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Predicted Effect of Climate Change on Mosquito Species *Culex pipiens* and

Aedes albopictus

by

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TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

ABSTRACT

Mosquito species *Culex pipiens* and *Aedes albopictus* are public health concerns due to their disease transmission capabilities. While much is known about their population dynamics, comparatively little is known about the impacts of current and impending climate change. The predicted climate patterns due to anthropogenic production of CO_2 have the potential for serious increases in dangers to human health. Many studies have established mosquitoes develop faster in warmer temperatures, and others predict the increase in mosquito populations. This study aims to understand how mosquito development and population growth respond to increases in both mean temperature and variability in future climate. To test these responses, I simulated the predicted increase via climate treatments in environmental chambers. Physiological responses of the mosquitoes in the chambers were monitored from first instar stage to adult emergence, at constant low-densities of single or mixed species cohorts. Competitive relationships between species were also monitored. Species success and fitness were evaluated through pupation rate, rate of adult emergence, and adult female size. Larval development was faster at warmer temperatures with little variability (29±10°C), however lower temperature with little variability produced the largest mosquitoes (24±4°C). Both species had negative physiological responses in treatments with high temperature variation.

INTRODUCTION

1. *Mosquito and Disease Risk*

The family of Culicidae, known more commonly as mosquitoes, has over 3,500 species as documented by the Mosquito Taxonomic Inventory (Harbach, 2008). They are found all over the world, particularly in temperate and tropical areas, though their range is known to expand beyond the Arctic Circle. The females of many mosquito species are known to take blood meals from vertebrates. Of the known mosquito species, four genera transmit viral diseases: *Aedes, Anopheles, Culex* and *Ochlerotatus* (Elbers *et al.*, 2015).

Mosquitoes are among the most effective vectors for parasitic and viral diseases, meaning they transmit diseases such malaria, dengue and West Nile virus (WNV) from an infected organism to another organism. Mosquito borne diseases have been known as a major cause of human disease for well over a century with epidemics as recent as the 1990's (Gubler 1998). As such, mosquitoes represent an ever present public health concern (Gubler 1998). The first case of West Nile virus in the western hemisphere occurred in 1999 around Queens, New York in the United States (Nash *et al*., 2001). Fifty-nine people with West Nile Virus were hospitalized between August and September of that year (Nash *et al*., 2001). It is likely that the outbreak of WNV in the U.S. was

introduced from the Middle East given the similarities of outbreaks in that region of the world (Jia *et al*., 1999).

From 1999 to 2007, over 11,000 cases of WNV were reported domestically, making it the most common arthropod-borne virus (arbovirus) in the United States (Reimann *et al*., 2008). Other frequently reported arboviral diseases were California (CAL) serogroup viruses, St. Louis encephalitis virus (SLEV), and eastern equine encephalitis virus (EEEV) all of which have mosquito vectors (Reimann *et al*., 2008).

The majority of WNV mosquito vectors in North America come from the genus *Culex,* with *Cx. pipiens* as the most frequent carrier during the time of the initial WNV outbreak, followed by *Cx. quinquefasciatus, Cx. restuans, and Cx. tarsalis* (Hayes *et al*., 2005; Turell *et al*., 2005*).* Females of *Cx. pipiens* are not only capable of transferring the disease from one organism to another, but they also transfers the disease vertically to the next generation (Turell *et al.*, 2001). Populations of mosquito vectors have also been shown to survive through cold winters, some with WNV detected after the population have overwintered, meaning that transmission of WNV can be reestablished when the weather conditions are favorable enough for the mosquito hosts (CDC 2000; Nasci *et al.*, 2001). In the northern part of the U.S., the WNV transmission season is considered to be from early spring to late fall with peak transmission during the warmer months (O'Leary *et al*., 2004).

Another consideration for the negative effects of mosquitoes is their role as invasive species. In general, invasive species are known to disrupt ecosystems by a variety of biological strategies including taking advantage of unoccupied niches, parasitic interactions, outcompeting the native species, or changing the environment around them (Williamson 1996). Many mosquito species introduced to North America from outside sources are opportunistic and establish populations wherever environmental conditions are suitable. They have been able to successfully establish and expand their range. As such, they are considered an invasive species (Juliano and Lounibos, 2005). Invasive mosquitoes are ones who have been introduced by humans and have the ability of impacting native ecosystems or human activity.

The history of mosquito species moving across the world is far more extensive than the body of knowledge which documents these invasions. Records of invasion vectors before the 1900s were poorly kept, though certain mosquito species have been expanding their geographic range since several centuries before (Lounibos, 2002). For example, the origin, introduction, and spread of *Aedes aegypti* and *Culex pipiens* is largely based on assumptions and drawing logical conclusions (Lounibos, 2002). The relatively recent invasion of mosquito species *Aedes albopictus* in the 1980s means that there has been a sufficient amount of attention given, resulting in ample documentation of its behavior and interactions with the surrounding native ecosystem (Juliano and Lounibos, 2005).

Many invasive species have been found to colonize areas affected by human disturbance (Richardson et al. 2000). With greater human disturbance comes more drastically altered ecosystems such that the native species are already at a competitive disadvantage (Moyle and Light, 1996). This is certainly true of mosquitoes where many invasive species have been shown to prefer areas with human populations more than the non-native and non-invasive species (Juliano and Lounibos, 2005). Establishing populations near humans also provides a benefit of having a direct source of blood meals. The establishment of an invasive mosquito in a particular area is commonly aided by transportation of juveniles on ships, though on a more local scale they can be transported through plant dishes or any other source of stagnant water (Lounibos, 2002).

Some mosquitoes also exhibit competitive advantages such as desiccation-resistant eggs, though this quality does not make them more likely to be an invasive species (Juliano and Lounibos, 2005). However it is a common trait among non-native mosquito species (Juliano and Lounibos, 2005). Juliano and Lounibos (2005) suggest that desiccation resistance makes them more likely to survive transportation. Another common advantage is that many non-native mosquitoes are fairly flexible when it comes to finding suitable habitat. They have the ability to develop in small containers at the larval stage, such as tree holes and manmade containers (Juliano and Lounibos 2005).

Species such as *Aedes albopictus* are superior competitors and demonstrate their harm as an invasive species by pushing out other mosquito

species or causing reductions in populations in an area of interspecific competition (Juliano, 1998). They are often found to be competitively superior to a variety of mosquito species, particularly *Ae. aegypti* (Barrera, 1996; Juliano, 1998; Daugherty *et al.*, 2000). In lab experiments, *Ae. albopictus* consistently outcompetes the native *Ae. triseriatus* as well (Livdahl & Willey, 1991; Novak, 1993). However *Ae. albopictus* does not always outcompete *Ae. aegypti* as seen in Rosen *et al.* (1976) and Braks *et al.* (2003). Several factors, such as climate and resource availability, could be at work to allow the success of one invasive species in one area, but limit it in another, as seen by the varying responses in the competition between *A. albopictus* and *Ae. aegypti* (O'Meara *et al.*, 1995). *Ae. albopictus* is particularly successful in man-made container habitats such as tires (Juliano, 1998).

Aedes albopictus may also have an impact on previously established populations of *Culex pipiens*. *Ae. albopictus* has been shown to be competitively superior to *Culex pipiens* at the larval stage. In Europe and North America, they are commonly found together in man-made containers (Carrieri *et al.,* 2003; Costanzo *et al.,* 2005). The interactions between the two invasives raise some concerns given that they are both vectors for West Nile virus and could increase disease transmission. *Cx. pipiens* is a well known vector for transmission among birds, though their feeding occasionally extends to humans (Fonseca *et al.,* 2004). *Aedes albopictus* is known as a bridge vector, meaning it has the potential to

connect West Nile Virus between humans and birds given that it feeds on both (Turell *et al.*, 2001).

2. *Mosquitoes as Ectotherms: sensitive to temperature and climate*

Like all ectotherms, the internal physiology of a mosquito is dependent on ambient temperatures. Numerous studies have demonstrated that mosquito development rates and survival are intricately linked to environmental temperature (Christophers 1960, Padmanabha *et al.* 2011). As such, understanding how mosquito population growth may be influenced by warming global temperatures is an important goal of empirical and model-based studies (Gong *et al.,* 2011; Wang *et al.*, 2011; Rochlin *et al.*, 2013; Ogden *et al.,* 2014).

The typical temperature range at the larval stage for maximum mosquito survival is dependent on the species, though between 16-29°C is typical for many species (Christophers, 1960; Beck-johnson *et al.*, 2013; Simoy *et al.,* 2015). Beck-Johnson *et al.,* (2013) determined that 20-26°C is the ideal range for *Anopholes* mosquitoes as determined by the low mortality of both larvae and adults. For short periods of time, *Aedes aegypti* larvae can survive exposure to 40-45°C, though long exposure to 35°C can be lethal (Christophers, 1960). Similarly, populations of *Culex eduardoi* in a laboratory setting experience 100% mortality by the pupal stage when constantly exposed to 30°C (Loetti *et al.* 2011). This same study estimated 28.1°C to be the ideal rearing temperature as determined by lack of mortality at each larval stage. Increased constant temperature within the

thermal limit boosts the vector competence and disease transmission for species such as *Cx. pipiens* (Dohm *et al.,* 2002).

Lab observations have shown that larval exposure to temperatures above 29°C result in smaller, and therefore less fit, adult *Ae. aegypti* mosquitoes (Christophers 1960). On the opposite end of the spectrum, the larvae of several mosquito species have been found to survive after being frozen in ice (Christophers 1960). However, in modeling experiments of *A. aegypti*, continuous exposure to 8°C rendered the larvae unable to pupate (Simoy et al., 2015). Some *Culex eduardoi* larvae were able to mature into adults while being subjected to a constant 7°C, albeit at a very high mortality rate throughout the later instar and pupal stages (Loetti *et al*. 2011).

3. Climate is Changing (temperature, variability, etc).

By analysis of several independent research groups, the Intergovernmental Panel on Climate Change (IPCC) has determined with 99% certainty that the troposphere has warmed due to human emissions of greenhouse gasses. In the last 120 years, the average global surface temperature has risen by 0.85°C (IPCC 2014). The last three decades have been the warmest decades the northern hemisphere has experienced in at least 800 years (IPCC 2014). Since around the industrial revolution, humans have progressively released more methane, carbon dioxide, and nitrous oxide into the atmosphere by the combustion of fossil fuels (IPCC 2014). These powerful greenhouse gases trap heat in earth's atmosphere,

resulting in a global warming trend. The effects of climate change have had and will have serious negative impacts on ecosystems and human ways of life. For example, the excess carbon dioxide in the atmosphere is partially absorbed by the ocean resulting in ocean acidification. Additionally, many species have had to alter their geographic range and migration patterns in order to find more suitable habitat (IPCC 2014). Species extinction patterns are beginning to be attributed to global warming due to habitat loss. Globally, the variability of climate and weather patterns has increased, even extending to increased temperature variation within a single day (IPCC 2014).

However the effects of climate change are not uniform across the globe (Easterling *et al.*, 1997). While the global average increase in temperature is predicted to be as high as 3.7°C by the end of the 21st century, northern latitudes will experience warming temperatures more drastically than tropical regions, including temperatures higher than an average 3.7°C increase (Easterling *et al.*, 1997; Hayhoe *et al.,* 2008; IPCC 2014). According to climate predictions from Hayhoe *et al.,* (2008), the current trajectory of carbon emissions will lead to an average increase of 5-6.5°C for the northeastern United States by the end of the 21st century (*Figure 1*). In an effort to quantify the effects of climate change on a more local scale, this climate study takes into account the temperature interactions between land, ocean, and atmosphere to construct predictions. The three models used for this study were atmosphere-ocean general circulation models (AOGCMs) created for the 2000 IPCC study. These models incorporate the combined effect

of human emissions such as greenhouse gases and aerosols in addition to natural emissions such as volcanic gases. The predictions given for the end of the 21st century are based on two scenarios of carbon emissions: one where the emission rate reflects the current trend of economic growth resulting in 970 parts per million (ppm) of CO_2 in the atmosphere, and another scenario where emissions are curbed resulting in 550 ppm (Nakienovi *et al.*, 2000, Hayhoe *et al.*, 2008). Furthermore, the growing season is expected to expand while the winters become warmer with fewer days below freezing (Hayhoe *et al*., 2007, Thibeault & Seth, 2014, Ning *et al.*, 2015).

