EFFECT OF TEMPERATURE AND NUTRIENT TYPE ON LONGEVITY OF *AEDES JAPONICUS, ALBOPICTUS,* AND *TRISERIATUS*

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Abstract. Mosquitoes are a public health concern to humans because they are vectors for many diseases. Vector competence, or a mosquito's ability to transmit disease, is strongly influenced by individual mosquito lifespans. Previous work has demonstrated that size of adult mosquitoes is a proxy for fitness and lifespan and can itself be influenced by environmental conditions experienced by juvenile stages. Additionally, adult longevity is further influenced by availability and quality of food resources during the adult life stage. Other studies have explored the effects of nutrient type on mosquitoes, but we strove to shed light on temperature *and* nutrient type throughout all life stages of understudied mosquito species. To study longevity in *Aedes japonicus, albopictus, and triseriatus*, we reared over 700 field caught mosquitoes and recorded juvenile development rate, adult longevity, and size. We reared juveniles at temperature regimes reflecting mean temperatures in June in Baltimore, MD (High Variable) and New York, NY (Low Variable) and at the MD daily high with no diurnal variation (High Constant). The greatest proportion of larva pupated in the high constant temperature, but there was no effect of temperature on the rate of pupation. Temperatures experienced at juvenile life stages also had an effect on adult size, across species. Adults reared in lower temperatures had smaller wings, suggesting that juveniles reared in lower temperatures emerge as smaller adults. In one species, *Ae. albopictus*, smaller wing length was significantly associated with shorter adult longevity. We also found a significant relationship between adult nutrient treatment and the days the mosquitoes were alive.

INTRODUCTION

Mosquitoes are a large public health concern across the globe, as they are responsible for the transmission of various diseases. In this study we collected mosquito eggs from the field in Baltimore, Maryland and received two invasive species, *Aedes japonicus*, and *Aedes albopictus*, and one native species, *Aedes triseriatus.* All three of these species are potentially important vectors for arboviruses, including chikungunya, dengue, Zika, West Nile and La Crosse viruses (Sardelis et al. 2002, Ibáñez-Bernal et al. 1997, Gerhardt et al. 2001).The range of *Ae. albopictus* has been steadily increasing northward, potentially due to their ability to live in containers with miniscule amounts of water, and or because climate change is causing northern locations in the United States to become warmer and more suitable for mosquito populations (Rochlin et al. 2013). The range of *Ae. japonicus* has been expanding southward as it is more adapted to cooler climates than warmer (Peyton et al. 1999). The increase in range is worrisome for humans because we know very little about these species in general and the northeastern US could potentially become exposed to the diseases that they can transmit. Baltimore, Maryland is an urban location where the range of these species are beginning to overlap.

Urban temperatures tend to be warmer than surrounding rural areas, potentially providing a more hospitable climate for mosquito species that are sensitive to cold temperatures. This phenomenon is called the urban heat island effect (UHI) and is defined as an urban area that is warmer than its surrounding rural areas by approximately 2-5°F due to the physical structures of cities and human activity (Environmental Protection Agency 2018). The effect of urban temperatures on mosquitoes is of interest to study because cities have high human population densities, there are many opportunities for standing water for mosquitoes to lay their eggs, and those sites have fewer predators such as dragonfly nymphs.

Mosquitoes normally feed on plant nectar, but when females are preparing to develop eggs, they require the proteins in animal blood. Depending on the species, once a female mosquito has a successful blood meal, she will lay her eggs in or at the edge of a stagnant body of water. Mosquito eggs hatch into larvae that feed on microorganisms present around them (Juliano et al. 2005). After molting several times in their larval stage, mosquitoes become pupae and then emerge as adults.

An adult mosquito's ability to acquire then pass on a pathogen to cause a disease is termed its "vector capacity". The factors that influence vector capacity include biting rate, vector immunity, vector density, and adult longevity (Ebrahimi et al. 2017). Adult longevity is not well studied, but plays a critical role in a mosquito's vector capacity for two reasons. First, a mosquito needs to live for a certain amount of time in its adult stage to be able to support pathogen replication. Second, the longer that a mosquito is alive, the more time it has to take blood meals, increasing the biting rate, and the more opportunities it has to lay eggs, and thus increasing the population density. Factors that might influence longevity are larval development rate and resource acquisition, both of which can affect adult size (Araujo, 2012). The size of emerging adult mosquitoes, often represented by wing size (Nasci 1986) has been shown to correlate with adult longevity.

