St. Edward's University

# Efficient Mosquito Vector Abundance Comparison Between Work and Natural Protected Areas

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## Introduction

With accelerating climate change, the world is experiencing a rise in emerging infectious diseases and a resurgence of once controlled vector borne diseases such as West Nile Virus and Dengue Virus (Gratz 54). The most recent headliner, the Zika Virus, is an arbovirus with a suspected link to microcephaly in newborns and numerous other diseases. Zika is only one example of the many viruses that are stirring public health concerns worldwide. The primary vector of dispersal for Zika, Chinkunkuya, West Nile, Dengue, and Malaria are mosquitoes. Thus far, researchers have identified two prime species, *Aedes aegypti* and *Aedes albopictus,* to be two of the most effective mosquito vectors for these viruses.

Mosquito populations are most sensitive to temperature, rainfall, predation, and competition. *Aedes aegypti* and *Aedes albopictus* inhibit tropical climates with a temperature range of tolerance between 26° C to 35°C with a significant decline in activity in temperatures below 25°C (Yasuno et al. 231). Precipitation is also an important factor for mosquitoes due to their complete metamorphic lifecycle which requires a source of water that will remain through the egg, larvae and pupae stages of development (Laird 1988). It is important to study mosquitoes and their ecosystems to model and predict the geographic range of potential and existing disease outbreaks.

# Literature Review

Four factors that have a direct impact on the abundance and distribution of mosquitoes are temperature, rainfall, predation and competition. Niche theory identifies these factors as constraints or natural controls of mosquito distribution. The invasive species *Aedes aegypi* has been present in the United States consistently since before the 1960s and monitored by public health officials ever since (Kaemer et al. 5) (Pratt et al. 1). They are notorious for carrying most vector borne diseases and continue to be highly efficient vectors for recurring and emerging infectious diseases (Githeko et al. 1137). *Ae. Aegypti* are diurnal bitters, meaning they bite during the day mostly in the mornings and in the evenings. They bite most frequently at optimal biting temperatures between 26-35°C with an observed decline in biting at temperatures below 25°C (Yasuno et al. 319). Multiple studies have found no seasonal variety in biting frequency though other studies support seasonal varieties in population dynamics (Yasuno et al. 319) (Sheppard et al. 193)(Camara et al. 8). This might be due to regional factors not yet completely understood including the interplay of weather and its immediate effects on breeding habitats (Scott et al. 89) (Camara et al. 8). *Aedes aegypti* is a container breeding species which favors artificial containers. For this reason, it is mostly found in highly urbanized areas, females feed almost exclusively on human blood, and is linked to the lack of seasonal variety (Scott et al. 89) (Camara et al. 9)( Azil et al. 1191). Changes in temperature are particularly important for arthropod vectors as they depend heavily on environmental temperatures during their immature life stages in aquatic environments and as adults. Mosquito larvae in particular mature faster in warmer water and thus have a greater reproduction capacity (Githeko et al. 1136, Azil et al. 1191). This observation is reinforced by studies that show patterns of rainfall to

be significant drivers of immature mosquito populations in dry areas of Puerto Rico (Barrera et al. 2). Key insights into the lack of seasonal variety of mosquito populations have been found by researchers studying the relationship between humans and the vectors. Researchers Azil et al. report “rainfall will fill breeding habitats (e.g. anthropogenic containers) for mosquitoes” which suggests a greater abundance closer to human settlements (1191).

*Aedes albopictus* is also an invasive species originating from Asia to the Americas. It was first introduced at the port of Houston, TX in 1985 and has since expanded and thrived along the east coast and the southeastern United States (Fournier et al 13). It is hypothesized *Ae. albopictus* is from a temperate part of Asia considering its success as far north as New York State where eggs are aptly prepared to overwinter (Fader 2). With warming climate patterns, its geographical distribution is expected to expand further north and west (Fournier et al 13). Frequently referred to as the Asian tiger mosquito, *Aedes albopictus* eggs struggle with heat. Long term populations have failed to establish themselves in areas with warm and dry winters as egg populations desiccate quickly (Fader 2). This species is also a container breeding mosquito but prefers more sub-urban areas and will often inhabit tree holes (Camara et al. 9). Lack of rain is a problem for mosquitoes in general since it dries up their breeding locations, whether anthropogenic or natural (TXSHD 37). *Ae. Albopictus* has been identified as highly competent vector for most vector borne diseases (Githeko et al. 1137).