The Hayhoe *et al.* (2008) climate model does not account for more specific variability caused by climate change, such as diurnal temperature variation or the frequency of extreme weather events. Changes in these types of variability are more difficult to predict via climate models. For daily temperature variation, some studies suggest it will change as a result of increased cloud coverage that leads to less fluctuation in temperature variations (Karl *et al*. 1991). Easterling *et al.* (1997) agrees, attributing greater cloud coverage to larger use of aerosols in the northern hemisphere, resulting in decreased diurnal temperature variations.

As for extreme weather events, Hayhoe *et al.* (2008) includes a predicted increase of frequency in extreme temperatures, such as days over 38°C. Precipitation in the northeast U.S. is expected to increase during the winter and decrease during the summer (Hayhoe *et al.*, 2007, 2008). The changes in

temperature and rainfall are also expected to alter hydrology patterns (Hayhoe *et al.*, 2007). Changes in soil moisture and stream flow predicted by AOGCMs is very likely to result in droughts ranging in frequency and severity with longer and more severe droughts becoming more common. In general, the northeast U.S. is expected to face more drastic and frequent climate extremes (Thibeault & Seth, 2014, Ning *et al.,* 2015).

The significance of climate change is beyond that of changing weather patterns and altered habitats. Humans will not go unaffected by these transitions. For example, the increase in frequency of high temperature days will contribute to heat related deaths (Thibeault & Seth, 2014; IPCC 2014). The warmer summers with increased frequency of drought will threaten the supply of food and water (IPCC 2014). Changes in hydrological cycles directly affect the availability of water for crops. The northeast U.S. is vulnerable in this way since it is an agriculturally intensive area (Hayhoe *et al.*, 2007). Even if the droughts are relatively short, less than a month, the effect on crop development can be devastating (Hayhoe *et al.*, 2007). The threat of food insecurity is not limited to agriculture; marine sources of food are in danger as well due to fishery collapses and habitats rendered unsuitable by warmer and more acidic waters (Doney *et al.,* 2012). As global temperatures rise, terrestrial ice melts, resulting in raised sea level. The IPCC prediction of a 4°C average increase for the 21st century could result in a sea level rise of 0.5-2.0 m. by 2100 (Nicholls *et al.*, 2011). This sea level rise could displace tens of millions of people who live on islands and coastal

areas, up to 2.4% of the global population (Nicholls *et al.*, 2011). In addition, people's livelihoods will be threatened by water shortages and expanding deserts, which could add to the count of potential climate refugees (Myers, 2002).

Climate change has the potential to indirectly affect the human population by affecting the spread of certain diseases. The main factors affecting the spread and intensity of vector transmission are the presence of an organism capable of transmitting the disease and presence of the disease itself (Dobson and Carper 1992). Changes in environmental conditions could affect either or both (Martens *et al.*, 1995; Epstein, 2001). Climate change can both directly and indirectly affect the vectors by 1) altering their physiology and 2) altering conditions of the surrounding environment to be more or less suitable to the organism and produce new areas of viable habitat (Epstein, 2001). Specifically, vector transmission and density is affected by factors such as temperature, precipitation, and humidity. Rising sea level caused by global warming can increase the availability of coastal breeding grounds (Epstein, 2001). However drought and expansion of global deserts will most likely restrict vector-borne disease transmission given that aquatic environments are necessary for vector growth and development. In general, warmer temperatures encourage parasite growth and transmission, so vectors are likely to spread to higher elevations and latitudes (Martens *et al.*, 1995). Future transmission of vector borne diseases such as malaria is dependent on climate change effects on a local scale. However Martens *et al.* (1995) estimate with their model that there will be a general risk increase of 2.9-10.1%

by 2050 with the assumption that there will be no significant cuts in greenhouse gas emissions.

There have already been observed changes in the distributions of certain insect populations as a result of climate change. For example, populations of the butterfly *Euphydryas editha* have shifted upward in elevation and northward by 92km along the western area of North America (Parmesan, 1996). Previously recorded populations were four times more likely to be extinct in the lower latitudes of Mexico than populations in the higher latitudes of Canada (Parmesan, 1996). In a study of 35 non migratory European butterflies, 63% shifted anywhere from 35-240km northward throughout the 20th century (Parmesan *et al*., 1999).

Mosquitoes are among the insect species reported to be expanding their ranges poleward, tracked by outbreaks of diseases carried by mosquitoes. Occurrences of vector borne diseases have been reported at higher latitudes and elevations than the diseases are typically associated with (ProMed 1997). In the high elevations of West Papua, several cases of malaria have been reported to expand past the previously reported elevation of 1700 meters to 2100 meters (ProMed 1997). The cause of this outbreak was hypothesized to be due to the average temperature increase of 2°C in the area (ProMed 1997). Malaria outbreaks in higher altitudes have been reported in Kenya and Tanzania as well (Matola, 1987; Some, 1994). Similarly, dengue fever in Mexico had risen to 1700 meters in altitude when it had previously been known to be only below 1000 meters (Koopman *et al.,* 1991).

In order to predict how mosquito populations and physiology might respond to climate change, past studies have compared mosquito growth at certain constant temperatures. Diurnal variations in temperature are often unaccounted for in these studies, which fails to reflect accurate conditions mosquitoes experience outside of a lab (Alto & Juliano 2001; Gong *et al*. 2011; Carrington *et al.* 2013). As such, predicted responses in population dynamics have the potential to be exaggerated given their more immediate physiological sensitivity to daily weather patterns rather than overall climate (Reiter, 2001). Physiological differences due to temperature variability are usually studied in the context of comparing species populations in urban areas (affected by urban heat islands) to more rural areas (LaDeau *et al*. 2015). It is unknown whether certain species will have specific physiological responses to climate change with the influence of temperature variation.

4. Aedes albopictus

The species used in this study are *Aedes albopictus* and *Culex pipiens. Ae. albopictus* is an invasive mosquito species native to Southeast Asia and is considered to be one of the most invasive mosquito species in the world (Hawley 1988). *Ae. albopictus* do not lay their eggs directly in water, but rather on the edge of small to mid-sized containers (anywhere from an ounce of water and greater) (Chan, 1971). They are typically found in man-made containers and tree holes (Chan, 1971; Hawley, 1988). The females typically rely on mammals, including humans, to obtain their blood meal (Hawley, 1988). The larval development time

is anywhere from 5-10 days depending on the temperature, food resources, and any other source of strain (Hawley, 1988). Larval development of *Ae. albopictus* cannot continue below temperatures of 11°C, however they are able to develop in temperatures in the low to mid teens (°C) albeit at a rate of several weeks (Udaka, 1959). Conditions of larval development are crucial because they affect the size of the adult, which is an important indicator for population success (Hawley, 1988). For example, smaller adult females tend to produce fewer eggs (Mori, 1979).

In the 1980s, *Aedes albopictus* was introduced to the U.S. first in subtropical areas and their range has since expanded northward (Hawley *et al.*, 1987). By 1985, they had become one of the most abundant invasive mosquito species in Houston, Texas. *Ae. albopictus* in the United States exhibited traits such as cold tolerance and photoperiod sensitivity, similar to the *Ae. albopictus* mosquitoes of China, Korea, or Japan, and unlike the *Ae. albopictus* mosquitoes of the more tropical areas of Asia (Hawley *et al.*, 1987). Hawley *et al.,* (1987) hypothesized that the relatively frequent shipment of car tires from these locations to the U.S. were what brought this strain of *Aedes* to the U.S., especially given that tires are frequently documented container habitats for mosquitoes (Reiter and Sprenger, 1987).

While their range in New York state is currently restricted to the southernmost part, Rochlin *et al*. (2013) have predicted the range to spread across the state in the coming decades, depending on the predicted trajectory of global carbon emissions and thus the severity of altered climates. Extreme cold weather

is most likely the main barrier for the northward expansion of *Aedes albopictus* along the eastern coast of the US (Teng & Apperson, 2000). As their range expands, an additional 30 million people will be at risk of exposure to *Ae. albopictus* and the diseases that it carries (Rochlin *et al.*, 2013).

In experiments subjecting *Ae. albopictus* mosquito larvae to different temperature regimes, their population rate of increase rose with temperature to the highest tested value of 26°C (Alto & Juliano, 2001). The rate of increase is likely driven by a more rapid developmental period and a higher rate of emergence as adults. In general, cooler temperatures slow the development and spread of *Ae. albopictus* resulting in population dynamics that differ between the southern and northern US (Alto and Juliano, 2001). However, with future warming temperatures on the rise, Alto and Juliano (2001) predict that the increased population size of northern *Ae. albopictus* colonies will not only result in an increase of its range, but that the warmer temperatures will also boost mosquito population sizes to a degree that enables them to colonize areas more successfully.

5. Culex pipiens

The origins of *Cx. pipiens* are of some debate, but it is certain that it has one of the widest geographic distributions for a mosquito species (Farajollahi *et al.,* 2011). *Cx. pipiens* has numerous subspecies that allow for the wide range, though the rapid occurrence of hybridization raises questions about if the taxonomic divisions are justified (Farajollahi *et al.,* 2011). The subspecies are

morphologically very similar, often visibly indistinguishable, though their behaviors and habitat can differ. The subspecies typically found in the temperate regions of the northeastern U.S. is *Culex pipiens pipiens* (Farajollahi *et al.,* 2011). *Cx. pipiens* typically takes its blood meals from birds, though it will occasionally take blood meals from mammals including humans (Farajollahi *et al.,* 2011). Given this information, the *Cx. pipiens* mosquitoes found in this region of the world are likely to be descended from the *Cx. pipiens* mosquitoes of Mediterranean areas which are known to take blood meals from both mammals and birds (Byrne and Nichols, 1999). Considering its origins, *Cx pipiens* is therefore a species of mosquito which is already accustomed to warmer temperatures. *Cx. pipiens* lays its eggs in rafts on the surface of water bodies such as ponds (Farajollahi *et al.,* 2011).

Mosquitoes of the genus Culex are one of the primary transmitters of West Nile Virus (WNV) (Dohm *et al.,* 2002). Temperature is among the factors that can influence vector capacity. Under lab settings in an experiment run by Dohm *et al*.(2002)*, Cx. pipiens* mosquitoes reared at 18°C transmitted WNV significantly less often than *Cx. pipiens* raised at higher temperatures. Of the four increasing temperatures in this experiment, *Cx. pipiens* were more likely to become infected with WNV after receiving an infected blood meal. The warmest temperature was 30°C and dissemination increased with temperature.

This relationship with disease transmission and temperature is what is hypothesized to be the reason behind a WNV outbreak in New York during the

unseasonably high temperatures of 1999 (Dohm *et al.,* 2002). However these experiments were conducted at a constant temperature which does not accurately reflect field conditions (Dohm *et al.,* 2002).

Climate-based population models provide further evidence for the expansion of *Cx. pipiens* throughout the northeast US (Gong et al., 2011). To understand the interactions of climate and the trajectory of vector-borne diseases, Gong *et al.* (2011) developed a model to predict the spread of vector-borne diseases with increasing average temperatures based on how 1) mosquito populations responded at a small scale and 2) applying the response to a large-scale prediction. This study predicts a vigorous development of *Culex* populations in the northeast for the future.

