

The Overwintering Physiology and Ecology of  
*Aedes aegypti* Mosquitoes in the Desert Southwest

by

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## ABSTRACT

The global spread of the *Aedes aegypti* mosquito, a competent disease vector, is occurring at an alarming pace. These invasive mosquitos have spread to latitudes once thought inaccessible due to thermal and hydric limitations, including Maricopa County, AZ, where strong increases in population size has occurred over the last decade. The *Aedes aegypti* in Maricopa County follow a highly seasonal pattern with populations all but disappearing each winter, only to return and build exponentially though the summer and fall. Maricopa County's winters are relatively mild, which raises the question of whether further global climate change will warm conditions enough to create a broadened seasonal breeding period, or worse yet, year-round mosquito activity within desert southwest cities.

This dissertation focuses on exploring the possible seasonal constraints on the egg, larva, and adult life stages of the *Aedes aegypti* mosquito within a suburban desert ecosystem. I explored whether climatic warming would raise temperatures enough to enable survival and development of these animals during the winter offseason. I determined that larval growth and adult flight are constrained by ambient winter temperatures in Maricopa County, explaining the currently observed winter crash in populations. However, warming by only a few degrees Centigrade could enable successful larval growth and development, as well as adult flight, even during the coldest desert months. I found that load and temperature interact to determine the flight cost of mosquitos, which can vary up to six-fold, with higher temperatures likely increasing their

flight performance but decreasing their distance capacities and increasing their need for fuel.

*Aedes aegypti* likely primarily overwinters as eggs. However, I showed that the vast majority of *Aedes aegypti* eggs die overwinter in Maricopa County. By manipulating humidity in eggs exposed to ambient air temperatures, I showed that desiccation, not cold temperatures, is the limiting factor in long-term survival of *Aedes aegypti* eggs. Together, my data suggests that humid, urban microhabitats may be essential for enabling overwinter egg survival in Maricopa County, providing a potential important pathway toward control of this disease vector.

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## Introduction

The World Health Organization (WHO) declared a state of emergency in 2016 regarding the rapid, global spread of the Zika (ZIKV), Dengue, and Chikungunya viruses ([www.who.int/en/](http://www.who.int/en/)). These pathogens are spread by the mosquito *Aedes aegypti*, an invasive day-time feeder which has specialized to extract blood meals from humans (Bargielowski, 2015). With the range that these diseases encompass expanding rapidly, including locally transmitted cases in Texas ([www.cdc/zika/intheus](http://www.cdc/zika/intheus)), it is becoming a question of not if, but when will ZIKV spread to Phoenix, AZ. At first glance, Phoenix's cold, dry winters and hot and dry summers should shield us from outbreaks of the largely tropical and sub-tropical *Aedes aegypti* mosquitoes. However, despite the dry heat, yearly catch rates by Maricopa County Vector Control are rising exponentially, especially during and after the monsoon season.

Addressing the issue of the ever-increasing *Aedes aegypti* population in the valley has proven difficult, and efforts to target the adults with chemical spraying has proven somewhat ineffective for Maricopa county (K. Smith, personal communication, March 2018). *Aedes aegypti* mosquitoes have demonstrated resistances to insecticides over time, including to some of the most commonly deployed agents such as pyrethroids, DDT and organophosphates, making traditional control efforts increasingly ineffective (Darriet & Chandre, 2013; Vontas et al., 2012).

Complicating matters more, the exponential rise seen in the Phoenix valley (Fig 1.) may be the result of the accelerated rate of urbanization in the valley. Increased urbanization directly correlates to a yearly increase in temperatures, and a decrease in day-night temperature range via the urban heat island effect (Yaun & Bauer, 2007; Kalnay & Cai, 2003), both of which could raise minimum winter temperatures, possibly reducing winter-deaths of mosquitos. Additionally, urbanization increases the prevalence of piped water, and the availability of protective microhabitats such as culverts, drains, swamp coolers, residences (especially potted plants) and refuse piles. In a study of breeding sites in the Tucson area, up to 13% of homes surveyed were found to have *Aedes aegypti* immatures in containers and each home surveyed had 2.2 water bearing containers on average, highlighting the ubiquity of container breeding grounds and difficulty in treating them (Walker, 2017). Storm drains have been implicated in the breeding and survival of *Aedes aegypti* mosquitos in Washington DC, Singapore, San Diego and Maricopa County, AZ (Lima et al., 2016; Metzger et al., 2017; Seidahmed & Eltahir, 2016a; Souza et al., 2017). These microhabitats and water sources likely shield mosquitos at varying life stages from unfavorable conditions during both the harsh summer months and the cooler winter periods. It is also possible that regional climate pressures have allowed *Aedes aegypti* populations to evolve new characteristics allowing them to thrive in temperate and subtropical climates (Chadee & Martinez, 2016; Marinho et al., 2016).

Determining the long-term trends for *Aedes aegypti* populations is important for determining potential arbovirus epidemic outcomes in the Phoenix area. Phoenix, the 6<sup>th</sup>

largest city in the U.S., is an important target for pest and disease mitigation. Strategies developed in the valley to ameliorate the presence of *Aedes aegypti* mosquitos may also be applied to other southwestern cities where *Aedes aegypti* are currently a problem or are at risk of becoming one. Climate predictions suggest that Arizona will become drier and warmer over the next decades (MacDonald, 2010). Fewer freezes, warmer winters, and earlier onset of warm, spring temperatures may allow for earlier than usual hatching of overwintering eggs, potentially leading to an early accelerated growth in populations and higher peak populations overall. Conversely, warm, dry conditions might reduce *Aedes aegypti* overwintering survival if *Aedes aegypti* eggs desiccate before water from rains or anthropogenic sources facilitate hatching. It may be that Maricopa County and similar cities in other xeric habitats around the Southwest have unique opportunities to reduce disease vector mosquito prevalence that are not available to more mesic subtropical cities that routinely have sufficient environmental rainfall to support mosquito reproduction. Plausibly, *Aedes aegypti* are unable to survive normal Phoenix winter conditions, which often include successive months without rainfall, and depend on escape habitats such as irrigation tunnels or buildings. If I knew that this were true, control measures that focused on management of such habitats could have major benefits for mosquito and disease control for Arizona in the long-term. Development of such control strategies will depend on developing an improved understanding of the physiological ecology of *Aedes aegypti*.

In this dissertation I examine three of the four life stages of the *Aedes aegypti* mosquito and determine how the physiology and ecology of the mosquito responds to our

unique thermal environments, urbanization, and also how possible climate change scenarios impact the life cycle of these invasive pests.

## **Climate change likely will enable *Aedes aegypti* larvae to develop during Maricopa County winter months**

### **Abstract**

Global warming trends have allowed poleward expansion of many species, but the specific mechanisms responsible for thermal mediation of range changes remain poorly understood. The spread of *Aedes aegypti* (L.)(Diptera: Culicidae) is predicted to be limited by abiotic factors including cold, but the mechanisms of such limitations and how climate change will affect range limits of *Aedes aegypti* remain unclear. In Maricopa County, Arizona, adult populations virtually disappear in winter and early spring, and *Aedes aegypti* populations increase exponentially through summer and early fall, suggesting that suppression of *Aedes aegypti* in winter likely reduces mosquito abundance through much of the year. *Aedes aegypti* larvae were not observed in artificial aquatic habitats in winter and spring but were abundant in summer and fall. I tested the capacity of *Aedes aegypti* larvae to successfully develop in current and warmer Maricopa County winter conditions. Larvae were reared outside at ambient local winter conditions, or outside with average temperature increased by 1.7°C to 12°C. Winter temperatures in Maricopa County fluctuate strongly; larvae were usually cold-paralyzed at night but active during the day. Larvae reared under ambient winter conditions were unable to develop to adulthood. However, warming average temperature by as little as 1.7°C allowed many larvae to successfully developed to adults. Because daytime highs in winter likely often allow adult flight, it is possible that relatively minor additional winter

warming may allow *Aedes aegypti* populations to avoid winter suppression in Maricopa County.

### **Introduction:**

Global warming is causing a spread of many species to higher latitudes and altitudes, with many ecological consequences (Au & Bonebrake, 2019; Parmesan et al., 1999; Rochlin et al., 2013). One of the most concerning of such trends is the ongoing poleward spread of disease-transmitting mosquitos that threaten to expand the range and impact of multiple tropical diseases (Gage et al., 2008; Githeko et al., 2000; Ryan et al., 2019; Wagner & Van Driesche, 2010). While the poleward spread of many tropical species is apparent, the specific mechanisms by which changing temperatures promote range changes remains unclear in most cases. Cold temperatures can limit poleward range expansion of species, but the sensitivity of specific life stages to thermal variation across realistic values relative to range limitation is poorly known. Here I investigate whether cold temperatures currently limit the development of *Aedes aegypti* larvae in Maricopa County, Arizona, a region in which this species has relatively recently invaded, as well as testing how much elevation in average winter temperature is required to avoid cold-suppression of larval development.

In the context of climatic warming, poleward range expansion likely occurs when temperatures rise sufficiently to prevent cold-, freeze-, or resource-limitation associated with winter. Insect life stages can vary dramatically in thermal and hydric tolerance, and therefore determining the relationship between temperature and life-stage specific performance is an important step in understanding the mechanisms determining range

limits and expansion (Crozier, 2003; Kingsolver et al., 2011). Theoretically, cold can limit all life stages of mosquitos. However, mosquitos have different thermal and hydric challenges at each life stage. In many mosquito species, the eggs are deposited above or at the waterline, causing them to be exposed to more variable and drier conditions than larvae (Day, 2016). *Aedes aegypti* eggs are generally believed to be resistant to cold and mild freeze damage (De Majo et al., 2017; Jass et al., 2019), which suggests that winter cold is most likely to be specifically suppressing the adult or larval stages. The larva and pupa stages are aquatic, and not vulnerable to hydric stress unless their habitat dries out, which is generally lethal (Christophers, 1960). Adult mosquitos are highly vagile and therefore can ameliorate thermal variation by solar basking or shade seeking behavior, potentially allowing them to avoid thermal extremes (Heinrich, 2013). The capacity of adults to thermoregulate and the generally observed high stress-resistance of the eggs suggests that cold-suppression of the larval stage may be critical in suppressing to mosquito development during winter; therefore, this research focuses on thermal variability and development of larval *Aedes aegypti*.

In 2016, the *Aedes aegypti* mosquito garnered global attention for its role in the Zika virus (ZIKV) epidemic transpiring across the sub-tropical regions of the world. *Aedes aegypti* is also host to a range of other diseases, including Dengue fever and Chikungunya, infecting around 96 million severely and killing 20 thousand per year (Gubler & Meltzer, 1999). *Aedes aegypti* are specialized to primarily feed on humans (Christophers, 1960). *Aedes aegypti* mosquitoes have demonstrated resistances to insecticides over time, including to some of the most deployed agents such as



pyrethroids, DDT and Temefos, making traditional control efforts increasingly ineffective (Darriet & Chandre, 2013; Vontas et al., 2012).

The range of the *Aedes aegypti* mosquito has been expanding poleward on all continents (Ryan et al., 2019; Shope R, 1991). Endemic populations of *Aedes aegypti* in temperate Ontario, Canada and Nagasaki, Japan have been documented; these populations are strongly limited by winter temperatures (Giordano et al., 2020; Khan Salah Uddin et al., n.d.; Tsuda & Takagi, 2001). *Aedes aegypti* has also expanded into the desert southwestern U.S., including Maricopa County, which includes Phoenix, Arizona (Pless & Raman, 2018). The rise in *Aedes aegypti* abundance seen in the Maricopa County valley is at least partly the result of urbanization, which has expanded the availability of water and increased temperatures, especially by decreasing the day-night temperature range via the urban heat island effect (Kalnay & Cai, 2003; Yuan & Bauer, 2007). Maricopa County has already experienced a 5°C minimum nightly temp increase in the last 50 years, along with a 3.1°C daily average increase over the same period, expanding the thermal window in which many arthropods can function by 30 days per year (Baker et al., 2002). An additional 2-4°C of warming is expected for the region in the next decade (Sprigg & Hinkley, 2000).

