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Potential of Increased Thermal Tolerance as a Response to Acute Heat Stress in a Disease-Transmitting Mosquito Species

Nicole Ashley Scanlon

Abstract

Each year hundreds of millions of people are infected with mosquito-borne illnesses. West Nile Virus is the leading cause of mosquito-borne diseases in the United States (McDonald et al., 2021). The first case of West Nile Virus was detected in the United States in 1999 (Sejvar, 2003). Since that time the number of cases has dramatically risen. Mosquitoes are ectotherms which means their body temperature is dependent on the thermal conditions outside of their body. Because of this they are very sensitive to changes in their environment. The Earth's average temperature has been slowly but steadily rising. This small but steady change in temperature will gradually have global altering effects. However, mosquitoes have the ability to quickly evolve and adapt with their short lifecycles, generation time, and ability to produce large amounts of offspring at once (Couper et al., 2021). Because of this it is important to determine if continued exposure to extreme temperatures will allow mosquitoes to gain thermal tolerance. If it possible, the world may see a rise in the number of mosquito-borne illnesses as the impacts of climate change become more severe. Research into mosquito thermal tolerance is important to demonstrate this insect's ability to adapt to a changing climate and the importance of developing and evolving vaccines and control efforts.

Keywords: West Nile Virus, mosquitoes, climate change, thermal tolerance

MONTCLAIR STATE UNIVERSITY

Potential of Increased Thermal Tolerance as a Response to Acute Heat Stress in a Disease-Transmitting Mosquito Species

By

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Introduction

According to the World Health Organization (WHO), each year about 700 million people are infected with mosquito-borne illnesses. Additionally, approximately one million of these people die. West Nile Virus is one such mosquito-borne illness.

Emily McDonald et al. (2021) in, *Surveillance for West Nile Virus Disease-United States, 2009-2018,* completed for the Centers for Disease Control and Prevention (CDC) states West Nile Virus, "…is the leading cause of domestically acquired arboviral disease in the contiguous United States" (McDonald et al., 2021). This report found that during the years of 2009-2018 the Centers for Disease Control and Prevention received reports of 21,869 cases of West Nile Virus (WNV) disease across all 50 states, the District of Columbia, and Puerto Rico. Out of these cases, 59% (12,835 cases) were classified as WNV neuroinvasive disease (McDonald et al., 2021).

West Nile Virus originates in birds. When mosquitoes feed upon infected birds they can become infected themselves and spread the disease to other animals and humans upon biting them. The West Nile Virus was first detected in the United States in 1999. Since that time, the number of cases in the United States has increasingly grown. Dr. James J. Sejvar, in his paper, *West Nile Virus: A Historical Overview*, reports that in 2000 (the year after West Nile Virus was initially detected in the United States) there were 21 cases of West Nile Virus detected in humans in the United States and the following year 66 total cases (Sejvar, 2003).

As an ectotherm, a mosquito's body temperature is determined by the thermal conditions outside the insect's body; making mosquitos sensitive to daily and seasonal fluctuations in temperature. As temperatures warm due to climate change their life and developmental cycle could potentially be altered. Generally, high temperatures kill mosquito populations because it dampens their ability to grow, reproduce, and survive. However, there is potential for adaptive evolution. Beard et al. (2016), writing for GlobalChange.gov of the U.S. Global Change Research Program studying Climate and Health, says that changes in season length due to climate change could impact mosquito survival rates however, "…mosquitoes are also able to adapt to changing conditions" (Beard et al., 2016).

This leaves the potential that through reoccurring exposure to high temperatures as larvae, adult mosquitos may have greater thermal tolerance. Greater thermal tolerance would allow adult mosquitoes to survive temperatures which would have killed past generations. Mosquito populations would soon grow, increasing the spread and infection-rate of mosquito-borne illnesses. For this reason, understanding mosquito thermal tolerance is important.

According to J.H. Porter and colleagues (1991), it has been found that there is a rise in the average annual temperature of the Earth's surface by approximately 0.3-0.7°C (Porter et al., 1991). This observed increase, which was noted 1991 when the paper was written, is supported today by scientists at NASA's Goddard Institute for Space Studies (GISS.) Earthobservatory.nasa.gov states that since 1880, the Earth's temperature his risen by 1.1°C (GISS Surface Temperature Analysis (GISTEMP), 2023).

While 1.1°C may not seem significant, the impact of this global temperature increase may have devastating consequences. J.H. Porter et al. (1991) focused on the many impacts of increased temperatures on insect pests. These results include an extension of geographical range, changes in population growth rates, increased number of generations, and extension of development season. It is not difficult to see how these consequences discussed by J.H. Porter et al. (1991) of global warming would allow mosquito populations to grow, flourish, and potentially spread mosquito-borne illnesses to more people in vast parts of the world at rates not seen before.