6. Culex and Aedes Raised in Cohabitation

Both species are prominent vectors for mosquito borne diseases (Hawley 1988, Farajollahi *et al*. 2011). The mid-Atlantic U.S. is a location where these two pest species experience habitat overlap and thus compete for resources (LaDeau *et al.,* 2013). This location provides temperature and habitat conditions that happen to be suitable to both species. There is sometimes an initial spatial separation of the species given the different oviposition methods. However, both species are able to oviposit in mid-sized container habitats (10-50 liters), common in urban areas (Carrieri 2003). These urban areas also allow for warmer microclimates that encourage larval growth (LaDeau *et al.,* 2013). Mosquito larvae commonly occupy containers such as tires, gutters, and planters (LaDeau *et al.,* 2013). Pupae

of both species have been observed to occupy the same containers (LaDeau *et al.,* 2013). The prevalent examples of cohabitation among these pest species pose a risk to the human population given the frequency they are found in urban areas (LaDeau *et al.,* 2015). Varying economic conditions in urban areas also drive the frequency of the container habitats suitable for mosquito habitat, resulting in lower income areas exposed to higher populations of mosquitoes and consequently a higher risk of contracting mosquito borne illnesses (LaDeau *et al.,* 2015).

Where habitat overlaps occur, *Ae. albopictus* is typically the superior competitor, as shown by many previous lab and field studies (Juliano, 1998; Carrieri *et al.,* 2003; Juliano, 2007; Costanzo *et al.,* 2011). In warmer temperatures (25°C), *Ae. albopictus* performs better than *Cx. pipiens* under resource competition, marked by a greater efficiency of converting food to biomass (Carrieri *et al.,* 2003). In the Carrieri study (2003), the *Aedes* mosquitoes were able to exploit food resources more efficiently and developed on average a day faster than the *Culex* mosquitoes. However in lower temperatures (such as 20°C), *Aedes* took 2.5 days longer than *Culex* to emerge as adults and the ability to convert food to biomass decreased. In conditions of food scarcity, the biomass of *Aedes* mosquitoes will decrease; however the percentage of adult emergence remains unchanged (Carrieri *et al.,* 2003). When the *Culex* mosquitoes were subjected to similar conditions, the larvae were unable to mature to the adult stage. When the ratio between *Cx. pipiens* and *Ae. albopictus* was altered, *Culex*

mosquitoes responded positively in weight and emergence to a lower percentage of *Aedes* mosquitoes (Carrieri *et al.,* 2003). The differential response to mosquito ratios plus the ability for *Aedes* mosquitoes to more efficiently convert food to biomass led Carrieri *et al.* (2003) to infer that *Ae. albopictus* mosquitoes have the ability to consume at a rate that leaves *Culex pipiens* with inadequate food resources, thus impacting their overall fitness.

In Costanzo *et al.* (2011), *Ae. albopictus* performance is marked by greater survivorship, larger wing length, and faster development time than *Cx. pipiens* when both species are competing for food. Even when given different types of food resources, *Ae. albopictus* consistently outcompetes *Cx. pipiens* when they are raised in cohabitation (Costanzo *et al.,* 2011). However, the type of food resource can alter how much of an advantage *Aedes* has over *Culex.* The Costanzo *et al.* (2011) study indicates that *Ae. albopictus* performs better in competition when the detritus mixture used as food has low microbial activity and low nutrient content. On the other hand, *Cx. pipiens* responded poorly regardless of the nutrient content or decomposition rate of the detritus material (Costanzo *et al.,* 2011).

MATERIALS AND METHODS

The data from the lab study were collected in Millbrook, New York and the Cary Institute of Ecosystem Studies. All climate data used for this study is therefore relevant to the state of New York.

Four climate scenarios were identified to represent expected changes in mean temperatures for the northeastern U.S. given the current trajectory of carbon emissions and varying levels of daily temperature variation. Percival environmental control chambers (model LT-36VL) were programmed for (1) current ambient conditions: climate average (24°C) from 1970-2000 for the month of July with low daily temperature variation (±4°C), (2) current mean with high daily temperature variation (±10°C), (3) predicted summer climate average for 2070 (29°C) with low daily variation (±4°C) and (4) future mean with high variation (±10°C). The climate predictions are from the data supplied by Hayhoe *et al.* (2008) and are based on the current trajectory of carbon emissions (*Figure 1*). The predictors were calculated through three different atmosphere-ocean general circulation models. Temperature changes were achieved through step increase peaks at 10AM-4PM and minimum temperatures at 10PM-4AM. All other times were set to the average temperature of the given treatment. All experiments were at constant 80% humidity and were under a 12 hour

photoperiod which fluctuated with the rising and falling temperature (*Figure 2*). Chambers were checked in person twice daily to be sure that they were at the correct climate condition. Alarms were established to go off if the temperature had varied more than $\pm2°C$ beyond the programmed temperature. No alarms went off during the course of this experiment.

The mosquito eggs were procured from the lab of Dr. Paul Leisnham at the University of Maryland. The *Cx. pipiens* eggs were field caught in Baltimore, MD. The *Ae. albopictus* eggs were the second generation of an initial field caught population. The *Aedes* mosquitoes had laid their eggs on seed paper placed near a source of water. Before hatching the *Aedes* eggs, the seed papers were kept in zipblock bags with a soaked cotton ball to maintain moisture. Bags were occasionally opened to replenish oxygen. I hatched the eggs by placing the seed paper in trays of shallow leaf tea (filtered incubation from dried oak and hickory leaves) with the addition of two pellets of shrimp food for extra protein. The trays were kept under artificial light during the day and the lights were turned off at night. The *Culex* eggs were sent as an egg raft in water which began to hatch during shipping. Each experiment began with first instar larvae (1-day old *Aedes,* 2-days old *Culex*), however no *Culex* mosquitoes were examined in the $29\pm10°C$ treatment. I transferred the mosquito larvae to the experimental jars using pipettes.

Mosquitoes were reared in 0.5L mason jars with gauze tops to ensure enclosure and maintain air circulation. There were 10 mosquito larvae per

mesocosm with 10 mesocosm replicates for each species treatment within the climate simulations (*Aedes albopictus, Culex pipiens,* or cohabitation with 5 of each per mesocosm).

In order to minimize any resource-limitation effects, each mesocosm was initiated with 300mL of leaf tea, similar to experiments such as Padmanabha *et al.* (2011). All mesocosms also received two pellets of shrimp food and were equilibrated with chamber conditions prior to adding larvae. The high humidity in the chambers was to maintain the water level within the jars. When the water evaporated below 300mL, more leaf tea was added.

After the larvae were placed in the incubators, each jar was checked once daily for pupae. Once pupation began, each jar was checked every 12 hours. During each check, the number of pupae per jar was recorded. Pupae were then transferred to breeding containers as soon as they were found, which allowed the pupae to emerge as adults while still being confined. Breeding containers, or breeders, are two-part containers with the bottom half housing the pupae until they emerge as adults, which are then funnelled into the top half. The breeders were also kept in the incubators and separated pupae by species treatment (*Aedes, Culex* or cohabitation) for each climate chamber. As adults began to emerge, the top halves of breeders were put in a freezer to kill the adults and a new top was put in its place so the remaining pupae could continue to emerge. This was done daily. The breeders were kept in the incubators until no living pupae were left. Frozen adults were separated into petri dishes by species treatment and date

emerged. After 24 hours of freezing, the now dead adults were air dried in order to keep moisture retention from affecting the dry weights.

Each female mosquito was weighed in milligrams on a mass balance. After being weighed, one wing per female mosquito was removed from the body and measured to the nearest quarter of a millimeter beneath a microscope. Each female mosquito that emerged as an adult has the following corresponding data recorded: wing length, weight, day emerged, chamber it was raised in, and whether it was raised in cohabitation or not. Mosquito population success is measured by adult biomass, wing length, time to pupation, general mortality, and percent emerged as adults. Biomass and wing length were only measured on the females as they are the ones who carry and transmit diseases through blood meals and have slightly different characteristics from the males. I measured the rate of mosquito larval development by determining the average day where 50% of the larvae had pupated in each climate treatment.

The treatments were analyzed between and within treatments using a series of ANOVA tests. While an ANOVA determines if there is an overall effect, it does not show which groups are different. I used a Tukey post-hoc test to show the differences between groups within an ANOVA test. The null hypothesis was that there were no differences and the alternate hypothesis is there is a difference. With my experiment, I tried to find evidence against the null hypothesis. The p-values indicate the probability of getting the observed conditions by chance alone. Treatments with only two groups of means were examined via a t-test. The

percentage of adults which emerged from the initial pupae had no statistical tests

performed. Statistical analyses and figures were conducted in R.

RESULTS

The data were primarily analyzed using a series of ANOVA tests to understand the average values of the mesocosms or of the individual mosquitoes within a climate treatment. Statistical significance between means within a single ANOVA test was analyzed using a Tukey post-hoc test.

Aedes albopictus

- Development time

  o There was a 7 day difference between the fastest and slowest pupation rates across the four climate scenarios tested (*Figure 3, Table 1*). *Ae. albopictus* pupated earliest in warmer conditions with low daily temperature variation (29±4°C) and pupation was slowest in the warmer average with high daily temperature variation (29±10°C). The *p* value of the ANOVA is less than 0.001. Only two of the temperature treatments were not significantly different from one another; however there is still a noticeable difference in favor of the treatment which reached higher temperatures (24±4°C-24±10°C, *p*= 0.09).

- Pupation

  o Average pupation rate decreased with increasing means and/or increasing daily temperature deviations. Average pupation rates

were between 69-92% success across climate treatments. The only treatments significantly different from one another were 24±4°C and 29±10°C (p=0.005, *Figure 4, Table 2*).

- Adult emergence

  o Of those that pupated, 75-93% emerged within each climate incubator (*Figure 5*). The climate treatment with the highest average and the lowest temperature deviation had the highest success of adult emergence.

- Wing Length: comparing cohabitation and single species containers

  o There were hardly any differences within a temperature treatment between the wing lengths of *Aedes* raised with *Culex* compared to wing lengths of *Aedes* raised on its own (*Figure 6, Table 3*). None were significantly different and the largest difference between wing lengths of cohabitation and single species mesocosms in the same climate treatment was less than 0.2mm.

- Wing length: combined cohabitation and single species containers

  o The average wing lengths per climate treatment of *Ae. albopictus* ranged from 2.55mm to 3.28mm (*Figure 7, Table 4*). All treatments were significantly different from one another and the *p* value of the ANOVA is less than 0.001. The climate treatment with the largest wings is 24±4°C and the treatment with the smallest wings is 29±10°C.

- Weight: comparing cohabitation and single species containers

  o There were hardly any differences within a temperature treatment between the weights of *Aedes* raised in cohabitation compared to weights of *Aedes* raised on its own (*Figure 8, Table 5*). None were significantly different and the largest difference between weights of cohabitation and single species mesocosms in the same climate treatment was 0.11mg.

- Weight: combined cohabitation and single species containers

  o All of the climate treatments resulted in weights that are significantly different from one another (*Figure 9, Table 6*). The *p* value of the ANOVA is less than 0.001. On average, *Aedes* mosquitoes were 0.64mg heavier at the lower temperature with low deviation compared to the lightest mosquitoes found in the high temperature with high deviation.

- Temperature deviation & temperature mean

  o For *Ae. albopictus* weight and wing length, the effect of temperature variation is very evident, even across different mean temperatures (*Figures 10 & 12*). However, the effects of temperature deviation (*Figures 10 & 12*) are more noticeable in the figures than the effects of mean temperature (*Figures 11 & 13*). *Aedes* mosquitoes were significantly larger in terms of weight and wing length when larvae were reared with low temperature

variation. On average, the *Ae. albopictus* mosquitoes in treatments with high temperature deviations are 0.26mg lighter (*Figure 13)* with wing lengths 0.34mm smaller (*Figure 11*) than the *Ae. albopictus* in treatments with low temperature deviations. The *Ae. albopictus* mosquitoes in treatments with the high average temperature are 0.1mg lighter (*Figure 12*) with wing lengths 0.13mm smaller (*Figure 10*) than the *Ae. albopictus* in treatments with low average temperature.

