Adaptations of *Stegomyia aegypti* L. and *Stegomyia Albopicta* Skuse to Water Current and pH

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Abstract:

Stegomyia aegypti (formerly *Aedes aegypti*) Linnaeus and *Stegomyia albopicta* (formerly *Aedes albopictus*) Skuse are the established vectors of dengue and chikungunya in populated areas worldwide. Its control is very difficult because they are adapted to the natural environment and fluctuations in the water quality. The aim of this work is the adaptability of the *S. aegypti* and *S. albopicta* eggs to water current generated by various ways and to pH.

Keywords: *Stegomyia aegypti*, *Stegomyia albopicta*, water current, pH

Introduction:

Mosquitoes belonging to the *Stegomyia* sps. Complex are highly adaptable to changing water quality characteristics, thus ensuring highest degree of survival and maximum fitness. Phenotypic traits such as growth rates and mass are affected by gene–environment interactions and a number of environmental factors include pH, salinity, temperature, density, food supplies and physical size and shape of the larval habitat (Clark *et al.*, 2004a, b; Nayar, 1968, 1969; Nayar and Sauerman, 1975; Trpis and Horsfall, 1969 and McGinnis and Brust, 1983).

S. aegypti eggs will survive longer at low relative humidity or at high temperature. The egg stage of *Aedes, Psorophora*, *Opifex* and *Hemagogus* species are capable of survive in dry conditions (Clements, 2000). In their natural environment, the eggs of *Stegomyia* sps. Mosquitoes are deposited in breeding places subject to periodic inundation (Gerberg *et al.*, 1994).

Eggs deposited by *S. aegypti* in the freshwater ecosystem occasionally are exposed to water currents and eddies eventhough the female takes care to deposit the eggs in shallow lentic waters. During inundation and heavy precipitation, the eggs as well as the larvae are exposed to unusual forces generated by swirling of water. High water current and flooding leads to *Anopheles* species larval deaths (Okogun, 2005). During winter and summer hatching numbers are lower than during spring and fall (Munga *et al.*, 2005).

Aedes mosquitoes oviposit its eggs which will be placed at varying distances above the water line and a female will not lay the entire clutch at a single site, but rather spread out the eggs over two or more sites (Foster and Walker, 2002), which is the adaptation of mosquitoes to make survive its offspring. Asynchronous egg hatching in *Aedes* mosquitoes permits overlap among individuals of different developmental stages within the same habitat, presenting possibilities for interactions among stages. In the laboratory, the magnitude of hatching suppression is influenced by interactions among resource availability, density and instars of larvae (Livdahl *et al.*, 1984).

pH is extremely important physical factor limiting the distribution and abundance of aquatic animals. Larval mosquitoes can tolerate ranges of ambient pH much greater than those tolerated by other aquatic animals can. The ability of *S. aegypti* eggs to survive wide pH ranges, is compounded by the ability of the gravid female to sense pH of the water in which it is about to oviposit. Maintaining pH is important for physiological processes that occur inside the larval midgut, thus ensuring survival of the larvae (Boudko *et al.*, 2001 and Corena *et al.*, 2002). The ovipositing female is able to identify major changes in water quality and lays egg accordingly (Seghal and Pillai, 1970). The adult female mosquito has a well-developed sensory system to assess water quality. This instinct increases the survival value of mosquitoes withholding eggs. From being laid in waters of an appropriate quality is a great survival strategy.

Material and Methods

Water swirling

(i) Centrifugation of eggs

S. aegypti and *S. albopicta* egg cards were cut into bits containing about 20 eggs. The card bits were placed inside centrifuge tubes in about 5 ml of water and centrifuged at 3000 rpm for 5 and 10 min. The timing started from the time the centrifuge attained a speed of 3000 rpm generating 2500g centrifugal force. The eggs were taken out from the centrifuge and placed in an empty bowl and allowed acclimate for about 30 min. before placing them in water for hatching. The eggs were subjected to strong centrifugal force generated in a centrifuge.

(ii) Rocking of eggs in a shaker

S. aegypti and *S. albopicta* egg cards were rocked in a vortex shaker for 5 and 10 min at 100 rpm. The eggs were placed in a 100 ml. conical flask in about 10 ml of water and the mouth of the conical flask was covered using cotton swabs. The shaker was stopped at the appropriate time and eggs taken out were placed in a dish for about 30 min. before placing them in water to initiate development.

