Impact of salinity, pollutants and organic debris in still water on the life cycle of larval *Culex sp* and the oviposition site preference of mosquitoes in Bryan-College Station, Texas

Elizabeth Anne Nevins, Emily Louise Young, Aryana Garza, Jeffrey Harris, Nicole Hartzoge, Kate Biebighauser, Kaetlyn Fuchigami, Taylor Cook

Editor Tatyanna Suggs

After major natural disasters, the number of vector-borne diseases increases significantly. After Hurricane Harvey, a study was created to examine the effects of organic and inorganic pollutants on the breeding site preference and life cycle of mosquitoes. For each part of this experiment, containers of water were prepared with various organic and inorganic pollutants. To study the breeding site preference, these containers were placed outdoors in three residential properties in the Bryan-College Station, Texas community. After a period of 27 days, no data was observed as the cold, frigid weather in the city was not conducive to breeding. In a second study involving the life cycle of mosquitoes, containers containing these same inorganic and organic pollutants were placed indoors. Larval *Culex sp* mosquitoes were observed for a period of eight days to see how each environment affected their progression. The containers with tap water, distilled water, and organic material saw the most growth of mosquitos, while the containers filled with oil and sodium chloride killed off all the larval mosquitoes. It was concluded that neither seawater nor an aqueous environment in which there is substantial pollution, specifically oil, are conducive to mosquito growth. However, an overall generalization cannot be made on the effect of pollutants on the life cycle of all mosquitos. Further research will be needed, as the sample size was relatively small and only included one genera of mosquito.

Key Words: Mosquitoes, *Culex sp*, pollutants, breeding, life-cycle

---

With the recent flooding that has occurred in Houston, Texas, due to Hurricane Harvey, the level of standing water has increased tremendously, increasing the

possibility of an influx in mosquitoes. Because mosquitoes are responsible for the transmission of various illnesses, natural disasters are often correlated with an increase in vector-borne diseases (Gagnon et al. 2002, Saeed and Piracha 2016, Wang et al. 2017). For example, the number of cases of West Nile virus (WNV) increased substantially following Hurricane Katrina (Caillouet et al. 2008). WNV is an extremely serious vector-borne disease that has become endemic to the United States since its introduction in 1999. This viral infection, which can cause a series of symptoms including neurological deficits, is vectored by *Culex spp* mosquitoes (DeLisi et al. 2017). Because *Culex spp* mosquitoes are extremely common in the United States, it is vital to further understand factors that affect their life cycle, and subsequently improve control efforts.

In addition to flooding, Hurricane Harvey has also caused the displacement of organic debris, oil, and other pollutants, which are settling in different parts of the Houston area. Proven by Hurricane Katrina, coastal cities that have a large amount of oil refineries surrounding them have an increased risk of water pollution (Esworthy et al. 2006). Urban centers such as Houston have a greater amount of polluted water than rural areas due to the increased infrastructure and placement of oil companies. Hurricanes have also been known to cause salinity intrusion in freshwater environments. Following Hurricane Rita, salinity levels rose as high as 30.9 ppm in regions closest to the coast (Steyer et al. 2005). Although some species of larvae have been shown to thrive in saltwater, not much is known regarding the effects of salinity on mosquitoes following a hurricane (Panigrahi et al. 2014). Additionally, different mosquito species prefer different water types and therefore the mosquito population specific to Houston may react differently to this change of environment (Shaman et al. 2002). Some research suggests that certain species of mosquitoes can adapt to pollutants (Fillinger et al. 2004). For example, pollution plays a major role in the breeding sites of *Anopheles gambiae*, a species of mosquitoes known to transmit Malaria (Awolola et al. 2007). However, questions have been raised as to what effects this salinity intrusion, pollution, and organic debris displacement may have on the mosquito population specific to Houston.

This study serves to provide insight into how various aqueous environments brought about by natural disasters (such as hurricanes) may affect larval *Culex spp* mosquitoes and the oviposition preference of female mosquitoes within the Bryan-College

Station (BCS) area. Due to its proximity to Houston, BCS serves as an adequate location to conduct this study. Two separate experiments were conducted. The first examines the preference of mosquitoes in BCS for specific breeding sites, while the second involves the tolerance for larval *Culex spp* mosquitoes in various environments. Hopefully, this research may provide a better understanding on how to combat the mosquito vector following natural disasters and prevent the transmission of vector borne diseases.