Lisa I Couper et al. (2021) studied mosquitoes directly and aimed to determine how mosquitoes adapt to climate warming. One of the most important traits of mosquitos to consider when studying thermal tolerance is their short generation time. “The generally rapid life cycles…favor rapid evolution” (Couper et al., 2021). Additionally, mosquitoes have large population growth rates, laying 100 eggs or more at a time. These traits reduce the risk of extinction as adaptation is facilitated.

Additional evidence that mosquitos may have the ability to gain increased thermal tolerance was found by Rocca et al. (2009) as cited by Couper et al. (2021). Rocca and colleagues (2009) discovered a link between a population’s thermal environment and its ability to tolerate heat. The researchers found that mosquitoes from warmer environments had higher upper thermal limits for respiration and survival after exposure to heat stress (Rocca et al., 2009). This gives credibility to the idea that if mosquitoes become accustomed to warmer temperatures over time, they may gain greater thermal tolerance allowing them to survive heat waves that once would have killed them.

Couper et al. (2021) believes that, to survive increasing global temperatures thermal acclimation is necessary for mosquitos. My research hypothesis is that, mosquito larva exposed to heat waves as they develop, will have greater thermal tolerance later in life. Such thermal acclimation in mosquitoes has been illustrated before. Emilie M. Gray conducted a study in 2013 that examined the impact of larval stage acclimation on the adult stage’s thermal relations in *Culex pipiens* (Gray, 2013). This paper is of particular importance to my research topic as *Culex pipiens* are major vectors of disease and the species studied in my thermal tolerance experiment.

Culex pipiens are found throughout North America and other temperate latitudes. These mosquitoes are dormant during the winter but as soon as temperatures are warm enough mated females prepare to lay eggs. The eggs are laid and hatch in aquatic habitats. The larvae stay in their aquatic habitat for several weeks then pupate. After, adults emerge into a terrestrial environment. Several generations occur throughout the summer until the weather becomes colder and the days shorten.

Gray (2013) discovered in their study that both larval and adult temperatures had significant impact on the CT_{max} (critical thermal maximum) of *Culex pipiens*. The results showed that higher temperatures during both the larval and adult stages led to an increase in CT_{max} (Gray, 2013). In this study, the mosquitoes that could withstand the highest temperatures or the CT_{max}, were the ones that had been developed in 26°C. The mosquitoes that had been reared at 18°C had the lowest CT_{max}, meaning they were least able to withstand heat wave conditions. The results of this study prove there is a connection between exposure to heat during the developmental stage and adult thermal tolerance.

Climate change and global warming are upon us with no end in sight. The consequences of this phenomena are seen far and wide, from large scale to small. Even a small increase in global temperature could drastically change an ecosystem. Global warming also has a dire impact on animals, especially ectotherms whose body temperature is determined by the thermal temperature outside their body. Changes in global temperature will potentially impact their lifecycles at every stage from growth to survival and reproduction. Mosquitoes are of particular concern because of their status as major disease vectors. While heat waves generally kill mosquitoes there is great potential and evidence for them to gain thermal tolerance. In doing so, the world will see their populations grow as well as disease transmission and death. Better understanding of mosquito thermal tolerance is vital because it will demonstrate a mosquito's ability to adapt to a changing climate and the importance of developing and evolving vaccines and control efforts.

Methods & Materials

Hypothesis

My research hypothesis is that, mosquito larvae exposed to heat stress as they develop, will have greater thermal tolerance later in life. For my first analysis, I set out to substantiate this hypothesis with a thermal tolerance experiment and chi-square analysis.

Mosquito Maintenance

The Chicago maintenance colony of *Culex pipiens f. molestus* (*Cx. Pipiens*) maintained in a lab at Montclair State University was utilized for this project. These mosquitos lay their eggs in rafts of 50-120 eggs directly on the water surface (Epstein et al., 2021.) Therefore, I used a small tray of water to collect *Cx. Pipiens* egg rafts. I isolated individual egg rafts, putting each one in a jar with 50 ml of dechlorinated water. I added food to the jars so that upon hatching, the larvae will have a nutrition source. I made a fish flake solution by blending .8 grams of TetraMin fish flakes in 200 milliliters of dechlorinated water for the food source. I then added 1000 microliters of the solution to each jar.

Afterwards, I placed the jars in the control incubator. The incubators are TriTech Research Digitherm 38-liter heating/cooling incubators. The control incubator maintained a constant temperature of 23°C. The lights turn on at 06:00 and turn off at 20:00. I then waited four days for the larvae to hatch.