(iii) Subjecting eggs to water current

S. aegypti and *S. albopicta* egg card bits were taken in a 100 ml. plastic bottle with a whole that fits into a half-inch tap. On one side of the bottle a vent smaller than the inlet was provided. The outlet was small and it did not allow egg card bits to escape. The tap was opened to allow the water to flow into the bottle. Since the outlet was at the top the water circulated within the bottle creating water current. The eggs of *S. aegypti* and *S. albopicta* were swirled in this current.

Egg Incubation

The treated *S. aegypti* and *S. albopicta* eggs along with control eggs were incubated by placing them inside water taken in small paper cups and placed under laboratory conditions of room temperature and humidity. The eggs hatched out were counted and hatching percentage was calculated.

Hatching of *S. aegypti* and *S. albopicta* eggs in different pH

Hatching of *S. aegypti* and *S. albopicta* eggs in different pH of 3-4, 4-5, 5-6, 6-7, 7-8, 8-9, 9-10, 10-11, 11-12 and 12-13 was studied. The pH of water was maintained at 10 different levels, the lowest being 3 and highest, 13. pH of the water was adjusted by adding HCl or NaOH and readings were measured by digital pH meter. Total number of eggs exposed to each pH level was 30.

S. aegypti eggs hatch in installments. Normally hatching takes 1-3 days. Number of eggs hatched in each day under different pH was recorded and calculated hatching percentage.

Results

Hatching of *Stegomyia* sps. eggs under different conditions of water was observed. Control *S. aegypti* eggs hatched in 1-2 days. Out of 20 eggs incubated 16 ± 1.2 eggs hatched on the first day and 3 ± 0.45 on the second day and the hatching percentage is 95. Control *S. albopicta* eggs hatched in 1-3 days. Out of 20 eggs, 11 ± 0.98 eggs hatched on the first day, 5 ± 0.42 on the second day and 4 ± 0.53 on the third day and the hatching percentage is 100.

The *S. aegypti* eggs centrifuged at 3000 rpm for 5 minutes, the hatching percentage was 84.17 ± 10.68. Out of 20 eggs, 11.16 ± 3.76 eggs hatched on the first day, 4.17 ± 1.47 on the second day, 1.5 ± 8.3 on the third day. Similar readings for *S. albopicta* was 8.33± 0.82 eggs on the first day, 5.66±1.96 eggs on the second day and 2.83±1.17 eggs on the third day.

Statistically significant deviations are observed in the hatchability of *S. aegypti* eggs subjected to external hydrological forces. Hatching on the first day was 30.25 percent less in eggs centrifuged for 5 minutes at 3000 rpm whereas on the second day the hatching was 39 percent higher in the experimental group compared to control. The hatching percentage was 11.4 less in the experimental group. In *S. albopicta*, hatching percentage was 15.83 less compared to control.

S. aegypti eggs centrifuged at 3000 rpm for 10 minutes, the hatching percentage was 95.83 ± 3.77. Hatching on the first day was 4.18 higher than control whereas on the second day the hatching was 22.33 percent less in the experimental group compared to control. For *S. albopicta* eggs, hatching percentage was 90.83 ± 3.76. Hatching on the first day was 3 percent higher than control whereas on the second day and third day 6.8 and 46 percent less respectively compared to control.

S. aegypti eggs, subjected to agitation in a shaker, at 100 rpm for 5 minutes, the hatching percentage was 96.6 ± 2.58. Out of 20 eggs, 14.5 ± 1.05 eggs hatched on the first day, 3.5 ± 1.05 on the second day and 1.33 ± 1.03 on the third day. In *S. albopicta*, the hatching percentage was 87.5 ± 8.21. Hatching on the first day was 22.73 percent less in eggs agitated for 5 minutes at 100 rpm whereas on the second day, the hatching was 16.6 percent higher and on the third day, 21 percent less in the experimental group compared to control.

