

Effect of pH on the Rate of Successful Egg Hatches in *Aedes aegypti*

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Abstract

This experiment sought to determine if there was a correlation between solution pH and the rate of successful hatches in *A. aegypti*. It was hypothesized that more neutral pHs would see the highest percentages of successful egg hatches, while hatch rates in more acidic or basic solutions would see diminished percentages. To test this, eggs laid from a single cohort of female *A. aegypti* were placed in five solutions of different pHs and the viability of the eggs was determined by counting the number of larvae hatched in each solution. Finally, the data was analyzed using a simple linear regression. No statistical significance was found between pH and hatching viability of *A. aegypti* eggs. Further research would have to be done to make a conclusion on the effect of pH on the rate of hatching for *A. aegypti* in their natural environment.

Introduction

Aedes aegypti are the primary vector for several deadly diseases, including Zika, dengue, chikungunya, and yellow fever (WHO 2018). Female *A. aegypti* often lay their eggs in artificial containers, like flower vases and automobile tires, where rainwater can collect (Guillena 2010). Public health efforts have often attempted to educate those in areas with poor sanitation, irregular water supplies, and inefficient waste management systems about these behaviors.

In many areas around the world, the pH of rainwater is becoming slightly acidic due to the interaction between sulfur dioxide and nitrogen oxides released by human activities with moisture in the atmosphere (Singh 2008, 15-24). Such pollution is a concern as it may affect the natural environment and harm the organisms that live within them. This experiment sought to examine if pH changes would ultimately make any significant impact on the ecology of *A. aegypti*.

Methods

In each trial, four cups containing solutions of pH 3, 5, 9, and 11 (± 0.5) were used. A control cup containing only deionized water was also included for each trial to represent the method typically used to hatch *A. aegypti* in the lab.

Solutions were made using Hydroponic Essentials pH Up and Hydroponic Essentials pH Down from Hydrofarm that contain Potassium Hydroxide and Phosphoric acid, respectively. pH was measured using a Vernier Tris-Compatible flat pH sensor attached to a Vernier LabQuest.

10-25 *A. aegypti* eggs that had been oviposited on small pieces of Whatman paper were placed in each cup. These cups, containing the eggs and 15 mL of the respective pH solutions, were placed in a vacuum chamber for exactly one hour in order to stimulate the hatching process. After one hour, the vacuum chamber was opened and the cups were removed.