Culex pipiens

- Development time

    o There was a 1.9 day difference between the fastest and slowest pupation rates across the three climate scenarios tested (*Figure 14, Table 7*). *Cx. pipiens* pupated earliest in warmer conditions with low daily temperature variation (29±4°C) and pupation was slowest in the cooler average with low daily temperature variation (24±4°C). However, there was no significant different between the two treatments with an average of 24°C. The *p* value of the ANOVA is less than 0.001. The 29±4°C is significantly different from the other two treatments.

- Pupation

    o Average pupation rates were between 87.1-92% success across climate treatments. There were no treatments significantly different from one another (ANOVA *p*=0.005, *Figure 15, Table 8*).

- Adult emergence

    o Of those that pupated, 92-97.5% emerged within each climate incubator (*Figure 16*). The climate treatment with the highest average and the lowest temperature deviation had the highest success of adult emergence.

- Wing Length: comparing cohabitation and single species containers

    o There were hardly any differences within a temperature treatment between the wing lengths of *Culex* raised with *Aedes* compared to wing lengths of *Culex* raised on its own (*Figure 17, Table 9*). None were significantly different and the largest difference between wing lengths of cohabitation and single species mesocosms in the same climate treatment was less than 0.1mm.

- Wing length: combined cohabitation and single species containers

    o The average wing lengths per climate treatment of *Cx. pipiens* ranged from 4.03mm to 4.33mm (*Figure 18, Table 10*). The 24±4°C treatment is significantly different from the other two treatments and the *p* value of the ANOVA is less than 0.001. The

climate treatment with the largest wings is 24±4°C and the treatment with the smallest wings is 24±10°C.

- Weight: comparing cohabitation and single species containers
  - There were hardly any differences within a temperature treatment between the weights of *Aedes* raised in cohabitation compared to wing lengths of *Aedes* raised on its own *(Figure 19, Table 11)*. None were significantly different and the largest difference between weights of cohabitation and single species mesocosms in the same climate treatment was 0.08mg.

- Weight: combined cohabitation and single species containers
  - All of the climate treatments resulted in weights which are significantly different from one another (*Figure 20, Table 12*). The *p* value of the ANOVA is less than 0.001. On average, *Culex* mosquitoes were 0.2mg heavier at the lower temperature with low deviation compared to the lightest mosquitoes found in the low temperature with high deviation.

DISCUSSION

The goal of this study was to better understand the physiological changes that occur among mosquito species *Aedes albopictus* and *Culex pipiens* as they are exposed to different climate regimes throughout their larval development. These physiological changes in turn can be used to infer individual fitness levels and vector competence. I measured the females of each species rather than all adult mosquitoes since 1) there are size differences between male and female mosquitoes of any species and 2) females are more relevant to disease transmission and population growth since they are the ones who take blood meals and lay eggs (Turell *et al.*, 2001). While the climate predictions used in this experiment were made for the state of New York, the implications are certainly applicable across New England.

Development Time

Both species developed significantly faster in the climate treatment with a warmer mean temperature and limited temperature variation (*Figures 3 & 14*). Rapid development time in warmer temperatures could lead to faster life cycles and population expansion. Warmer temperatures would be particularly advantageous for survival in temporary habitats. For example, faster development could mean that the mosquitoes are able to emerge before their habitat evaporates. This is especially true for *A. albopictus* given that they often lay eggs in small

container habitats found in urban areas (W. A. Hawley, 1988). Given the heat islands and the container habitats found in urban areas, there is increasing evidence that urban areas will be more susceptible to rises in *A. albopictus* populations (Rochlin *et al*. 2013). The additional increase in temperature due to global warming could exacerbate these effects.

Pupation & Emergence

For both species, the average percentages of pupae from the initial larval populations were generally not statistically significant across climate treatments (*Figures 4 & 15*). The only area of statistical significance was in the *Aedes* 29±10°C which was significantly different from the lower climate mean with low temperature variation. The lower percentage of pupation in the treatment with high temperature variability is likely due to the stress of larvae being exposed to a wide range of temperatures. In terms of adult emergence, both species responded positively to warm temperatures with limited daily temperature variation (*Figures 5 & 16*). While no statistical significance can be determined from these figures, it is still interesting to see greater adult emergence in the 29±4°C treatment as it supports the pupation successes also seen in this climate treatment.

Competitive Effects

Given that *A. albopictus* is typically the superior competitor to *C. pipiens,* I was not expecting to find such a minimal statistical difference between the species reared in competition (Costanzo *et al.*, 2011) (*Figures 6, 8, 17, & 19*). A possible explanation for the lack of competition effects is that there were more

than enough food resources in the mesocosms so competition for resources was not necessary. Another possibility is that the number of mosquitoes within the mesocosms was small enough to have no noticeable density dependent effects. It was important to assign a known density of mosquitoes to the mesocosms in order to even out any density dependant population effects. This is a key consideration given that density dependant effects are greater at higher temperatures (Juliano, 2007).

It is difficult to compare the results of the *Ae. albopictus* wing lengths to the standard *Ae. albopictus* wing length due to how their range as an invasive mosquito has expanded, resulting in regionally specific differences (Vargas *et al*., 2013). That being said, even though the differences between the treatments are significant, they are well within the documented range of *Ae. albopictus* wing lengths.

Adult Physiology

Differences between treatments were overall more remarkable with the mosquito weights rather than wing lengths (*Figures 7, 9, 18, & 20*). The most dramatic changes seen in the *Aedes* mosquitoes are between the 29±10°C climate treatment compared to all the other treatments. The 29±10°C produced dramatically lighter *Aedes* females with noticeable smaller wing lengths compared to the other treatments.

What was surprising about the climate treatments in terms of adult physiology was that the lower average temperature with low temperature variation

(24±4°C) produced heavier mosquitoes of both species with larger wing lengths than the warmer average temperature with low temperature deviation (29±4°C). This contradicts the successes seen in the larval development where *Aedes* and *Culex* pupated faster at the 29±4°C treatment. With increasing means and variation, physiological responses in terms of weight and wing length decreases significantly, though mosquitoes of both species tend to be significantly heavier and *Aedes* has larger wing lengths at the 29±4°C compared to the 24±10°C. However, hardly any difference is noticeable in *Culex* wing lengths between these temperature treatments. Given that past research has shown that smaller adult females lay fewer eggs, the faster larval development may not be enough to boost mosquito populations as temperatures increase (Mori, 1979).

Temperature Mean and Temperature Variation

Despite the varying mean temperatures across the treatments, the temperature variability itself appeared to greatly influence mosquito physiological responses (*Figures 10-13*). While all of the differences between high and low variation or high and low means were significantly different for both weight and wing length, the differences were more pronounced for weight rather than wing length. Though I only have two levels of temperature variability, the results show that greater temperature variation likely causes greater stress on developing mosquito larvae resulting in adults with smaller, lighter bodies than those reared in treatments with less variability. These results are particularly concerning when considering that many past studies, such as Gong *et al.* 2011 and Alto & Juliano

2001, are based on how mosquitoes respond to a constant temperature representing the mean temperature of the climate regime in question. Similar results were found in a study of *Aedes aegypti* mosquitoes in Thailand where the treatments with larger temperature variation resulted in higher rates of larval mortality and delayed larval development time (Carrington *et al*., 2013).

Implications and Future Studies

It would be beneficial to repeat this study while also having a treatment at the constant mean temperature. This way I can measure the rate of change in mosquito physiology relative to increasing temperature fluctuations. As such it is regrettable that I was not able to include a *Culex* comparison at the warmer temperature with higher variation (29±10°C) and be able to compare how *Culex* fares with the changes in both average means and daily variations as I was able to do with the *Aedes* mosquitoes. It would also be interesting to repeat the study with same species, but different generations or eggs obtained in different areas of the country to account for genetic diversity. Further areas of exploration would be to see how sensitivity to temperature changes with mosquitoes who are infected with viral diseases.

What this study does show is that while we face the extreme likelihood of an average increase in temperature, how mosquito populations respond to climate change is highly dependent on the specifics of daily temperature fluctuations. Taking this into consideration, past modeling studies which rely on temperature means to determine population dynamics may not yield accurate results

(Carrington *et al.,* 2013, Gong *et al*., 2011, Alto & Juliano 2001). In order to create modeling parameters which reflect real-world conditions, a more thorough understanding of mosquito physiological changes as a result of temperature variation is needed.

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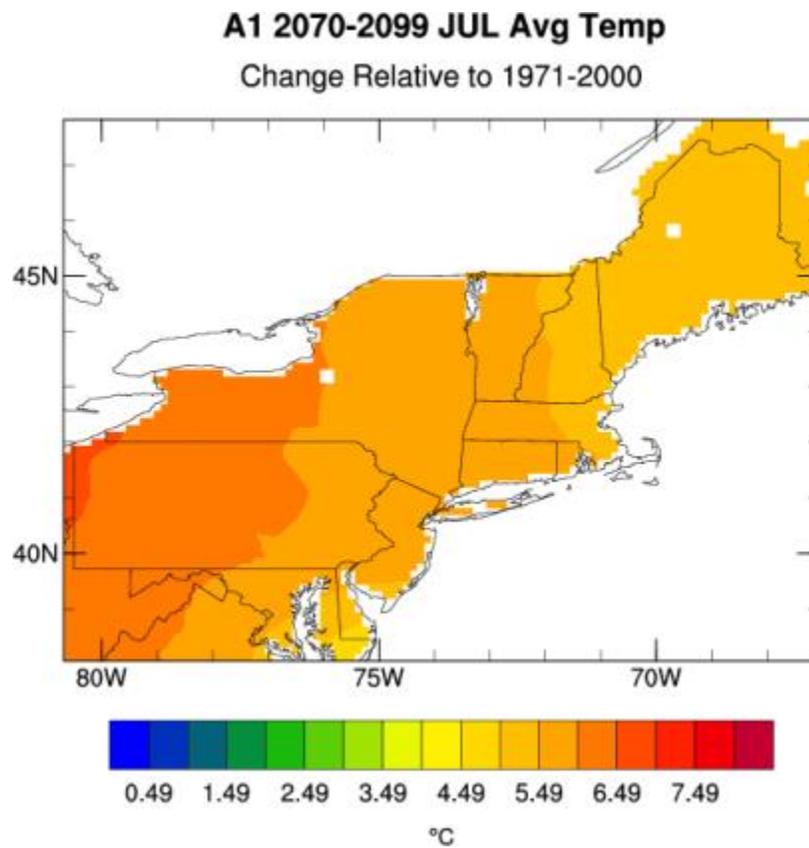Williamson, M. (1996). Biological Invasions, *Department of Biology, University of York, UK.* p 244

