Available online at: www.cbioresearch.com/journal.html ISSN No. 0973-8207

Impact of pH range on the hatchability of *Aedes aegypti* (L.) eggs

L. Charlet Bhami and S. Sam Manohar Das*

Department of Zoology and Research Centre, Scott Christian College (Autonomous), Nagercoil, Tamilnadu, India – 629 003

*Correspondence e-mail: sambiocontrol@gmail.com

Abstract

Aedes aegypti eggs are highly sensitive to changes in the hydrogen ion concentration (pH) of the water in which they are allowed to incubate. Acidic pH ranges are ovicidal whereas, eggs hatch and survive to the larval stages in pH higher than 4. The ability of *Ae. 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. Except at very low pH ranges of 2-4, hatching of *Ae. aegypti* eggs and survivability of the hatched larvae have been observed.

Keywords

Aedes aegypti, pH, ovicidal

Introduction

Larval mosquitoes are remarkably tolerant of extreme pH, allowing them to exploit a wide variety of habitats in nature (Clements, 2000 and Clark *et al.,* 2004). Larvae of *Aedes aegypti* (L.) and *Ochlerotatus taeniorhynchus* Wied. complete development in waters ranging from pH 4 to 11 in the laboratory. Across this range haemolymph pH of acclimated larvae varies by only 0.1 pH units or less (Clark *et al.,* 2004).

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 pH of the luminal contents in mosquito larvae increases from near neutrality in the foregut to a value that exceeds 10 in anterior midgut then drops to 7.5 in the posterior midgut (Dadd, 1975 and Ramsay, 1950).

The eggs of *Aedes* mosquitoes are elongate oval in shape. The outer shell of the eggs are patterned with small reticulations (Goma, 1966). The *Aedes* sp. eggs are white in colour and soft when they were newly laid but later the eggs turn black and become quite hard (Schlager and Fuchs, 1974 and Christopher, 1960). Eggs of *Ae. aegypti* are laid in batches by the female (Kettle, 1984 and Rhodain and Thosen,1997) above the water surface. It can remain dry for months but still remain viable and hatch when they become flooded with water. (Harwood and James,1979). Eggs of *Aedes* sp. mosquito hatch in instalments and may require repeated immersions in water followed by short periods of desiccation (Gillett, 1951).

pH is regulated in two major extracellular compartments, the haemolymph and the midgut lumen. The cellular mechanism involved in the generation of highly alkaline conditions within the midgut of lepidopteran mosquito larvae have received considerable attention. The malpighian tubules secreted acidic fluid but their contributions to acid –base homeostasis were minor (Harrison *et al.,*1991; Phillips *et al.,* 1993 and Harrison, 2001). Aquatic insect rely primarily on epithelial transport across renal systems for acid base homeostasis (Cooper, 1994 and Harrison, 2001). Larval mosquitoes can tolerate ranges of ambient pH much greater than those tolerated by other aquatic animals. 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 (*Ochlerotatus taeniorhynchus* Wied.), 4.4- 9.3 (*Aedes geniculatus* Olivier), 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). Prolonged drought may cause smaller habitats to desiccate rapidly, within the family Culicidae,

species have evolved various strategies to survive these adverse conditions. For example, the egg stage of *Aedes, Psorophora*, *Opifex* and *Hemagogus* species are capable of surviving dry conditions for several months (Clements, 2000), whereas of the malaria vector *Anopheles gambiae* Giles s.i. can survive up to 2 week in dry conditions (Beier *et al.,*1990). Larvae of latter species are capable of surviving for several days in desiccated habitats. Several studies have demonstrated a strong relation between water acidity and composition of the macroinvertebrate community (Raddum *et al.,* 1988; Mulholland *et al.,* 1992; Herrmann *et al.,* 1993; Winterbourn and Mc Diffett, 1996; Earle and Callaghan, 1998). The present study is designed to find out the influence of different pH levels on the hatchability rate of *Ae. aegypti* eggs.

Materials and methods

Procurement of *Aedes aegypti* eggs

Ae. aegypti were raised from eggs obtained on egg cards from Centre for Research in Medical Entomology (CRME), ICMR, Madurai.

Laboratory culture

The egg cards were placed in ion – free water and allowed to hatch out. The hatched out larvae were grown in the laboratory by allowing them in water taken in 100 ml plastic cups. The larvae were fed with powdered soya biscuits and yeast in the ratio 3:1. The larvae were occasionally transferred to fresh cups to bring down the mortality of developing larvae.