The temperatures that larvae are exposed to may influence a mosquito's longevity (Padmanabha et al. 2011). Larvae do not pose any public health concerns in that stage, but quicker emergence into adults could result in a higher vector capacity (Ebrahimi et al. 2017). Studies have shown that this life stage determines the size of the adult mosquito the larva will become (Xue et al. 2010). Adult mosquito size is an indication of fitness and therefore a longer lifespan, higher rates of biting and ability to travel (Padmanabha et al. 2011).

What mosquitoes eat in their adult life stage also may be an important factor in determining their longevity. Xue and colleagues (2015) have shown that Ae. albopictus lives longer when given sucrose than on just water or water and blood alone. Sugar sources that originate from different plants can also have biological implications on mosquito survival and fecundity (Hien 2016).

In this study we gathered data to answer three questions: 1. Does the temperature that juvenile mosquitoes develop at influence larval survival, adult size or longevity? 2. Does flower type available for nectar resources influence adult longevity? 3. Is there an underlying resource that these species could be receiving from plants that may enable them to live longer or do they simply require the carbohydrates from the nectar? To explore these questions, we collected *Ae. japonicus, Ae. albopictus, and Ae. triseriatus* eggs from Maryland, and reared them in constant or variable temperature regimes that reflect mean June conditions in New York City and Baltimore, MD. We reared the adults that emerged from the juvenile experiment in habitats with one of four resource treatments, including water or a sucrose dilution alone and two species of flower: an opportunistic species (*Trifolium repens*), and a common potted species (*Impatiens walleriana*).

METHODS AND MATERIALS

Egg acquisition

Seed germination paper was placed out for one week in standardized plastic ovitraps with water and grass at two locations in Baltimore City, Maryland. After collection, 12 seed germination papers with visible eggs were dried and transferred to the Cary Institute in Millbrook, NY.

The seed germination papers were randomly divided and soaked in untreated well water at one of the assigned temperature treatments. First instar larvae (within 48 hours of hatching) were randomly divided into 400 ml glass jars at a population density of 18-25 larvae in 200 mL of water. Only first and second instars were put into the jars to maximize the amount of their life that underwent the temperature treatment. We defined our density treatment based on previous work by McCrea and LaDeau (unpublished data) that found little evidence of competitive effect at this ratio.

Temperature treatment during larvae stage

The mean maximum and minimum temperatures in June from 2012 to 2018 were gathered from the National Oceanic and Atmospheric Administration and we determined that there was about a 5°F difference between the diurnal temperatures in the weather stations of Baltimore, Maryland and Central Park, New York New York. (NOAA, 2018).We reared juveniles at temperature regimes reflecting mean temperatures in June in Baltimore, Maryland (High Variable, 29.4 C then 21.3 C,) or New York, NY (Low Variable, 26.5 C then 18.4 C) and at the MD daily high with no diurnal variation (High Constant, 29.6 C).

Ten jars were exposed to the high variable temperatures, 10 jars were exposed to the low variable temperatures, and 10 jars were exposed to the high constant temperature. Percival Environmental Chambers held the relative humidity constant at 75% and switched between light (14 hours) and dark (10 hours) each day. The temperatures that we used were determined from weather stations inside each city. The extra larvae that were not in the treatment jars were kept in a separate high density jar for each temperature treatment.

To feed the larvae we placed 223.7 mg of Kaytee brand rabbit food into every jar at the beginning of the first week that the larvae emerged. After the first week, many larvae in the treatment jars died, most likely due to the ratio of rabbit food to the amount of water. It is well known that larvae need to be able to break through the surface tension of the water with their siphons to breathe air, but when the water is highly saturated it becomes more difficult for small larvae to penetrate (Lee et al. 2018). After 3 days of the rabbit food, the larvae were moved to jars with clean well water and the population density in each jar in each treatment was restored to 18- 25 by using the extra larvae in the stock jars. From this day on the larvae were fed 1 mL, then 2mL (after 1 week) of fish food every day. 1.5 g of Tetra Min brand Tropical Flakes fish food was dissolved in 200 mL of well water and any undissolved fish food was discarded and each day the 2mL were taken from this stock.

We monitored the incubator temperature and relative humidity and checked for pupae every 12 hours. Once pupae were observed we moved them via pipet to a separate habitat for the nutrient source treatments. We ended the incubation phase of the experiment the 20^{th} day of the experiment.

Four nutrient type treatments during adult stage

All of the pupae that developed in a 24 hour period, within a given temperature treatment, were randomly distributed in groups of up to 6 pupae to an adult habitat with a specified nutrient treatment. In order to ensure that we could keep track of when each pupa became an adult and died in each habitat, once one pupa out of the 6 became an adult, any pupae that did not emerge in the same 24 hour period were removed and put into a separate habitat with the same nutrient treatment. The room temperature in which the habitats were kept ranged from 70-72 °F.