In the *S. aegypti* eggs subjected agitated, at 100 rpm for 10 minutes, the hatching percentage was 95 ± 4.47. Statistically significant deviations of hatching on the first day was 39.56 percent less in eggs agitated for 10 minutes at 100 rpm whereas on the second day, the hatching was 55.66 percent higher in the experimental group compared to control. In *S. albopicta,* the hatching percentage was 86.66 ± 4.08. Out of 20 eggs, 9.5 ± 1.87 eggs hatched on the first day, 4.83 ± 1.72 on the second day and 3 ± 1.41 eggs on the third day.

S. aegypti eggs, subjected to water current during flow from tap for 5 minutes, the hatching percentage was 96.67 ± 2.58. Hatching on the first day was 55.19 percentage less in eggs subjected to water current during flow from tap, for 5 minutes whereas on the second day, the hatching was 227.6 percentage higher in the experimental group compared to control. In *S. albopicta,* the hatching percentage was 93.33 ± 6.83. Out of 20 eggs, 8.66 ± 1.21 eggs hatched on the first day, 7.66 ± 2.06 on the second day and 2.33 ± 0.82 eggs on the third day.

In the *S. aegypti* eggs, subjected to water current during flow from tap for 10 minutes, the hatching percentage was 95.83 ± 2.04. Out of 20 eggs, 8.17 ± 1.67 eggs hatched on the first day, 8.17 ± 1.17 on the second day and 2.83 ± 1.17 on the third day. In *S. albopicta*, the hatching percentage was 90 ± 7.75. Hatching on the first day was 24.27 percent less, whereas on the second day, the hatching was 36.6 percent higher and on the third day, 29.25 percent less in the experimental group compared to control (Table 1) (Figure 1).

S. aegypti and *S. albopicta* eggs were exposed to pH ranges 3-13. At the pH 3-4, the eggs did not hatch. In the pH range 4-5, hatching percentage of *S. aegypti* and *S. albopicta* was 37.33 ± 1.56 and 49 ± 2.7 respectively. In *S. aegypti*, the number of egg hatched in the first day of incubation was 3.1 ± 0.8, on the second day, 2.1± 0.6 and 6.0± 0.49 eggs on the third day. In *S. albopicta*, 6.4± 0.7 eggs hatched on the first day and 8.3± 0.7 eggs on the second day.

At the pH 6-7, hatching percentage of *S. aegypti* and *S. albopicta* was 71.66 ± 3.5 and 68.33± 5.2 respectively. In *S. aegypti*, the number of egg hatched in the first day of incubation was 8.3± 0.7, on the second day, 7.5± 0.8 and 5.7± 0.81 eggs on the third day. In *S. albopicta*, 9.6 ± 0.46 eggs hatched on the first day and 6.4 ± 0.7 eggs on the second day and 4.5± 0.75 eggs on the third day.

At the pH 7-8, total number of egg hatched was 30 ± 2.34 and 26.3± 2.41 for *S. aegypti* and *S. albopicta* and its hatching percentage was 100 ± 2.1 and 87.66± 3.4 respectively. In the pH range of 8-9, *S. aegypti* and *S. albopicta* egg hatched on the first day was 6.4 ± 0.9 and 9 ± 0.46, on the second day, 9.6 ± 0.46 for both species and 10 ± 0.89 and 6.4 ± 0.7 respectively on the third day.

At the pH 9-10, the hatching percentage was 64 ± 5.3 and 28.33 ± 0.12. At the pH 10-11, the hatching percentage was 61.66 ± 4.8 and 9 ± 0.7 for *S. aegypti* and *S. albopicta* respectively. At the pH 11-12, *S. aegypti* egg hatched on the first day was 5.7 ± 0.81, on the second day, 5 ± 0.46 and 3.4 ± 0.66 on the third day. *S. albopictus* eggs was not hatched beyond this pH level. The results of ANOVA showed that there was a significant difference in egg hatching between different pH levels. But species level, there is no much significance (Table 2 and Table 2a) (Figure 2).