Understanding how *Aedes aegypti* populations are increasing rapidly in this urban desert region requires an examination of the abiotic factors that limit population growth, and the life-stages at which these limitations occur. In most years, *Aedes aegypti* adults virtually disappear from Maricopa County in winter and spring (December – April), but are very abundant in summer and fall. Maricopa County winters are relatively mild, with

average temperatures in January being  $\sim 12^{\circ}\text{C}$ , but with high circadian variation in temperature, often ranging from nighttime lows near  $0^{\circ}\text{C}$  to daytime highs above  $18^{\circ}\text{C}$  (Horel et al., 2002). It is unknown whether *Aedes aegypti* larvae can develop under these highly thermally-variable conditions. Based on prior studies, the minimum temperatures required for larval development in *Aedes aegypti* mosquitos may vary with study location, suggestive of local adaptation. In Nagasaki, Japan, the minimum ambient temperature for development of *Aedes aegypti* larvae was found to be  $8-9^{\circ}\text{C}$ , based on an estimate using monthly temperature records vs. development rate and survival to pupation (Tsuda & Takagi, 2001). However, several other studies, using constant temperature lab rearing methods, have found that  $13^{\circ}\text{C}$  is the minimum temperature at which larvae can successfully develop (Bar-Zeev, 1958; Carrington et al., 2013; Tun-Lin et al., 2000). Total development time for larvae quickly lengthens at low temperatures, potentially leading to mortality if their habitat dries before pupation and eclosion can occur. Field studies examining both acute, and fluctuating temperature regimes have found that *Aedes aegypti* larvae can take up 31 days to develop during winter periods with a mean ambient temperature ranging from  $14-15^{\circ}\text{C}$ , with complete lethality occurring in the lab at  $12^{\circ}\text{C}$  or lower for one week or longer (Carrington et al., 2013; Chang et al., 2007). Together these constant-temperature studies suggest that larval development may be impossible due to cold-suppression throughout most of the Maricopa County winter. However, since on many winter days air temperatures are above  $15^{\circ}\text{C}$  for many hours, and local adaptation may occur, it is uncertain.

It has become increasingly clear that use of constant-temperature studies to predict thermal effects on field performance can be inaccurate (Angilletta, 2009). Constant-temperature regimes likely misrepresent the effects of thermal periods on animals outside the laboratory for many reasons (Brakefield & Mazzotta, 2002). Slow, cyclical changes in temperatures can facilitate the acclimation of physiological traits which might protect insects from damage at low temperatures, including via the expression of protective proteins, peptides and the synthesis of cryoprotectant carbohydrates (Lubawy et al., 2020; Rinehart et al., 2007). *Aedes aegypti* larvae have been shown to adapt to cold in part by maintaining ion balance, attenuating the effects of hyperkalemia (Jass et al., 2019). An organism capable of fast acclimation can adjust to uncertainty in climatic variation, even within the course of a day (Angilletta, 2009). If *Aedes aegypti* larvae experience temperatures above the developmental threshold for a least a portion of the day, they may be able to develop during that time, while pausing development at night when temperatures are depressed, which has been found to be the case in some butterfly larvae (Brakefield & Mazzotta, 2002). Analysis by Carrington et al. (2013) found that fluctuations in temperatures, as well as the magnitude of the fluctuations, significantly affected development time of *Aedes aegypti* mosquitoes, suggesting that constant temperatures are likely not a reliable measure of field development.

In this study, I report long-term and seasonal trends for *Aedes aegypti* in Maricopa County, and assess larval development in field realistic conditions winter conditions for Maricopa County, with natural fluctuating temperatures and natural winter photoperiod. I

used *Aedes aegypti* larvae that were hatched from wild-caught eggs originating in the late fall, as populations may have local or seasonal adaptations. Additionally, I applied an offset to the ambient temperature fluctuations to create a range of warmer conditions, in the context of fluctuating circadian temperatures.

## **Methods:**

### **Maricopa County Vector Control long term trapping survey:**

The long-term adult trapping data was collected by Maricopa County Vector Control field teams from 2006 to 2016. The data was generated from a variable number of sampling sites (604 to 750) throughout Maricopa County, checked weekly by a team of 25 field surveyors. Bioquip Heavy Duty EVS mosquito traps (Rancho Dominguez, CA) with dry ice bait and no artificial lighting were primarily used for trapping. Biogents BG-Sentinel (Regensburg, Germany) automated mosquito traps, along with Biogents BG-GAT traps (Regensburg, Germany) were employed for additional coverage when clustered *Aedes aegypti* populations were identified. The contents of the traps were identified to the species level and totaled by sex. The trap locations are publicly available and integrated into an ArcGIS interactive database ([maricopa.maps.arcgis.com](http://maricopa.maps.arcgis.com)). To calculate the seasonal trends in mosquito trapping, I analyzed the data by summing the number of males and females in each trap, and plotting the median number of mosquitos per trap over the ten-year period, To calculate the ten-year trend, the total number of

mosquitos trapped from all locations was added together for each year, then plotted over the decade.

### **Effect of season on *Aedes aegypti* larval presence:**

From September of 2017, till August of 2019, water temperatures and *Aedes aegypti* larva presence was recorded in nine, 19-liter buckets placed in the backyard of a house in Tempe, Arizona (33.339, -111.924) known to experience high abundances of *Aedes aegypti*. Onset HOBO Pendant® UA-002-08 data loggers (Bourne, Massachusetts) were used to record temperature levels, and larval presence was observed every 1-10 days depending on season (frequently in the summer, less so in winter). If mosquito larvae were observed, they were collected from the bucket with a net and their species identity confirmed with a dissection scope.

### **Larval rearing for field experiments**

The parents of larvae used in the field overwintering experiments were reared from Maricopa County, AZ, origin eggs collected by Maricopa County Vector Control from September to November of 2019. These were eggs were placed in a 500ml beaker, submerged, and hatched in a solution of 0.25 g/L baker's yeast (Byttebier et al., 2014). As the 1<sup>st</sup> instar larvae emerged, they were fed TetraMin fish flakes every 1-2 days, making sure than an excess amount of food was visible in the container. The rearing density for the larvae was maintained at fewer than 500 animals per liter of water. As pupae began to appear, the beaker of larvae was placed in a 95-liter polymer cage to contain the expected adults. Cotton balls saturated with 10% sucrose solution were made

available for the adults as they began to emerge, however these were taken away two days prior to blood feeding. One week after emerging, the adults they were blood fed using mice (IACUC protocol: 18-1662R). After a three-day gestation period the females were supplied with moist seed germinating paper to encourage oviposition. Once the females had finished ovipositing, the eggs were kept moist for an additional 48 hours before being dried, and placed in open zip lock sandwich bags which were stored at 100% humidity and 24°C. High humidity in the egg storage containers was achieved by storing damp paper towels along with the opened egg bags within a larger 3.8L bag. These eggs were kept for less than one month before the hatching procedure was repeated to produce the larvae for the experiment. In the lab, across all life stages, the mosquitos were exposed to a 12:12 L/D photoperiod at 24°C.

After hatching, the 2<sup>nd</sup> instar larvae were moved to their outdoor experimental enclosures. The larvae were randomly distributed between three treatments: control (no heating), mildly-warmed (1.7 - 7.0°C increase in average temperature), and strongly-warmed (5.8 - 12.0 °C increase in average temperature). For each treatment, there were three 150 ml clear plastic containers filled with 125 m/l dechlorinated tap water, each with twenty larvae. TetraMin fish flakes were supplied in each cup to start, with more added every three days or when food was completely consumed.

### **Manipulation of thermal conditions for larval outdoor rearing**

All larvae containers were placed on a table one meter above the ground and protected from rain, wind, and sunlight by a roof and individual small lidless pine box (10x10x14 cm, 0.95 cm thick walls), but were exposed to normal fluctuations in air temperature.

Each cup of larvae in the mild and moderate warming treatments was placed on 40mm<sup>2</sup> thermoelectric plates with 40mm<sup>2</sup> aluminum heatsinks attached using thermally conductive adhesive on each side. The warming orientation of the thermoelectric plate was positioned upwards, towards the water cups, to ensure adequate energy transfer from the heating units to the water. Each thermoelectric device was powered by two KORAD KD3005D 30V, 5A power supplies (Shenzhen, China). The thermoelectric plates were wired in parallel using insulated speaker cable. Temperatures were measured in the cups using HOBO Pendant® UA-002-08 data loggers submerged in each cup. Temperatures were logged each hour in each low temperature and high temperature treatment cup and one ambient treatment cup.

#### **Assessment of larval survival and development:**

The larva cups were monitored every three days in the early afternoon. Due to cycling temperatures, larvae were generally motionless at night, in cold stupor, but they were often active in the afternoon. The number of live and dead larvae and pupae in each cup were counted using a 30-second video of each cup taken with an Apple iPhone 7 Plus (Cupertino, CA) analyzed frame by frame using Microsoft QuickTime media player (Redmond, WA). Counts were made from video recordings because it was difficult to count living larvae in the cup due to their activity. Larvae and pupae were counted as alive if any movement was detected; otherwise they were scored as dead. Adults were counted by noting the number of exuvia present in each cup.

## **Statistical Analysis:**

To determine the effects of ambient and increased temperature on development of pupae under outdoor conditions, I used a chi squared test to compare the number of pupae that emerged vs the expected at the ambient, mildly warmed, and strongly warmed temperatures. The probability of survival for animals across all life stages was tested using a Kaplan-Meier survival analysis. To analyze the effects of temperature on adult production I used a generalized linear model (GLM) with the presence of each individual adult modeled as a binomial distribution with a logit link function, with the minimum, maximum and mean container temperatures used as continuous predictor variables. I confirmed homoscedasticity of model data by plotting fitted values vs. residuals. The analysis was carried out using R coding environments with the “lme4” package (Bates, 2007; R Core Team, 2013).

## **Results**

### **Effect of year and month on trap rate of *Aedes aegypti* adults in Maricopa County**

The number of *Aedes aegypti* trapped in Maricopa County has increased dramatically over the past decade (Fig. 1A). While this may be partly due to trapping effort, from 2013 to 2014 the total number of adult *Aedes aegypti* trapped yearly in Maricopa County increased strongly despite only a slight increase in the deployment of trapping stations (604 to 660). The median number of larvae trapped per month was highly seasonal, with a dramatic drop in populations from December through April each year (Figure 1B).



### **Effect of season on *Aedes aegypti* larval presence and water temperatures**

During October and November, 2017, May – November 2018, and May – November, 2019, *Aedes aegypti* larvae were commonly found in my trapping buckets, with 44% of buckets sampled having *Aedes aegypti* larvae over these years. *Aedes aegypti* larvae were not detected in my buckets from late November 2017 to April of 2018. The animals disappeared again in early November 2018, reappearing the following May. Water temperatures in the buckets varied strongly within and across days as well as seasonally (Fig. 1B, Table 1). In November through March, when larvae had disappeared, minimum water temperatures in the buckets dropped as low as 1°C and averaged as low as 10.5°C in January 2019 (Table 1). Water temperatures in April, when larvae were never found, averaged 21°C, with an average low of 10°C. During the duration of the experiment, no *Aedes aegypti* larvae were ever discovered in the four out of nine buckets that received sunlight, regardless of season. Sun exposed buckets displayed higher mean temperatures, as well as higher peak temperatures than their shady counterparts (Table 1, A, B).

### **Overwintering larvae development and survival**

The mildly-warmed and strongly-warmed treatments produced significantly more pupae than the ambient treatment ( $\chi^2 = 51.74$ ,  $N = 180$ ,  $p < 0.005$  (Fig. 3B). Mosquitoes reared at ambient winter temperatures (January 31<sup>st</sup> to March 3<sup>rd</sup>) experienced higher mortality than animals reared at in the mildly warmed or strongly-warmed treatments (Kaplan-Meyer,  $\chi^2_{180} = 40.10$ ,  $p < 0.0001$ , Fig.3C). The number of adults was significantly affected by temperature and the interaction of temperature and day for mean, minimum and maximum temperatures (general linear models, Table 3), with zero adults produced

in the ambient treatment containers, and the number of adults appearing unrelated to temperature within the warmed containers (Fig. 4).

### **Discussion:**

The number of *Aedes aegypti* adults trapped in Maricopa county has increased strongly over the past decade (Fig. 1A), but the populations remain nearly completely suppressed in winter (Fig. 1B, Fig. 2). I found that *Aedes aegypti* larvae could not develop in the strongly fluctuating ambient winter conditions, but that relatively small increases in mean temperature allowed larval development to occur. These data suggest that the strong suppression of *Aedes aegypti* in Maricopa County winter is at least partly due to cold-suppression of the larval development, but that relatively minor additional warming could release this suppression.

Cold suppression of performance may occur for multiple life stages of *Aedes aegypti* in the Maricopa County winter. The lack of larvae in the winter months could be due to adults being incapable of low temperature flight and feeding in winter. Several studies have reported that *Aedes aegypti* cannot fly at temperatures below 10°C, and that flight performance is limited at temperatures below 15°C (Rowley & Graham, 1968b). However, air temperatures above 15°C occur on many winter days in Maricopa County (Horel et al., 2002), so occasional adult flight and feeding seems possible in the Maricopa County winter. Thus, my finding that larva development was entirely suppressed in the ambient treatments (Figs. 3, 4), suggests that cold-suppression of larval development is the major reason that these mosquitos disappear in the Maricopa County winter (Fig. 1).