A flower in water, or water or sucrose dilution alone was put in to each habitat to determine if the mosquitoes live longer when provided with flowering plants versus just water or sucrose dilution. Clover (*Trifolium repens)* is typically found in non-managed lawns, while impatiens (*Impatiens walleriana*) is typically found in managed gardens. We obtained both plants locally and kept flowers alive in water. Each container had water for pupae to be placed in until they emerged as an adult, and either a clover flower, impatiens flower, a cotton ball soaked with 10% sucrose (Xue et al. 2015), or sterile water. The concentration of sucrose used is a common concentration among studies similar to ours.

We noticed that in the habitats with sucrose, the limbs of some of the mosquitoes would become sticky and they would not be able to walk or fly correctly. It most likely was due to the viscosity of the sucrose on the over-saturated cotton ball. In some cases, they died, presumably prematurely inside of the sucrose tub. Because this was documented several times, one week after the initial adult emerged we decided to change the way the sucrose was arranged in the habitats. For the habitats that already had the tubs filled with sucrose, we emptied out the excess sucrose so that the cotton ball was only slightly moist. For some of the pupae that emerged and were assigned to receive sucrose, they were given a vial with sucrose filled to $3/4^{th}$ of the way up stuffed with a cotton ball. The vial method did not allow the cotton ball to become overly saturated and prevented mosquitoes from falling into liquid. Since the water treatment was administered in a similar way to the sucrose, we changed some of the tubs to vials as well.

The sex and species of the mosquito were identified as adults because it is easiest to do during this stage anatomically. The majority of the mosquitoes that survived to adulthood were *Aedes japonicus*, therefore most of our results are focused on this species.

Measuring Longevity

Development rate from larvae to their transition to pupae was recorded as the number of larvae pupated each day. Adult longevity was recorded as the number of days that the adult mosquito was alive from emergence from the pupae stage to death. As each mosquito died, they were collected, their species was identified, and their wing length was measured.

Wing Measurements

Each mosquito wing was measured from the jugal fold to the end of the first vein (subcostal) in millimeters. Measurements were done using Cell Sans computer program at a magnification times 60.

Mosquito disposal

After the mosquitoes died and their measurements were taken, they were archived properly according to standard New York State and Cary Institute lab procedures.

Statistical Analysis

All of our data was imported into a well-organized excel sheet. Using RStudio, we ran linear models and t-tests used standard packages to analyze our data (RStudio, 2016). Differences in proportions of larvae that pupated from different temperature treatments were compared using chi-square tests (prop.test function), while differences in continuous means were compared using the functions t.test or lm for standard linear regression. Post-hoc comparison tests with bonferoni correction were used for pairwise tests. R was used to make the boxplots and the correlational model figures. For the boxplots, the solid black line represents the median of the data and the data inside of the box is where 95% of our data is located. Any circles on the boxplots indicate that the data point is an outlier.

RESULTS

Temperature Treatment at Larval Stage

Across all of the temperature treatments the mean proportion of larvae that pupated was 0.78 (Figure 3). The adult mosquitoes that were kept in the incubators their entire lives are labeled breeder.temp and they are included in these results as well as all other appropriate figures except Figure 8. The proportion of larvae that pupated was significantly different across temperature treatments that they came from (X-squared = 24.789, df = 2, $p < 0.001$, Figure 3). The proportion of larvae that pupated in the high constant treatment and the high variable treatment differed by 0.21. The difference in proportion for the high variable treatment and the low variable treatment was 0.12. The high constant treatment had the highest proportion of its larvae pupate compared to the high variable and low variable treatments. The rate of pupation, which was an mean of 17.8 days until 50% of the pupae in that temperature treatment emerged, was not significantly different across all temperature treatments ($F_{(2,26)}$= 0.289, p-value =0.7514).

Effect of Temperature Treatment at Larval Stage on Wing Length and Adult Longevity

Since the majority of the mosquitoes that we collected and reared were *Ae. japonicus*, we have chosen to report only the adult data from *A. japonicus* mosquitoes. Our population of *Aedes triseriatus* was very low in comparison to *A. japonicus* and our *A. albopictus* population was interestingly exclusively present only in the low variable temperature treatment. At the end of our study we reared, identified, and measured 73 *A. albopictus*, 283 *A. japonicus*, and 40 *A. triseriatus* mosquitoes. The mean wing length across all temperatures of the A. japonicus mosquitoes that were measured was 2.78 mm (Figure 5). *A. japonicus* that emerged from the high constant was significantly bigger by a mean of 0.4 mm than the ones that emerged from the high variable treatments ($F_{(2,280)}$ = 5.385, p< 0.001, Figure 4), (Pairwise test, p=0.0042, Figure 4). The low variable temperature treatment was not significantly different than the other two treatments (t=-1.137, df=280, p=0.25658). We expected to see similar results for the relationship between the temperature treatments and days alive since size is a proxy for longevity. We did see similar results between these two parameters. The mean days alive across temperature treatments for *A. japonicus* was 4.9 days. The number of days that *A. japonicus* spent alive in their adult stage in the high constant treatment was significantly longer than the high variable temperature treatment ($F_{(2,280)}$ = 4.067, p-value =0.01815, Figure 5), (Pairwise t-test, p= 0.021, Figure5). High constant *A. japonicus* lived 1 day longer than the high variable mosquitoes. The low variable temperature treatment was not significantly different than the other two treatments (t= -0.669, df= 280, 0.50426).