In human dominated landscapes mosquito populations tend to thrive. The common link between high mosquito populations and high human populations are water holding containers (Gratz 54)(Githeko et al. 1137)( Barrera et al. 2). This is true specifically for container breeding

species *Aedes aegypti* and *Aedes albopictus*. They are incredibly successful around humans because not only do they have reliable food sources, such as humans and domestic animals, but they also have a plethora of breading sites including water meters, septic tanks, and other development projects (Barrera et al. 1,2)(Gratz 54)( Githeko et al. 1137). In a comparative study of dengue between the Mexican city of Nuevo Laredo and the American city of Laredo, researchers have found that though there are higher rates of dengue transmission in Nuevo Laredo, there is a higher mosquito vector density in Laredo, TX due to a larger quantity of mosquito saturated containers (Reiter et al. 87).

From a public health perspective, mosquito species of importance are greatly concentrated within *Culex quinquefasciatus, Aedes aegypti, and Aedes albopictus* in Texas (Pratt, H. et al. 2). It is important to note that even though an effective vector in sufficient numbers does increase the chances of vector borne disease outbreaks, the determining factors are much more complex and include heavy socio-economic factors (Gratz 54)(Reiter et al. 88). Since these species have similar larval ecosystems, overlap in geographical distribution and share microhabitats they are subject to interspecific competition pressures (Camara et al. 2). *Aedes albopictus* is a very effective competitor against both *Aedes aegypti* and *Culex quinquefasciatus* (Fader 2). Researchers Camara et al. have observed negative effects on *Aedes aegypti* population densities in areas where they coexisted with *Aedes albopictus (2).* Same result has been found with *Aedes albopictus* outcompeting *Culex quinquefasciatatus* in artificial environments (Fader 4).

Mosquitoes compete with other species for resources, but do not stand a chance against predators. Top water minows from the genus *Gambie* have been recorded as mosquito larvae predators in Texas (TXSHD 37). Mosquito populations are also adversely affected by mites and other parasites (Fader 4). Mosquito larvae of the *Toxorhynchites* genus feed on larvae of different species especially of the container breeding kind (Fader 4)( Chadee, D.D. 376)( Bradshaw & Holzapfel 507). These organisms aid in mosquito population control where they are present.

## Objectives

The purpose of the mosquito survey is to gain insight into the diversity and abundance of mosquito populations in Central Texas between preserved ecosystems and developed ones. It is of public health importance to understand key spatial areas people should be aware of when mosquito populations are booming. The study will also explore environmental and anthropogenic effects on mosquito abundance and the theory of anthropogenic change on natural environments exacerbating the population densities of viable arthropod vectors of disease.

## Hypothesis

I hypothesized that there will be a greater abundance of mosquitoes found within an obstructed work area as opposed to a natural protected area where the ecosystem is intact.

## Study Sites

This study used a geographical survey method in two sites: a developed area and a protected natural area. The St. Edward's University campus consists of recreational areas,

education and administrative areas, and residential areas. Wild Basin Wilderness Preserve is divided into reception and educational areas, a creek valley area and two steeper slopes on either side of the creek. The preserve consists of predominantly native flora and fauna with regular invasive plant species removal by volunteers. The university campus is a mix of native plants and some exotic ornamental flowering plants with some smaller wildlife spotted throughout, mostly on the south west and east end of the property. The criteria used to define potential breeding and resting areas were proximity to standing water, areas of high humidity (near sprinkler systems), shade vs. direct sunlight, proximity to buildings and foliage density. The comparisons between sites were mosquitoes found near people versus mosquitoes found away from people.

