Survey of Mosquito (Diptera: Culicidae) Species Inhabiting Bryan & College Station, Texas

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Abstract: Mosquitoes have long been known as a nuisance of an insect. Their role as vectors has increased the importance of them in both the medical and veterinary. Mosquito-borne diseases are a serious threat to the health and well-being of all human beings. The re-emergence of serious diseases such as West Nile virus, yellow fever, malaria, and Zika virus have reinforced the importance of studying mosquitoes and their distribution across environments. Researchers have spent much time and money investigating the distribution of mosquitoes worldwide. While it is commonly understood that certain species are more prevalent in particular areas of the world, the distribution of mosquitoes within smaller communities of the United States, such as Bryan/College Station (BCS), is less studied and understood. The purpose of this survey was to gain a greater understanding of how environmental factors influence the prevalence and type of mosquitoes present in the Bryan/College Station vicinity. Mosquito larvae were collected from four different locations once a month from September to November, 2016. The larvae were identified using a wet mount slide. Additionally, some larvae were allowed to develop into mature adults, subsequently killed, and analyzed for identification. Environmental conditions were noted at each collection. Mosquitoes from the genera *Aedes, Psorophora,* and *Culex* were identified with *Aedes* being the most prevalent. Analysis of environmental conditions showed pH had a negligible effect on larval development while water and air temperature did indeed impact larvae numbers. Furthermore, mosquito numbers were greater in populated, brushy areas with standing water.

Keywords: Culicidae, survey, environment, *Aedes*, *Culex*

Mosquitoes are some of the most threatening insects inhabiting earth due to their ability to vector many deadly diseases. Although lacking physical strength, mosquitoes have been credited with killing more people than all of the wars in history combined. For example, mosquitoes transmitting malaria kill 2-3 million people and infect another 200 million people every year. Tens of millions more are infected with other mosquito-borne diseases, including encephalitis, dengue, yellow fever, and filariasis (Illinois Department of Public Health). Because maintenance of body fluid ionic composition and pH is extremely important for homeostasis, the ionic composition and pH of the larval habitat water are major factors, which limit the distributions of many mosquitos.

Surprisingly, there is no evidence that pH significantly limits the habitats of larval mosquitoes in nature (Clements, 2000). Some reported pH values for larval habitats range from 3.3 to 8.1 (*Ochlerotatus taeniorhynchus*), 4.4–9.3 (*Aedes geniculatus*), 3.3–9.2 (*Psorophora confinnis*), and 4.4–9.3 (*Anopheles plumbeus*). *Aedes flavopictus* has even been reared in waters ranging from pH 2–9, and *Armigeres subalbatus* in the pH range of 2–10 (Keilin, 1932; Kurihara, 1959; MacGregor, 1921; Peterson and Chapman, 1970). This data demonstrates the ability of larvae to adapt and develop under highly acidic or basic conditions. Although it is known that larvae can survive in these conditions, one purpose of this experiment was to see whether or not larval numbers were significantly more or less abundant based on pH, water temperature, air temperature, and other geographic landmarks. This study also sought to explore the various species inhabiting the Bryan/College Station area, as this is an important aspect of disease control.

Materials and Methods

This experiment focused on collection of larval mosquitoes from various bodies of water in the Bryan/College Station area in order to determine the optimal water pH, water and air temperature, and other geographical factors for optimal growth of the mosquito population. For the experiment, the quick dipping method was used for the collection of the larval mosquitoes; a technique that is the most efficient amongst most mosquito species. When collecting the water samples, we completely submerged mosquito breeder containers, as larvae are known to descend to the bottom of a pool of water upon sensing shadows. Collections were performed at John Crompton Park (30.5921036° N, -96.3362333° W), Lake Bryan (30.7120° N, -96.4730° W), the Texas A&M Equestrian Center (30.6169511° N,-96.3738448° W), and the Southside Historic District neighborhood (31.53417° N, -96.8503376° W) (**Figure 1**). Collections were made at three different times in each location—during the months of September, October, and November. Physical structures serve as protection and a microhabitat for larvae. Therefore, collections occurred near these physical structures to ensure the effective collection of the specimens.

Using mosquito breeders provided by the Texas A&M University Entomology Department, we collected mosquito larvae samples from water around the Bryan/College Station area. At the time of collection, air temperature, water temperature, and water pH were recorded. Additionally, a note of other characteristics, such as the presence of livestock, open versus brushy areas, area activity (the presence or absence of human activity), and other attributes of the surroundings. To optimize the capture of larval species, a couple of containers were left with the tested water to create a suitable breeding environment for mosquitoes. Using pH strips (Micro Essential, Brooklyn, NY), the pH of the water was measured in each location of collection by dipping the strip into the water and recording the color change. A small portion of mosquito larvae were then transferred into a mosquito breeder (Bioquip, Rancho Dominguez, CA) before being left out in a warm, dry environment to develop into adults. In the laboratory, the larval specimens were transferred with a disposable pipette dropper (Globe Scientific Inc., Paramus, NJ) and stored in Eppendorf tubes (Fisherbrand, Pittsburgh, PA) containing