FIGURES

A1 2070-2099 JUL Avg Temp

Change Relative to 1971-2000

Figure 1: A figure from the Hayhoe et al. (2008) study which predicts increases in temperature for the northeastern U.S. region. Increases are predicted to be between 5-7°C. These predictions are based on the current trajectory of global carbon emissions.
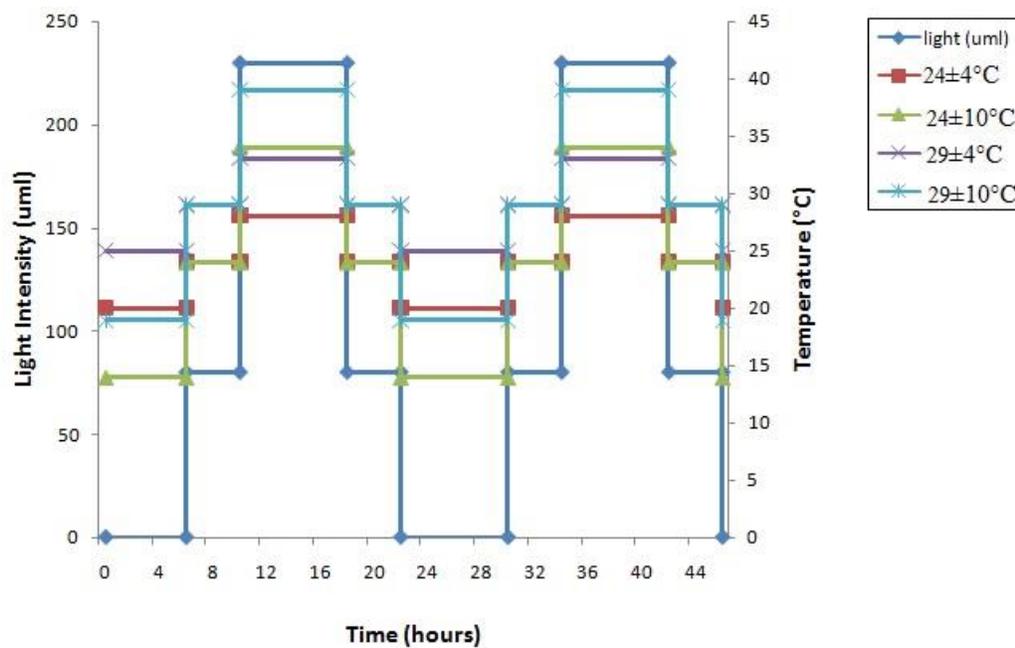
Figure 2: Graphical representation of the step-increases in temperature and light intensity throughout the experiment. This cycle repeated every 24 hours until the completion of the experiment.
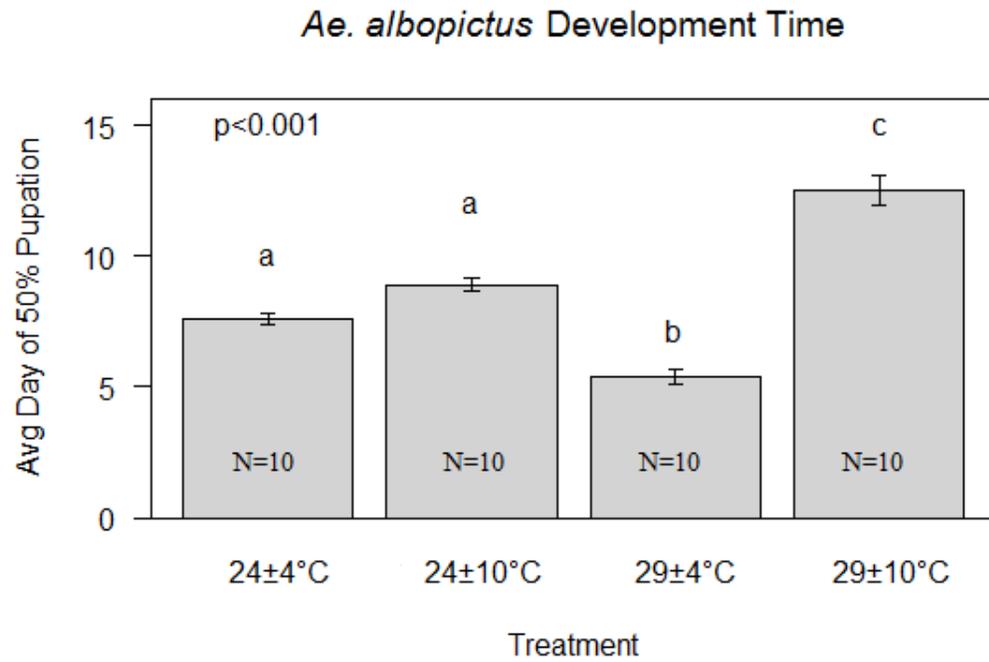
Figure 3: ANOVA bar chart depicting the average number of days per temperature treatment for 50% of the *Aedes albopictus* larvae to pupate. Bars sharing a letter are not significantly different from one another. Error bars are ± the standard error. *P<0.001.*
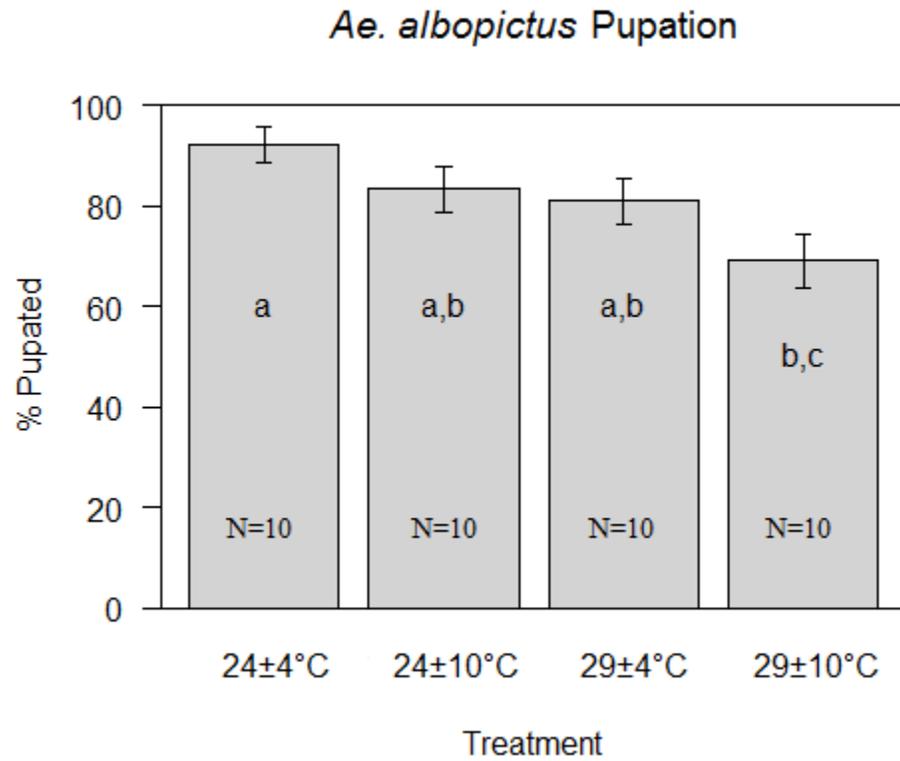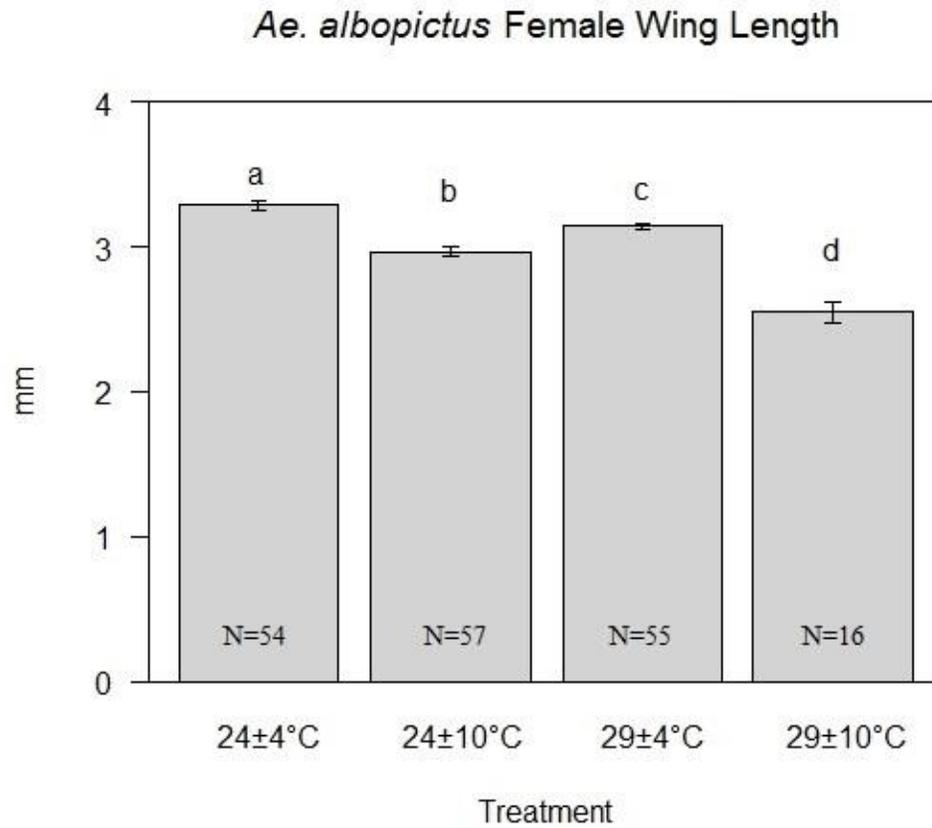
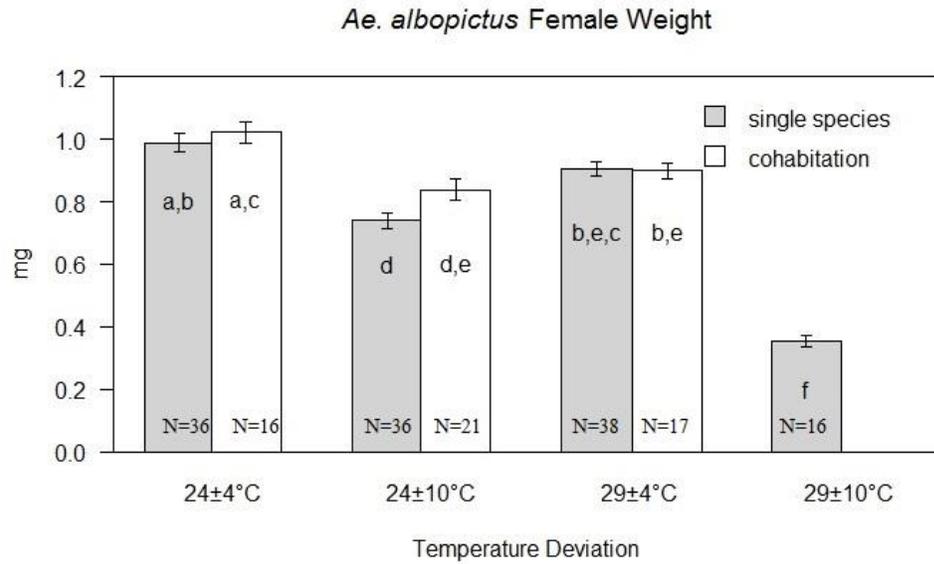Figure 4: ANOVA bar chart depicting the average total percent of pupation per temperature treatment for *Aedes albopictus*. Bars sharing a letter are not significantly different from one another. Only the first and the last treatment were significantly different. Error bars are ± the standard error. .
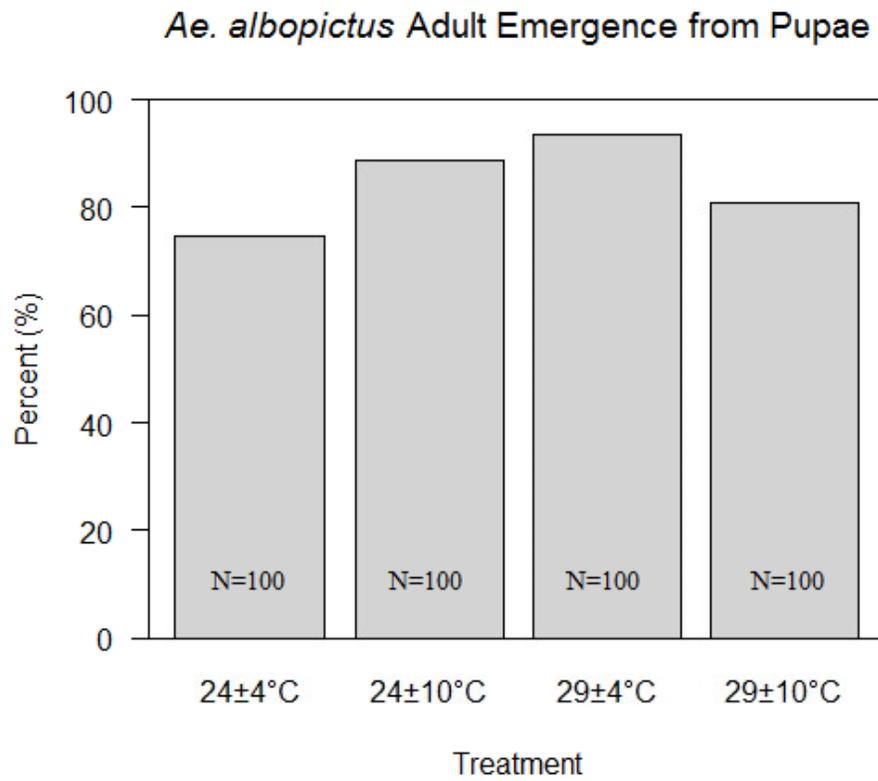
Figure 5: Bar chart depicting the percentage of *Aedes albopictus* pupae which emerged as adults for each climate treatment.
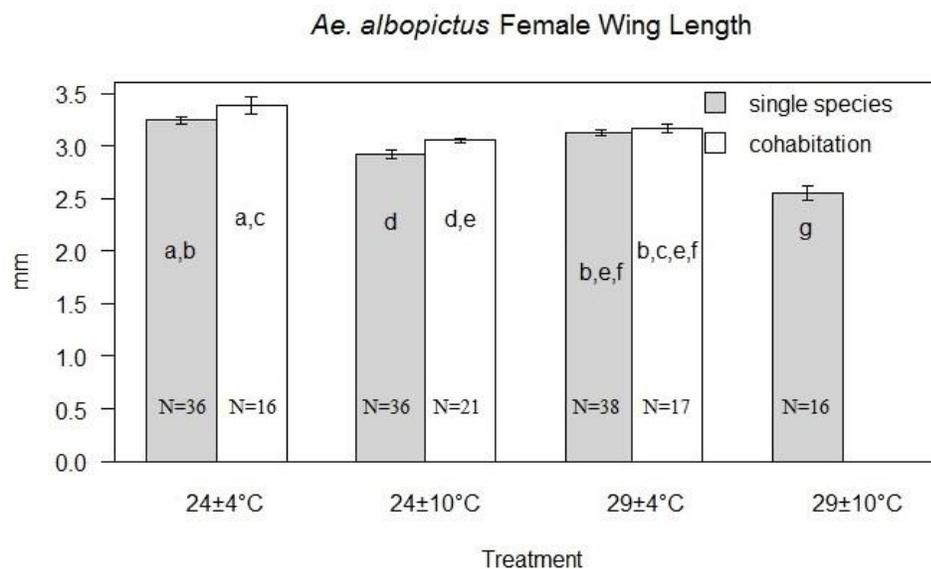
Figure 6: ANOVA bar chart depicting the average wing length of *Aedes albopictus* adult females per temperature treatment. Grey bars represent *Ae. Albopictus* reared only with members of the same species. White bars represent *Ae. Albopictus* reared in cohabitation with *Culex pipiens*. Bars marked with the same letter are not significantly different from one another. Error bars are ± the standard error. *P<0.001*.