I suspect that cold-suppression of larval development occurred due to cold-injury associated with the cold night-time temperatures. When I checked larvae in the morning, they were almost always paralyzed, though hours later when water temperatures warmed, they were swimming and feeding. Average water temperatures for shaded buckets (Table 1) in the months of December, January and February fell below the known threshold of 12°C for constant-temperature studies (Carrington et al., 2013). Additionally, minimum temperatures fall well below 12°C from November to March (Table 2). Cold coma onset was found to occur at ~6°C and lower in cold and warm-acclimated larvae (Jass et al., 2019). Cold damage in insects is primarily the result of ionic and osmotic imbalances that occur when cold temperatures inhibit active ion transport (Košťál et al., 2004). The ion imbalance created in the hemolymph causes a sharp drop in the excitability of the muscle tissues, placing the larvae in an immobilizing chill coma (Jass et al., 2019; MacMillan & Sinclair, 2011). While repeated cold exposure may help to harden the larvae against lethal effects through cryoprotectant and heat shock protein upregulation (Marshall & Sinclair, 2012; Wang et al., 2006), such hardening responses are apparently insufficient to allow *Aedes aegypti* larvae to develop in Maricopa County winters. Chill coma may be a particular problem for aquatic insects such as *Aedes aegypti* larvae since maintaining their position in the water column is essential for respiration at the surface. I often observed the ambient treatment larvae motionless near the bottom of the containers during the night and morning, with activity beginning in the late afternoon. This same effect was noted in another similar study on *Aedes aegypti* cold response and chill tolerance, and it is possible that hypoxia generated by the lack of access to air provides a further disruption ion balance in the these larvae (Campbell et al., 2018; Jass et al., 2019).

In the warmed containers where larvae could develop, the developmental rates I observed at cycling field temperatures were similar those observed in laboratory studies, most of which have used constant temperature regimes, despite the wide circadian variation in temperatures the larvae in our experiments experienced. My mildly-warmed containers, for which the mean temperature was  $18.2 \pm 4.4$  °C (average range = 10°C), had a mean time to pupation of ~25 days. A similar, laboratory study found no significant difference between constant and cyclical temperature regimes on larvae development, and found that a mean temperature of 16°C with a daily temperature range of 7.6°C was associated with a developmental time of 32 days (Carrington et al., 2013).

It is important to note that regional variation in temperatures or microhabitats may also allow *Aedes aegypti* to overwinter. My data suggest that both adult and larvae populations are suppressed during the winter months, so eggs are likely the most common overwintering reservoir due to their well-documented hardiness and resistance to cold (Byttebier et al., 2014; Farnesi et al., 2015; Sota & Mogi, 1992a). However, it is also possible that adults or larvae are able to overwinter in protected microhabitats such as inside buildings or in underground habitats such as irrigation tunnels and sewage storage (Arana-Guardia et al., 2014; Bermudi et al., 2017; Chadee & Martinez, 2016; Lima et al., 2016).

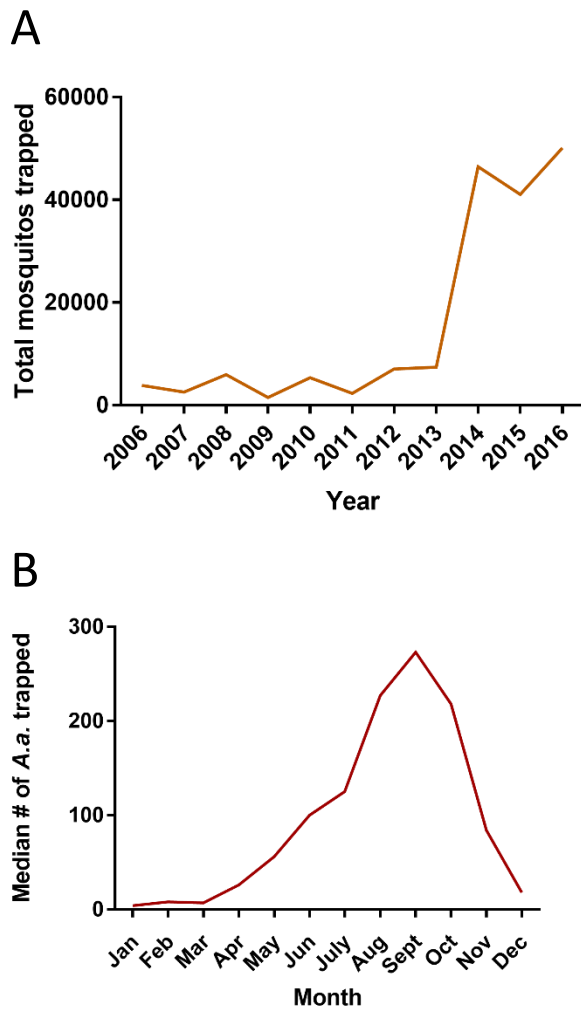
*Aedes aegypti* adult numbers are very low in April, and larvae were not observed in April, even though water temperatures averaged over 20°C and minimum water temperatures were higher than 10°C (Table 1 A, B). Many other studies (refs), and results from my warmed treatments (Figs. 3, 4) suggest that *Aedes aegypti* larvae should be able

to develop under such thermal conditions. Thus, it seems unlikely that suppression of this mosquito in the late spring in Maricopa County is not due to suppression of larval development, but rather represents a lag, likely due to the time required for populations to recover from winter suppression. It seems likely that eggs that have successfully overwintered begin to hatch at this time, leading to larval and adult appearance in May.

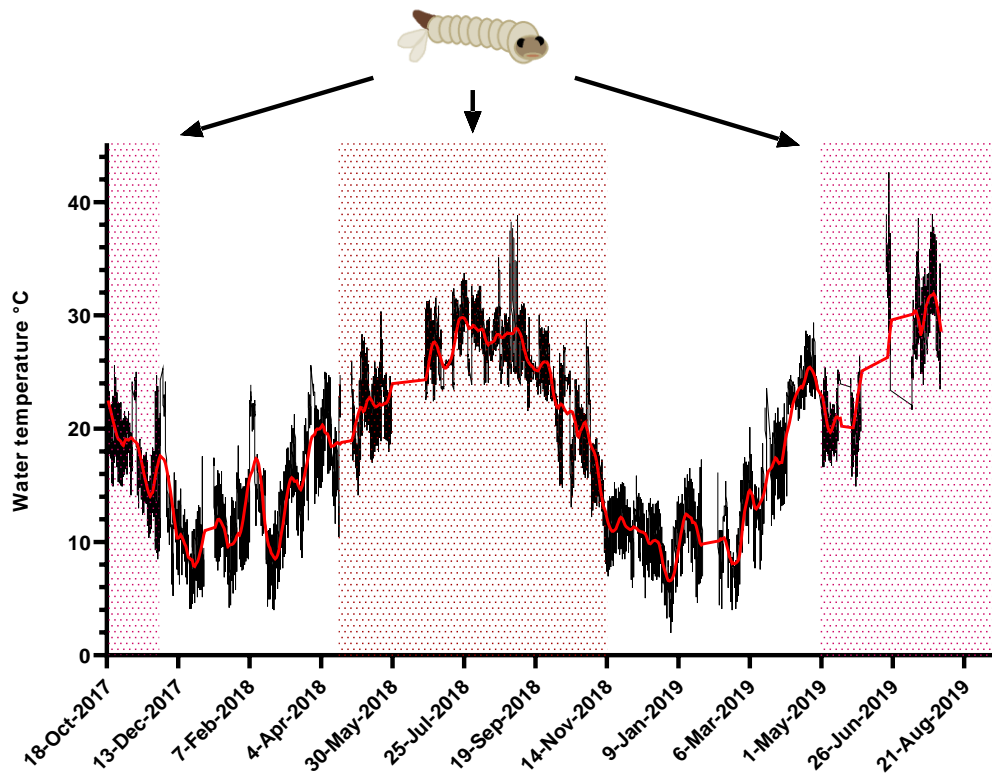
Over the full two years of observation, buckets exposed to sunlight more than 50% of the day never showed any *Aedes aegypti* larval presence, regardless of season or the occurrence of many larvae in shady buckets, suggesting that *Aedes aegypti* adults in Arizona actively avoid sunny locations. This contrasts with results from two studies from tropical locations. In north-eastern Peru and Puerto Rico, *Aedes aegypti* adults were more likely to deposit eggs in containers with more sunlight exposure (Barrera et al., 2006; Wong et al., 2011). This is interesting as in winter, utilization of sunlit waters could increase reproductive fitness of *Aedes aegypti* when evaporation is not a concern (Kearney et al., 2009). Humidity is much lower in Arizona than the tropical locations previously studied, and maximal summer temperatures are higher, so it is possible that locally adapted Maricopa County *Aedes aegypti* mosquitoes have been selected for shady oviposition behavior due to faster water evaporation rates or warmer water temperatures which might hit upper critical thermal limits in the hottest summer months. Sunny summer bucket temperatures measured as high as 50°C in July, averaging  $34.85 \pm 7.3^\circ\text{C}$  (Table 1A), exceeding the known upper critical temps of the larvae (Carrington et al., 2013), suggesting that local populations may have been selected to avoid sunny habitats for egg-laying.

The most surprising aspect of this study was the observation that with just 1.7°C of mean warming, *Aedes aegypti* larvae were able successfully develop, pupate and eclose during the Maricopa County winter season. With current climate trends suggesting that the city of Maricopa County will experience an additional 2-4°C of mean warming by the end of the 2030s (Sprigg & Hinkley, 2000), the possibility of an extended, or a broadened seasonal active period for the mosquitos seems likely. Mosquitos developing during the winter months could lead to higher peak population levels, as well as cause concern for increased periods of disease transmission if local Dengue Fever or Zika virus transmission becomes a reality.

**Figures:**



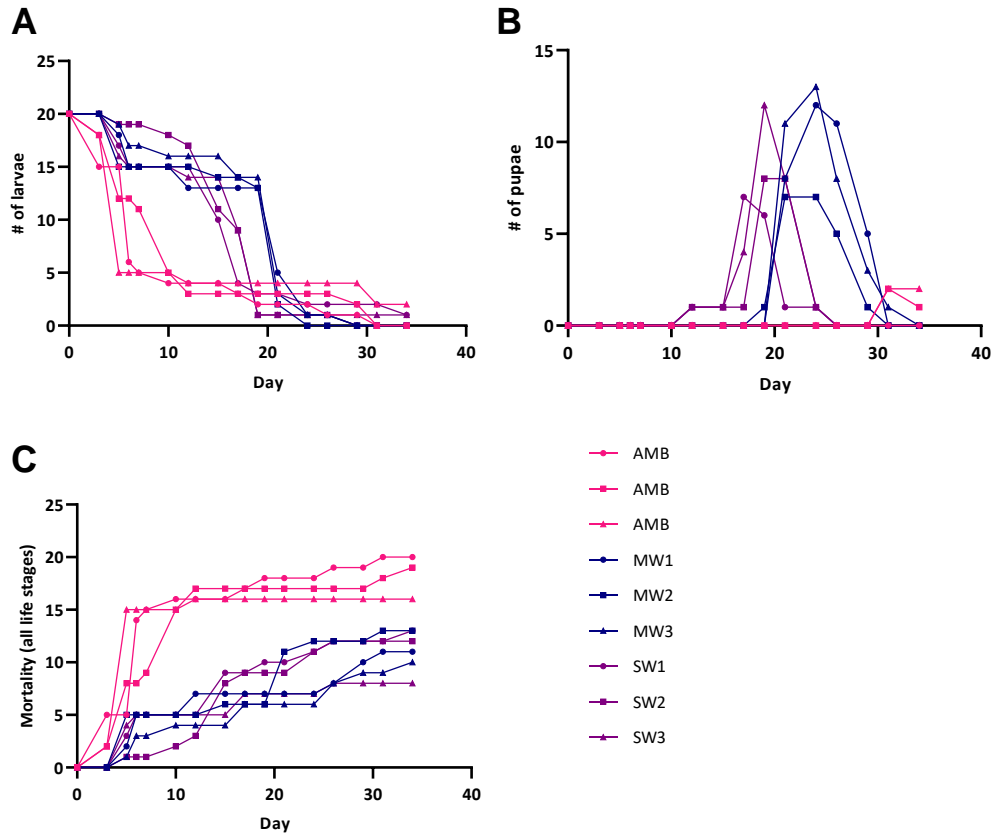
**Figure 1. A:** Median number of *Aedes aegypti* adults trapped per year in the Maricopa County metroplex. **B:** Median number of *Aedes aegypti* adults trapped per month from 2006-2016. Data courtesy of Maricopa County Vector Control.