Effect of Nutrient Type on Adult Longevity

As the pupae became adults they were given different nutrient treatments. The results from our nutrient treatment were not exactly what we anticipated. The standard deviation in the number of days alive for *A. japonicus* was 2.05 days, which is quite a bit of variance within one species. There is a similar deviation of 2.07 for males and 1.87 for females when the data is further split by sex. None of the other treatments were significantly different from each other. Based on the medians of each treatment, many of the mosquitoes that fed on water lived slightly longer than the floral nutrient sources, which is not what we had anticipated. We did expect the median for the sucrose treatment to be higher than the control and breeder temp.

We had two different methods of administering water and sucrose (in a vial and in a tub) and there was a significant difference across all species in the days alive for the mosquitoes that had water in a tub versus water in a vial (t = -6.5072, df = 125.45, p-value <0.001, Figure 7). The mosquitoes that had water in a vial lived a mean of 2 days longer than the mosquitoes that had water in a tub but they were also specimens that emerged later in the experiment. There was no significant difference between the days alive when the mosquitoes were given water in a vial and sucrose in a vial. (t=-0.669, df=280, p = 0.50426).

Other Exploratory Figures

There are a few differences between species that is suggested by our data. The number of days alive differed significantly among the species (F-statistic: $28.72_{(2,351)}$, p-value<0.001, Figure 8). A. albopictus lived for a median of 4 days, A. japonicus lived for a mean of 5, and A. triseriatus lived for a mean of 2.

We anticipated that wing length would have a significant positive relationship with days alive because the relationship has been reported in other similar studies. In our data set there indeed was a significant positive correlation between wing length and the days that the mosquito spends alive as an adult ($T_{(1,281)} = 8.2901$, p-value <0.001, Figure 9). The association only explained 20% of the variation in the data, however.

There was a significant positive relationship between the number of days that the mosquitoes spent in their larval stage and the number of days the mosquitoes spent as adults ($T_{(1,397)} = 7.11$, *p-value <0.001,* Figure 10). The association explains 11% of the data.

DISCUSSION

Temperature Treatment at Larval Stage

The highest proportion of pupae were found in the high constant temperature treatment. This trend is to be expected, as higher temperatures are conducive for the growth of most kinds of bacteria that do not inhabit extreme environments on earth (Willey et al 2008). Bacteria proliferate at higher rates when they are in a warmer environment, and since larvae consume bacteria to grow and move on to their next life stages it is not surprising that the temperature treatment with a high temperature for 24 hours a day yielded the most pupae. The median of the larvae that pupated in the high variable temperature treatment was lower than the low variable median, which is not what we had expected because lower temperatures seemed as though they would be less conducive for bacteria growth. We expected the high variable to be lower than the high constant because the HV has cooler nights. It is worth noting that the data for the high variable treatment is more variable than the other treatments, suggesting that some of the data followed the expected trend, but some other unknown factor caused lower proportions of pupation in each jar.

The rate of pupation for mosquitoes in the field is important because it is beneficial for mosquitoes that live in hotter climates to transition through their aquatic life stages at a quick rate because the body of water that they are in can dry out quickly in hot temperatures. Despite this notion, we found no difference in the rate of pupation across the treatments. Perhaps the temperatures were not different enough from each other, or perhaps *Aedes japonicus*, which was the most abundant species, is more versatile and does not need to pupate more quickly or slowly in different temperatures.

Effect of Temperature Treatment at Larval Stage on Wing Length and Adult Longevity

Despite the proportion of larvae that pupated being different, the wing length of *A. japonicus* mosquitoes that emerged from the temperature treatments had an expected trend. The high constant mosquitoes were the largest, the high variable was significantly smaller, and the low variable were smaller than the HV. Since the size of a mosquito is determined by the amount of food it gets in its larvae stage this is to be expected. The species distribution was not completely even across the temperature treatments so there could have been a slight species effect on the wing length. *A. japonicus*, *albopictus*, and *triseriatus* are known to be different sizes. There was a similar trend for the days that the adult mosquitoes were alive. The positive correlation that we found between wing length and days alive further support data that has been found in other similar studies.