Figure 7: ANOVA bar chart depicting the average wing length of *Aedes albopictus* adult females per temperature treatment. Bars sharing a letter are not significantly different from one another. Error bars are ± the standard error. *P*<0.001.
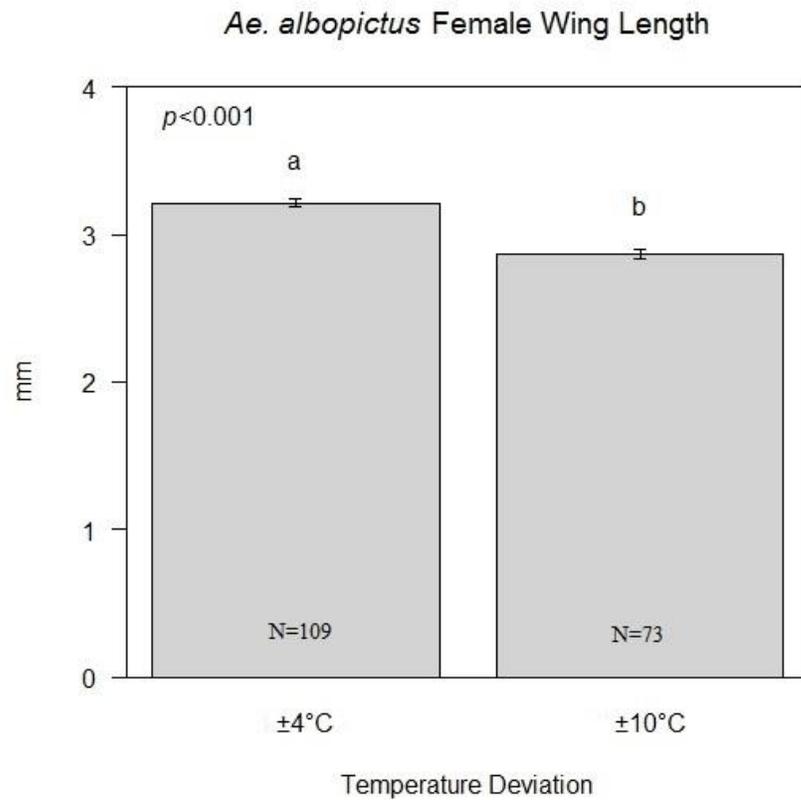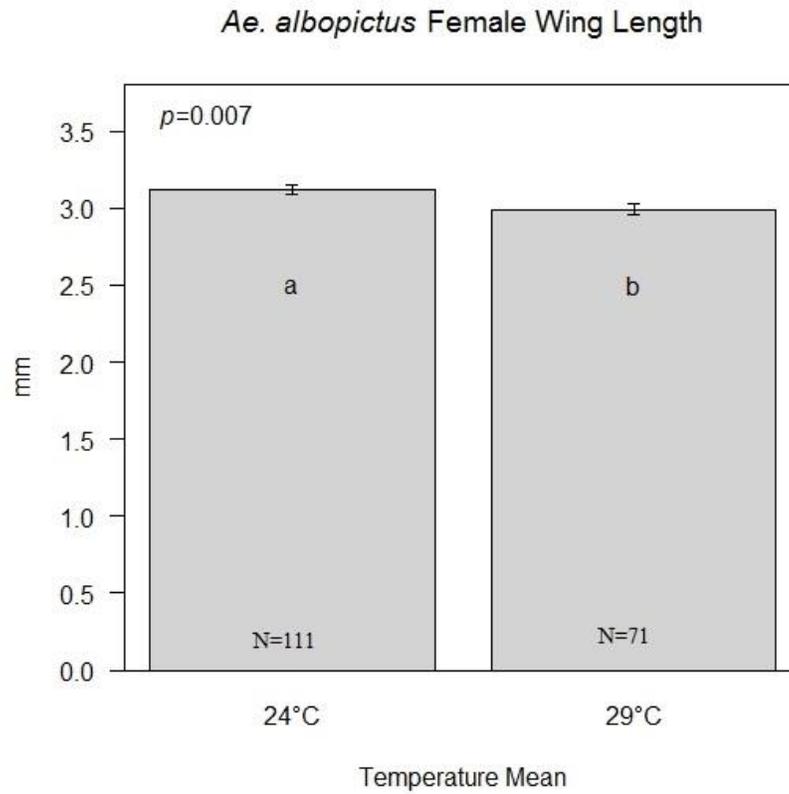
Figure 8: ANOVA bar chart depicting the average weight of *Aedes albopictus* adult females per temperature treatment. Grey bars represent *Ae. Albopictus* reared only with members of the same species. White bars represent *Ae. Albopictus* reared in cohabitation with *Culex pipiens*. Bars marked with the same letter are not significantly different from one another. Error bars are ± the standard error.

Ae. albopictus Female Weight
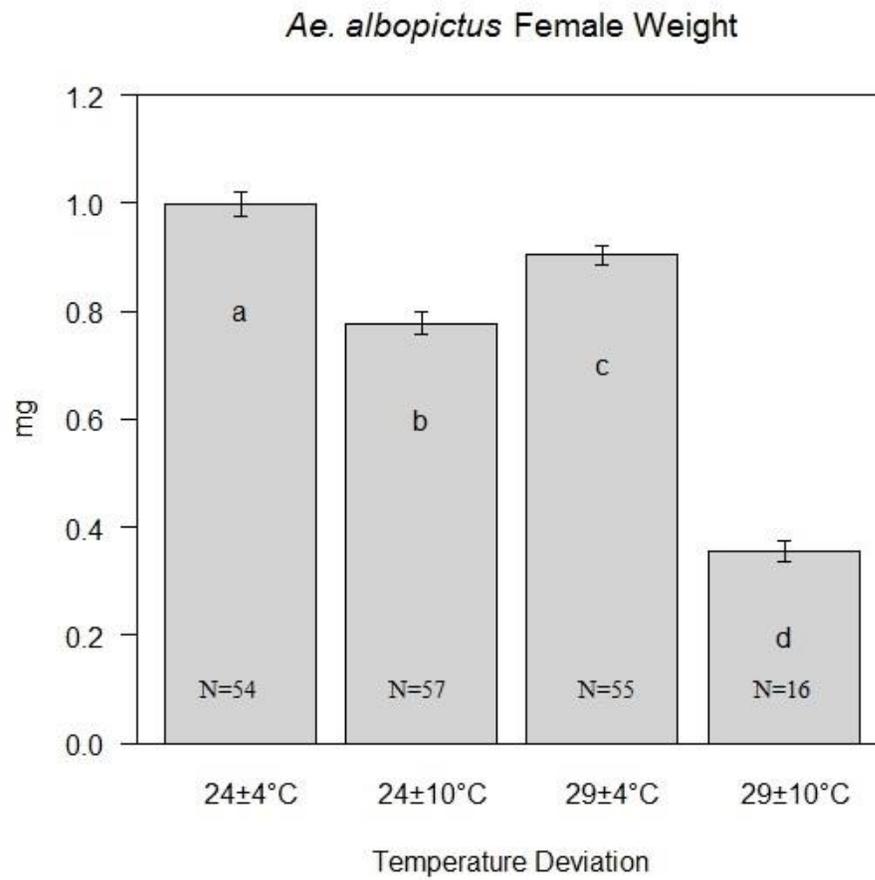
Figure 9: ANOVA bar chart depicting the average weight of *Aedes albopictus* adult females per temperature treatment. Bars sharing a letter are not significantly different from one another. Error bars are ± the standard error. *P*<0.001.
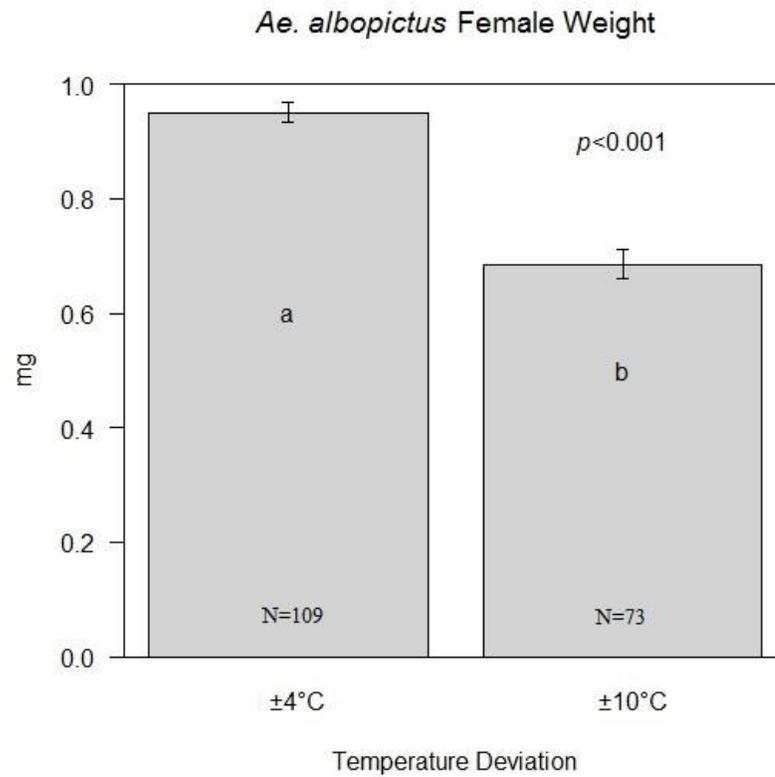
Figure 10: T-test bar chart depicting the average wing length of *Aedes albopictus* adult females per temperature deviation. Bars marked with the same letter are not significantly different from one another. Error bars are ± one standard error.
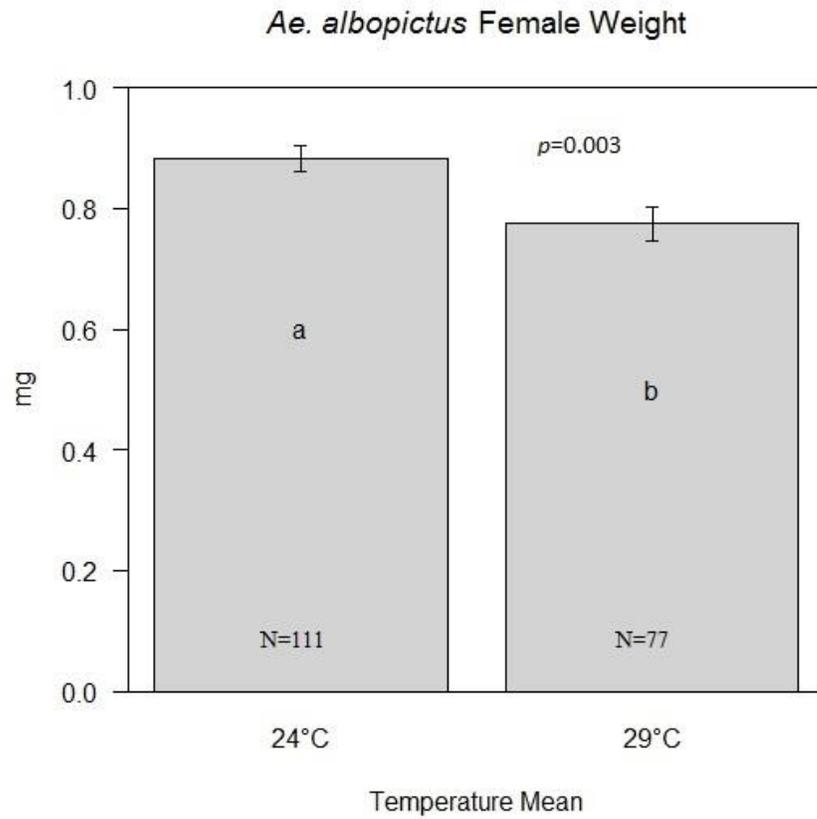
Ae. albopictus Female Wing Length

