = 30.0153, p < 0.05 in case of pupae, Table 4). The highest duration was observed in control (*viz.*, 114.6 hours in larvae and 41.8 hours in pupae) and gradually decreased as the temperature exposure periods increased (*viz.*, 87.4, 85.5, 83.0, 81.6, 80.5, and 76.4 hours, respectively of larval duration and 40.4, 39.6, 38.9, 37.1, 35.2, and 33.1 hours, respectively of pupal duration (Table 4).

At 27°C the duration of the complete larval period of *Ae. aegypti* was found seven days (168 hours)⁽²²⁾ and at 23°C the complete duration of the larval period of the mosquito species was nine days (216 days)⁽²³⁾. At 22°C the duration of the pupal stage of *Ae. aegypti* required three - four days (72 - 96 hours)⁽²⁴⁾. Age of pupation increased as temperature decreased from 30 to 27°C⁽²⁵⁾. In the laboratory at 23°C, the larval development time of *Ae. albifasciatus* was around nine days (216 hours) and adults emerged within one week⁽²⁶⁾.

Table 3. Mortality of the pupae of *Ae. aegypti* developed from the eggs treated at 35°C in different exposure periods and control in an ambient condition of the laboratory (at 28±4°C and 80% RH).

Exposure period (hrs)	Total no. of larvae pupated	Total no. of pupae died	Number of pupae died (Mean ± SD)	Mean pupal mortality (%)	F value
Control	134	5	1.66 ± 0.577	3.32	0.649446
0.5	110	14	4.66 ± 2.516	9.32	
1	100	17	5.66 ± 4.041	11.32	
2	100	18	6.00 ± 4.358	12.00	
4	85	21	7.00 ± 5.29	14.00	
8	77	15	5.00 ± 3.464	10.00	
24	71	14	4.66 ± 2.886	9.32	

Insignificant at 5% level (F = 0.649446, p > 0.05).

Table 4. Mean duration of the larvae and pupae of *Ae. aegypti* obtained from the eggs treated at 35°C in different exposure periods and control in an ambient condition of the laboratory at 28 ± 4°C and 80% RH.

Stage	Control (hrs)	Exposure period in hours (Mean ± SD)						F value
		0.5	1	2	4	8	24	
Larvae	114.6 ± 3.34 ^b	87.4 ± 4.79 ^b	85.5 ± 5.31 ^{bd}	83.0 ± 5.08 ^{bcd}	81.6 ± 5.44 ^{de}	80.5 ± 7.46 ^e	76.4 ± 1.075 ^a	64.4427*
Pupae	41.8 ± 0.966 ^{ab}	40.4 ± 1.349 ^b	39.6 ± 1.37 ^b	38.9 ± 1.791 ^c	37.1 ± 2.299 ^d	35.2 ± 2.923 ^e	33.1 ± 0.421 ^a	30.0153*

Significant at 5% level (F = 64.4427, p < 0.05 for larvae and F = 30.0153, p < 0.05 for pupae); same letters in the column show insignificance at 5% level in DMRT.

The time between pupation and emergence of *Ae. aegypti* at 23 - 27°C was 45 hours for males and 60 hours for females, and the mean periods of the mosquito from eclosion to pupation at 27°C was 6.4 days (154 hours) and seven days for this period at 23 - 26°C, respectively⁽²⁷⁾. The larval developmental time of *Ae. albopictus* from egg hatching to

pupation was inversely correlated with temperature, lasting seven days at 32°C and the duration of pupal period varied between two and three days at that temperature⁽²⁸⁾. This indicates that with the decrease of temperature, the larval and pupal period becomes prolonged. The larval and pupal duration of *Ae. albopictus* depends also on the nature of containers in which they are developing; the total time from egg hatching to adult emergence in tree hole, bamboo stump and auto tyre in a temperature range between 18 and 22°C were 19.6, 27.3 and 37.5 days, respectively⁽²⁹⁾.

The lengths of the 2nd, 3rd and 4th instar larvae of *Ae. aegypti* at 35°C for various exposure periods and control are shown in Table 5. Significant differences in length among different exposure periods and control were observed in the 2nd instar larvae ($F = 4.314$, $p < 0.05$) and 3rd instar ($F = 3.786$, $p < 0.05$). The lengths of the 2nd and 3rd instar larvae were the highest (*viz.*, 4.52 and 6.46 mm, respectively) in control. The 3rd and 4th instar larvae developed from the eggs treated at 35°C in different exposure periods showed no significant difference in mean body length among the exposure periods. High temperature showed insignificant ($F = 1.138424$, $p > 0.05$) effect on the length of the 4th instar larvae in different exposure periods and control.