Table 1
Hatching of *Stegomyia* sps. eggs under different conditions

Sl. No.	Conditions	Incubation period (days)						Total egg hatched		Hatching percentage	
		1		2		3					
		No. of egg hatched / 20 eggs									
		S. aegypti	*S. albopicta*	*S. aegypti*	*S. albopicta*	*S. aegypti*	*S. albopicta*	*S. aegypti*	*S. albopicta*	*S. aegypti*	*S. albopicta*
1	swirled for 5 min in a centrifuge (3000 rpm)	11.16 ± 3.76 (-30.25) *	8.33± 0.82 (-24.27) *	4.17 ± 1.47 (39) *	5.66±1.96 (13.2)	1.5 ± 0.83 (0)	2.83±1.17 (-29.25) *	16.83 ± 2.14 (-11.41) *	16.83 ± 1.72 (-15.85) *	84.17± 10.68 (-11.40) *	84.17 ± 8.61 (-15.83) *
2	swirled for 10 min in a centrifuge (3000 rpm)	16.67± 1.21 (4.188)	11.33 ± 1.21 (3)	2.33 ± 0.82 (-22.33) *	4.66 ± 1.03 (-6.8)	0.5 ± 0.55 (0)	2.16 ± 0.75 (-46) *	19.12 ± 0.75 (0.63)	18.17 ± 0.75 (-9.15)	95.83± 3.77 (0.874)	90.83 ± 3.76 (-9.17)
3	shaker swirled (100 rpm) for 5 min	14.5± 1.05 (-9.38)	8.5 ± 1.05 (-22.73) *	3.5 ± 1.05 (16.67) *	5.83 ±1.47 (16.6)*	1.33 ± 1.03 (0)	3.16 ±0.75 (-21) *	19.3 ± 0.52 (1.58)	17.5 ± 1.64 (-12.5) *	96.6± 2.58 (1.68)	87.5 ± 8.21 (-12.5) *
4	shaker swirled (100 rpm) for 10 min	9.67± 1.03 (-39.56) *	9.5 ± 1.87 (-13.64*)	4.67 ± 0.82 (55.66) *	4.83 ± 1.72 (-3.4)	4.67 ± 1.21 (0)	3 ± 1.41 (-25) *	19 ± 0.89 (0)	17.33 ± 0.82 (-13.35) *	95± 4.47 (0)	86. 66 ± 4.08 (-13.34) *
5	eggs subjected to water current during flow from tap for 5 min	7.17± 0.75 (-55.19) *	8.66 ± 1.21 (-21.27) *	9.83 ± 0.75 (227.6) *	7.66 ± 2.06 (53.2) *	2.33± 0.82 (0)	2.33 ± 1.21 (-41.75) *	19.33 ± 0.52 (1.74)	18.66 ± 1.37 (-6.7)	96.67± 2.58 (1.76)	93.33 ± 6.83 (-6.67)
6	eggs subjected to water current during flow from tap for 10 min	8.17± 1.67 (-48.94) *	8.33 ± 1.03 (-24.27) *	8.17 ± 1.17 (172.32) *	6.83± 1.47 (36.6) *	2.83± 1.17 (0)	2.83 ±1.17 (-29.25) *	19.17 ± 0.41 (0.89)	18 ± 1.55 (-10)	95.83± 2.04 (0.87)	90 ± 7.75 (-10)
7.	Control	16 ± 1.2	11 ± 0.98	3 ± 0.45	5 ± 0.42	-	4 ± 0.53	19	20	95	100

Note: Readings in parenthesis indicates percent change over control values * Deviations significant at P ≤ 0.05 (t-test)