p=0.007

Figure 11: T-test bar chart depicting the average wing length of *Aedes albopictus* adult females per mean temperature. Bars marked with the same letter are not significantly different from one another. Error bars are ± one standard error.

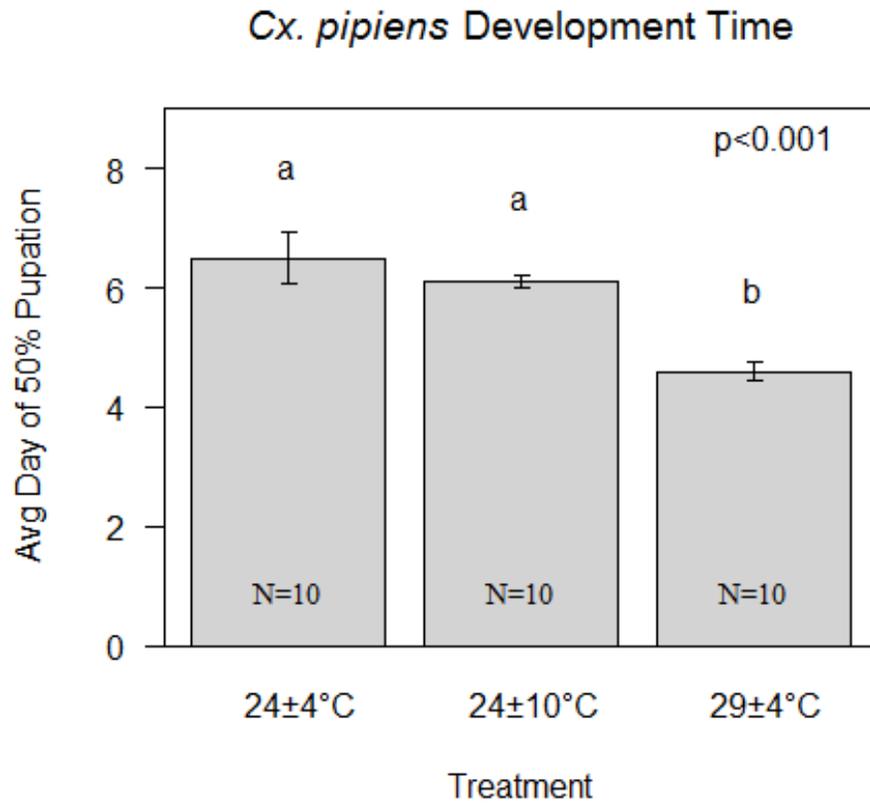Figure 12: T-test bar chart depicting the average weight of *Aedes albopictus* adult females per temperature deviation. Bars marked with the same letter are not significantly different from one another. Error bars are ± one standard error.

Figure 13: T-test bar chart depicting the average weight of *Aedes albopictus* adult females per mean temperature. Bars marked with the same letter are not significantly different from one another. Error bars are ± one standard error.