Table 5. Mean length (mm) of 2nd, 3rd and 4th instar larvae of *Ae. aegypti* from the eggs treated at 35°C in different exposure periods and control in an ambient condition of the laboratory at 28 ± 4°C and 80% RH.

Larval instar	Control	Mean larval length (mm) in exposure period (hrs)					
		0.5	1	2	4	8	24
2 nd	4.518 ± 0.587 ^b	3.845 ± 0.237 ^a	3.65 ± 0.579 ^b	3.88 ± 0.475 ^b	3.855 ± 0.484 ^b	3.625 ± 0.457 ^b	3.615 ± 0.442 ^b
	6.455 ± 0.613 ^a	5.74 ± 0.445 ^b	5.915 ± 0.464 ^b	5.935 ± 0.393 ^b	5.715 ± 0.455 ^b	5.635 ± 0.498 ^b	5.575 ± 0.481 ^b
3 rd	7.616 ± 0.557	7.62 ± 0.534	7.52 ± 0.493	7.31 ± 0.471	7.35 ± 0.522	7.37 ± 0.527	7.13 ± 0.506

Significant at 5% level ($F = 4.314312^*$, $p < 0.05$ for 2nd instar larvae; $F = 3.785768^*$, $p < 0.05$ for 3rd instar larvae); and insignificant at 5% level ($F = 1.138424$, $p > 0.05$ for 4th instar larvae.). Same letters in the columns show insignificance at 5% level in DMRT.

The transverse diameter of the head capsules of 1st and 4th instar larvae was significant ($F = 6.6512$, $p < 0.05$ and $F = 4.5209$, $p < 0.05$, respectively), but of 2nd and 3rd instars showed insignificance ($F = 0.353$, $p > 0.05$ and $F = 0.17$, $p > 0.05$, respectively) (Table 6).

Temperatures ranging from 15 - 31°C significantly affect the size of head capsule widths of the larval instars of *Ae. albopictus* and *Ae. triseriatus* in laboratory⁽³⁰⁾.

Table 6. Mean diameter (mm) of the head capsule of all four larval instars of *Ae. aegypti* obtained from the eggs treated at 35°C in different exposure periods and control in an ambient condition of the laboratory at 28 ± 4°C and 80% RH.

Larval instar	Control	Mean diameter (mm) of head capsule in exposure period (hrs)					
		0.5	1	2	4	8	24
1 st	0.336±	0.332±	0.302±	0.293±	0.285±	0.275±	0.243±
	0.350 ^a	0.0373 ^{ab}	0.041 ^{bc}	0.056 ^{bc}	0.0368 ^c	0.0263 ^c	0.018 ^c
2 nd	0.476±	0.456±	0.467±	0.45±	0.436±	0.417±	0.387±
	0.127	0.146	0.182	0.199	0.196	0.167	0.108
3 rd	0.695±	0.684±	0.684±	0.69±	0.681±	0.672±	0.644±
	0.12	0.135	0.131	0.141	0.132	0.13	0.11
4 th	0.907±	0.871±	0.853±	0.811±	0.77±	0.745±	0.723±
	0.039 ^a	0.041 ^{ab}	0.053 ^{abc}	0.156 ^{bcd}	0.141 ^{cd}	0.123 ^d	0.085 ^d

Significant at 5% level ($F = 6.6512^*$, $p < 0.05$ and $F = 4.5209^*$, $p < 0.05$, for 1st and 4th instar larvae, respectively; insignificance ($F = 0.353$, $p > 0.05$ and $F = 0.17$, $p > 0.05$ for 2nd and 3rd instar larvae, respectively). Same letters in the row show insignificance at 5% level in DMRT.

The length of the cephalothorax of the pupae of *Ae. aegypti* is shown in Table 7. The length of the cephalothorax of the pupae developed from the eggs treated with high temperature and different exposure periods showed significance at 5% level ($F = 2.343$, $p < 0.05$). It was also seen that the eight and 24 hours exposure periods showed similarities with control, but half an hour, one, two, four hour exposures of high temperature had no effect among them. However, they differed from the control.

The body length of adult males and females is also shown in Table 7. High temperature showed no effect ($F = 0.353$, $p > 0.05$ for the males and $F = 0.614$, $p > 0.05$ for the females) on the body length of adult males and females *Ae. aegypti* emerged from the eggs treated with high temperature at 35°C. Adult size increased as temperature decreased.