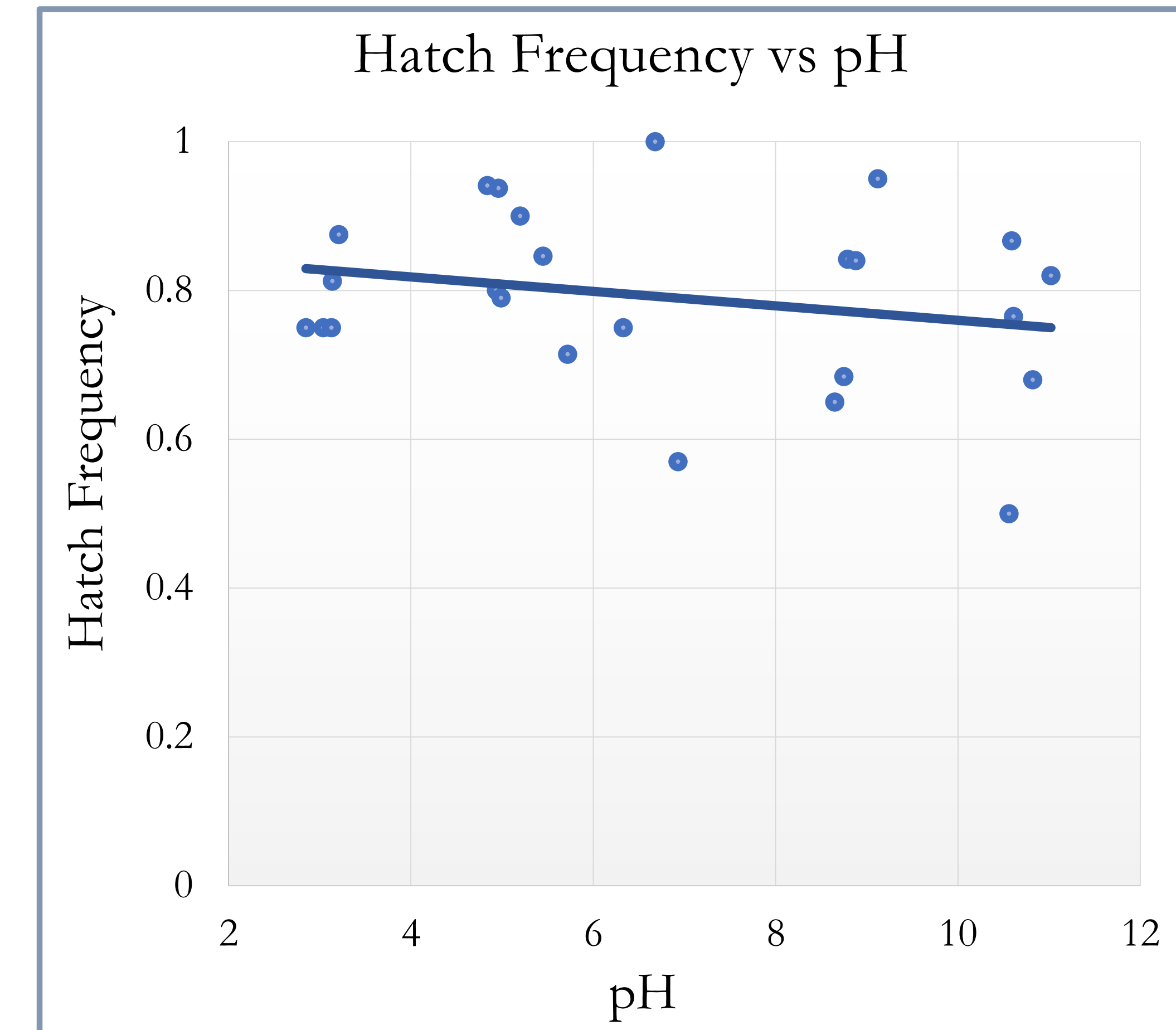
Using a transfer pipet, the liquid and larvae in the solution were transferred one mL at a time to a small black well that allowed the white-colored larvae to stand out under the dissecting microscope. The larvae in each cup were counted and recorded, and a ratio between the number of larvae to the number of eggs in each cup was obtained. A total of five trials were conducted for this experiment.



▲ Figure 1: Larvae seen under the microscope

◀ Figure 2: Samuel separating the *Aedes aegypti* eggs

Results



▲ Figure 3: A simple linear regression was utilized to determine the correlation between pH and hatch frequency of *A. aegypti* eggs. Although the line of best fit in Figure 3 shows a slight negative correlation, there was no statistical significance between the two variables ($p=0.271$).

pH (± 0.5)	# of eggs	Amt of soln.	Length of time in water	# of living larvae	Hatch Frequency
3	17	15 mL	1 hr	14	0.875
5	17	15 mL	1 hr	16	0.941
Control	13	15 mL	1 hr	11	0.846
9	20	15 mL	1 hr	13	0.65
11	17	15 mL	1 hr	13	0.765

▲ Figure 4: This figure is an example of the variables observed and recorded. Shown above is the data obtained from Trial 4.

Conclusion

Analysis of the data from the experiment did not suggest any significant difference in hatch rates between the various pHs. Further research would have to be done in order to prove a relationship between pH and the actual success rate of hatching in *Aedes aegypti* eggs.

Future experiments could involve a greater sample size in order to gain more conclusive results. Some concern was also raised about the PO_4^{3-} and K^+ ions in the acidic and basic solutions, respectively, which could have influenced the data. Efforts to minimize the variety of ions in each solution were taken.

As much research focuses on the larval stage and its interaction with pH, finding a relationship between the egg stage and pH could be a novel approach to public health efforts (Clark 2004, 2297–2304). Areas where rainwater pH values come near the ideal pH for *Aedes aegypti* can be prioritized in an effort to limit the growth of these mosquitoes at a very early stage.

Literature Cited

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Acknowledgements

A special thanks to Dr. Marty Harvill, Dr. Jason Pitts, Dr. Joshua Patrick, Shan Ju Shih, Jack Stutz, and the Baylor Biology Department for their insight, diligence, and resources.