Figure 14: ANOVA bar chart depicting the average number of days per temperature treatment for 50% of the *Culex pipiens* larvae to pupate. Bars sharing a letter are not significantly different from one another. Error bars are ± one standard error. *P<0.001*.
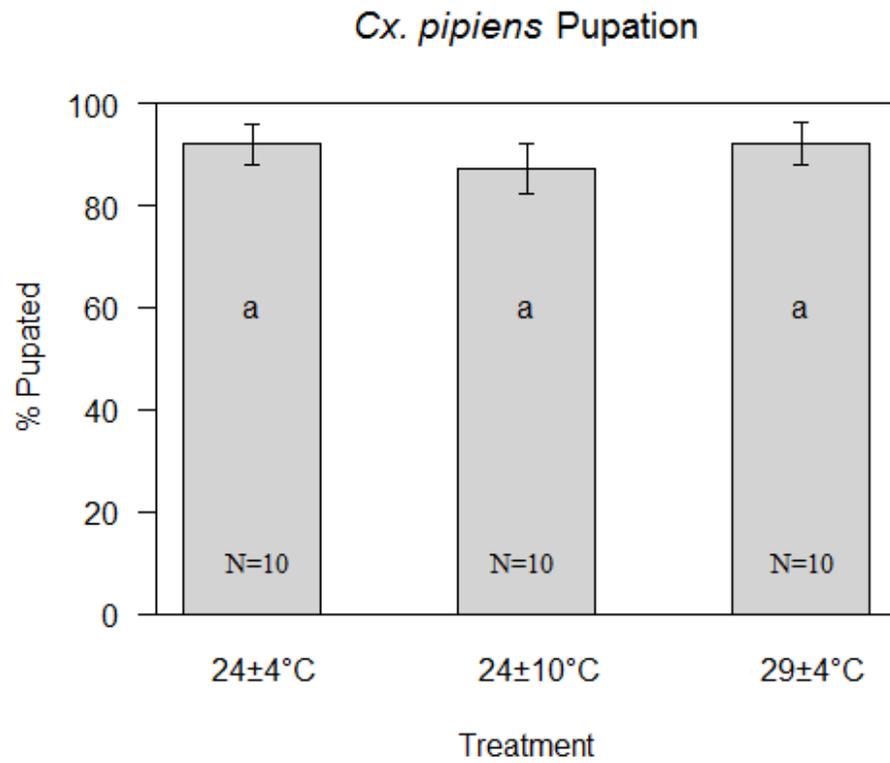
Figure 15: ANOVA bar chart depicting the average total percent of pupation per temperature treatment for *Culex pipiens*. Bars sharing a letter are not significantly different from one another. Error bars are ± the standard error. *P*=0.663.
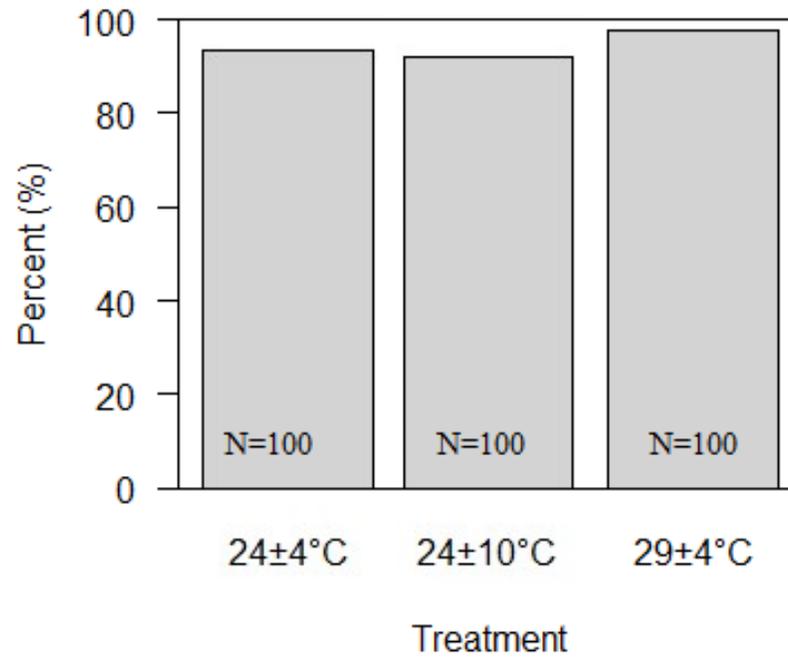
Figure 16: Bar chart depicting the percentage of *Culex pipiens* pupae which emerged as adults for each climate treatment.
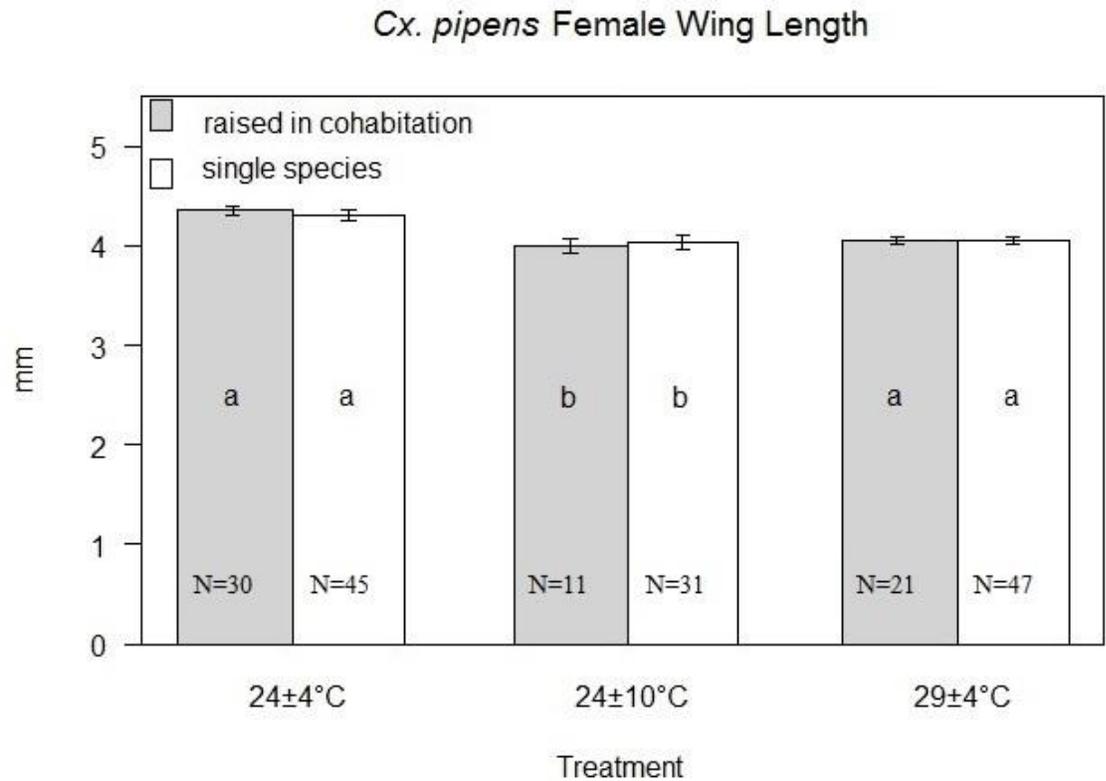
Figure 17: ANOVA bar chart depicting the average wing length of *Culex pipiens* adult females per temperature treatment. White bars represent *Cx. pipiens* reared only with members of the same species. Grey bars represent *Cx. pipiens* reared in cohabitation with *Aedes albopictus*. Bars marked with the same letter are not significantly different from one another. Error bars are ± the standard error. *P*=0.216.
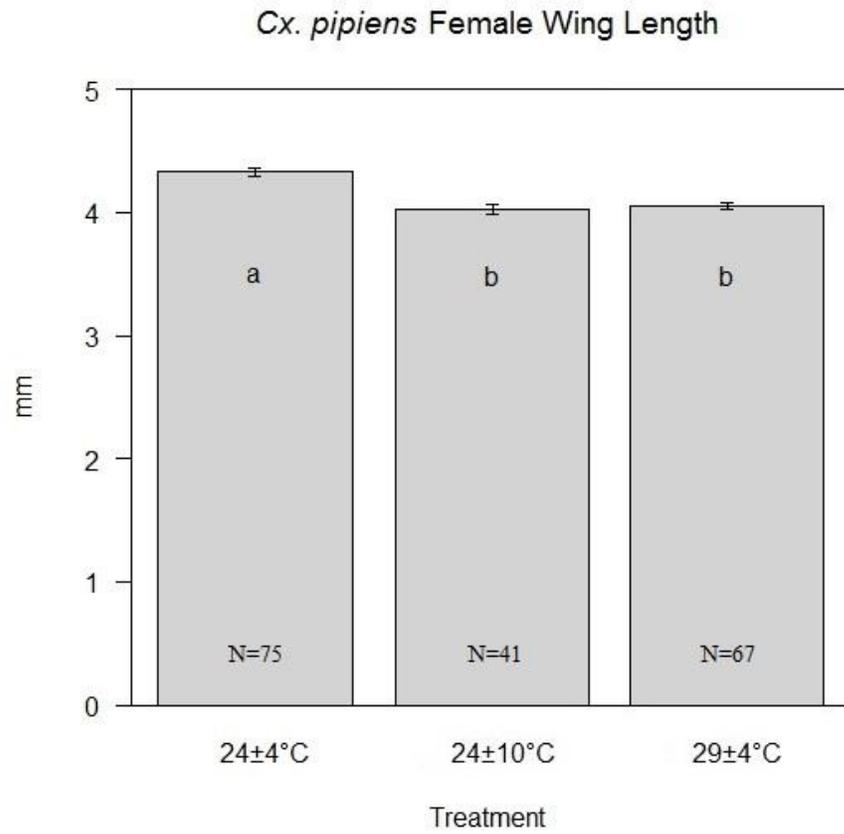
Figure 18: ANOVA bar chart depicting the average wing length of *Culex pipiens* adult females per temperature treatment. Bars sharing a letter are not significantly different from one another. Error bars are ± the standard error. *P*<0.001.
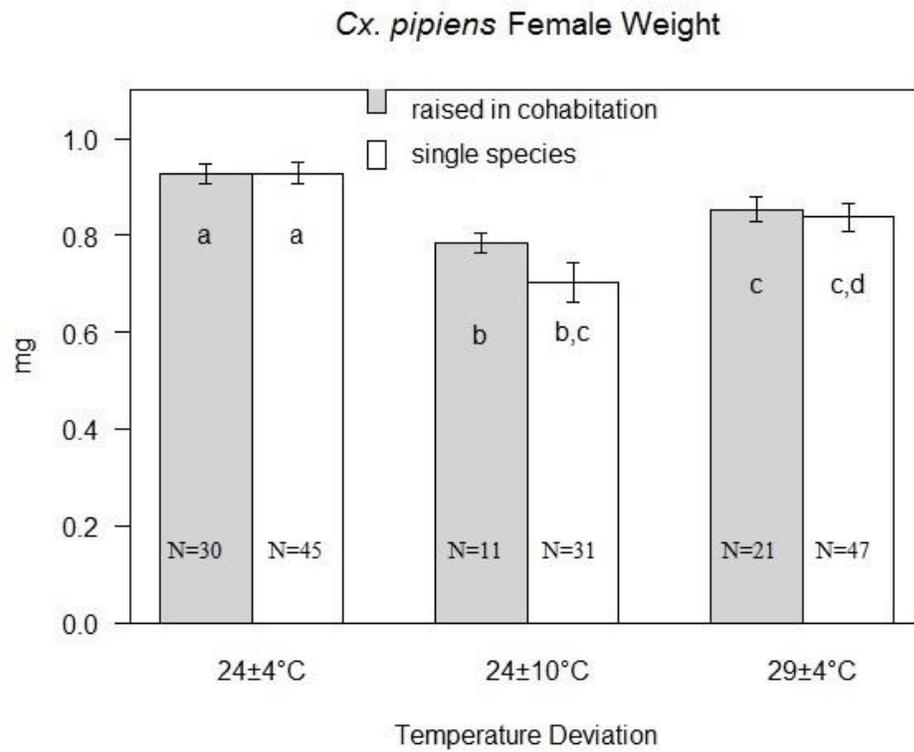
Figure 19: ANOVA bar chart depicting the average weight of *Culex pipiens* adult females per temperature treatment. White bars represent *Cx. pipiens* reared only with members of the same species. Grey bars represent *Cx. pipiens* reared in cohabitation with *Aedes albopictus*. Bars marked with the same letter are not significantly different from one another. Error bars are ± the standard error.
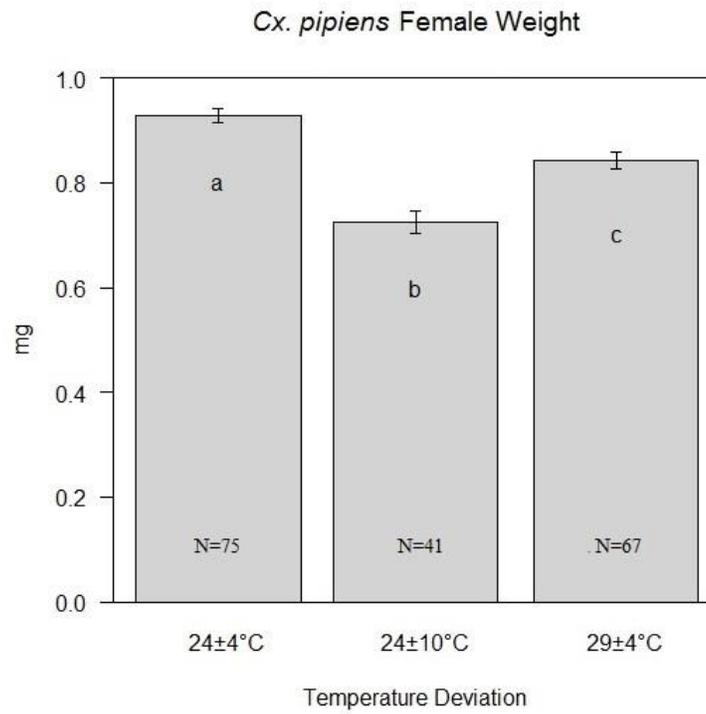
Figure 20: ANOVA bar chart depicting the average weight of *Culex pipiens* adult females per temperature treatment. Bars sharing a letter are not significantly different from one another. Error bars are ± the standard error.

TABLES

Table 1: ANOVA table depicting the strength of differences between the average number of days per temperature treatment for 50% of the *Aedes albopictus* larvae to pupate.

	Df	Sum Sq	Mean Sq	F value	P value
Climate treatment	3	265.25	88.42	67	<0.001
Residuals	35	46.19	1.32		

Table 2: ANOVA table which depicts the average total percent of pupation per temperature treatment for *Aedes albopictus*.

	Df	Sum Sq	Mean Sq	F value	P value
Climate treatment	3	2696	898.6	4.405	0.0099
Residuals	35	7140	204		

Table 3: ANOVA table depicting the average wing length of *Aedes albopictus* adult females per temperature treatment. "Competition" refers to the comparison between *Aedes* reared on its own or with *Culex pipiens.*

	Df	Sum Sq	Mean Sq	F value	P value
Competition	1	0.908	0.9075	18.658	<0.001
Climate treatment	3	6.975	2.3250	47.799	<0.001
Competition & climate	2	0.080	0.0402	0.826	0.44
Residuals	161	7.831	0.0486		

Table 4: ANOVA table depicting the average wing length of *Aedes albopictus* adult females per temperature treatment.

	Df	Sum Sq	Mean Sq	F value	P value
Climate treatment	3	7.521	2.5072	49.7	<0.001
Residuals	164	8.273	0.0504		

Table 5: ANOVA table depicting the average weight of *Aedes albopictus* adult females per temperature treatment. "Competition" refers to the comparison between *Aedes* reared on its own or with *Culex pipiens.*

	Df	Sum Sq	Mean Sq	F value	P value
Competition	1	0.335	0.3351	15.124	<0.001
Climate treatment	3	4.901	1.6336	73.719	<0.001
Competition & climate	2	0.068	0.0342	1.542	0.2171
Residuals	160	3.545	0.0222		

Table 6: ANOVA table depicting the average weight of *Aedes albopictus* adult females per temperature treatment.

	Df	Sum Sq	Mean Sq	F value	P value
Climate treatment	3	5.166	1.7219	76.18	<0.001
Residuals	163	3.684	0.0226		

Table 7: ANOVA table depicting the differences between the average number of days per temperature treatment for 50% of the *Culex pipiens* larvae to pupate.

	Df	Sum Sq	Mean Sq	F value	P value
Climate treatment	2	20.7	10.033	13.68	<0.001
Residuals	27	19.80	0.733		

Table 8: ANOVA table which depicts the average total percent of pupation per temperature treatment for *Culex pipiens*.

	Df	Sum Sq	Mean Sq	F value	P value
Climate treatment	2	157	78.64	0.418	0.663
Residuals	27	5083	188.27		

Table 9: ANOVA table depicting the average wing length of *Culex pipiens* adult females per temperature treatment. "Competition" refers to the comparison between *Culex* reared on its own or with *Aedes albopictus.*

	Df	Sum Sq	Mean Sq	F value	P value
Competition	1	0.103	0.1023	1.541	0.216
Climate treatment	2	3.626	1.8130	27.246	<0.001
Competition & climate	2	0.045	0.0226	0.339	0.713
Residuals	178	11.844	0.0665		

Table 10: ANOVA table depicting the average wing length of *Culex pipiens* adult females per temperature treatment.

	Df	Sum Sq	Mean Sq	F value	P value
Climate treatment	2	3.721	1.8606	28.31	<0.001
Residuals	181	11.897	0.0657		

Table 11: ANOVA table depicting the average weight of *Culex pipiens* adult females per temperature treatment. "Competition" refers to the comparison between *Culex* reared on its own or with *Aedes albopictus.*

	Df	Sum Sq	Mean Sq	F value	P value
Competition	1	0.0615	0.0615	3.650	0.0577
Climate treatment	2	1.0387	0.5194	30.8	<0.001
Competition & climate	2	0.045	0.0226	0.339	0.713
Residuals	178	11.844	0.0665		

Table 12: ANOVA table depicting the average weight of *Culex pipiens* adult females per temperature treatment.

	Df	Sum Sq	Mean Sq	F value	P value
Climate treatment	2	1.082	0.5411	32.04	<0.001
Residuals	175	2.956	0.0169		