## Methods

I used two different types of traps, BG Sentinel 2 traps (Biogents AG, Germany) to sample the aerial population, which includes "females seeking blood meals", of mosquitoes and sticky ovitraps that targeted egg laying females (Service, M.W. 1976)(Ritchie et al. 2003)(Hahn et al. 5). A BG Sentinel trap was used at mosquito resting sites with three in the developed area and three within the natural preserve area. BG Sentinel traps are recommended to *Aedes aegypti* and *Aedes albopictus* surveillance programs measuring distribution and abundance of vectors as well as ovitraps (Hahn, M.B. et al. 4). Thus, I employed 30 handmade modified ovitraps that were divided between the two sites. The ovitraps were placed near potential breeding sites such as grassy shady areas where water was observed to pool naturally or near bodies of water. Ovitraps are frequently used for larval monitoring which directly translates to

breeding sites (Fournier et al. 15) (Hahn et. al. 4). Since this study focused on adults, the ovitraps were lined with double sided tape to capture ovipositing females.

BG Sentinel 2 traps were placed in areas suspected to have high levels of human activity. Human activity was measured by observation counts at each of the trap locations for a duration of 15 minutes over a period of two days. The total number of people observed per site was then divided by the total time spend observing. Human proximity and activity data is necessary for disease transmission monitoring. The ecological overlap of humans and mosquito vectors increases the likelihood of transmission, especially if competent vector species also inhabit the area (Smith et al. 191). On average, the traps were placed 100 ft. from buildings and sources of water (human activity and breeding sites). The number of mosquitoes captured were recorded as well as their genera on a daily basis. Identification to the species level was made when possible. Though gender is critical in disease transmission, there was no discrimination between males and females to gather an overall abundance. Other variables collected were weight of samples as a measure of biomass, temperature and precipitation during the sampling period. For data analysis, a two-tailed T-test and ANOVA statistical test were employed.

## Results

The variety of mosquito genera collected between the sites was limited to *Aedes, Anopheles, Culex* and *Toxorhynchites*. The T-test reflects a mosquito abundance four times greater on the St. Edward's University campus than that of Wild Basin Wilderness Preserve on average. It also found significant differences between sites for mosquito abundance, human

activity and temperature variables. Approximately 95% of mosquitoes collected were either *Culex quinquefasciatus* or *Aedes albopictus.* No *Aedes aegypti* were collected in either site.

**Group Statistics**

| | location | N | Mean | Std. Deviation | Std. Error Mean |
|---|---|---|---|---|---|
| canopy | 1.00 | 20 | 64.30 | 48.416 | 10.826 |
| | 2.00 | 18 | 83.67 | 13.814 | 3.256 |
| human | 1.00 | 32 | 10.9531 | 6.68093 | 1.18103 |
| | 2.00 | 18 | 4.8333 | 4.31141 | 1.01621 |
| mosquito | 1.00 | 32 | 26.5938 | 20.74711 | 3.66761 |
| | 2.00 | 18 | 7.6667 | 16.14639 | 3.80574 |
| temperature | 1.00 | 32 | 95.7188 | .99139 | .17526 |
| | 2.00 | 17 | 95.0588 | .89935 | .21812 |

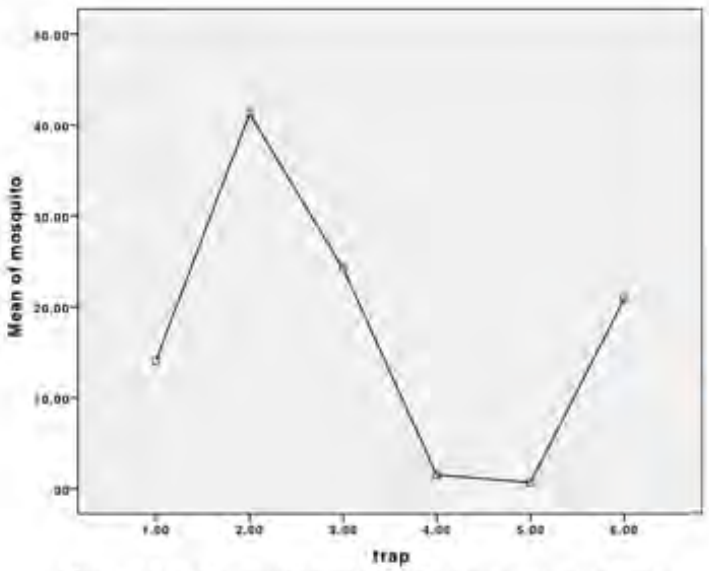
Table 1.1 Comparison of variable means between Wild Basin and St. Edward's campus, p<.05