**Figure 2**

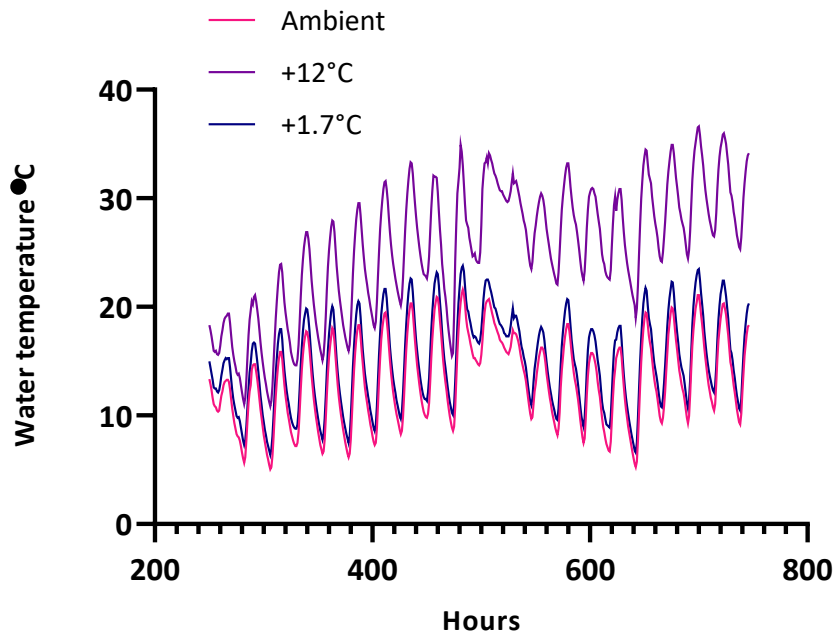
Temperatures and presence (red grid) or absence (white) of *Aedes aegypti* larvae in a bucket of water placed outdoors in Tempe, Arizona over 22 months in 2017-2019. Nine buckets were observed every 1-10 days, and temperature values from loggers in the buckets were offloaded monthly. The presence/absence data applies to all nine buckets. Temperature data shown is from a single, representative bucket which was constantly shaded. The bold central line was generated with a smoothing function (2<sup>nd</sup> order polynomial, nearest neighbor, single pass), while jagged, vertical lines connect daily high and low temperatures.





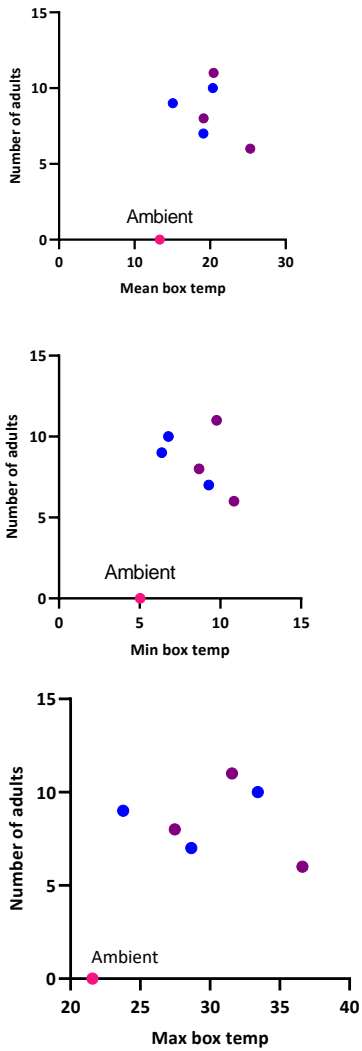
**Figure 3**

Number of larvae (A), pupae (B), and number of larvae + pupae + adults vs. time for *Aedes aegypti* larvae kept in ambient outdoor conditions or with mean temperatures elevated by mild (MW) or strong warming (SW). All containers started with 20 2<sup>nd</sup> instar larvae. Treatment significantly affected the number of pupae (Chi squared test,  $P < 0.05$ ), and survival of all life stages (Kaplan Meyer test,  $P < 0.05$ ).



**Figure 4**

Water temperatures (one hour averaged) recorded for a representative ambient treatment box, the maximally heated treatment container in the strongly-warmed treatment group, and the container with the lowest increase in mean temperature in the mildly-warmed treatment group, during the larval rearing experiment.



**Figure 5**

Number of adults produced vs mean (A), minimum (B) and maximum (C) box temperatures. The pink dot shows the three ambient treatment containers, blue dots the containers from the mildly-warmed treatments, and brown dots the containers from the strongly-warmed treatments.

**Tables:**

**A**

<b>Sunny</b>	<b>Jan</b>	<b>Feb</b>	<b>Mar</b>	<b>Apr</b>	<b>May</b>	<b>Jun</b>	<b>Jul</b>	<b>Aug</b>	<b>Sep</b>	<b>Oct</b>	<b>Nov</b>	<b>Dec</b>
	12.41	13.67	18.27	23.23	25.17	30.39		34.85	29.56	23.91	18.55	
<b>Mean</b> (°C)	± 5.19	± 5.97	± 6.43	± 5.67	± 7.02	± 6.76	± 32.61	± 7.35	± 5.91	± 6.24	± 5.93	± 11.15 ± 4.79
<b>Min</b> (°C)	0.78	2.52	5.35	9.28	13.08	19.28	21.76	27.08	21.19	11.82	6.37	1.98
<b>Max</b> (°C)	28.95	29.75	36.84	39.73	46.59	45.45	48.29	50.04	42.76	38.49	35.33	26.88

**B**

<b>Shade</b>	<b>Jan</b>	<b>Feb</b>	<b>Mar</b>	<b>Apr</b>	<b>May</b>	<b>Jun</b>	<b>Jul</b>	<b>Aug</b>	<b>Sep</b>	<b>Oct</b>	<b>Nov</b>	<b>Dec</b>
<b>Average</b> (°C)	± 10.55	± 11.32	± 15.07	± 21.29	± 21.22	± 28.37	± 28.78	± 28.22	± 26.41	± 20.22	± 16.09	± 10.39 ± 3.44
<b>Min</b> (°C)	1.98	4.00	5.86	10.46	14.13	19.76	21.66	23.48	21.57	13.08	6.98	3.26
<b>Max</b> (°C)	18.52	23.87	25.61	29.35	30.36	37.27	38.94	38.27	37.71	29.65	25.03	25.32

**Table 1:**

Average, minimum, and maximal temperatures for a Sunny (A) and Shaded (B) 19L bucket in Tempe, Arizona by month from 2017 to 2019. Standard deviations listed with each mean.

	<b>Mean</b>	<b>Min</b>	<b>Max</b>	<b>Range</b>
<b>Ambient (combined)</b>	13.34	5.037	21.57	16.53
<b>Mildly-warmed (combined)</b>	18.18	9.025	27.77	18.75
<b>Strongly-warmed (combined)</b>	21.65	10.32	31.27	20.94

	<b>AMB</b>	<b>SW1</b>	<b>SW2</b>	<b>SW3</b>	<b>MW1</b>	<b>MW2</b>	<b>MW3</b>
<b>Minimum</b>	5.037	10.85	8.68	9.768	6.37	9.275	6.775
<b>Maximum</b>	21.57	36.62	27.47	31.57	23.77	28.66	33.43
	13.34 ±	25.33 ±	19.14 ±	20.46 ±	15.06 ±	19.12 ±	20.35 ±
<b>Mean</b>	3.99	5.98	4.57	5.08	4.20	4.52	5.95

**Table 2:** (A) Container water temperature values (mean, minimum, maximum, °C) for the ambient, mildly-warmed and strongly-warmed treatment groups. Data were logged once per hour, from day 8 to 31, and averaged across containers. (B) individual container temperature (mean, minimum, maximum, °C) values demonstrate the variation that was observed.

	Estimate	Std. Error	z value	Pr(> z )	
(Intercept)	-8.93624	1.464336	-6.103	1.04E-09	***
Max temp	0.19108	0.046663	4.095	4.22E-05	***
Day	0.593113	0.271884	2.181	0.0291	*
Max temp:Day	-0.00612	0.008829	-0.693	0.4884	

	Estimate	Std. Error	z value	value Pr(> z )	
(Intercept)	-10.0908	1.33666	-7.549	4.38E-14	***
Min temp	0.78934	0.14197	5.56	2.70E-08	***
Day	0.97884	0.23301	4.201	2.66E-05	***
Min temp: Day	-0.0636	0.02536	-2.507	0.0122	*

	Estimate	Std. Error	z value	Pr(> z )	
(Intercept)	-9.00638	1.28166	-7.027	2.11E-12	***
Mean temp	0.29317	0.06114	4.795	1.63E-06	***
Day	0.70537	0.2379	2.965	0.00303	**
Mean temp:Day	-0.01477	0.01164	-1.269	0.20457	

**Table 3:** GLM outputs for min, maximum and mean box temperature vs adult production during the outdoor field development experiment.

**Desiccation stress is the primary limitation on egg overwintering of *Aedes aegypti* in Maricopa County, Arizona, USA**

**Abstract:**

The mosquito *Aedes aegypti* is a vector of multiple diseases and is currently spreading rapidly on a global scale. A prime example is the desert-embedded Maricopa County, Arizona, where populations of *Aedes aegypti* have been increasing exponentially over the past decade. In Maricopa county, *Aedes aegypti* virtually disappear each winter, with populations building up exponentially through summer and fall. As winters in Maricopa County are relatively mild, an important question is whether climatic warming might *Aedes aegypti* to survive the winter and possibly reproduce year-round. Most mosquitos, including *Aedes aegypti*, are thought to survive cold winters in the egg stage. I found that *Aedes aegypti* eggs had very low survival rates in Maricopa County winter conditions. To determine whether egg mortality was a result of cold stress or desiccation I exposed eggs to local field temperatures but at higher humidities or elevated temperatures. Warmer conditions did not significantly improve egg survival. However, high humidity greatly improved egg survival, suggesting that desiccation is the primary source of *Aedes aegypti* egg mortality in Maricopa County winters. Other potential abiotic limits on mosquito egg survival can be freezing or cold damage. *Aedes aegypti* eggs had high survival at -3°C for three hours but were killed by exposure to -8°C. Because Maricopa County winter temperatures have not dropped below -3°C in recent history I conclude that freezing is unlikely to limit *Aedes aegypti* winter survival here. However, eggs exposed to one week

at 3°C had reduced survival relative to other treatments. In some years, Maricopa County experiences prolonged temperatures in the 2-10°C range, so such cold conditions may reduce egg survival. My data suggests that climactic warming over the winter months has some potential to increase egg survival, but that availability of humid urban microhabitats are likely necessary for *Aedes aegypti* eggs to overwinter in this desert city habitat.



## Introduction

Global climate change has driven the range of tropical insect species further poleward in recent years, introducing species into areas well beyond their historical habitats (Au & Bonebrake, 2019; Parmesan et al., 1999; Rochlin et al., 2013). The fallout from these species moving into nonnative environments has been far-reaching for not only the displacement and destruction of native species, but also with regards to vector-borne diseases (Gage et al., 2008; Githeko et al., 2000; Ryan et al., 2019; Wagner & Van Driesche, 2010). *Aedes aegypti* mosquitos, an important vector of multiple diseases, is a prime example of this trend. While historically confined to the tropics and sub tropics, in recent years populations of *Aedes aegypti* have spread as far north as Europe and Canada (Giordano et al., 2020; Liu-Helmersson et al., 2019), and caused local outbreaks of Dengue fever in Croatia and France in 2010 (Gjenero-Margan et al. 2010; Bouzid et al., 2019). While this northward expansion is well documented, the limiting physiological mechanisms remain poorly understood.

Like many organisms, the life stages of insects can vary dramatically both in physiology and habitat, influencing the thermal and hydric conditions they are exposed to. The varying effects of climate change on the fitness of different life stages has been well-documented in plants, vertebrates and invertebrates (Crozier, 2003; Kingsolver et al., 2011; Rivas et al., 2019; Shipley et al., 2020; Walck et al., 2011). One major common difference among life stages in insects is that thermoregulatory behaviors such as basking or shade-seeking are often possible for adults but not immobile stages (usually eggs or pupae (Bonebrake et al., 2014). Pupal and egg stages of insects are usually immobile, and

so must cope with their local thermal and hydric conditions. Harsh seasonal conditions can induce diapause in any developmental stage of insects, causing a suspension of development and greatly enhanced environmental tolerance. The lack of diapause was thought to confine many insects such as *Aedes aegypti* to the tropics, but even in mosquitos that lack egg-diapause, the eggs are well-known to be the life-stage most capable of surviving cold winters (Lima et al., 2016).