Materials and methods

Study site. BCS is a metropolitan area located in Eastern Texas, approximately 90 miles northwest of Houston. It is situated at 30º36'5N latitude and 96º18'5 W longitude. The climate is subtropical with an average temperature of 20.6ºC. The city is located at an elevation of 93 meters above sea level and receives approximately 40 inches of rain per year (US Climate Data 2017). This study site was chose chosen due to its proximity to Houston.

Study design. Two experiments were involved in our study design. The first, Experiment A, involved filling five 5.7 L (6 qt) clear containers with 3.8 Liters (1 gallon) of water. Each contained various inorganic and/or organic contaminants and was set outside near each other. Container #1 contained 3.8 L of tap water, which acted as the control. The other four containers were filled; one with distilled water, one with polluted water (simulated by the addition of canola oil and some trash), one with organic debris in water (stimulated by the addition of leaves, dirt and grass), and the last with salt water (simulated by the addition of NaCl). The tap water was drawn from the same residential faucet for all the containers in a single location. Container #2 was filled with 3.8 L of distilled water. Approximately 240 mL (1 cup) of canola oil was added to 3.8 L tap water to simulate pollution in container #3, in addition to three items of trash. The oil was used to mimic the effects of motor oil, while remaining environmentally safe. Organic material was added to the 3.8 L (1 gallon) of tap water for container #4. This organic material container contained approximately 500 g of leaves, grass and dirt from each respective location. Container #5 was setup to simulate saltwater. Approximately 133 g of Sodium Chloride (NaCl) was added to 3.8 liters of tap water to replicate the concentration of salt in seawater (a salinity of 3.5%). Three sets of these containers were created, and each placed in a different location in the Bryan-College

Station area. These containers were all allowed to sit uncovered so that mosquitoes could deposit their eggs. Every three days, water was replenished if needed and mosquito eggs/larvae counted. Subsequently, Experiment B was completed indoors. Five smaller containers containing 0.5 L of each of the solutions described above were placed in doors. These containers were labeled #1-5 respectively and 100 *Culex sp* larval mosquitoes were placed in each. Mesh netting was taped over each container to disallow any newly hatched mosquitoes from escaping. These containers were checked daily for the progression of larvae into pupae and adults.

Figure 1. Experiment A setup.

Collection method. For Experiment A, eggs were to be removed if present using a pipette and subsequently placed in a petri dish. They were then to be counted using an image software called *ImageJ* to estimate the number of eggs present. This technique has been described previously (Mains et al. 2008). After the eggs/larvae are removed and counted, they were to be recorded in the data sheet.

Controls and locations. To increase chances of mosquito activity for Experiment A, three different locations in the BCS area were chosen to perform the first experiment. All three locations were situated in residential areas that have high levels of mosquito activity. By keeping location constant, the temperature, humidity, and rainfall were able to act as controls. However, only one location was used for Experiment B. Because this experiment was performed indoors, temperature and humidity were able to remain constant throughout its progression. The genera of mosquito, *Culex sp*, remained constant throughout the study. Additionally, the number of larvae present at the beginning of the experiment (100) was the same in all five containers observed.

Statistical Analysis. There was no analysis performed for Experiment A, due to lack of results. A Chi-Square Test was performed on the data from Experiment B in order to determine the statistical significance of any difference in data collected between the control and four variables. This test was

completed under the assumption that the data follows a standard normal probability distribution. The following formula was used:

$$X^2 = \sum \frac{(o-e)^2}{e}$$

Our null hypothesis (H_0) states that there is no relationship between our control group (Container #1) and our variable groups (Container #2-5). In order to reject this null hypothesis, a value of $p \leq 0.05$ must be calculated between the control and specified variable group.

Results

Experiment A. This experiment was conducted during the months of October through November of 2017. Each set of five containers were set up at the three different locations in the College Station area. Over the 27 day period that this experiment was conducted, no data was observed. It was hypothesized that the colder weather during the time period that this experiment was conducted may have discouraged mosquito breeding. Observation began on October 23, 2017 when there was a mean temperature of approximately 19ºC. Three days into the experiment, a cold front blew into Brazos county, driving the temperatures down to 15-20ºC during the day, and just above 0ºC at night. While temperature varied throughout this 27-day period, mean temperature steadily dropped. It is typical of mosquitoes to dramatically decrease and stop breeding after the first freeze of winter (Estallo et al. 2015). Despite the temperature not reaching freezing, temperatures did reach a low of 1.11ºC on October 29, 2017. This could have been cold enough to make mosquito breeding patterns slow, or even stop altogether. Despite the weather, the containers were checked at minimum every three days for a total of 27 days. At all three experiment locations, there was no eggs laid/observed in any of the water filled containers. At the third location site, all bugs observed in any of the five containers were dead, and of all the dead bugs in the containers, only three of them were mosquitoes. No tables are needed for this experiment, because exactly no data was collected.