After 5 – 7 days larvae pupated and the pupae were transferred to cups inside cages. After 1 ½ to 2 days adults emerged. Males were fed with 10% sucrose and females were given blood feed. Paper cups containing filter paper strips were kept in the cage for the mosquitoes to lay their eggs. After 3-5 days, eggs were laid and these eggs were used for the experiments. Hatching of *Ae. aegypti* eggs in different pH of 2-3, 3-4, 4-5, 5-6, 6-7, 7-8, 8-9, 9-10, 10-11, 11-12 was studied. The pH of water was maintained at 10 different levels, the lowest being 2 and highest, 12. 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.

Hatching percentage of *Ae. aegypti* eggs was calculated by the following formula.

$$\text{Percentage of hatching} = \frac{\text{Number of eggs hatched}}{\text{Total number of eggs}} \times 100$$

Ovicidal percentage of *Ae. aegypti* egg was calculated by the following formula

$$\text{Ovicidal percentage} = \frac{\text{Number of eggs not hatched}}{\text{Total number of eggs}} \times 100$$

Ae. aegypti eggs hatch in instalments. Normally hatching takes 1-3 days. Number of eggs hatched in each day under different pH was recorded.

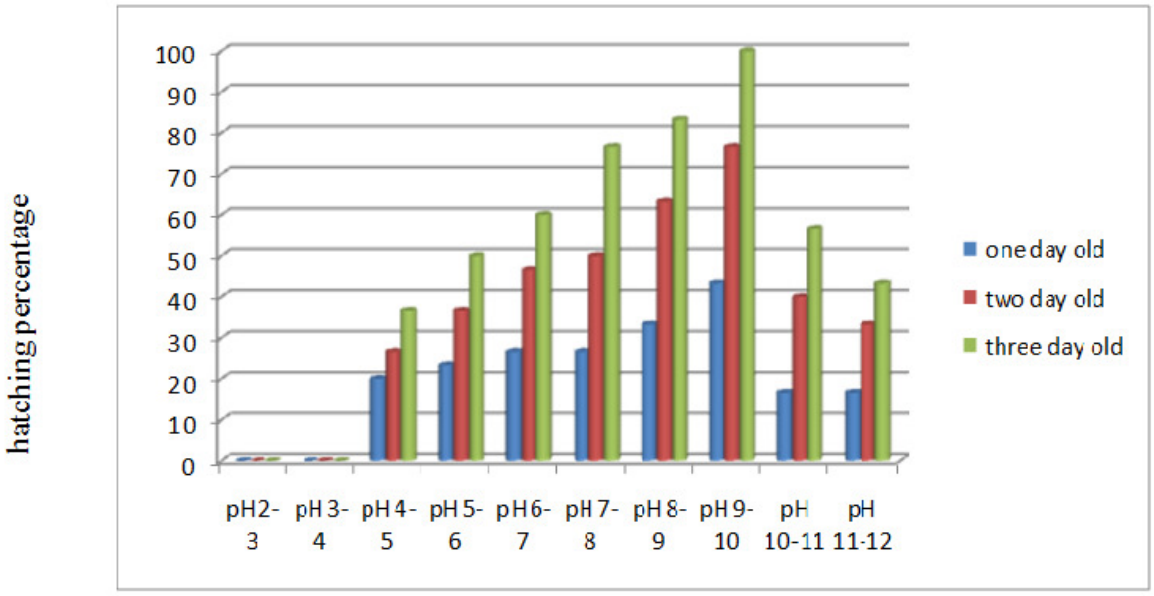
***Aedes aegypti* egg card**

Results

One day old *Ae. aegypti* eggs exposed to pH range 2-3 and 3-4, did not hatch and the ovicidal rate was 100%. In the pH range 4-5, the hatching percentage was 20 and only six eggs hatched out of the 30 eggs exposed. At the pH range at 5-6, 23 eggs were unhatched, the hatching percentage was 23.3 and the ovicidal rate was 76.6%. At the pH range 6-7 the hatching percentage increased to 26.6 and the ovicidal rate was 73.3. At the pH 7-8 the hatching percentage 26.6 and the ovicidal rate was 73.3%. At the pH range 8-9, the hatching percentage increased to 33.3 and the ovicidal percentage was 66.6. In the pH range 9-10 the hatching percentage was increased to 43.3% and ovicidal rate was 66.6%. From pH range 10-11 the hatching percentage slowly decreased and the hatching percentage was 16.6 and the ovicidal percentage was 83.3. At the pH range 11-12 the hatching percentage decreased to 16.6 and the ovicidal rate was 83.3%.