Anthropogenic climate change has had major consequences for habitats globally (Travis, 2003). Anthropogenic development of the Maricopa County, AZ has led to localized warming, partly as a result of the urban heat island effect (Kalnay & Cai, 2003; Yuan & Bauer, 2007). This has contributed to Maricopa County experiencing an increase of 5°C in minimum nightly temperature, and a 3.1°C daily average temperature increase over the last 50 years, increasing the number of months per year that arthropods can be locally active (Baker et al., 2002). An additional 2-4°C of warming is expected for the region in the next decade (Sprigg & Hinkley, 2000).

Despite having some of the highest yearly recorded temperatures in North America, Maricopa County's winter months intermittently drop below freezing, with nightly minimums averaging from 4°C to 9°C from November to March (Table 1). Corresponding with these temperature decreases, Maricopa County Vector Control trapping data indicates *Aedes aegypti* populations decline sharply, starting in November, slowly recovering when average minimum nightly temperatures have begun to climb above 10°C in March (Table 1). *Aedes aegypti* eggs are known to be fairly resistant to cold weather, but sensitive to desiccation stress, suggesting that desiccation may be a

more significant problem than cold damage for overwintering populations (Byttebier et al., 2014; Farnesi et al., 2015; Sota & Mogi, 1992a, 1992b). However, precipitation for egg hatching and larval habitat remains intermittently available through the winter in Maricopa County, and humidity is higher than in the summer and fall months, arguing against desiccation being a major limitation on egg survival. Here I examine the physiological limitations on egg survival of *Aedes aegypti* in Maricopa County. Do eggs have high survival under ambient conditions? If not, is desiccation or cold/freezing the major cause of egg death? To test whether desiccation is a major cause of egg death in the Maricopa County winter I compared egg hatch rate for eggs kept outside at ambient temperatures, either at ambient or elevated humidity. To test for potential effects of cold, I tested the effect of plausible freezing conditions or continuous cold, non-freezing exposure on egg hatch rate, in both cases choosing temperatures and durations approximating the coldest conditions reported for Maricopa County over the last ten years. Finally, I ask whether the warmer winters predicted by increased urbanization and climate change are likely to improve or reduce survival of *Aedes aegypti* eggs in Maricopa County. If cold is the major threat to egg survival, then warming should improve egg survival. Conversely, since warmer winters likely increase evaporative water loss, warmer winters could reduce egg survival.

## Methods

### **Wild-capture, lab rearing, and egg generation and pre-experiment treatment of *Aedes aegypti***

Eggs used in the 2018/2019 winter egg viability experiments were third generation offspring reared in the lab from wild-caught larvae collected in the late fall from field sites in Maricopa County, AZ. The larvae were generated by netting from a 19L bucket that was continuously monitored for *Aedes aegypti* activity. The adults from each generation were fed using anesthetized laboratory mice (IACUC protocol: 18-1662R) and kept on a 12:12 hour light/dark cycle at 24°C. Seventy-two hours after each feeding, adult females were given wet seed germination paper and allowed to lay eggs for 12 hours before the paper was removed. This was repeated until egg laying ceased. Each paper was then kept damp for an additional 72 hours while the attached eggs developed. At the end of the 72-hour period the egg paper was dried and stored in an 80% humidity-controlled container at approximately 24°C. Eggs used in the trials were stored for a maximum of one week before the experiments. The eggs hatched for adult rearing and population maintenance were hatched using yeast solution immersion (0.25g/L) for 24 hours (Byttebier et al., 2014). At the end of 24 hours the egg paper was removed, and unhatched eggs were discarded. Larvae were reared no more than 100 per 500mL of water and fed a diet of Purina<sup>®</sup> *Cat-chow Complete*. Food was provided every one to two days, with more food only added when existing food had been consumed from the bottom of the containers.

The *Aedes aegypti* used in my analysis of egg exposure at freezing temps were third generation eggs generated from wild, Maricopa county AZ-area larvae collected from the 19L outdoor buckets in the late September to October 2019. The feeding and rearing of the adults, eggs and larvae were identical to those techniques used in the 2019 experiment.

The *Aedes aegypti* eggs tested in the one-week cold exposure experiment were generated from wild-caught eggs in Tempe, AZ in November 2020 that were laid on seed paper by wild adults in 19L buckets. The larvae were reared to adulthood, fed, and then provided materials to lay eggs according to the procedures used in the 2018/2019 experiment. The eggs were reared in the lab for only one generation before use in the experiment.

### **Hatch rate of eggs exposed to Maricopa County winter conditions: 2018**

I tested the effect of humidity on the hatch rate of *Aedes aegypti* eggs exposed to ambient Maricopa County winter weather in 2018. Eggs were exposed to ambient temperatures from February to May, either at ambient humidity or at a humidity elevated by a saturated NaCl solution, with egg hatch rate tested every 30 days.

Eggs were placed five to each of 44, 1.5 ml open plastic vials. Forty eggs (eight vials) were tested at the start of the experiment (February 8<sup>th</sup>). Half of the remaining vials of eggs were deployed in racks in an open top container allowing for ambient humidity exposure, while the other half of the eggs were sealed in an airtight container humidified to ~75% relative humidity (RH), maintained using a super saturated (357g/L) NaCl

solution (Young, 1967). Saturated sodium chloride solutions maintain a very stable (73-76% RH) air humidity across a temperature range of 0-80°C. Vials were shielded to avoid direct sun, UV exposure, or rain. A total of 6 vials of 5 eggs were hatched per treatment, per month, with the last hatch on May 8<sup>th</sup>, 2018. Hatching was measured by immersing the eggs in a 0.25g/l yeast solution, followed by one week of observation for emerging larvae.

### **Hatch rate of eggs exposed to Maricopa County winter conditions: 2019**

In 2019, I tested the effect of humidity and warming on egg hatch rate for eggs exposed to ambient Maricopa County winter temperatures. Eggs were either exposed to ambient temperatures and humidities, ambient temperatures with 75% RH, or ambient humidities with air temperatures warmed 1°C. In total, 588 eggs were deployed, five to each 1.5 ml microcentrifuge tubes. Again, experiments ran from February to May, and egg hatch rate was measured initially, and every 30 days after for each group.

Warming was accomplished by placing the egg vials in covered wood boxes (10x10x14 cm, 0.95 cm thick walls), warmed by a thermoelectric generator under the box. Each thermoelectric generator had a 40 mm aluminum heat sink attached to each side with thermal adhesive, with the heating side of the thermoelectric generator positioned upward through a cutout in the bottom of the box. Each thermoelectric generator was wired in parallel and connected to a 12-volt, 5-amp laboratory power supply which was adjusted to achieve the desired warming. The ambient temperature/humidity vials and the ambient temperature/high humidity vials were handled as described above for the 2018 experiments. I monitored the temperature and

humidity in the treatment containers with HOBO® U23 V2 (onsetcomp.com) outdoor temperature and humidity monitors with their probes inserted into the boxes through small holes drilled into the sides.

### **Effects of exposure to three hours of freezing temperatures on egg hatch rate**

Subzero temperatures occasionally occur, but over the past ten years, the coldest temperature recorded at two weather stations was  $-2^{\circ}\text{C}$  for several hours (Table 1).

Therefore, I tested the effects of three hours exposure to  $0^{\circ}\text{C}$  or  $-3^{\circ}\text{C}$  as representative tests of the coldest freezing temperatures likely to occur. I also tested the effect of  $-8^{\circ}\text{C}$ , as literature data suggests that this temperature should have high lethality for *Aedes aegypti* eggs (Byttebier et al., 2014; Hanson & Craig, 1995).

A total of 600 eggs, five eggs per 1.5 ml vial, were divided into two pre-treatments: half of the eggs were “hardened” by exposure to  $3^{\circ}\text{C}$  for three hours; the other half of the eggs were maintained at  $24^{\circ}\text{C}$  for three hours. For both treatments, during pretreatment, RH was maintained near 100% using open water beakers within air-tight plastic containers. Seventy-five eggs (15 1.5 ml vials of 5 eggs) from each treatment were hatched at room temperature to test for an effect of the hardening treatment on egg hatch rate. To expose eggs to potentially freezing conditions, vials containing the eggs were submerged for three hours in a water bath filled with propylene glycol set to  $0^{\circ}\text{C}$ ,  $-3^{\circ}\text{C}$  or  $-8^{\circ}\text{C}$ . A thermocouple positioned in a “dummy” centrifuge tube confirmed that the air inside the tubes reached the treatment temperature within 20 minutes. At the end of the three hours, the eggs were removed and allowed to sit at room temperature for 24 hours before the vials were flooded with 0.25g/L yeast water to stimulate hatching. To ensure that no late

hatching eggs were missed, egg hatch rate was counted 12 hours after simulated hatching, and again at 48hrs, 72hrs and one-week post yeast water exposure.

### **Effects of exposure to one week of cold non-freezing temperatures on egg hatch rate**

In terms of sustained non-freezing cold exposure, the coldest average temperatures over a month averaged over the past ten years was 11°C (Table 1). However, minimum temperatures in the coldest months average 4°C (Table 1), and there have been weeks with average temperatures below 5°C. Therefore, as the maximal possible ecologically-realistic cold temperature test for Maricopa County I tested the effect of one week of 3°C. As a more realistic test of cold exposure, I tested the effect of cycling temperatures between 18°C and 3°C for one week, as representative realistic conditions for the coldest conditions in a Maricopa County winter (Table 1).

Forty eggs (8 vials of 5 eggs) were hatched on day zero to determine the baseline hatch rate. A total of 250 eggs, five each per 1.5 ml vials, were divided into four equal groups, and exposed to 3°C, 25°C, 18.5°C and 3/18.5°C temperatures at a RH of 75%, with a 12:12 light: dark cycle. The 3°C /18.5°C group was transferred to 3°C for 12 hours at night and returned to 18.5°C for 12 hours during the day. Ten vials (50 eggs) were used for each treatment-time point combination. Egg hatch rate was tested after seven days at each temperature treatment by immersing the eggs in a 0.25g/L yeast hatching media. These eggs were monitored at 12, 48 and 72hrs, and finally one-week post immersion to ensure that no late hatches were missed.



## **Statistics**

I tested the effects of humidity and/or temperature on egg hatch rate under field conditions separately for the 2018 and 2019 experiments using a generalized linear mixed model (GLMM) with egg hatch as a binary outcome (assuming a binomial distribution and using a logit link function), treatment as a fixed effect and vial as a random effect. Similarly, the effect of exposure to three hours of freezing temperatures was evaluated with a general linearized model, using temperature and hardening status as treatment variables, and egg hatch as a binary outcome. To evaluate the effects of one week of exposure to cold, non-freezing temperatures, I used a GLMM with egg hatch as a binary outcome, treatment as a fixed effect, and vial as a random effect. For all models, homoscedasticity was confirmed by plotting the residuals vs the fitted values. All statistical analyses were performed using R, with the “lme4” package installed (Bates, 2007; R Core Team, 2013).

## **Results**

### **Hatch rate of eggs exposed to Maricopa County winter conditions: 2018**

In 2018, the egg hatch rates in the ambient temperature/humidity treatment declined strongly over the 3-month period, while the egg hatch rate in ambient temperature/high humidity treatment remained high and did not vary significantly from the initial 90% day zero hatch rate (Fig 1, Table 2). In the ambient temperature/humidity treatment, egg hatch

rate declined to 3% by day 90 (Fig. 1). Humidity averaged  $83.0 \pm 2.6\%$  in the elevated humidity treatment, while humidity in the ambient treatment averaged  $34.0 \pm 16.4\%$ .

### **Hatch rate of eggs exposed to Maricopa County winter conditions: 2019**

In 2019, the hatch rate of eggs exposed to ambient temperature and humidity declined strongly with time, dropping from 82% to 7% after 90 days (Fig. 1, Table 3). Elevated humidity significantly increased egg hatch rate, with a 55% hatch rate after 90 days (Fig. 1, Table 3). Increasing mean temperature by 1°C did not affect egg hatch rate (Fig. 1, Table 3). Both air temperatures and humidities varied circadianally, with daily temperature range often being  $> 15^{\circ}\text{C}$  and daily relative humidity range  $> 5\%$  (Fig. 2). Early in the winter, average temperatures were below  $10^{\circ}\text{C}$ , with minimum temperatures sometimes dropping below  $0^{\circ}\text{C}$ . Late winter/spring average temperatures rose to over  $20^{\circ}\text{C}$ , with minimum temperatures approximately  $15^{\circ}\text{C}$  (Fig. 2). Relative humidities averaged 40-50% early in winter, declining to about 25% in late winter/spring. In the containers with high regulated humidity, relative humidity averaged about 75% (Fig. 2).