Preparation

Upon hatching I separated the larvae from each family into three jars; 2 experimental and 1 control. In each jar I placed 10 larvae. I then made a fish flake solution to feed the larvae by blending 2.0 grams of TetraMin fish flakes into 200 milliliters of dechlorinated water and placing 2000 microliters of the solution into each jar. The control and experimental populations were then placed into separate incubators for 48 hours. The control population was placed in the control incubator which was set to 23°C. The lights in this incubator turn on at 06:00 and turn off at 20:00. The experimental population was placed in the experimental incubator. In the experimental incubator the temperature remained at 35°C from 08:00 until 18:00 and then 27°C from 18:00 until 08:00. The lights in this incubator were on from 06:00 until 20:00. These settings were meant to simulate a heat wave.

After 48 hours both control and experimental populations were fed a fish flake solution created by blending 2.0 grams of TetraMin fish flakes into 200 milliliters of dechlorinated water and placing 2000 microliters into each jar. I then placed all jars, experimental and control, into the control incubator for 48 hours. The control incubator was set to 23°C and the lights turn on at 06:00 and turn off at 20:00.

Trials

Once both populations spent 48 hours in the control incubator, I ran the thermal tolerance experiment. To examine thermal tolerance, I removed individual larva from each jar and placed each individual in a 13x100 mm culture tube with 2 ml of dechlorinated water. Each tube was designated with either blue, yellow, red, or green tape. Tubes designated as blue remained in the bath for 15 minutes, yellow tubes remained in the bath for 30 minutes, red tubes for 45 minutes, and green tubes for 60 minutes. I began by taking one larva from one family and placing it in a blue tube, the next larva from that family was placed into the yellow tube, the next larva after that red, and so on until there were no larva left from that particular family. I then repeated that step with the next family. These steps were completed twice and in total 327 larvae were included in the experiment.

To being the thermal tolerance experiment, all of the tubes were immersed in a water bath that was set at 28°C for 30 minutes to acclimate the larvae. After 30 minutes all of the tubes were immersed in a second water bath which was set to 38°C. The tubes were removed after each of their respective times had passed. Following the removal of the last set of tubes at 60 minutes all of the tubes were returned to the initial water bath that was set to 28°C for 30 minutes. Finally, I placed all of the tubes back in the control incubator.

After twenty-four hours in the control incubator the larvae were assessed for an immediate mortality response. For the larvae that survived, my focus was on their ability to pupate and reach adulthood; these additional factors help to determine possible long-term effects of heat stress on the larvae's' ability to develop and survive. Upon the collection and recording of all data, a chi-square statical test was used to compare the number of living and dead larvae from the control population to the number of living and dead larvae from the experiment population to determine if there is a relationship between the values.

Results

Thermal Tolerance Statistical Analysis

Upon completion of the thermal tolerance experiment and the following twenty-four hours in the control incubator, the number of alive and dead mosquitoes for both the control and experimental populations, at each time designation, was tallied. I began by totaling the results of the control population. Beginning with the larvae that had spent 15 minutes (blue designation) in the water bath I found that 47 larvae were alive while 6 had died. After 30 minutes (yellow designation) in the water bath a total of 42 larvae survived and 6 died, after 45-minutes (red designation) 31 larvae had survived while 10 died, finally, after 60 minutes (green designation) 22 larvae were found alive and 12 had died.

Next, I tallied the results of the experimental population. For the larvae that were in the water bath for 15 minutes (blue) a total of 42 lived while 9 died, for the larvae that spent 30 minutes in the water bath 34 survived and 7 died, the 45-minute designation resulted in 22 alive larvae and 17 dead, and at 60 minutes 16 larvae survived and 4 died.

After compiling the data, I needed to establish if there was a statistical significance in the results of my thermal tolerance experiment data. This was completed with a chi-square calculator 2 x 2 contingency table. In total I created four tables, one for each time designation; 15 minutes, 30 minutes, 45 minutes, and 60 minutes. I designated Column 1 of the table for the control population, and column 2 for the experimental population. Row 1 of the table held the number of larvae alive twenty-four hours after the thermal tolerance experiment and row 2 for the number of larvae dead after the experiment. I set the significance level to .05.

I began by creating the table for the larvae that spent 15-minutes in the water bath. For these larvae 47 of the control population survived while 6 died and for the experimental portion 42 had survived while 9 died. Upon running the chi-square calculator, the chi-square statistic was 0.8427, the p-value was .358612. The chi-square calculator determined the result was not significant at $p<.05$. Next, I created a table for the larvae that spent 30, 45, and 60-minutes in the water bath. For the larvae that spent 30-minutes in the water bath the chi-square statistic was 0.3708 and the p-value was .54259. The larvae at the 45-minute designation had a chi-square statistic of 3.2952 and a *p*-value of .069484. Finally, the data from 60-minutes showed a chi-square statistic of 1.4127 and a *p*-value of .23461.