Effect of Nutrient Type on Adult Longevity

We expected all of the adult treatments to have a significant relationship to the mosquito's days alive as an adult, but we did not expect there to be no differences between the majority of the nutrient treatments. We did not expect the mosquitoes to do just as well on water as they did with the two floral resources or the 10% sucrose. This is potentially because white clover and impatiens naturally do not have high nectar levels to make a difference to the mosquitoes, or that once the flower stems are cut, the nectar level decreases significantly and the mosquitoes do not get any nutrients from the flowers (Zimmerman 1988). Nectar characters can vary with plants, among- and within flowers, plant size, inflorescence or flower size, flower age, sexual stage and flower position in an inflorescence (Rathcke 1992).

Longevity Across Species

Figure 4 is intriguing because it can be interpreted as a sign that if we had a larger sample size of *Aedes albopictus* and *triseriatus* to compare well enough to *Aedes japonicus*, we may be able see a trend in how long each species lives in comparison to each other.

The mosquitoes that emerged as pupae later in the experiment tended to live longer as adults. We do not have any possible theories as to why this is, and the association only explained 11% of the variation in the data, but the relationship was significant. This relationship, however could explain or could be explained by some of the differences in the longevity of the mosquitoes that were administered the vial nutrient treatments, since we gave those to pupae that emerged later in the experiment. In addition, there was a significant difference between how long adult *A. japonicus* males and females lived ($t = -3.6583$, $df = 223.03$, p-value <0.001). Females lived longer, which is in accordance to what many other studies have found.

Future Directions

In the larval stage of our experiment we did not have a clear idea of how much food to administer to the larvae in proportion to the amount of water they were in and the population density. We administered too much rabbit food. Because of this, many larvae died in the treatment jars within the first several days and the population needed to be replaced by taking larvae from higher density stock jars held at the same temperature treatment. It is possible that we did not get as many *Aedes albopictus* survive in the treatment jars to be used in the larval and adult treatments because they did not survive the initial overload of food. It is also possible that the distribution of species in each temperature treatment that we received was simply a correct sample of the distribution present in the field sites that they were collected from. As stated in our methods, we had some mosquitoes die early because of the way that we had initially administered the sucrose, the lack of significant difference between the sucrose and control regardless of mode of administration could be a result of this.

Both the temperature treatment that the mosquitoes were subject to as larvae as well as the nutrients they were provided as adults could have influenced their longevity, but future studies may be able to parse out the contributions of both of these factors on mosquito longevity. In the future we would like to conduct this experiment more times so that we hopefully get a more even sample size and distribution of species and so that we minimize the changes that we make throughout the duration of the experiment. In addition to this, another study could test a wider range of flower types that are perhaps potted so that we can better understand how the species of the flower impacts the longevity of these species.

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FIGURE 1. **Wing measurement using Cell Sans computer program**. Wings were measured from the jugal fold to the end of the first vein labeled on the figure. Magnification: x5.25.

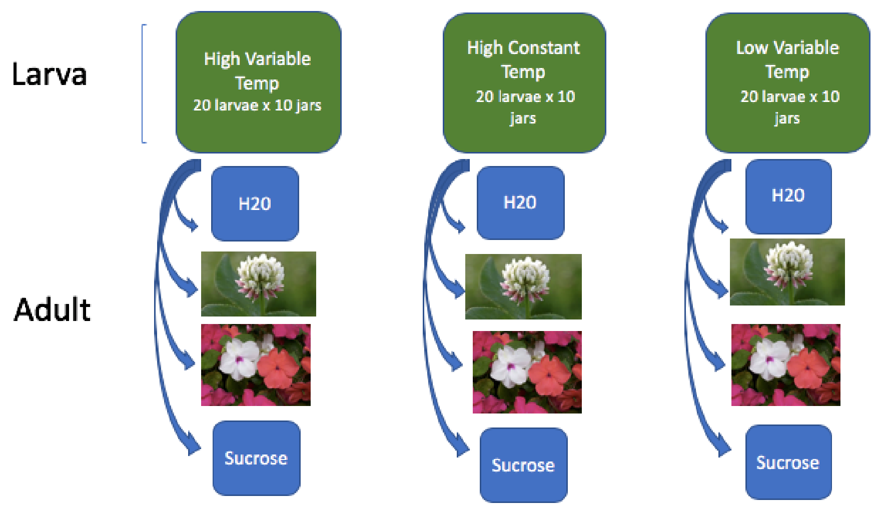

FIGURE 2. **Treatment schematic**. Each mosquito was reared as an egg until its death as an adult. The adult treatments from top to bottom are water (control), white clover, impatiens, and 10% sucrose.