### **Effects of exposure to three hours of freezing temperatures on egg hatch rate**

Exposure to three hours of  $-8^{\circ}\text{C}$  treatments almost eliminated all egg hatching (GLM, Table 4, Fig. 3) in both the hardened and unhardened groups. Three hours of exposure to  $0^{\circ}\text{C}$  or  $-3^{\circ}\text{C}$  reduced egg hatch rate by about 40% relative to eggs maintained continuously at  $24^{\circ}\text{C}$  (Fig. 3, Table 4). Hardened eggs had had lower initial survival, but hardening did not affect survival at  $-3^{\circ}\text{C}$ . Survival in the hardened eggs dropped from

63% initially, to 50, 55.4, and 6.6% at 0°C, -3°C and -8°C respectively. The unhardened eggs saw a reduction in survival from 77.2% at room temp, to 50.3%, 45.5% and 1.3% across the 0°C, -3°C and -8°C intervals.

### **Effects of exposure to one week of cold non-freezing temperatures on egg hatch rate**

Compared with the initial, 24°C treatment, I found a significant decrease in survival for the 3°C, and the 3 /18.5°C 12/12hr variable treatments (GLM, Table 5, Figure 4). The initial, day zero 24°C treatment had 84% survival, but dropped to 14% after one week at 3°C. The final survival for the 3°C/18.5°C treatment was 58%, and 68% for the 24°C treatment after one week.

### **Discussion**

My finding that increasing relative humidity to over 75% eliminated or greatly reduced declines in egg hatch rate overwinter indicates that water loss is the predominant abiotic challenge to the overwintering survival of *Aedes aegypti* mosquito eggs in Maricopa County. The vulnerability of *Aedes aegypti* mosquito eggs to desiccation has been well documented (Byttebier et al., 2014; Farnesi et al., 2015; Sota & Mogi, 1992a, 1992b), but it is interesting that after more than ten years in this region, that overwintering egg survival remains highly limited by water loss rates. Egg death due to desiccation during winter and spring is like the primary mechanism ensuring low numbers of *Aedes aegypti* in Maricopa County during spring and summer when temperatures are otherwise

excellent for larval development and adult reproduction, given that irrigation and other anthropogenic sources of water should allow sufficient water for larval growth.

I found that the eggs were largely resistant to acute exposures to the coldest freezing conditions that they are likely to experience in Maricopa County. In the last ten years, only once have temperatures dropped as low as  $-5^{\circ}\text{C}$ , and generally the very coldest temperatures of the year are within a few degrees of  $0^{\circ}\text{C}$  (Table 1). I found that *Aedes aegypti* eggs survived  $0^{\circ}\text{C}$  or  $-3^{\circ}\text{C}$  for three hours moderately well, experiencing about a 40% decrease in hatch rate (Fig. 3). On the other hand, exposure to  $-8^{\circ}\text{C}$  was highly lethal (Fig. 3). *Aedes aegypti* eggs supercool to  $-24^{\circ}\text{C}$ , with death occurring much earlier than the freezing point (Hanson & Craig, 1995; Hatchett, 1946). However, duration of exposure to subfreezing temperatures is also important, as exposure to  $-3^{\circ}\text{C}$  for 24 hours greatly reduced egg survival (Hanson & Craig, 1995; Hatchett, 1946). However, such extended subfreezing temperatures have not been documented in Maricopa County, at least in recent years. However, at higher elevations around Maricopa County much colder temperatures occur, and freeze-damage seems likely to be a major limitation on overwintering survival of *Aedes aegypti* eggs in such regions.

In general, temperate insects are at a greater risk of death from chilling injuries than from freeze related damage (Bale, 2002), Insects can also experience damage from prolonged exposure to low, non-freezing temperatures, or to repeated exposures to low temperatures (Marshall & Sinclair, 2012). Such cold-damage can occur due to inhibition of ion-motive ATPases, resulting in loss of ionic gradients and membrane potentials, with subsequent osmotic damage and sometimes apoptosis or necrosis (Overgaard &

MacMillan, 2017). I did find that one week exposure to 3°C significantly reduced egg hatch rate (Fig. 4). However, these conditions are much colder than typically experienced in a Maricopa County winter (Fig. 2, Table 1). The coldest days I documented in my two years of monitoring had fluctuations between daytime highs of 10-30°C and nighttime lows of 0-10°C. To test the effect of something similar I exposed eggs to one week of temperatures fluctuating between 18.5°C in the day to 3°C at night. While there was a trend toward lower egg hatch rate in this treatment, it was not significant (Fig. 4), so I conclude that exposure to ecologically-realistic above-freezing cold temperatures are unlikely to have a major effect on the hatch rate of overwintering *Aedes aegypti* eggs. In colder regions, such cold damage may be highly relevant and more limiting to survival of *Aedes aegypti* eggs (Giordano et al., 2020; Khan Salah Uddin et al., 2020; Lima et al., 2016)

The very low numbers of *Aedes aegypti* found in early summer are consistent with the hypothesis that dry conditions cause death of most overwintering eggs. The low survival of eggs in ambient humidities that I documented suggest that eggs that do survive the winter likely do so in protected, humid microhabitats. This raises the possibility that education and urban planning efforts to minimize such suitable habitat could be very effective in greatly reducing the number of eggs able to overwinter in desert cities, thereby taking advantage of the local dry conditions to control mosquitoes in a pesticide-free manner.

Urban communities can create humid microhabitats for mosquitoes in a variety of ways. *Aedes aegypti* mosquitos are adept at completing their life cycle indoors, where

humidity values are often higher (Dzul-Manzanilla et al., 2017; Kampen et al., 2016). A survey of Tucson, Arizona neighborhoods in August 2012 found that 64% of houses city wide had at least one water bearing container, and 13% of houses had immature *Aedes aegypti* present. Flowerpots were found to be the most common container for *Aedes aegypti* larvae (Walker et al., 2018). Thus, *Aedes aegypti* eggs may be overwintering in such indoor habitats. There may also be specific regions of local high humidity which may facilitate overwintering egg survival. Relative humidities near a golf course averaged over 50% during winter, compared to under 10% near the airport (Table 1).

*Aedes aegypti* eggs could also possibly overwinter in the underground water distribution infrastructure. *Aedes aegypti* breed in rain water storage systems in California and Brazil (Bermudi et al., 2017; Metzger et al., 2017). Maricopa County possesses extensive underground flood irrigation and storm drain networks, set up to deal with the yearly Monsoon season rains. These systems are closed, with the exception of open-air flood gates where entry and oviposition could occur. Humidity in these specific environments is likely high enough to sustain any potential eggs through the winter months.

Maricopa County is fortunate to possess dry conditions that can be harnessed for mosquito control, at least in winter. With suitable public awareness of the possibility of mosquito eggs overwintering in such humid microhabitats, actions could be taken. Dumping indoor water containers or bleaching them can eliminate indoor breeding (Bhat & Krishnamoorthy, 2014; Saleeza et al., 2011). Adding screens, ventilation, or better drainage systems to underground water storage facilities could greatly reduce the ability

of mosquitoes to utilize such habitats. Identifying and treating high-humidity areas such as golf courses could also be very effective in further reducing the ability of *Aedes aegypti* eggs to overwinter in Maricopa County. The movement of the Zika, Dengue and Chikungunya viruses to the US may provide the impetus needed to bring about such changes.

## Tables and figures

<b>KPHX (Sky Harbor Airport)</b>	<b>November</b>	<b>December</b>	<b>January</b>	<b>February</b>	<b>March</b>
Average temp (°C)	18.81 ± 1.65	13.37 ± 1.09	14.04 ± 1.69	15.42 ± 2.09	19.47 ± 2.29
Average max (°C)	25.57 ± 1.68	19.81 ± 1.32	20.41 ± 1.99	21.99 ± 2.55	26.13 ± 2.65
Average min (°C)	12.67 ± 1.37	7.86 ± 1.14	7.93 ± 1.72	9.1 ± 1.79	12.63 ± 1.8
Average relative humidity (%)	1.6 ± 3.36	8.22 ± 3.95	6.71 ± 4.98	4.3 ± 5.06	0.07 ± 4.47
Average absolute min (°C)	6.57 ± 1.92	2.02 ± 0.96	2.26 ± 3.02	3.43 ± 2.62	7.34 ± 2.28

<b>Phoenix Encanto Golf Course</b>					
Average temp (°C)	15.91 ± 1.53	11.06 ± 1.34	11.57 ± 1.12	13.61 ± 1.61	17.88 ± 0.97
Average max (°C)	25 ± 2.28	19.44 ± 0.94	20.14 ± 1.84	21.71 ± 2.54	25.81 ± 1.64
Average min (°C)	8.43 ± 1.53	4.44 ± 1.34	4.17 ± 1.12	6.16 ± 1.61	9.7 ± 0.97
Average relative humidity (%)	47.55 ± 2.28	56.64 ± 0.94	52.75 ± 1.84	46.58 ± 2.54	39.18 ± 1.64
Average absolute min (°C)	2.17 ± 5.07	-1.72 ± 7.65	-1.44 ± 6.85	0.56 ± 7.59	3.94 ± 6.13

**Table 1:**

Average, average minimum, average maximum, absolute minimum temperatures, and relative humidity collected at the Phoenix Sky Harbor and Encanto weather station from 2010 to 2020. The absolute minimum temperature was calculated by averaging the lowest 30-day temperature for each month over the ten-year period. Standard deviations listed.



	<b>Estimate</b>	<b>Std. Error</b>	<b>z value</b>	<b>Pr(&gt; z )</b>	
(Intercept)	-0.03553	0.84559	-0.042	0.96648	
Initial hatch	2.23276	0.9964	2.241	0.02504	*
High humidity	-0.08293	1.05111	-0.079	0.93712	
Day	-0.03984	0.01764	-2.259	0.02391	*
High humidity: Day	0.06185	0.02063	2.998	0.00272	**

**Table 2:**

GLM output for the 2018 egg field exposure, examining the effect of raising humidity on over-wintering egg hatch rate. All values are referenced to the ambient temperature and humidity treatment. There was a significant interaction of humidity and day on egg hatch rate.

	<b>Estimate</b>	<b>Std. Error</b>	<b>z value</b>	<b>Pr(&gt; z )</b>	
(Intercept)	2.704815	0.566996	4.77	1.84E-06	***
1°C warming	0.796488	0.726772	1.096	0.27311	
Initial hatch	-1.190687	0.657566	-1.811	0.07018	.
High humidity	-1.92953	0.741336	-2.603	0.00925	**
Day	-0.055302	0.009821	-5.631	1.79E-08	***
1°C warming: day	-0.0122	0.012543	-0.973	0.33073	
Initial hatch: day	NA	NA	NA	NA	
High humidity: day	0.050185	0.012277	4.088	4.36E-05	

**Table 3**

GLM output for the 2019 egg field exposure, examining the effect of raising humidity and raising mean temperature by 1°C on egg hatch rate. All values are referenced to the ambient temperature and humidity treatment. There was a significant interaction of humidity and day on egg hatch rate ( $p < 0.00005$ ).

	Estimate	Std. Error	z value	Pr(> z )	
(Intercept)	0.21706	0.23387	0.928	0.35333	
-3°C	-0.41453	0.25984	-1.595	0.11064	
-8°C hardened	-2.87031	0.51844	-5.536	3.09E-08	***
-8°C	-4.52113	1.03282	-4.377	1.20E-05	***
0°C hardened	-0.24373	0.32869	-0.742	0.45837	
0°C	-0.08353	0.32904	-0.254	0.79959	
24°C hardened	0.32193	0.33353	0.965	0.33443	
24°C	1.00151	0.30463	3.288	0.00101	**

**Table 4. A**

GLM table testing the effect of acute freezing exposure on egg hatch rate with -3 hardened eggs as a reference.