The results of each of the four chi-square tables found the results to be not significant at $p<.05$. The result concludes that there is insufficient evidence to reject the null hypothesis that the groups are different. This means that, with this data alone, there is no strong evidence to support the presence of an association between the control and experimental datasets.

Temperature and West Nile Analysis

A mosquito's survival and life cycle are greatly affected by the thermal conditions of their environment. Therefore, it is important to establish if there is a possible connection between temperature and the rate of West Nile Virus cases reported. My hypothesis is that, over time, due to rising temperatures, there will be a rise in the number of reported cases of West Nile Virus.

To determine if my second hypothesis is true, I chose to focus on two different locations in New Jersey; the Newark Liberty International Airport in Newark, New Jersey and the Atlantic City International Airport in Atlantic City, New Jersey. I chose these two sites because of their locations and distance from one another. The airports are significant because they are where the weather stations (from which the data was obtained) reside. The weather stations are maintained and the data is collected by the National Oceanic and Atmospheric Administration. The location of the weather stations within the state will provide a range of temperatures for the state; Newark Airport being in northern New Jersey and the Atlantic City Airport in southern New Jersey. Both locations can be seen in Figure 6 on page 25.

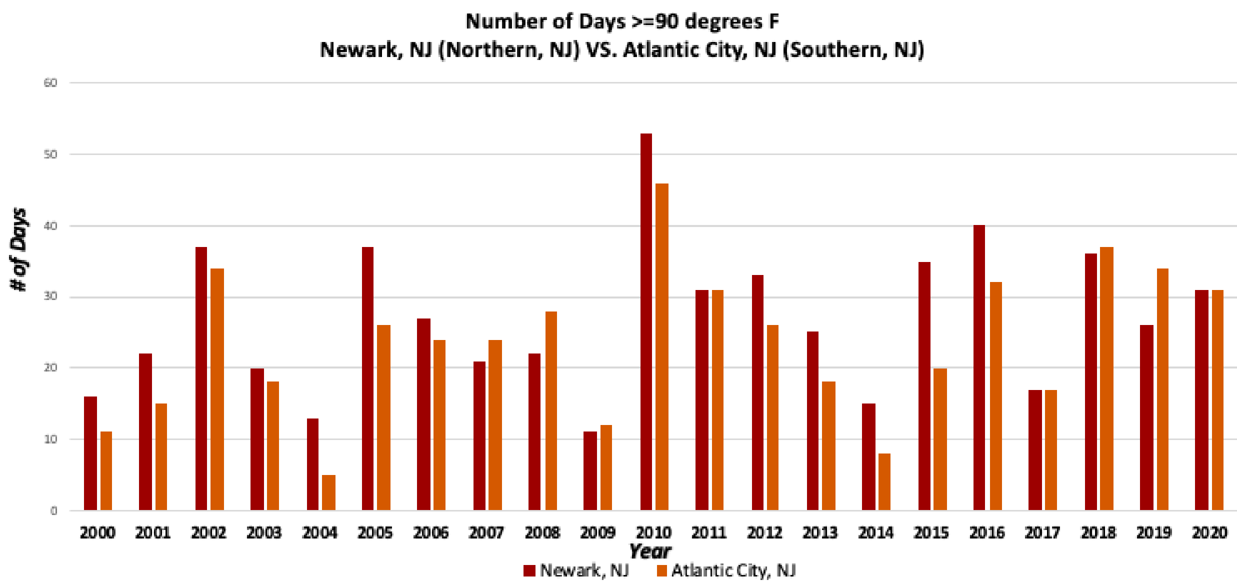
For this second analysis, I downloaded monthly summarized data reports for the months of May through September from 2000-2020. From weather.gov, which is maintained by the US Department of Commerce National Oceanic and Atmospheric Administration. I used their

NOWData tool to download monthly summarized data, for the months of May through September, for the year range of 2000-2020, a variable of maximum temperature, and a threshold of >= 90°F; summarized by number of days.

I chose the months of May through September because that is when temperatures generally begin to rise and mosquito activity increases. The 20-year span of 2000- 2020 provides a large data set to more easily see possible trends. The data provided the monthly number of days the maximum temperature was >= 90°F. I then totaled the number of days >=90°F per year for each location and plotted them side-by-side.