Two day old *Ae. aegypti* eggs exposed to pH ranges 2-3 and 3-4 did not hatch and the ovicidal rate was 100%. In the pH range 4-5, the hatching percentage was 26.6% and eight eggs hatched out of 30 eggs exposed. The pH range 5-6, 19 were unhatched, the hatching percentage 36.6. At the pH range 6-7, the hatching percentage slowly increased to 46.6% the ovicidal percentage was 53.3. At the pH range 7-8, 15 eggs were hatched and 15 eggs were unhatched and the hatching percentage was 50. At one pH range 8-9 hatching percentage increased to 63.3% and the ovicidal percentage was 36.6. At the pH range 9-10, 76.6% of eggs were hatched, 23.3% were unhatched. At 10-11 hatching percentage decreased 40. At the pH range 11-12, 33.3 percentage of eggs only hatched and 66.6 percentage eggs were unhatched.

Three day old *Ae. aegypti* eggs were exposed to pH ranges 2-3 and 3-4 did not hatch and the ovicidal rate 100%. In the pH range 4-5, hatching percentage was 36.6 and the ovicidal percentage was 63.4. At the pH range 5-6, hatching percentage was 50 and one ovicidal percentage 50. At the pH range 6-7, hatching percentage was 60 and the ovicidal percentage was 40. In the pH range 7-8, hatching percentage was 76.6 and the ovicidal percentage was 23.3. At the pH range 8-9, hatching percentage was 83.3 and the ovicidal percentage was 16.6%. At the pH range 9-10, hatching percentage was 100, the ovicidal percentage was nil. The highest hatching percentage was in the pH 9-10. At pH 10-11 the hatching percentage decreased slowly and was 56.6, the ovicidal percentage was 43.4. At pH 11-12 the hatching percentage was 43.3 and the ovicidal percentage was 53.6.



Discussion

Mosquitoes belonging to the *Aedes* complex are highly adaptable to changing water quality characteristics, thus ensuring highest degree of survival and maximum fitness. The different life stages egg, larvae and pupae are capable of tiding over drought condition (Clements, 2000), temperature extreme (Huffakers, 1994), turbidity (American Public Health Association (APHA), 2006), salinity (Woodhil, 1938) and pH levels (Umar and Pedro, 2008).

Pupae are highly tolerant due to their impervious external covering known as the pupal case. The pupal stage extends for about 3-4 days and once the larva has pupated, its chances of getting affected by changes in water quality condition is extremely limited (Agnew and Koella, 1999). If it all, certain physico, chemical characteristics should affect *Aedes* sp. life stages, the impact has to be during the egg or larval stage.

In the present study the impact of changes in the hydrogen ion concentration of water was tested on *Ae. aegypti* eggs. The female *Ae. aegypti* lays eggs in clean water of high pH and low salinity. The ovipositing female is able to identify major changes in water quantity and lays eggs accordingly (Seghal and Pillai, 1970). The adult female mosquito has a well developed sensory system to assess water quality and this instinct increases the survival value of mosquitoes. Withholding eggs, from being laid in waters of an appropriate quality is a great survival strategy.

In this study the eggs laid by *Ae. aegypti*, were mechanically transported 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. In waters of pH 2-4, the ovicidal percentage was 100 and all the exposed eggs remained unhatched. The extreme pH level affected the eggs. The hatching percentage increased as the acidic pH turned basic. Ovicidal effect was minimum at pH 9 and 10. At this pH, 100 percentage hatching was recorded in *Ae. aegypti* eggs. Umar and Pedro (2008) conducted some laboratory bioassays in which the results indicated that maximum survival of both field and laboratory strains of the larvae of *Ae. aegypti* occurred between the pH values of 6.5 and 8.0. Outside these range, the mosquito larvae suffered high mortalities in 24 hours of exposure. Addition of different types of effluents into water bodies damages the fresh water ecosystem turning water samples acidic.

The highly acidic water samples are injurious to the larvae and hence the adult female prefers to lay eggs in another area where pH is normal. Rao *et al.* (2011) showed that *Aedes albopictus* breeding increased in waters of high pH and it was evident that *Ae. albopictus* favoured a specific pH range for breeding. This could be an important factor in breeding site selection and larval survival. Navarro *et al.* (2003) found increasing salinity in the laboratory also decreased oviposition.

Acknowledgement

Funding by UGC (UGC Major Project No. F.40-377/ 2011(SR)) is gratefully acknowledged.

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