70% ethanol. The species of the mosquitos were identified using the entomology laboratory manual provided by Texas A&M University, microscope slides (Fisher Scientific, Hampton, NH), and microscopes provided by the Texas A&M University Entomology lab. A wet mount slide was prepared by placing a drop of the larvae/ethanol mixture with the disposable pipette dropper between a microscope slide and a coverslip (Fisher Scientific, Hampton, NH). A paper towel was used to soak the excess liquid from the slide and clear nail polish (OPI, North Hollywood, CA) was used to permanently affix the cover slide to the microscope slide. The larvae specimens were analyzed by their differing characteristics, as illustrated in the laboratory manual as a reference. Adult mosquitoes were killed in the freezer and placed in glass vials for identification using dissecting microscopes provided by the Texas A&M Entomology Department. Identification of the larval specimens was compared with identification of the adult mosquitoes from the same collection. These results were further analyzed.

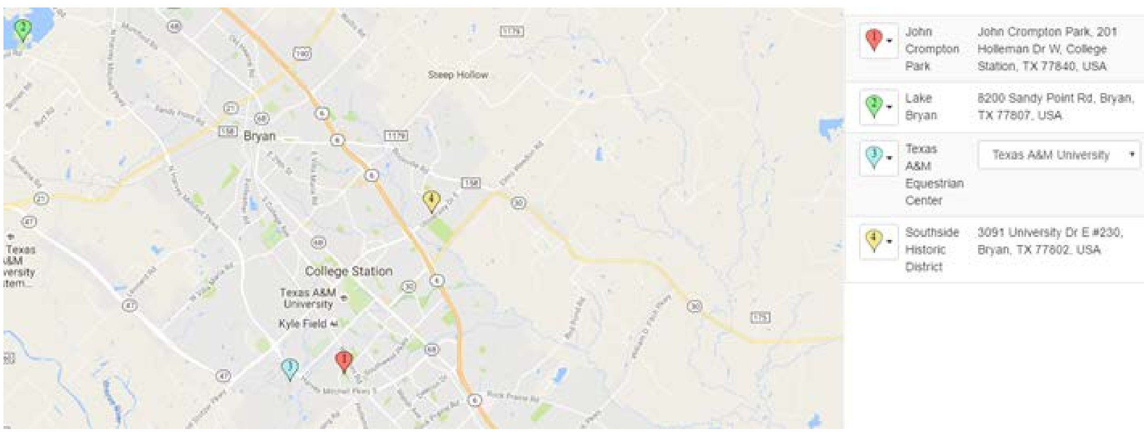

Figure 1. Map of the Mosquito Collection Locations

Results

Collections were successful at least once in each location. Results from each collection were recorded (Table 1). Quick dipping from the main pond of John Crompton Park produced a total of 12 larvae; four developed into adults. Species found at John Crompton park included *Aedes aegypti* and *Aedes albopictus.* A total of four larvae came from Lake Bryan, which developed into two adults. Identification of these species revealed *Aedes vexans* and *Aedes albopictus* were present. Species found at the Texas A&M Equestrian Center included *Culex restuans, Aedes albopictus,* and *Culex quinquefasciatus.* A total of nine larvae were collected here, six of which survived to adulthood. Collection from the Southside Historic District in College Station produced 15 larvae from three species (*Culex quinquefasciatus*, *Culex restuans*, and *Aedes aegypti*). Of these 15 larvae, five successfully developed into adult mosquitoes. For the September collection, air temperatures at each location were recorded at 91°F and water temperatures ranged from 83°F to 85°F. In October, air temperature was recorded at 79°F at the time of collection, while water temperature of collection sites ranged from 70°F to 75°F. At the final collection in November, air temperature was 73°F and water temperature ranged from 67°F to 70°F. The pond at John Crompton Park was slightly basic at each collection (pH 7.5-8), while Lake Bryan was slightly acidic at each collection (pH 6.5). Standing water at the Equestrian Center was either slightly basic or neutral, with recorded pH values of 8 and 7. Collection sites in the Southside Historic district ranged from slightly acidic (pH 6) to slightly basic (pH 7.5). Unique geographic characteristic of each location are recorded in Table 1.

Date	Location	Air temp/Water temp (F)	Water pH	Distinctive environmental characteristics	# larvae	Larvae ID	# adults reared	Adults ID
09/17	John Crompton Park	91/83	7.5	Open area pond with trees bordering one side; relatively high human traffic	5	*Aedes aegypti*, *Aedes albopictus*	2	*Aedes aegypti*
09/17	Lake Bryan	91/80	6.5	Freshwater lake; brushy; low human traffic	4	*Aedes vexans*, *Aedes* albopictus	2	*Aedes albopictus*
09/17	Equestrian Center	91/85	8	standing water pond; livestock (horses); many trees	5	*Culex restuans*, *Aedes albopictus*	3	*Culex restuans*, *Aedes albopictus*
09/17	Southside Historic District	91/85	6	Bird bath in residential neighborhood	6	*Culex quinquefasciatus*, *Culex restuans*	2	*Culex restuans*
10/15	John Crompton Park	79/72	8	Open area pond with trees bordering one side; relatively high human traffic	5	*Aedes aegypti*, *Aedes albopictus*	2	*Aedes aegypti*, *Aedes albopictus*
10/15	Lake Bryan	79/70	6.5	Freshwater lake; brushy; low human traffic	0	N/A	0	N/A
10/15	Equestrian Center	79/72	7	standing water pond; livestock (horses); many trees	3	*Culex restuans*, *Culex quinquefasciatus*	3	*Culex restuans*, *Culex quinquefasciatus*
10/15	Southside Historic District	79/75	7	Bird bath in residential neighborhood	5	*Culex quinquefasciatus*	2	*Culex quinquefasciatus*
11/12	John Crompton Park	73/68	7.5	Open area pond with trees bordering one side; relatively high human traffic	2	*Aedes albopictus*	0	N/A
11/12	Lake Bryan	73/67	6.5	Freshwater lake; brushy; low human traffic	0	N/A	0	N/A