Figure 1

No. of Days >=90℉ in Newark, NJ and Atlantic City, NJ

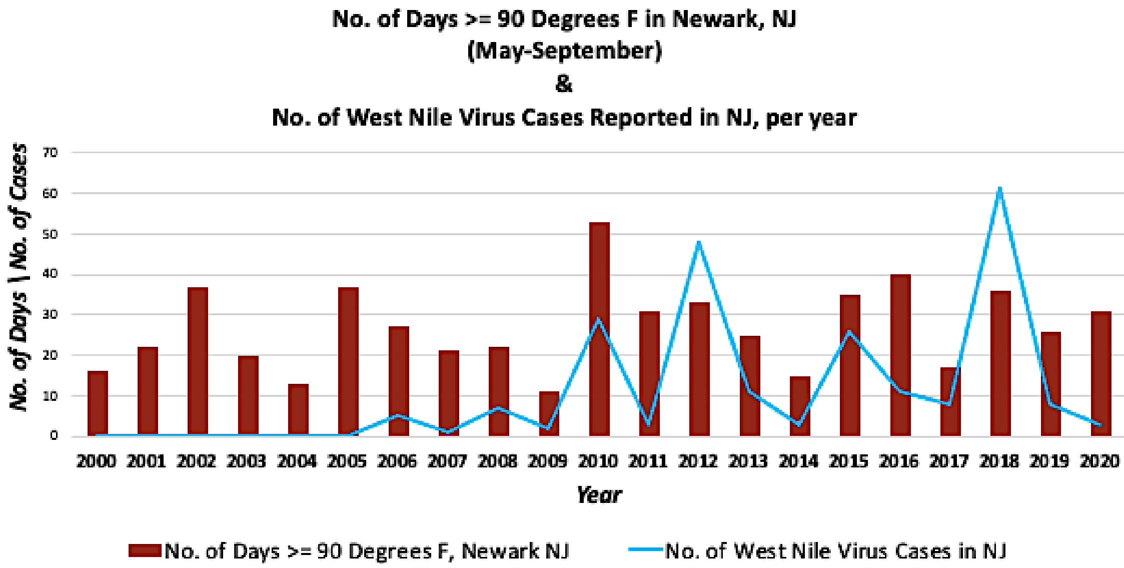


Note. The number of days the maximum temperature was >= 90°F for the years of 2000-2020 for the Newark Liberty International Airport, Newark, NJ and the Atlantic City International Airport, Atlantic City, NJ.

For data on the prevalence and frequency of West Nile Virus in New Jersey I downloaded Vector-borne Surveillance Reports from the New Jersey Department of Health for the years of 2006-2020, data for years before 2006 was not available. I then plotted the number of West Nile Virus cases per year in New Jersey against the number of days >=90°F in Newark, NJ and Atlantic City, NJ

Figure 2

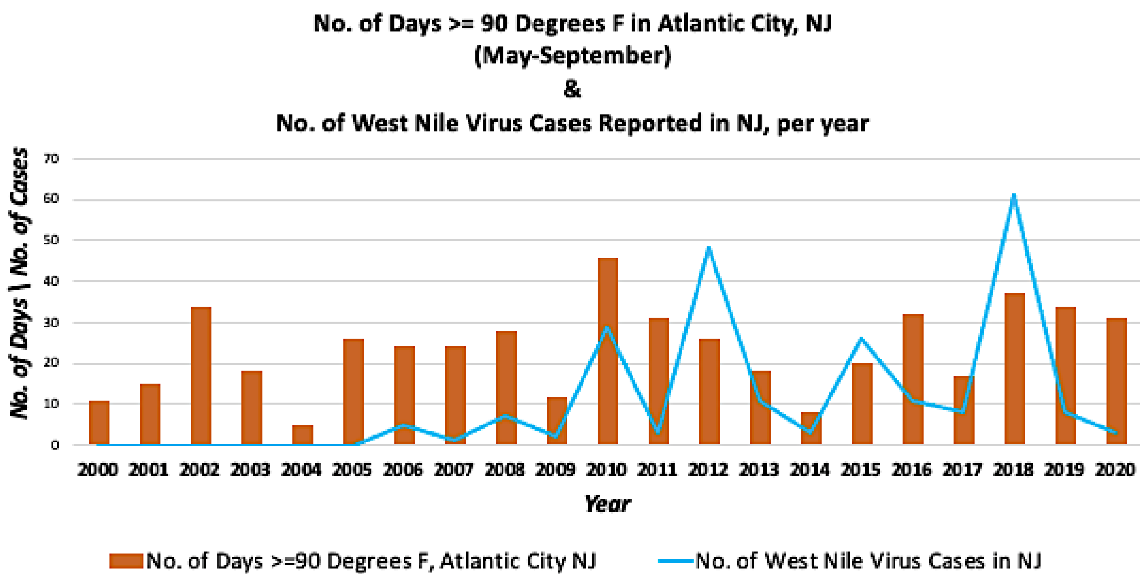
Newark, NJ No. of Days >=90 ℉



Note. The number of West Nile Virus cases in New Jersey per year for the years of 2006-2020 (data was not available for years before 2006) plotted against the number of days the maximum temperature was >= 90°F for the years of 2000-2020 for the Newark Liberty International Airport, Newark, NJ.