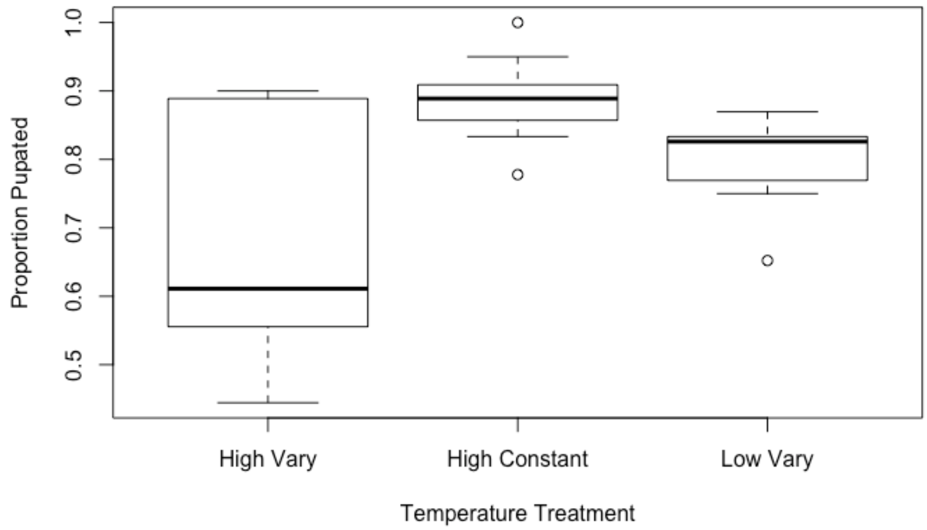

FIGURE 3. **Proportion of larvae of all species and sexes that pupated from each temperature treatment** (n=399). There was a significant relationship between proportion pupated and the temperature treatment X-squared = 24.789, df = 2, $p < 0.001$).

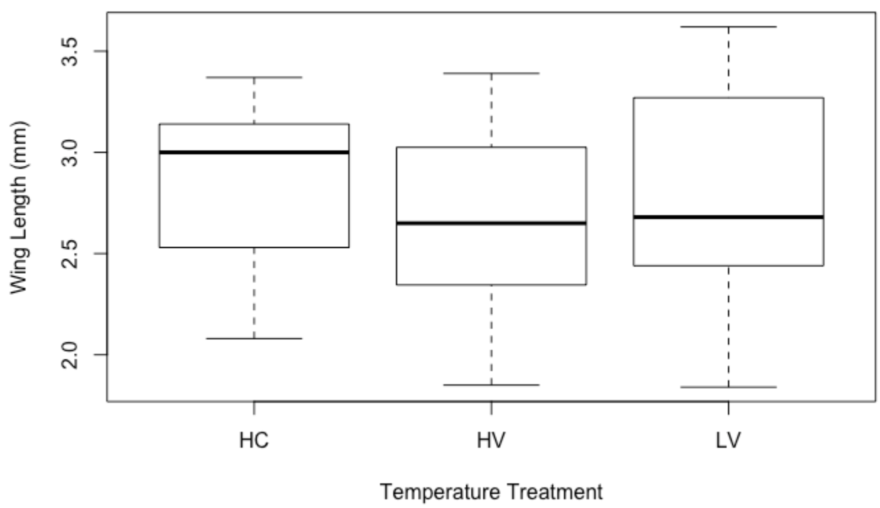

FIGURE 4. **Wing length in millimeters of *Aedes japonicus* of both sexes from each temperature treatment** (n=399). There was a significant relationship between the High constant and High variable temperature treatments and the wing length ($F_{(2,280)} = 5.385$, $p< 0.001$).