Experiment B. This experiment was conducted indoors over a period of eight days. Two of the containers were determined to be inhospitable to the *Culex sp* larvae used in this experiment. 100% of the larvae in these containers (#3 and #5) were dead within 12 hours from initiation of the experiment. By day eight, container #1 (tap water only) contained 83% of live larvae, 4% dead larvae, 7% pupae, and 6% adult mosquitoes.

Comparatively, container #2 had very similar results with 84% live larvae, 4% dead larvae, 5% pupae, and 7% adult larvae by the last day of the experiment. Container #4, which contained residential organic material, had 87% live larvae, 4% dead larvae, 7% pupae, and 2% adult mosquitoes. Container #4 had the highest survival rate, but container #2 had the greatest growth and development within the mosquito population. A Chi-Square Test was performed on each data set for day eight of the experiment. Container #1 acted as the expected range. Containers #3 and #5 expectedly yielded a value of $p = 0.00$. Container #2 yielded a value of $p = 0.958$ and container four a value of $p = 0.414$. With this information, we were unable to reject our null hypothesis for containers #2 or #4. Therefore we cannot conclude that the data yielded for these containers is statistically significant. However, we may be able to reject the null hypothesis for containers #3 and #5. This data is statistically significant and should be analyzed more thoroughly to understand the reasoning behind our results. The results for all eight days are shown in the table below.

Difference in Results from Experiment A to Experiment B. A change of location was incorporated in order to carry out Experiment B. Experiment A was performed outside in the hopes that the mosquito season would carry on into the late September and October months. However, no data was observed for Experiment A. The difference in time frame between the two experiments was substantial. Experiment A was performed over a period of 27 days, while Experiment B lasted only 8 days. In addition to length of time the containers were set up, Experiment B was checked more frequently than the containers from Experiment A. While the containers in Experiment A were checked daily for traces of mosquitoes, factors such as weather, animals, and climate played significant roles in the upkeep of the containers. Because Experiment B was developed in response to a lack of results in Experiment A, modifications were made which led to a significant change in results. Possibly the most important shift in results was due to the addition of larvae during Experiment B. Thus, the outcome of Experiment B was more focused on living mosquitoes and their transition to adulthood rather than the simple presence of mosquitoes in any form. Overall, a time frame of 27 days gave no results in Experiment A, while a period of only 8 days gave decent recordable data in Experiment B.

Container #		Day 0	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8
1	Larvae (Alive)	100	97	94	92	89	86	85	85	83
	Larvae (Dead)	0	0	0	0	0	3	4	4	4
	Pupae	0	3	6	8	9	8	8	6	7
	Adults	0	0	0	0	2	3	3	5	6
2	Larvae (Alive)	100	98	96	94	92	88	87	83	83
	Larvae (Dead)	0	0	0	0	0	2	2	4	4
	Pupae	0	2	4	6	7	8	8	8	6
	Adults	0	0	0	0	1	2	3	5	7
3	Larvae (Alive)	100	0	0	0	0	0	0	0	0
	Larvae (Dead)	0	100	100	100	100	100	100	100	100
	Pupae	0	0	0	0	0	0	0	0	0
	Adults	0	0	0	0	0	0	0	0	0
4	Larvae (Alive)	100	99	97	96	94	92	90	88	87
	Larvae (Dead)	0	0	0	0	0	1	2	3	4
	Pupae	0	1	3	4	5	6	7	7	7
	Adults	0	0	0	0	1	1	1	2	2
5	Larvae (Alive)	100	0	0	0	0	0	0	0	0
	Larvae (Dead)	0	100	100	100	100	100	100	100	100
	Pupae	0	0	0	0	0	0	0	0	0
	Adults	0	0	0	0	0	0	0	0	0

Table 1. The Results of Experiment B.

