Effects of Water Temperature on Development and Survival to Adulthood in *Culex quinquefasciatus* (Say)(Diptera:Culicidae) and *Psorophora ferox* (Humboldt)(Diptera:Culicidae) Larvae

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Abstract Due to the ability of mosquito species to survive in wide variety of temperatures, an experiment was conducted to test how water temperature affects the maturity rate of *Culex* and *Psorophora* species larvae and adults. The two genera are commonly found in College Station, Texas. Larval mosquitoes were reared in different temperature environments ranging from 9°C to 34°C to ascertain the effects of water temperature on the maturity and survival rates of mosquitoes. The data collected from this experiment revealed that mosquito larvae mature very quickly in water temperatures of approximately 21°C. The larvae can survive for extended periods of time, specifically 5 weeks during this experiment, in water that is below 10°C. This data implicates that, during the colder months of November and December in Texas, large populations of mosquitoes could survive in their larval state in breeding pools and rapidly mature to adults during periods of uncharacteristic warm weather. These large influxes in mosquito populations coupled with reduced cold-weather mosquito control measures could result in higher disease transmission rates between humans and mosquitoes.

Keywords: *Culex quinquefasciatus, Psorophora ferox, mosquito development, water temperature*

Members of the family *Culicidae* are effective vectors of numerous human diseases, including Dengue, Yellow Fever, and various encephalitis' (Center for Disease Control 2015), making them vastly important in terms of medical entomology. College Station, Texas is known to be the home of numerous members of the family Culicidae, most notably *Culex quinquefasciatus* and *Psorophora ferox*, which are the mosquito species used in this experiment. Moreover, in College Station, West Nile Virus, a disease known to be effectively vectored by *C. quinquefasciatus*, exists at an endemic level (City of College Station 2015). Mosquitoes are considered a pest species, whose bites cause considerable annoyance and mild pain for those on which they are inflicted, especially if the bite elicits a significant allergic response. For these reasons, it is of significant importance to discern the density and chances of survival of local mosquito populations. The developmental and survival rates of mosquito larvae is known to be more successful in warmer climates during warmer times of the year (Mosquito Magnet 2015). In the United States, mosquito populations tend to peak in the months between April and October. The density of active adult mosquitos also increases as the location nears the equator, where temperatures are known to be higher. College Station is located in the southern region of the U.S., causing high average temperatures year round, but especially in the early spring through late fall time of year. The preferred water temperature range of *C. quinquefasciatus* and *P. ferox* as larvae is near 30°C (Malheur Vector Control District 2015), which is consistently attained during the summer months in Texas. However, though it is prone to a warmer climate, due to its geography and topography, College Station is also prone to large fluctuations in temperature throughout the year, especially in the colder months of November and December. For this reason, mosquitoes at every stage of development must be able to withstand seasonal and spontaneous temperature variation in order to survive and continue to propagate. This experiment was designed to simulate the natural temperature fluctuation in College Station and illustrate the survival ability of local mosquito populations.

Materials and Methods:

Source of Mosquitoes

Culex quinquefasciatus and *Psorophora ferox* used in this experiment were field collected larvae from College Station, Texas. A commercial fifty-liter cooler (Igloo Products Corporation, Katy, TX) was filled with 35 liters of pure water and a cup of local soil. It was then placed in the back corner of a residential backyard under hanging vegetation, which would serve to provide the necessary substrate for larval consumption as well as serve to shield the site from extreme daytime temperatures. The cooler was then left untouched for a period of two weeks, during which time it became an oviposition site for local adult female *C. quinquefasciatus* and *P. ferox* mosquitos. Larvae of the same age (two to three days since eclosion) from this colony were used for this experiment.

Experimental Protocol

After a period of two weeks, during which time adult female mosquitoes were given a chance to oviposit multiple clutches of eggs, researchers removed the cooler from its isolated location to begin the experiment. Mosquito larvae were transferred to a 5.5L plastic bowl (Walmart, Bentonville, AR) by dipping the bowl into the cooler, allowing water to settle and allowing the larvae to get into the bowl then removing it from the cooler. This random selection served as our experimental sample. Three larval rearing containers (FisherSci, Chicago, IL) were then procured and filled with 500 mL of the same nutrient-rich water that was previously in the cooler. Into each of the three containers, twenty random larvae were deposited. Larvae at the same stage of development (based upon size and time since eclosion) were placed in each of the three larval rearing containers by picking them up individually with a ⅓ cup measuring spoon (Walmart, Bentonville, AK). Once the larval rearing containers were filled with water and larvae and assembled, they were placed in one of three environments that vary based upon temperature. The first container was placed in a dim-lighted refrigerator (Frigidaire, Martinez, GA) at a constant temperature of 9°C. The second container was placed in a dim room at a room temperature of 22°C. The third and final container was placed in a dim room with a heat lamp (Fluker Farms, Port Allen, LA) outfitted with a 60W bulb (Exo Terra, Mansfield, MA) placed directly above it, ensuring even distribution of heat. The equilibrium temperature of the water in the heated container was 34°C. These containers were left completely untouched for a period of six weeks (September 29th, 2015 to November 10, 2015), with the exception of weekly checks by the researchers, during which time the number of larvae, pupae, and adult mosquitoes in each container was recorded.