**Independent Samples Test**

| | | Levene's Test for Equality of Variances | | t-test for Equality of Means | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | | 95% Confidence Interval of the Difference | |
| | | F | Sig. | t | df | Sig. (2-tailed) | Mean Difference | Std. Error Difference | Lower | Upper |
| canopy | Equal variances assumed | 80.795 | .000 | -1.636 | 36 | .111 | -19.367 | 11.836 | -43.372 | 4.639 |
| | Equal variances not assumed | | | -1.713 | 22.388 | .101 | -19.367 | 11.305 | -42.789 | 4.055 |
| human | Equal variances assumed | 16.095 | .000 | 3.491 | 48 | .001 | 6.11979 | 1.75322 | 2.59470 | 9.64488 |
| | Equal variances not assumed | | | 3.928 | 46.958 | .000 | 6.11979 | 1.55805 | 2.98533 | 9.25426 |
| mosquito | Equal variances assumed | .962 | .332 | 3.338 | 48 | .002 | 18.92708 | 5.66979 | 7.52721 | 30.32695 |
| | Equal variances not assumed | | | 3.581 | 42.933 | .001 | 18.92708 | 5.28536 | 8.26766 | 29.58650 |
| temperature | Equal variances assumed | .001 | .979 | 2.288 | 47 | .027 | .65993 | .28843 | .07968 | 1.24018 |
| | Equal variances not assumed | | | 2.359 | 35.656 | .024 | .65993 | .27981 | .09226 | 1.22759 |

Table 1.2 Comparison of variables for significant differences, p<.05

The ANOVA test showed significant differences between traps one and two on the St. Edward's University campus.




| Trap # | Location |
|---|---|
| 1-3 | Campus |
| 4-6 | Wild Basin |

Figure 1.1 Mosquito collection means per trap

Discussion

The greatest abundance at both sites appeared to be near decorative fountains. Though the water is not stagnant in these areas, it does provide the dark protected areas for eggs to mature. In areas where native plant species dominated, both on campus and at the nature preserve, mosquito abundance was relatively low. Amongst both of these predominantly native areas, *Toxorhynchites rutilus septentrionalis,* or elephant mosquitoes were found. The presence of these mosquitoes are significant due to their mosquito larvae consumption in their immature life stages. Research conducted in Trinidad and Tobago and Florida notes the decline in container breeding mosquito populations when larvae and pupae of the *Toxorhynchites* genus is present (Chadee 376)( Bradshaw & Holzapfel 507). Previous studies support predation on mosquito larvae in their aquatic environments to reduce the abundance of mature mosquito vectors (Fincke et al. 1997). Other mosquito predators present were spiders, which affected the mosquito sample counts. I hypothesize there was a greater quantity of mosquitoes captured on

some days, but had been consumed before collected since spiders would frequently be present in the catchbags from the preserve. Often times, the spiders could be observed feeding under the microscope. Spiders are known to prey on mosquitoes, though not many species specialize in mosquitoes (Cross, F.R. & Jackson, R.R. 123). Niche theory delineates competition and predation to be critical factors in an organism's 'realized niche' (Pianka E. R. 170-171). Previous studies support the existence of competition between *Aedes aegypti* and Aedes albopictus. It has been suggested that *Aedes aegypti* prefers urban areas while *Aedes albopictus* prefers suburban and rural vegetated areas since they have been commonly found in greater quantities in their respective sites (Camara et al. 2). Niche theory and competition might identify this difference in spatial preference to be a way to reduce interspecific competition ( Pianka E.R. 176). It is likely that temperature and precipitation were not factors that inhibited the capture of *Aedes aegypti* since neither of the sites were residential urban areas. Sticky ovitraps were successful in collecting larvae, but not adults. Repeatedly, the adult female was observed landing on the side of the cup but completely avoiding the sticky tape. Other limitations of the study included time constraints and technological malfunctions. A third, urban collection site would possibly influence the diversity of mosquito samples collected to include *Aedes aegypti* at minimum.

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