	Estimate	Std. Error	z value	Pr(> z )	
(Intercept)	1.2186	0.1952	6.242	4.31E-10	***
-3°C hardened	-1.0015	0.3046	-3.288	0.00101	**
-3°C	-1.416	0.2257	-6.275	3.50E-10	***
-8°C hardened	-3.8718	0.5022	-7.71	1.26E-14	***
-8°C	-5.5226	1.0248	-5.389	7.08E-08	***
0°C hardened	-1.2452	0.3024	-4.118	3.83E-05	***
0°C	-1.085	0.3028	-3.584	0.000339	***
24°C hardened	-0.6796	0.3077	-2.209	0.027185	*

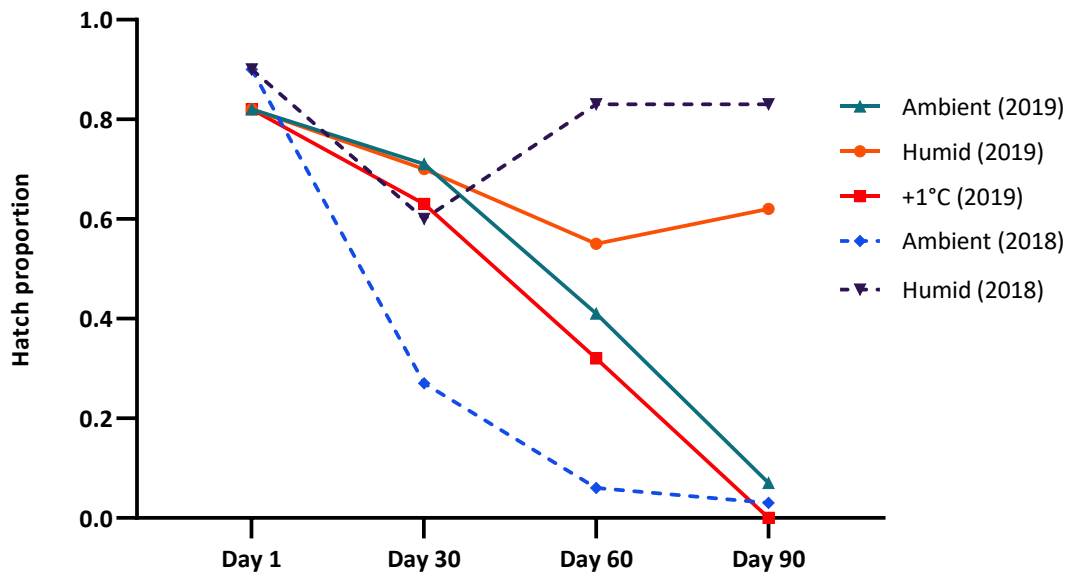
**Table 4. B**

GLM table for the effects of acute freezing exposure on egg hatch rate with room temperature eggs as a reference.

	Estimate	Std. Error	z value	Pr(> z )	
(Intercept)	1.8353	0.4841	3.791	0.00015	***
24°C	-0.9724	0.6236	-1.559	0.11889	
18.5°C	-0.5472	0.6357	-0.861	0.38941	
3°C	-3.902	0.7453	-5.236	1.64E-07	***
Variable	-1.477	0.6166	-2.396	0.0166	*

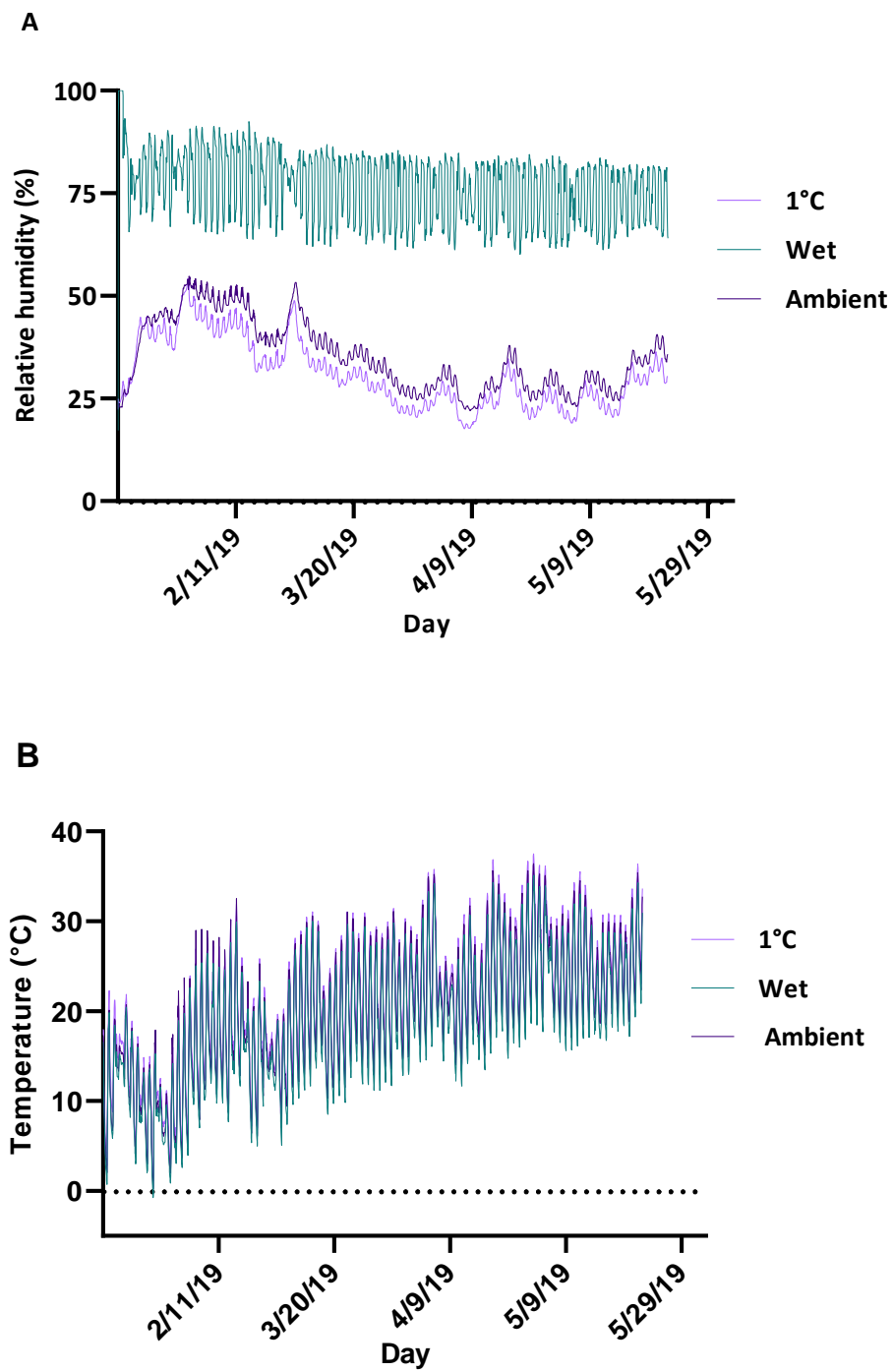
**Table 5**

GLM table testing the effect of cold exposure on egg hatch rate, with relative humidity = 75% for all treatments. One week at 3°C and one week of temperature fluctuations (Variable treatment, 12/12 L/D, 18.5°C/3°C) significantly reduced egg hatch rate.



**Figure 1**

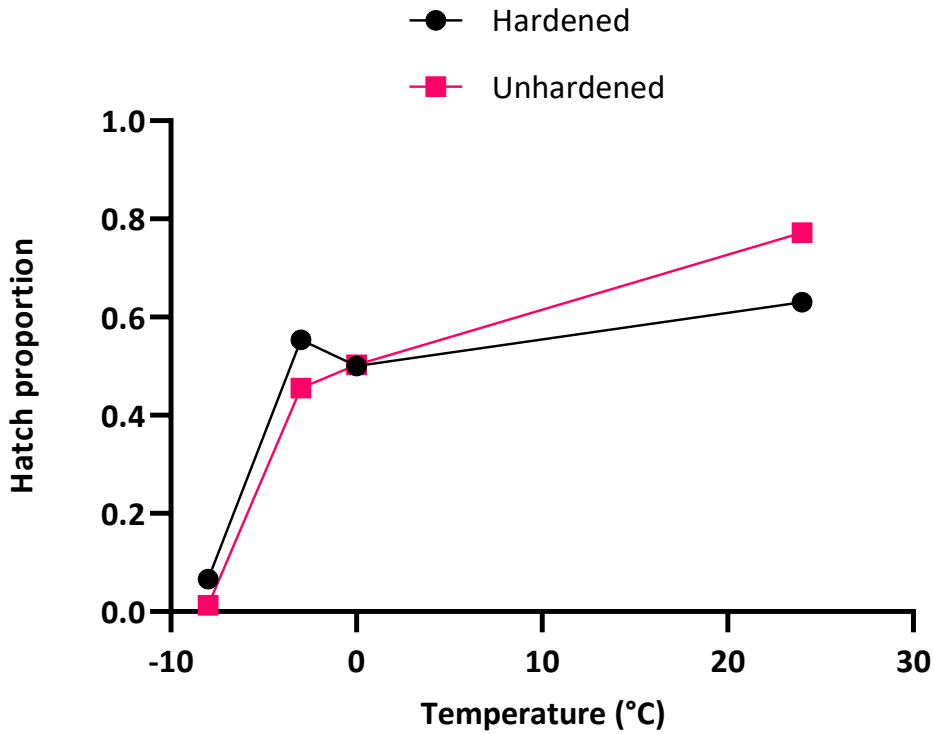
Hatch rate of *Aedes aegypti* eggs after various durations of winter exposure from February to May in 2018 and 2019. Hatch rate of *Aedes aegypti* eggs exposed to ambient temperatures and humidities in Maricopa County declined strongly through the winter in both years (Tables 2, 3), with very low hatch rates of eggs at winter's end. In 2018 (Table 2) and 2019 (Table 3), the hatch rates of eggs exposed to ambient humidity were significantly lower than for eggs exposed to elevated humidity. In 2019, raising mean temperature by 1°C did not change egg hatch rate through the winter (Table 3).



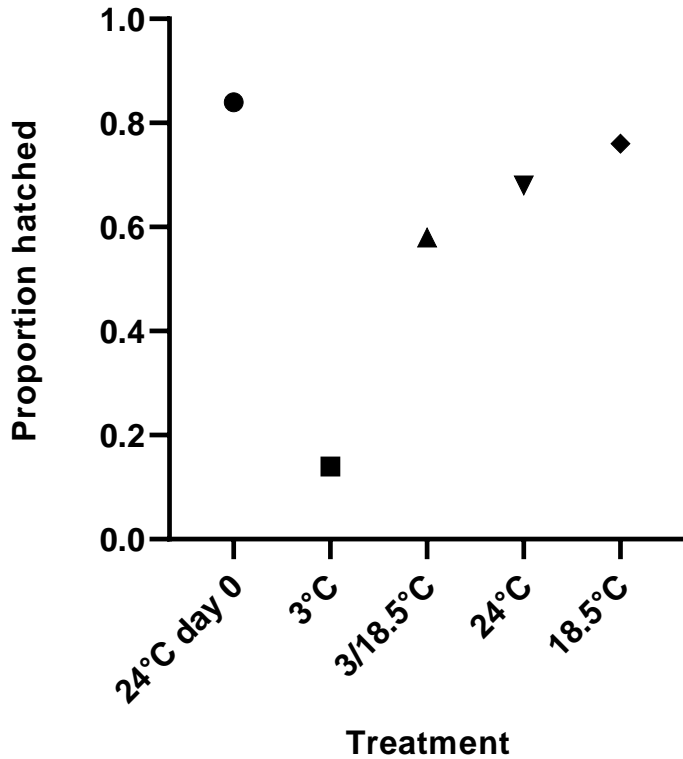
**Fig. 2.** Field conditions for 2019 experiment. (A) Temperature and humidity averages for +1°C, high humidity, and ambient treatments from February to May 2019. Relative humidity in the high humidity treatment was regulated to  $76.7 \pm 8.2\%$ . Ambient and 1°C

treatments averaged  $35.3 \pm 8.7\%$  and  $30.9 \pm 30.9\%$  relative humidity, respectively. (B) Temperatures experienced by the eggs during the 2019 winter exposure. Mean temperatures averaged  $18.9 \pm 7.0^\circ\text{C}$  for the high humidity treatment,  $20.2 \pm 7.1^\circ\text{C}$  for the ambient treatment, and  $21.0 \pm 7.1^\circ\text{C}$  for the  $+1^\circ\text{C}$  eggs. However, for all treatments, there was strong circadian variation in temperature, with an average range of  $35.6^\circ\text{C}$ . All temperature and humidity values were collected hourly.





**Fig 3.** Exposure to 0°C and -3°C for 3 hours slightly, but significantly, decreased egg hatch rate compared to unhardened, room temperature eggs ( $p < .05$ ). Exposure to -8°C resulted in near complete mortality in both the hardened and unhardened eggs ( $p < .001$ ). Hardening by exposure for one hour at 3°C did not significantly change egg viability at cooler temperatures, however it did significantly depress room temperature hatching. A total of 5 eggs were placed in each vial, and 15 vials hatched per treatment (GLM, Table 4 A,B).