Table 2

Hatching of *Stegomyia* sps. eggs exposed to various pH ranges

Sl. No	pH	Incubation period (days)						Total egg hatched		Hatching percentage	
		1		2		3					
		No. of egg hatched / 20 eggs									
		S. aegypti	*S. albopicta*	*S. aegypti*	*S. albopicta*	*S. aegypti*	*S. albopicta*	*S. aegypti*	*S. albopicta*	*S. aegypti*	*S. albopicta*
1	3-4	0	0	0	0	0	0	0	0	0	0
2.	4-5	3.1 ± 0.8	6.4± 0.7	2.1± 0.6	8.3± 0.7	6.0± 0.49	0	11.2 ± 0.7	14.7 ± 0.56	37.33 ± 1.56	49 ± 2.7
3.	5-6	4.2 ± 0.63	7.2± 0.65	4.5± 0.75	9.6± 0.46	7.2± 0.65	1.2± 0.75	15.9± 1.35	18 ± 1.3	53 ± 2.7	60± 3.3
4.	6-7	8.3± 0.7	9.6± 0.46	7.5± 0.8	6.4± 0.7	5.7± 0.81	4.5± 0.75	21.5± 2.1	20.5 ± 1.5	71.66 ± 3.5	68.33± 5.2
5.	7-8	7.2± 0.23	10.4± 0.7	10.1± 0.9	9.7± 0.81	13.1± 0.98	6.2 ± 0.24	30 ± 2.34	26.3± 2.41	100± 2.1	87.66± 3.4
6.	8-9	6.4± 0.9	9± 0.46	9.6± 0.46	9.6± 0.46	10± 0.89	6.4± 0.7	26± 1.23	25± 2.7	86.66± 5.8	83.33± 3.45
7.	9-10	8.3± 0.56	2.4 ± 0.24	6.4± 0.7	6.1± 0.81	4.5± 0.87	0	19.2± 0.98	8.5± 0.79	64 ± 5.3	28.33± 0.12
8.	10-11	5.6± 0.84	1.9 ± 0.24	7.2± 0.65	0	5.7± 0.39	0	18.5± 0.45	1.9 ± 0.24	61.66± 4.8	9± 0.7
9.	11-12	5.7± 0.81	0	5± 0.46	0	3.4± 0.66	0	14.1± 0.5	0	47± 2.67	0
10	12-13	1.8 ± 0.8	0	0	0	0	0	1.8± 0.6	0	6± 0.3	0

Table 2a

Analysis of Variance (ANOVA) of egg hatching

Anova: Two-Factor Without Replication						
SUMMARY	*Count*	*Sum*	*Average*	*Variance*		
pH 4-5	2	25.9	12.95	6.125		
pH 5-6	2	33.9	16.95	2.205		
pH 6-7	2	42	21	0.5		
pH 7-8	2	56.3	28.15	6.845		
pH 8-9	2	51	25.5	0.5		
pH 9-10	2	27.7	13.85	57.245		
pH 10-11	2	20.4	10.2	137.78		
pH 11-12	2	14.1	7.05	99.405		
pH 12-13	2	1.8	0.9	1.62		
S. aegypti	9	158.2	17.57778	68.57944		
S. albopicta	9	114.9	12.76667	110.75		
ANOVA						
Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
rows	1226.571	8	153.3214	5.89515	0.010719	3.438101
columns	104.1606	1	104.1606	4.004934	0.080363	5.317655
Error	208.0644	8	26.00806			
Total	1538.796	17				

Figure 1

Hatching of *S. aegypti* and *S. albopicta* eggs under different conditions of water