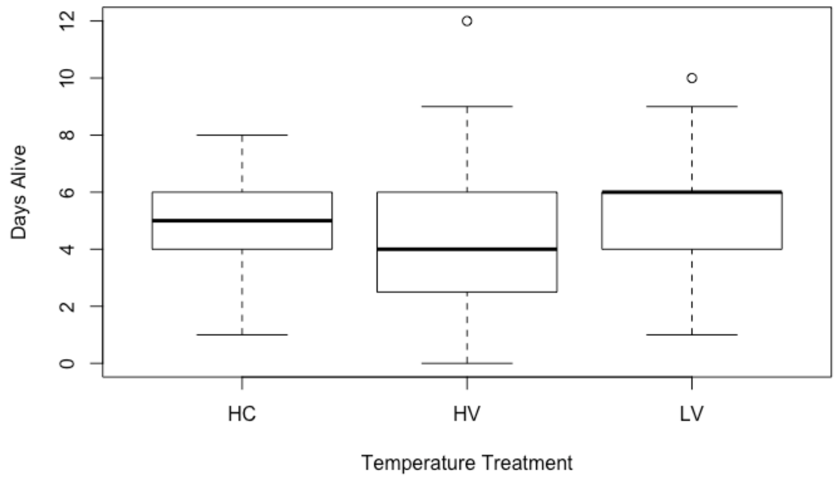

FIGURE 5. **Days alive for adult *Aedes japonicus* of both sexes from each temperature treatment** (n=399). There was a significant relationship between the HC and HC temperature treatments and the days that the mosquitoes spent alive in their adult stage ($F_{(2,280)} = 4.067$, p-value =0.01815).

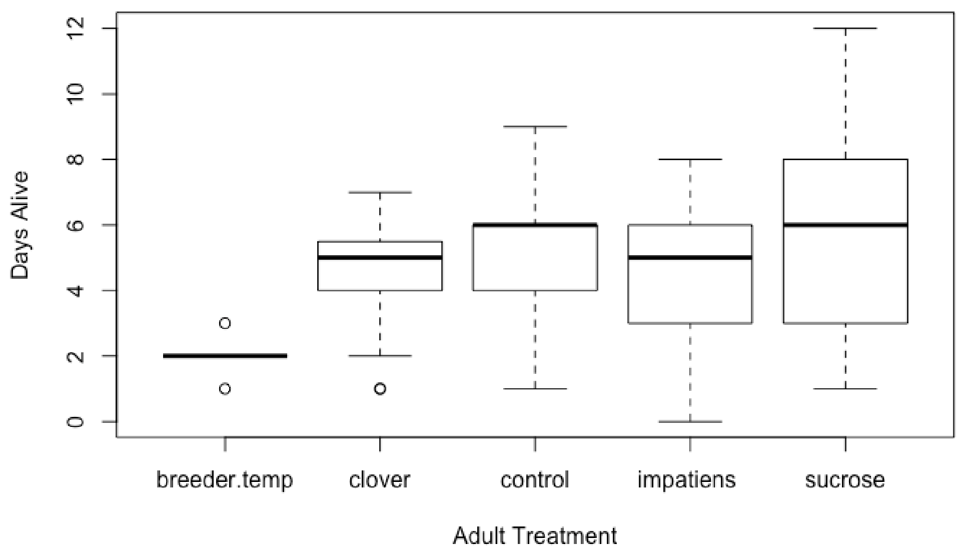

FIGURE 6. **Days alive for adult *Aedes japonicus* of both sexes from each nutrient treatment** (n=399) There is a significant relationship between all of the nutrient treatments and the days that the adult A. japonicus mosquitoes were alive ($F_{(4,278)} = 6.498$, $p < 0.001$). (breeder.temp)= the stock pupae that were left in the incubators in a breeder to live as adults without any nutrients.

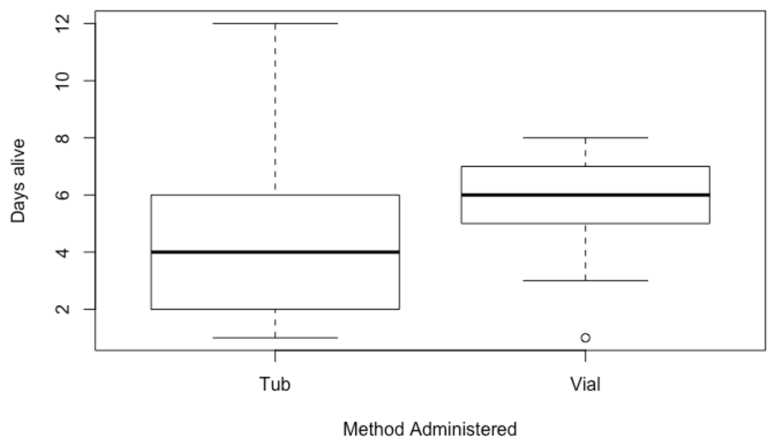

FIGURE 7. Days alive across all species and both sexes of adults that had water (control) administered from a tub and from a vial as nutrients (n=105). There was a significant difference in the days alive for the mosquitoes that had water in a tub versus water in a vial ($t = -6.5072$, $df = 125.45$, p-value <0.001).