Data Collection and Analysis

The containers were left undisturbed during which time the larvae were allowed to either fully develop or die at an undeveloped stage. At the end of these six weeks, the containers were taken from their respective environments for analysis. The specimens (both dead and alive) from each container were counted and their final stage of development recorded.

Specimen Preservation

Using forceps (BioQuip, Rancho Dominguez, CA), nine adult mosquitoes were randomly selected from each of the three larval rearing containers to be preserved. First, the mosquitoes were identified based on their species using taxonomic keys and separated. They were then submerged in unscented hand sanitizer (Gojo Industries, Akron, OH) in one of three specimen vials (2 dram 17 x 60 mm), using laboratory forceps to transfer. Six mosquitoes that died in a larval state were selected randomly from each of the three containers. A pot (Calphalon, Bowling Green, OH) was placed on a stove (Frigidaire, Martinez, GA) and filled with water. The water-pot combination was then heated to 102°C, at which time it attained a consistent, light boil. Using laboratory forceps, the six randomly selected, deceased larval mosquitoes were transferred to the pot. After a period of thirty seconds, the mosquito larvae were removed from the pot, once again via laboratory forceps and transferred to a countertop, where they were identified by species using taxonomic keys. The mosquito larvae were then separated by species and, using laboratory forceps, placed in specimen vials (2 dram 17 x 60 mm) containing ethanol for preservation.

Results:

The contents of each rearing container were observed and recorded on a weekly basis starting on the first Tuesday after collection. The last recording was taken on November 10th when all larvae, pupae and adults had perished. The 34°C water had a survival rate of 40 percent with eight out of twenty larvae developing completely to adulthood. Two out of the twenty larvae originally placed in the 34°C water fully pupated and half of the original twenty perished as larvae. The 22°C, room temperature water had a survival rate of 100 percent with twenty out of twenty larvae developing completely to adulthood. All twenty larvae in the room temperature water completed their life cycle to adulthood within the first five days of the experiment. This is not surprising because this was the nearest temperature to the mosquitoes' ideal development water temperature. The 9°C water had a survival rate of zero percent with none of the twenty larvae developing to adulthood and only one progressing to the pupae phase. However, three of the larvae did survive for over a month with almost half surviving for four weeks.

Discussion:

In Texas, temperatures in November and December can be highly variable from day to day. Predicting insect population fluctuations during the winter is more difficult than in the summer months when it is expected that the temperature will be around 38°C consistently for months at a time. This experiment was designed to simulate the possible temperatures that could be experienced in Texas during this time and the effects it has on the development of larval mosquitoes. As expected, the temperature closest to the mosquitoes' optimal development temperature had the highest percent of survival. A 100% rate of survival in the 22°C water was expected because the optimal water temperature for larval development in mosquitoes is 30°C (Malheur Vector Control District 2015). The 9°C water, a temperature close to what would be felt on a cold November day in Texas, held the mosquito larvae in a state of almost suspended animation. These larvae survived for weeks, rarely moving and only known to be alive after being pestered into activity. Three larvae were observed to survive in this state for over 5 weeks and were presumed to have died from starvation, due to being unable to move, therefore unable to hunt. These data implicate that, after a long cold spell in November or December, many mosquito larvae could potentially be revived from inactivity on a particularly warm day in late November and a large swell in adult mosquito populations could be observed a few days after. This coupled with the decrease in mosquito control measures could potentially create a serious problem in places with previously large populations of mosquitoes. A lack of personal mosquito prevention measures and increased mosquito populations in rural areas could lead to increased transmission of mosquito born diseases. West Nile Virus has been gaining traction in East Texas over the last decade (Dillard 2012) and is effectively vectored by *C. quinquefasciatus*, one of the mosquitoes studied in this experiment. The potential outbreak of West Nile Virus that could come from an unexpected influx of *C. quinquefasciatus* could have a devastating effect on the at risk populations of East Texas.

Figure 1- Alive and deceased Culicidae count in 34°C Water

34C	Sept. 29	Oct. 4	Oct. 11	Oct. 18	Oct. 25	Nov. 1	Nov. 10
Live Larvae	20	10	10	2	0	0	0
Live Pupae	0	2	2	0	0	0	0
Live Adult	0	2	0	0	0	0	0
Deceased Larvae	0	0	0	8	10	10	10
Deceased Pupae	0	0	0	2	2	2	2
Deceased Adult	0	6	8	8	8	8	8

Figure 2- Alive and deceased Culicidae count in 22°C Water

22C	Sept. 29	Oct. 4	Oct. 11	Oct. 18	Oct. 25	Nov. 1	Nov. 10
Live Larvae	20	0	0	0	0	0	0
Live Pupae	0	0	0	0	0	0	0
Live Adult	0	4	0	0	0	0	0
Deceased Larvae	0	0	0	0	0	0	0
Deceased Pupae	0	0	0	0	0	0	0
Deceased Adult	0	16	20	20	20	20	20

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