The temperature at 35°C and different exposures have negative impacts on mosquito development as these have been observed in egg hatching, larval and pupal mortality, duration of larval and pupal periods, morphometry of larvae, pupae and adults, and fecundity of *Ae. Aegypti* in the laboratory. In a different approach other than the above aspects, Alto and Juliano⁽³¹⁾ observed that the populations of *Ae. albopictus* at 26°C had greater intrinsic rates of increase and lower asymptotic densities than populations at 22 and 24°C; the populations at high temperatures initially had greater daily per capita

emergence rates, and steeper declines in per capita emergence rate as density increased over the course of the experiment. They also observed that there was no temperature effect on the size of adult females nor on the per capita daily mortality rate of adults. In concluding remarks they said that in regions with relatively high summer temperatures, the populations of *Ae. albopictus* are likely to have high rates of population growth with adult populations peaking early in the season and these populations may attain relatively low peak densities of adults; whereas, the populations occurring in regions with low summer temperatures are likely to experience slow, steady production of adults throughout the season with population size peaking later in the season, and may attain higher peak densities of adults. They also opined that high temperature conditions, associated with climate change, might increase the rate of spread of *Ae. albopictus* by increasing rates of increase and by enhancing colonization due to rapid population growth. As the two mosquito species, *Ae. aegypti* and *Ae. Albopictus*, are very close, we may therefore expect the similar effects on *Ae. aegypti* as well which may be worth trying to investigate in the laboratory and field conditions in the country.

Table 7. Mean cephalothoracic length of pupae and adult body length of *Ae. aegypti* obtained from the eggs treated at 35°C in different exposure periods and control in an ambient condition of the laboratory at 28 ± 4°C and 80% RH.

Pupa and adult	Control	Mean length (mm) in exposure period (hrs)					
		0.5	1	2	4	8	24
Cephalothorax (mm)	1.907 ± 0.317 ^a	1.49 ± 0.346 ^b	1.605 ± 0.28 ^b	1.6 ± 0.3 ^b	1.505 ± 0.345 ^b	1.7 ± 0.217 ^{ab}	1.715 ± 0.246 ^{ab}
Male body length (mm)	3.00 ± 0.349	2.98 ± 0.332	2.98 ± 0.339	2.97 ± 0.34	2.97 ± 0.34	2.94 ± 0.333	2.9 ± 0.362
Female body length (mm)	3.51 ± 0.251	3.50 ± 0.258	3.46 ± 0.231	3.44 ± 0.231	3.41 ± 0.242	3.38 ± 0.248	3.33 ± 0.346

Significant at 5% level ($F = 2.343166^*$, $p < 0.05$ for pupal cephalothoraxes; insignificance ($F = 0.353$, $p > 0.05$ and $F = 0.614$, $p > 0.05$ for adult males and females, respectively). Same letters in the row show insignificance at 5% level in DMRT.

Man has been altering the atmosphere through its various activities. Continuous increase in the concentration of various green house gases, such as carbon dioxide, carbon monoxide, methane, various oxides of nitrogen, and the chloro-floro-carbons (CFCs), which absorb infrared radiation in the spectral bands where there are gaps in the carbon dioxide and water spectra, thus closing the atmospheric window leading to an increased radiation absorption by the atmosphere, and its retention increases the temperature of the earth; as a consequence, large-scale changes in the temperature-

climate pattern of the earth-atmosphere system are expected⁽³²⁾. Since 40% of the global warming is caused by carbon dioxide, as the concentration of the gas in the atmosphere rises, increased radiation trapping will lead to an increased greenhouse effect and consequent global warming; as the temperature rises, more water will evaporate and pass into the atmosphere; water vapour is even more effective than carbon dioxide in preventing the long-wave radiations of the earth from escaping⁽³²⁾. Thus, the effects of man's interference with the carbon cycle through fossil fuel consumption, deforestation, agricultural practices, industrial emission and nuclear testing has already started to show an impact on the biosphere⁽³²⁾.

In conclusion, from the above discussion it is very much indicative that temperature changes have influential impacts on mosquito life and its population dynamics. To know more about the long term effects of temperature (global warming) on the mosquito population of Bangladesh, both laboratory and field experiments should be initiated, as further attempts, on the sustenance of the effects of high temperature on the subsequent generations of the mosquito. If possible, both genetic and ecological approaches may be included in this focused area.

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