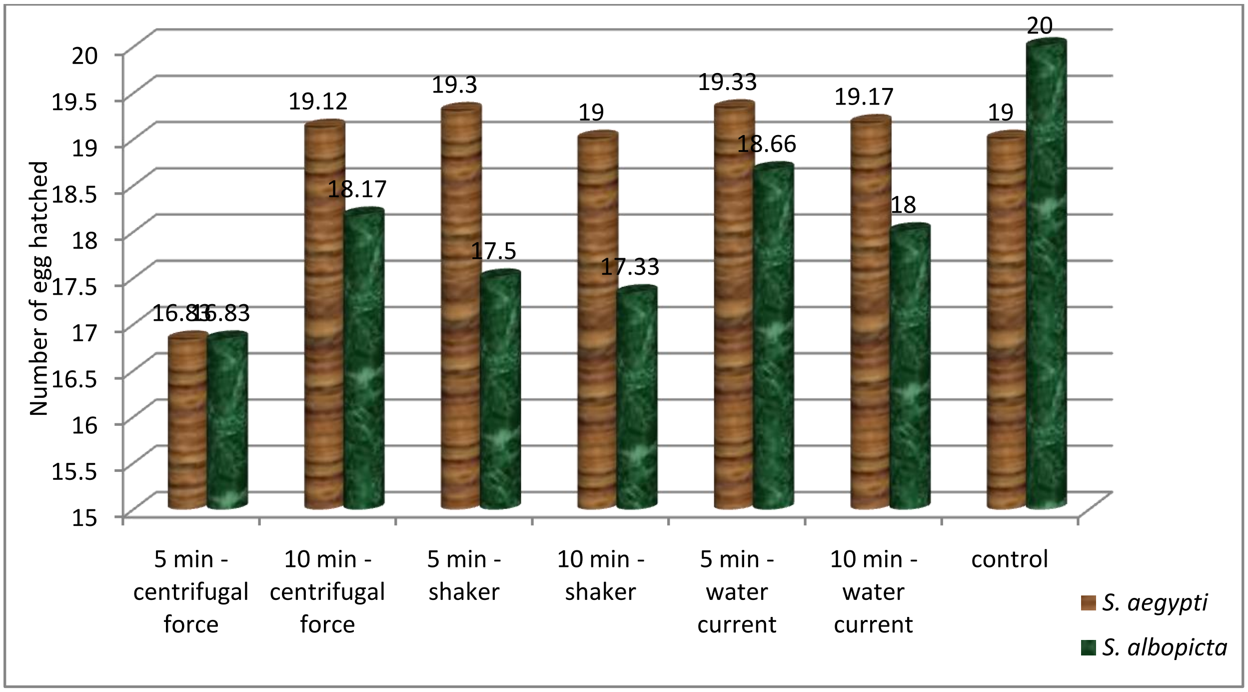
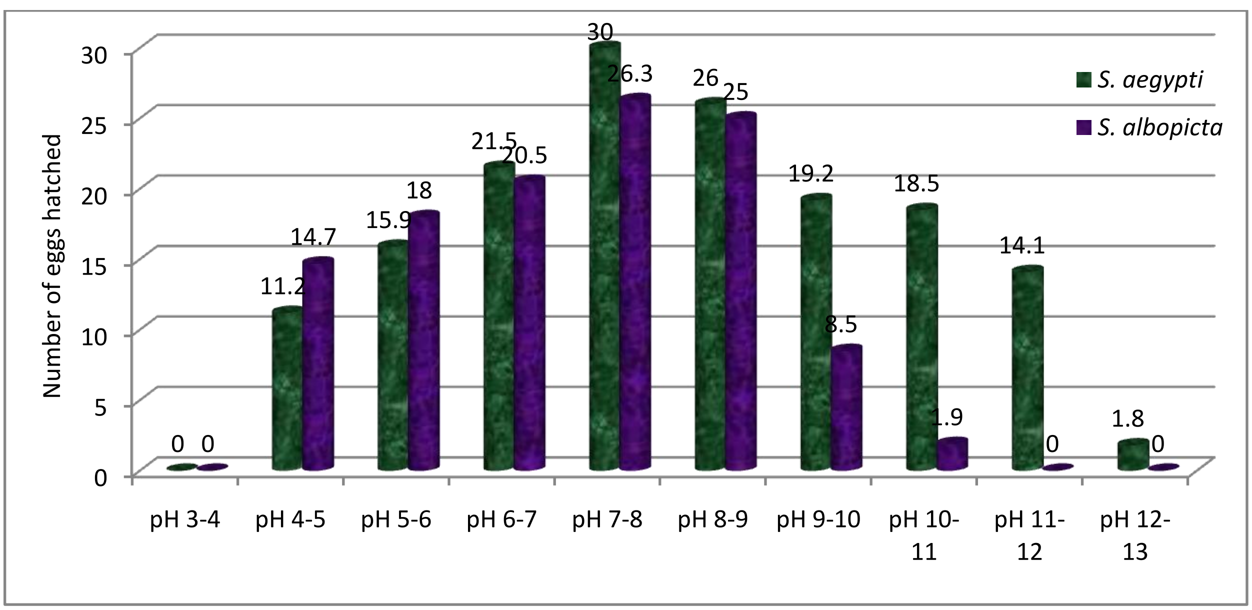


Figure 2

Hatching of *S. aegypti* and *S. albopicta* eggs exposed to different pH ranges



Discussion

S. aegypti eggs, larvae and pupae are adapted to tide over pH levels (Umar and Pedro, 2008), salinity (Jude *et al*., 2012), temperature extremes (Huffakers, 1994a) and drought conditions (Clements, 2000).