Figure 3

Atlantic City, NJ No. of Days >=90 ℉

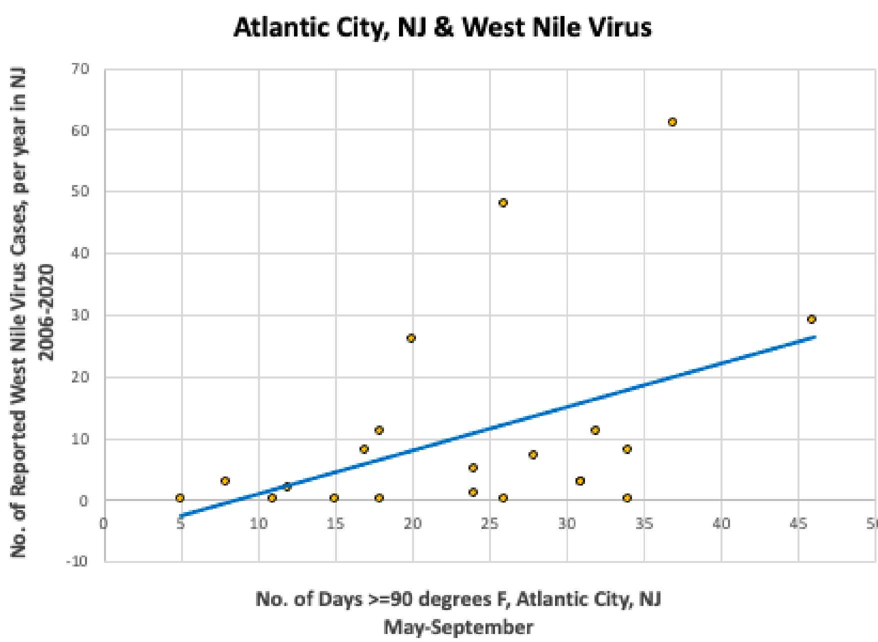


Note. The number of West Nile Virus cases in New Jersey per year for the years of 2006-2020 (data was not available for years before 2006) plotted against the number of days the maximum temperature was >= 90°F for the years of 2000-2020 for the Atlantic City International Airport, Atlantic City, NJ.

The next step was to establish if there is any statistical significance within the data. To do this, I ran a regression analysis using R version 4.2.3 (Shortstop Beagle) computer software for statistical computing. First, I ran the regression analysis for the number of West Nile Virus cases in New Jersey for the years of 2006-2020 against the number of days >=90°F in Atlantic City, New Jersey for the years of 2000-2020. The regression analysis showed an adjusted R-squared value of 0.1526 and a *p*-value of 0.0451.

Figure 4

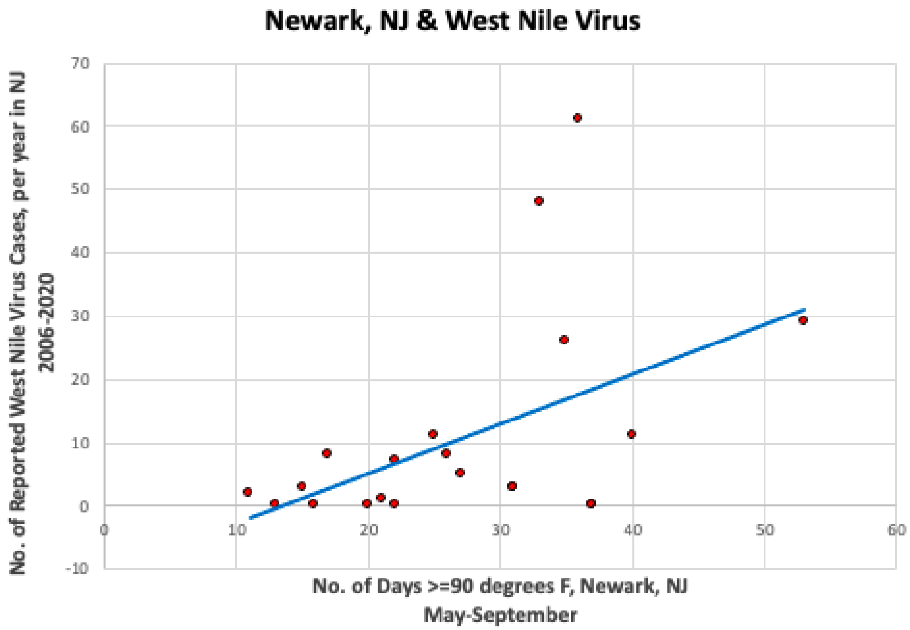
Atlantic City, NJ temperatures vs. West Nile Virus cases: A Regression Analysis



Note. Regression analysis comparing the number of days >=90°F in Atlantic City, NJ for the years of 2000-2020 to, the number of West Nile Virus Cases in NJ for the years of 2006-2020 (data was not available for the years before 2006).