Discussion

Experiment A failed to produce any substantial results due to the cold weather that was seen in BCS for the duration of data collection (Oct. 2017- Nov. 2017). As mentioned previously, mosquitoes are known to stop breeding after the first freeze of winter (Estallo et al. 2015). Although it did not freeze in College Station, the temperature did fall as low as 1.11ºC. Because of this, it was inferred that mosquitoes were unable to breed and deposit eggs into the containers therefore resulting in a lack of data. Further research is suggested in order to explore mosquito preference during a more suitable season. This potential data could provide vital insight for combating the specific mosquito population of Houston, and controlling an increase following flooding events.

In Experiment B, environments with a salinity of 3.5% NaCl and those containing a substantial amount of inorganic pollution, notably oil, were found to be inhospitable to the larval *Culex sp.* Containers #3 and #5 caused 100% larval death within a 12- and 24-hour period, respectively. Previous research suggests that the presence of canola oil can prevent larval mosquitoes from being able to breath via the siphon (Riccuiti, 2016). The oil most likely dispersed across the water to form a film in which the larvae were unable to penetrate. It was hypothesized that this may have been a major factor in the inability of the *Culex sp* larvae to survive in container #3, which held 240 mL of canola oil. Larval death was also heavily noted in container #5, which consisted of an aqueous

3.5% NaCl solution. In this instance, 100% larval death occurred within a 24-hour period. Even dilute NaCl solutions can stunt the growth of some species due to destruction of food sources and salt has been used as a larvicide in certain instances. (Lee, 1973). However, the presence of NaCl alone, does not always cause rapid larval death. Other studies have shown that some species of larval mosquitoes can successfully grow in the presence of low NaCl concentrations (Wigglesworth 1932). For example, various *Aedes spp* and *Culex sitiens* have been shown to thrive at higher levels of salinity (Jonusaite et al 2017, Roberts and Irving-Bell 1997). While our experiment shows that this *Culex sp* larvae cannot thrive in the presence of a 3.5% NaCl solution, this does not ultimately disprove the idea that some species can adapt to levels of high salinity. Further research is suggested to determine if *Culex spp,* and other species of medical importance, can thrive at lower levels of salinity.

The larvae in containers #1, #2, and #4 saw the only growth within the eight days that the experiment was conducted. In containers #1 and #2, larvae were able to mature due to the absence of any external stimulants or factors present that could potentially inhibit growth. Of the containers that possessed surviving larvae, container #4 produced the least number of adults. In container #4, which was filled with 3.8 L of tap water along with approximately 500 g of organic debris (leaves and grass), only 2 larvae matured into adults within the eight-day period of data collection. Whereas, in containers #1 and #2 approximately 7 larvae were able to surpass the pupal stage. This could have resulted from the organic debris forming a layer of scum on the surface of the water, potentially mimicking (to a lesser extent) results of container #3 (Imam et al. 2014). Although, this subtle difference in growth may not be due to the presence of organic debris at all. Due to the difficulty involved in separating larvae in various stages of their life cycle, all five containers had varying amounts of larvae in different stages. It is possible that container #4 received comparatively more larvae in earlier stages. Consequently, there would be less mosquitoes able to mature into adults over an eight day period. Additionally, not much can be inferred about the susceptibility of mosquitoes from earlier to later life stages. Questions were raised as to whether mosquitoes is earlier larval stages may be less resistant to pollutants in their environments. Also due to this lack of separation, we were not able to determine if the various environments affected the rate at which

larvae matured. For example, it is unknown if larvae in container #4 matured more slowly than those in container #1 or #2, and vice versa.

This study provides a basis for further research regarding how natural disasters, such as hurricanes, may affect the life cycles of medically important mosquitoes. It was concluded that an aqueous solution of 3.5% NaCl was enough to cause larval death. Additionally, the addition of 250 mL of canola oil to 0.5 L of tap water acted in a similar manner and was also an adequate, environmentally friendly, means of killing the larval *Culex sp.* As mentioned previously, more research is desperately needed to draw any legitimate conclusions regarding how varying levels of salinity intrusion, organic debris, and inorganic pollutants may affect the life cycle of *Culex sp* and other species of relevance.

References

Awolola, T.S., A.O. Oduola, J.B. Obansa, N.J. Chukwurar, and J.P. Unyimadu. 2007. Anopheles gambiae s.s. breeding in polluted water bodies in urban Lagos, southwestern Nigeria. J. Vector. Borne. Dis. 44: 241-4.