11/12	Equestrian Center	73/69	7	standing water pond; livestock (horses); many trees	1	*Aedes albopictus*	0	N/A
11/12	Southside Historic District	73/70	7.5	Old tire filled with water	4	*Aedes aegypti*, *Culex quinquefasciatus*	1	*Aedes aegypti*

Table 1. Collection sites and conditions of larvae along with identification of larvae and adults

Discussion

Analyzation of results suggests pH values have no significant impact on larval development. High numbers of larvae were found in both acidic and basic conditions with no extreme outliers under either circumstance. However, there does appear to be a direct relationship between air or water temperature and larvae numbers. As both air and water temperatures decreased, less larvae were collected at each site. It is assumed that this correlation is not purely linear because extreme temperatures, both hot and cold, would decrease viability of larval and adult mosquitoes.

The pH appeared to have no significance on the larval population and development. The pH readings that were observed fell within the range of 6-8, which indicates that the pH of the water was either neutral, slightly basic, or slightly acidic. The influence of pH displays significance in that natural conditions can provide a pH environment that either has a favorable or unfavorable association of biological and chemical factors for the breeding locations upon which there is either successful or unsuccessful development of larva (MacGregor 1921). Some species of mosquitoes find their optimal environmental conditions for development in water sources that are acidic while others may prefer more alkaline. The various localities where the larva are found containing various species can show a restriction of a pH index within a short range that allow for the proper development of the particular species.

Various mosquito species are sensitive to temperature. Mosquitoes are categorized as ectotherms in which each of their life stages is dependent on the temperature in the developmental and mortality rates (Beck-Johnson et al. 2013). Adult population abundance was highest in environments where the temperatures were suitable for juvenile mosquitoes. These areas possess optimal environmental conditions that lead to successful completion of developmental stages. Using the data collected, the viability of the larva and adult mosquitoes could be assessed. It appeared that the larval population was highest when the seasonal temperatures were in lower 90s to the mid-80s. The larval populations appeared to decrease with seasonal temperatures in the upper 60s to mid-70s.

Larvae collection numbers were highest in Southside Historic District and John Compton Park. Since these two areas experience the most human traffic, it can be suggested that mosquitoes favor areas where humans reside or visit frequently. The Lake Bryan collection site produced the lowest larval numbers perhaps due to the rough water conditions and less frequent human interaction. The vector’s ability to actively seek out its host depends on olfactory receptors located on the antennae, maxillary palpi, and labellum (Lu, 2007). The greatest larvae collection amounts in areas of higher human traffic is likely explained by mosquito attraction to human carbon dioxide production. Carbon dioxide interacts with human skin odorant, lactic acid, to produce a strong attractive response in mosquitoes (Guerenstein 2008). Carbon dioxide is a general olfactory cue for all mosquitoes, however, mosquitoes such as *Ae. aegypti* can locate lactic acid which is an excretory substance in humans (Dekker, 2002). Thus, it is more common for higher larvae production to exist in areas with denser human population.

Mosquito host preference depends on multiple factors. These factors include host availability, nutritional value of blood, the energy needed for digestion, defensive behavior of the host, season, and physiological factors (Lyimo 2009). The presence of mosquitoes at the Equestrian Center suggests mosquitoes are will feed on and attracted to livestock. *Culex restuans* and *Culex quinquefasciatus* were found at the Equestrian Center. It is common for North American *Culex* species to transition from feeding on birds in early spring and summer to mammals in late summer and fall (Lyimo 2009). The underlying cause for variation in mosquito host choice is likely linked to host availability; however, lower larval numbers than John Compton Park and Southside Historic District suggest a preference for human blood over livestock blood (Lyimo 2009).

In conclusion, mosquito larvae collection was performed in areas where water was standing rather than moving. The highest overall collection site was a birdbath in Southside Historic District. Mosquitos prefer to lay their eggs in habitats with standing water, as it is difficult for larvae to survive in fast-flowing water conditions. Studies show that female mosquitoes also prefer to lay their eggs in water collected in man-made containers (Zika, Mosquitos, and Standing Water, 2016). This would explain a higher number of larvae collected in a tire filled with water in the Southside Historic District and a higher collection trend in birdbaths.

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