I ran the analysis again, this time for the number of West Nile Virus cases in New Jersey against the number of days >=90°F in Newark, New Jersey. This time the analysis showed an adjusted R-squared value of 0.2038 and a *p*-value of 0.02296. For both analyses, Newark and Atlantic City, there was a significance level of .05.

Figure 5
Newark, NJ temperatures vs. West Nile Virus cases: A Regression Analysis



Note. Regression analysis comparing the number of days >=90°F in Newark, NJ for the years of 2000-2020 to, the number of West Nile Virus Cases in NJ for the years of 2006-2020 (data was not available for the years before 2006).

The results of both regression analyses determine that there is statistical significance between the number of West Nile Virus cases and the number of days >= 90°F in Newark, NJ and Atlantic City, NJ. However, because the adjusted R-squared value for Newark is larger than that of the R-squared value for Atlantic City, Newark displayed the greatest statistical significance.

Although the data from my analyses looks like it may fit a more complex model, such as an exponential relationship, I could not run one because there was no West Nile Virus data for the years of 2000-2006. Therefore, zeroes had to be used as a placeholder.

Discussion

The results of my thermal tolerance experiment did not show a relationship between the control population of larvae and the experimental population. However, for the 45-minute thermal tolerance experiment, although the results were not statistically significant at $p>.05$ it appears there may be differences; with a greater sample size those differences may become more apparent.

The results of the experiment may not have proven my original hypothesis, however, there is evidence of a connection between rising global temperatures and mosquito-borne illnesses, as my regression analysis shows.

Through the comparison of the number of West Nile Virus cases, to the number of days with temperatures >= 90°F in Newark, NJ and Atlantic City, NJ, a connection was found between rising temperatures and an increase in the number of West Nile Virus cases. This answers the question, are heat waves a contributing factor to West Nile Virus? My data shows that heat waves are a contributing factor but there are other contributing factors to consider.

One reason may be the land use differences between Newark, NJ and Atlantic City, NJ. Newark is an urban environment surrounded by lots of infrastructure and is located right outside the metropolis of New York City. Atlantic City on the other-hand, is along the coast, and has more open and green spaces. In a place such as Atlantic City, there is greater opportunity for a more diverse ecosystem. A more diverse ecosystem means potentially more predators and competition for mosquitoes. However, in Newark, there are fewer natural predators of mosquito such as birds, bats, other insects, and fish. This is because an urban environment isn't generally where these animals' habitats are found. Mosquitoes, unlike fish, or birds can survive in almost any habitat and most only need a small pool of water to reproduce. Other important contributing factors to include in future studies; population density, age of population, precipitation, water bodies, and bird migration patterns. All of these factors together contribute to a larger picture of climate change and West Nile Virus.

Couper's (2021) analysis indicates that certain broad characteristics of mosquito populations, along with significant selective pressures imposed by rising temperatures, can aid in their quick evolution and adaptation to climate warming. Specifically, the typical time it takes for a mosquito generation to develop and the rate at which mosquito populations increase are comparable to those of species that have already displayed adaptative responses to climate change (Couper et al, 2021).

Unfortunately, the thermal tolerance experiment I ran was limited by time. I was only able to test one generation of each family. Barring time limits, if I was to run the experiment again, I would like to run the experiment along distinct family lines. After running the initial heat stress test, I would allow the surviving mosquitoes to develop to adulthood. Eventually, the experimental and control populations would be bred and the experiment will run again with their offspring. This would allow me to examine the potential for changes in thermal tolerance across generations. Potentially substantiating Couper's research suggesting mosquitoes' ability to quickly evolve and adapt to climate change.

Conclusions

The results of my thermal tolerance experiment did not find a statistical significance. Nevertheless, previous research studying mosquitoes and my own regression analysis find evidence of a correlation between rising temperatures and mosquito-borne illness rates.

However, there is much more research into the subject to be done. Future studies may consider generational adaptation and evolution. Over time, one may see higher rates of survival among larvae whose parents survived and reproduce despite heat stress.