Caillouet, K.A., S.R. Michaels, X. Xiong, I. Foppa, and D.M. Wesson. 2008. Increase in West Nile neuroinvasive disease after Hurricane Katrina. Emerg. Infect. Dis. 14: 804-807.

DeLisi, N., J. Ottea, and K. Healy. 2017. Susceptibility of *Culex quinquefasciatus* (Diptera: Culicidae) in southern Louisiana to larval insecticides. J. Econ. Entomol. 20: 1-6.

Estallo, E.L., F.F. Luduena-Almeida, M.V. Introini, M. Zaidenberg, and W.R. Almiron. 2015. Weather variability associated with *Aedes* (stegomyia) *aegypti* (Dengue vector) oviposition dynamics in northwestern Argentina. Plos. One. 10: e0127820.

Esworthy, R., L. Schierow, C. Copeland, L. Luther, and J.L. Ramseur. 2006. Cleanup after Hurricane Katrina: environmental considerations. Library of Congress and Congressional Research Service, DC, USA.

Fillinger, U., G. Sonye, G.F. Killeen, B.G. Knols, and N. Becker. 2004. The practical importance of permanent and semipermanent habitats for controlling aquatic stages of Anopheles gambiae sensu lato mosquitoes: operational observations from a rural town in western Kenya. Trop. Med. Int. Health. 9: 1274-1289.

Gagnon A.S., K.E. Smoyer-Tomic, and A.B. Bush. 2002. The El Nino southern oscillation and malaria epidemics in South America. Int. J. Biometeorol. 46: 81-89.

Imam, H., Zarnigar, G. Sofi, and A. Seikh. 2014. The basic rules and methods of mosquito rearing (*Aedes aegypti*). Trop. Parasitol. 4: 53-55.

Jonusaite, S., A. Donini, and S.P. Kelly. 2017. Salinity alters snakeskin and mesh transcript abundance and permeability in midgut and malpighian tubules of larval mosquito, *Aedes aegypt*i. Comp. Biochem. Physiol. A. Mol. Integr. Physiol. 205: 58-67.

Lee, F.C. 1973. Effect of various sodium chloride concentrations on the development of the mosquito *Culiseta incidens* (Thomson) (Diptera: Culicidae). Mosq. News. 33: 78-83.

Mains, J.W., D.R. Mercer, and S.L. Dobson. 2008. Digital image analysis to estimate numbers of *Aedes* eggs oviposited in containers. J. Am. Mosq. Control. Assoc. 24: 496-501.

Panigrahi S.K., T.K. Barik, S. Mohanty, and N.K. Tripathy. 2014 Laboratory evaluation of oviposition behavior of field collected *Aedes* mosquitoes. J. Insects. 2014: 1-8.

Riccuiti, E. 2016. GAT mosquito traps can be effective even without pesticides. *Entomology Today*. Entomological Society of America, retrieved September 28, 2017, from https://entomologytoday.org

Roberts, D.M. and R.J. Irving-Bell. 1997. Salinity and microhabitat preferences in mosquito larvae from southern Oman. J. Arid. Environ. 37: 497-504.

Saeed, U. and Z.Z. Piracha. 2016. Viral outbreaks and communicable health hazards due to devastating floods in Pakistan. World. J. Virol. 5: 82-84.

Shaman, J., M. Stieglitz, C. Stark, S. Le Blancq, M. Cane. 2002. Using a dynamic hydrology model to predict mosquito abundances in flood and swamp water. Emerg. Infect. Dis. 8: 8-13.

Steyer, G.D., B.C. Perez, S. Piazza, G. Suir. 2007. Potential consequences of saltwater intrusion associated with hurricanes katrina and rita, pp. 137-146. *In* G.S. Farris, et al. (eds.), Science and the storms-the USGS response to the hurricanes of 2005. U.S. Geological Survey, LA.

US Climate Data. 2017. Climate College Station - Texas. Retrieved September 28, 2017, from https://www.usclimatedata.com.

Wang, Y., W. Pons, J. Fang, and H. Zhu. 2017. The impact of weather and storm water management ponds on the transmission of West Nile virus. R. Soc. Open. Sci. 4: 170017.

Wigglesworth, V.B. 1932. The adaptation of mosquito larvae to salt water. J. Exp. Biol. 10: 27-36.