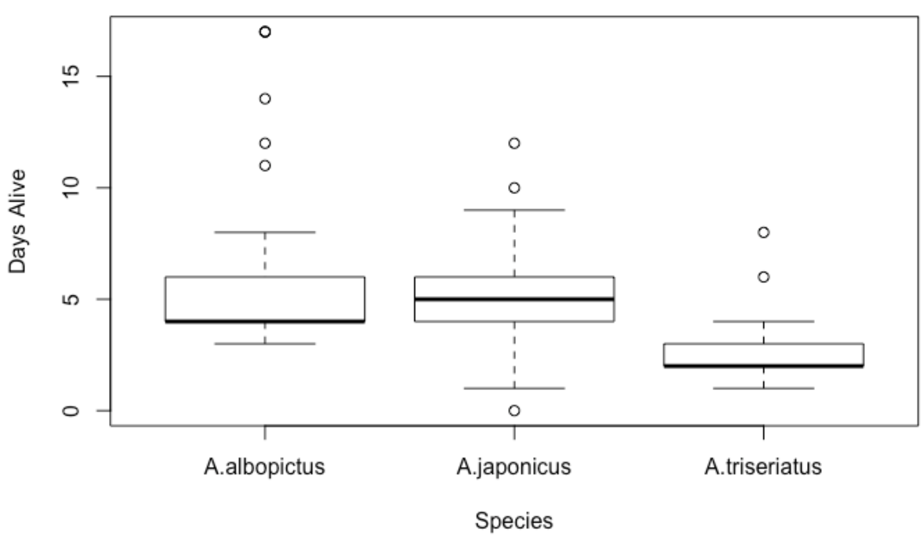

FIGURE 8. Days alive of each species and sexes that were collected (n=399). The *Aedes albopictus* mosquitoes that were not given any food or water (breeder.temp) were not included from this figure because the all of those mosquitoes were *A. albopictus* and there was not a large population of *A. albopictus* mosquitoes to begin with. Therefore we excluded them to make the species more comparable. The number of days alive differed significantly among the species ($F= 28.72_{(2,351)}$, p-value<0.001).

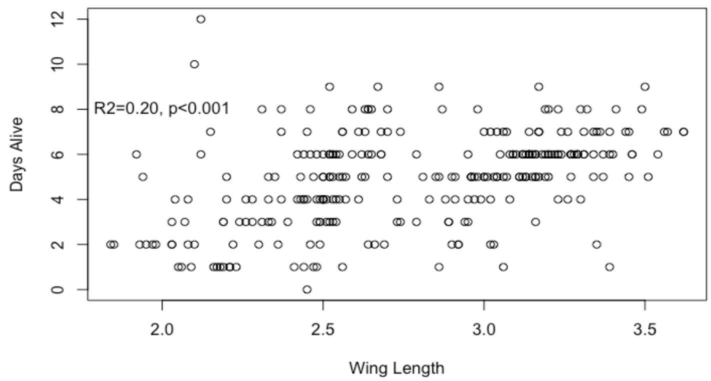

FIGURE 9. **Correlation between both sexes of *Aedes japonicus* wing length in millimeters and their days alive as an adults.** Each circle represents one mosquito. There was a significant positive relationship between the wing length and days alive ($T_{(1,281)} = 8.2901$, p-value <0.001).

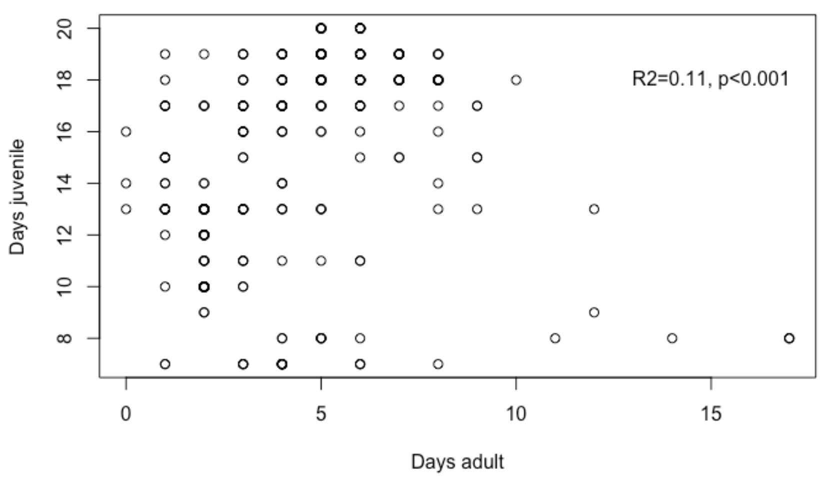

FIGURE 10. **Correlation between wing length of all species and both sexes of mosquitoes and their days alive as an adults.** Each circle represents one mosquito and many circles overlap which is indicated by the thicker circles. There was a significant positive relationship between the number of days that the mosquitoes spent in their larval stage and the number of days the mosquitoes spent as adults ($T_{(1,397)} = 7.11$, p-value <0.001).