In the aquatic environment, the eggs of dipterans and other insects are subjected to various external environmental factors as well as several hydrological forces. The impact of such factors results in swirling, churning and spinning of the eggs. *Stegomyia* species eggs are deposited along the fringes of water bodies by the gravid females. But during deluges water wells up and the eggs are subjected to severe water currents. It is not easy to follow the fate of dipteran eggs under natural conditions of inundation. *Stegomyia* species eggs showed that timing of hatch varies not only among eggs within the same batch but also among strains within species.

The present study simulated conditions in which the eggs of mosquitoes are subjected to water current and hydrological forces. The eggs centrifuged for a maximum period of 10 minutes and during the procedure eggs were subjected to very high centrifugal force. It is likely that the egg contents are pushed to one side of the egg, as is expected in eggs exposed to strong eddy currents. The results show that swirling in a centrifuge did not significantly affect the hatchability of the eggs. The mean hatching percent was about 96 percent. This indicates that the insect eggs are highly adapted to resist all possible hydrological forces impacting on them.

When the eggs were rocked in a shaker for a maximum period of 10 minutes they were subjected a force different from centrifugal force. The shaker made circular movements and it was expected to churn the inner contents of the egg. But the hatching percentage as high as 97 percentage indicating in-ability of the external forces in affect the inner egg contents. The water flow from tap also fails to produce any considerable change in the hatchability of *S. aegypti* and *S. albopicta* eggs. So, it is clear that *S. aegypti* and *S. albopicta* eggs well adapted to escape different hydrological forces that may affect during certain specific season.

S. aegypti eggs seem to have a cushioning system that prevents external shocks from affecting the nucleus of the egg. The centrolecithal eggs of insects seem to be highly stable resisting forces acting on the nucleus. The eggs of *S. aegypti* and *S. albopicta* are too small and thus they may escape the centrifugal or circular force acting on them.

S. aegypti and *S. albopicta* are highly adaptable to wide fluctuations in the physico – chemical characteristics of water in which they breed. Addition of different types of effluents into water bodies damages the fresh water ecosystem turning water samples acidic. In the present study, the impact of changes in the hydrogen ion concentration of water was tested for its hatching ability of *S. aegypti* and *S. albopicta* eggs. The eggs laid by *S. aegypti* and *S. albopicta* were mechanically transport to water samples of different pH. If the adult mosquito were allowed to lay eggs in low pH samples, they would not have deposited their eggs. Because, the highly acidic water samples are injurious to the larvae and hence the adult female prefer to lay eggs in another area where pH is normal.

In waters of pH 3-4, the ovicidal percentage was 100 and all the exposed eggs remained unhatched. The extreme pH level affected the eggs. The hatching percentage as the acidic pH turned basic. At pH 7 and 8, 100 percentage of hatching was recorded for *S. aegypti* eggs and 88 percentage for *S. albopicta* eggs. This study correlated with the study of Umar and Pedro (2008), they conducted laboratory bioassays, indicated that maximum survival of both field and laboratory strain of the larvae of *S. aegypti* occurred between the pH values of 6.5 and

8.0. Rao *et al.* (2011) showed that *Ae. albopictus* breeding increased in waters of high pH, resulted in a significant decreases in larval density. *S. aegypti* and *S. albopicta* are successful in moderately alkaline pH for the hatching of their eggs.

Ae. aegypti complete development in waters of pH ranging from 4 to 11 (Clark *et al*., 2004a). There is no evidence that pH ever limits the habitats of larval mosquitoes in nature (Clements, 2000) where reported pH values for larval habitats range from 3.3 to 8.1 (*O. taeniorhynchus*), 4.4- 9.3 (*Ae. geniculatus*), 3.3-9.2 (*Psorophora confinnis* Fab.) and 4.4- 9.3 (*Anopheles plumbeus* Stephens). *Aedes flavopictus* Yamada has been reared in waters ranging from pH 2-9 and *Armigeres subalbatus* Coquillett in the pH range of 2-10 (Keilin,1932; Kurihara, 1959; Mac Gregor,1921 and Peferson and Chapman, 1970). So, this study revealed that *Stegomyia* sps. eggs are well adapted to tide over varying conditions of water.

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