Another factor to consider is location, each environment offers unique biome; habitat availability, biodiversity, predators or lack thereof, precipitation, altitude, human population and more. As mosquitoes are greatly affected by thermal conditions outside of their bodies any one of these factors could potentially play a role in their survival, rates of reproduction, thermal tolerance, and rates of disease transmission.

In a world where the impact and effects of climate change are becoming impossible to ignore, research into mosquito thermal tolerance is important to demonstrate this insect's ability to adapt to a changing climate and the importance of developing and evolving vaccines and control efforts in an uncertain climate future.

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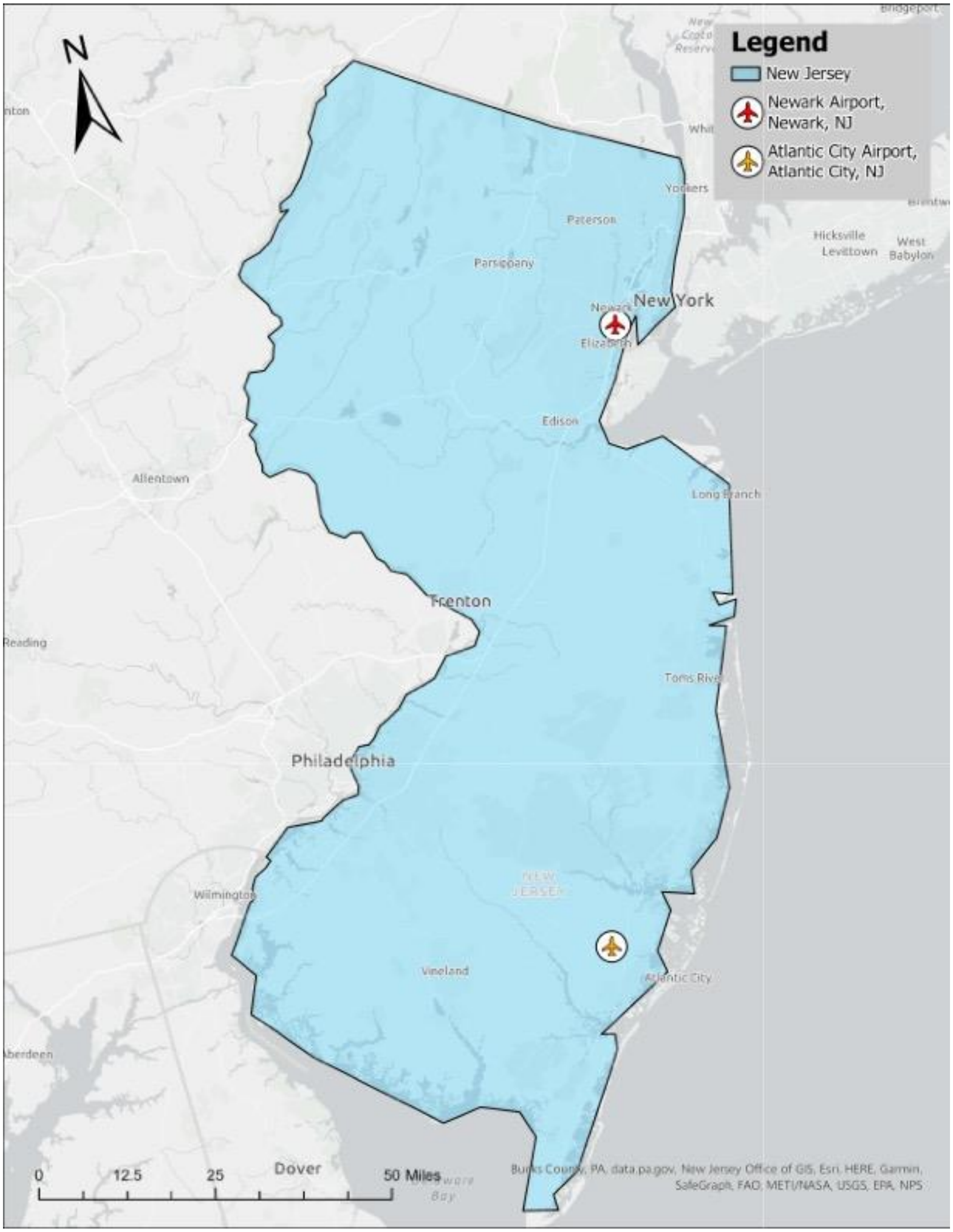
Figure 6
Location Map



Note. Map of New Jersey displaying the location of the two weather stations where temperature data was retrieved; Newark Liberty International Airport, Newark, NJ & Atlantic City International Airport, Atlantic City, NJ. I created this map using ArcGIS Pro 3.1.1. with data found on ArcGIS Pro Online.