**Figure 4**

The effect of seven-days of exposure to 24°C, 3°C, or 18.5°C, or temperatures that cycled between 3 and 18.5°C every 12 h on egg viability. All treatments were at 75% RH. A GLM analysis showed that acute exposure to 3°C significantly decreased survival, as well as exposure to 12hr alternating 3°C/18.5C temperatures (Table 4). None of the other treatments were significantly different than the initial hatch proportion. Five eggs were placed in each tube, and there were 10 tubes per treatment. Egg viability was induced by hatching the animals in a yeast solution and monitoring for larvae for one week.

## **Temperature and loading effects on the flight performance and energetics of the invasive Yellow Fever mosquito, *Aedes aegypti***

### **Abstract:**

Temperature strongly effects the performance of ectotherms, and most insects are small ectothermic flyers, yet we know little about how temperature effects their ability to fly. Here I investigated the effect of temperature on the willingness to fly, and the flight metabolic rate of sugar-water-fed female *Aedes aegypti* mosquitos. After one hour of exposure to a temperature, willingness to fly showed a thermal performance curve, with no flight at 10°C or 40°C, suppressed flight below 15°C or above 35°C, and a high percentage of flight between 18.5 and 35°C. Flight  $V_{CO_2}$  increased approximately linearly with air temperature, more than tripling as air temperature rose from 18 to 38°C. Across a more than two-fold range, body mass was strongly correlated with abdominal height, but not wing length, suggesting that mass variation was primarily due to carriage of different amounts of sugar water. At 29°C, flight  $V_{CO_2}$  increased approximately linearly with abdominal height, and body mass. Flight  $V_{CO_2}$  of the most heavily loaded mosquitoes was about 3x greater than the lightest animals. Lightly-loaded mosquitoes (lightest 30%) only weakly increased their  $V_{CO_2}$  with temperature while heavily loaded mosquitoes (heaviest 30%) had a more than 3x greater slope of  $V_{CO_2}$  on air temperature. Air temperature and load interact to determine the flight cost of mosquitoes, which can vary more than six-fold, with implications for their distance capacities and need for fuel.

**Introduction:**

Temperature is generally considered the most important abiotic factor determining organismal performance (Buckley & Huey, 2016; Huey & Berrigan, 2001), and climate change increases our need to understand how temperature affects organisms. Most insects are small and depend on flight for dispersal, and often feeding, yet there have been very few studies of thermal effects on flight performance of small insects. Small insects (under 50 mg) generally have body temperatures closely matched to air temperature due to their large surface-to-volume ratios (Kingsolver, 1983; Roberts & Harrison, 1998; Stevenson, 1985a, 1985b). Higher temperatures are well-known to increase many aspects of flight performance of insects including tendency to fly, wing beat frequency, load-lifting capacity and flight durations (Dillon & Frazier, 2006; Tsuji et al., 1986). However we currently lack measures of the effect of temperature on flight metabolic rates in small ectothermic insect fliers, likely due to technical limitations. In this study I examine the effect of air temperature on the flight metabolic rate of one of the world's most medically important insects, the yellow fever mosquito, *Aedes aegypti*.

Load-lifting is theoretically predicted to isometrically increase the metabolic power output and requirements of flight due to the need to proportionally increase lift (Ellington & Lighthill, 1984; Marden, 1994). Most but not all empirical data have supported this hypothesis (Feuerbacher et al., 2003; Wolf et al., 1989), and this question has not yet been examined in small ectothermic insects. The interaction between temperature and loading on flight metabolic rate is of particular interest, as it has been shown in several species that higher body temperatures increase flight muscle or

organismal flight performance (Curtsinger & Laurie-Ahlberg, 1981; Josephson, 1981; Mronz & Lehmann, 2008). In *Drosophila*, higher body temperatures increase load carriage capacities, with high body temperatures allowing carriage of a load approximately equal to body weight (Lehmann 1999). However, as yet, we lack data on how load affects the metabolic rate during loaded flight in small, free-flying ectothermic insects.

*Aedes aegypti* mosquitos are small ectotherms, weighing 0.5 to 1.6 mg as adults (Arrivillaga & Barrera, 2004), that fly over a broad range of air temperatures (Capinha et al., 2014). Adults feed on nectar and fly to search for human hosts for blood meals. An important question is how air temperature affects the cost of flight, as this will determine the need of mosquitos for nectar or other sugar sources while searching for hosts. The blood meal can impose a load of 1-2x body mass, which must be carried away (Christophers, 1960); thus mosquitos are likely among the most capable insects for lifting loads during flight, rivaling the dance fly and beewolf (Marden, 1989; Strohm & Linsenmair, 1997). Here I investigate the effects of air temperature and loading on the carbon dioxide emission rate ( $V_{CO_2}$ ,  $\mu\text{l hr}^{-1}$ ) of flying female *Aedes aegypti* mosquitos flight, as well as the effect of air temperature on tendency to fly.

## **Methods:**

### **Animal rearing**

Mosquitos used in this study were reared from larvae field-collected in Tempe, Arizona, USA, in November of 2020. In the lab, both larvae and adults were maintained at a 12:12 L/D photoperiod at 24°C. These field-collected larvae were kept in 0.5 liter containers at under 500 larvae per liter, and fed a diet of Tetramin Fish flakes every other day to ensure a surplus of food was available. Water containing pupae were moved into 95 liter cages; adults eclosed and could fly within the cage and feed on cotton balls soaked with a 10% sucrose solution. Adult density was less than 500 adults per cage. A portion of these female adults, one to two weeks of age, were used in the experiments testing the effect of air temperature on the tendency to fly.

The remaining adults were blood-fed on mice (IACUC protocol: 18-1662R). Gravid females were supplied moist seed germination paper three days after feeding for egg oviposition habitat. The egg oviposited on the paper remained there for three days, after which the paper was dried until no longer wet to the touch (12 hours) and then placed in bags stored in a large container kept at near 100% humidity using damp paper towels. Hatching was induced by immersion in a solution of 0.25 g/L baker's yeast (Byttebier et al., 2014). Larvae and adults were reared as described above. The female adults used for measurements of flight metabolic rate were one to two weeks past eclosion and had continuous access to cotton balls saturated with 10% sucrose solution.

### **Effect of air temperature on the tendency to fly**

The tendency of *Aedes aegypti* female adults to fly was measured at 10, 12.5, 15, 18.5, 24, 30, 37 and 40°C. Thirty animals were tested at each air temperature, in three trials of ten mosquitos each. Mosquitos were placed in translucent polymer cages (18.5x15.75x11.26cm) inside an incubator set at the test temperature for one hour. Incubator temperature was independently checked with a BAT-12 Physitemp thermocouple reader (Clifton, NJ). Then, a test tube vortexer was used to vibrate the cages while video was taken by a GoPro Hero 8 Black (San Mateo, CA) recording at 1440p resolution and 60 frames per second. Animals who exhibited hovering flight behavior and were able to reach seven centimeters above the floor of the container were counted as successful flyers. Nonflyers either remained on the chamber bottom or jumped but were not able to sustain flight in the upper portions of the chamber. Measurements took three days to complete; replicate temperature trials occurred on different days to mitigate age effects. The video data was uploaded directly from the GoPro camera to a computer and analyzed in Apple QuickTime media player.

### **Effects of load and temperature on adult flight carbon dioxide emission rates:**

The  $V_{CO_2}$  during flight was measured for mosquitos at temperatures ranging from 15°C to 40°C. Mosquitos were captured from the colony in an aspirator, and transferred to the flight respirometry chamber, which was a custom rounded 50 ml glass container with two fittings for gas flow and a stopper-sealed opening for animal entry. The flight respirometry chamber was within an incubator (VWR Drosophila Refrigerated Peltier

incubator, Randor PA), set to the test temperature. To allow handling of the mosquito in the incubator the incubator door was often open, and plastic sheeting was attached to the incubator frame that covered most of the door space, aiding in maintaining the incubator at its set temperature during the measurements. Flight behavior was induced using a test tube vortexer pressed directly to the glass flight chamber. Most mosquitos hovered well within the flight chamber for several minutes. After each animal was measured, they were removed from the flight chamber with an aspirator and anesthetized with 25% CO<sub>2</sub> air from a compressed air tank. This allowed later handling of the animals for weighing and image captures.

The V<sub>CO<sub>2</sub></sub> was measured with flow-through respirometry, using a Li-Cor 6252 CO<sub>2</sub> gas analyzer. CO<sub>2</sub> free, dry air was supplied to the system via a Parker Balston Purge FT-IR gas generator (Lancaster, NY). Flow rates were set at 250 ml min<sup>-1</sup> using two 500 ml min<sup>-1</sup> Tylan Fm-380 V mass flow (USA) controllers regulated by a Tylan RO-28 (USA). The Li-Cor 6252 gas analyzer was configured in differential measurement mode, with one mass flow controller supplying a constant stream of CO<sub>2</sub> free, dry air to the Li-Cor 6252 (Lincoln, NE) reference cell, while the other Tylan unit supplied air to the flight chamber before returning gas to the Li-Cor 6252 sample cell. The differential readout was converted from analog 0-5V to digital using a linear transformation in Sable System's Expedata software system, with 5 V output from the Li-Cor calibrated to 50 PPM of CO<sub>2</sub> to minimize digitization noise. The 95% washout time of the system was 36 seconds.



Air temperatures during flight were measured using a thermocouple thermometer inserted into the respirometry chamber. To vary the air temperature in the flight chamber, the incurrent air stream from the Parker Balston FT-IR Purge Gas generator (Lancaster, NY) was routed into the flight incubator, and directed through approximately two meters of coiled, copper tubing (0.64 internal diameter) which were submerged in several liters of water in a container within the incubator. To speed temperature equilibration at the lowest and highest temperatures, this water was sometimes cooled with ice or warmed with hot water. The CO<sub>2</sub> fraction in excurrent air from the flight chamber was recorded while the mosquito was flying for approximately one minute, with the highest 15 sec peak subsampled in Expedata (Las Vegas, NV) and reported as the mean fractional CO<sub>2</sub> in excurrent air during flight. The CO<sub>2</sub> trace was baselined and drift-corrected before and after each flight measurement by flowing dry, CO<sub>2</sub>-free air through the empty flight chamber. The V<sub>CO2</sub> was calculated by multiplying STP flow rate times the baseline-corrected mean fractional CO<sub>2</sub> during flight.

After measurements of carbon dioxide emission rate, animals were weighed using a Mettler Toledo XP2U (Columbus, OH) with a resolution of 0.0001 mg. To measure wing length and abdominal diameter, a top-view image of the mosquito was taken using a VWR dissecting microscope (Rador, PA), with the image recorded using an iPhone 12 Pro Max (San Mateo, CA) camera through the eyepiece. The mosquito was placed on a 1 mm grid that was simultaneously imaged to provide a reference scale for measurement calibration (Abramoff et al., 2004). Abdominal diameter was assessed as a measure of feeding status. Wing lengths and abdominal diameters were measured from the images

using ImageJ open source software (Abramoff et al., 2004). Abdominal diameter was measured at its widest point.

### **Statistics analysis:**

To model temperature effects on the tendency to fly, I tried fitting multiple non-linear models and report the model with the lowest Akaike information criteria value and highest  $R^2$ . Linear regressions were used to determine the relationship between  $V_{CO_2}$  and body mass, body mass vs. air temperature,  $V_{CO_2}$  vs air temperature,  $V_{CO_2}$  vs. body mass at 29°C, and wing length and abdominal diameter vs. body mass. As I did not know load precisely, I tested for an interactive effect of load and temperature on flight  $V_{CO_2}$  using just the heaviest 30% and lightest 30% of animals, as these seemed likely to differ strongly in load amount. For this data set I used a GLM with  $V_{CO_2}$  as a dependent variable, top 30% or bottom 30% of masses as a dichotomous predictor, and temperature as a continuous predictor. To analyze the effects of temperature and animal mass on adult flight metabolism I used a generalized linear model (GLM) with air temperature (°C) and body mass (mg) as continuous predictors for  $V_{CO_2}$ . I confirmed homoscedasticity of all GLM models by plotting fitted values vs. residuals. All analysis was carried out using R coding environments with the “lme4” package (Bates, 2007; R Core Team, 2013).

## Results

### **Adult take off temperature threshold:**

The tendency to take off was modeled reasonably well with a second order polynomial function (Fig. 1, AIC = 14.39,  $R^2 = 0.917$ ). At 10°C, no mosquito in any of the replicates was able to fly. The animals were generally immobile, and unable to right themselves once the shaking procedure began. The percent of mosquitos able to fly was 20% at 12.5°C, increasing to 50% at 15°C. Between 18.5°C and 29°C, more than 90% of mosquitos flew. At 37°C, about half of mosquitos were able to fly, and at 40°C, only 7% of animals were capable of flight.

### **Body masses and morphology**

Body masses of mosquitos selected to fly did not vary with air temperature (Fig. 2). It appeared that variation in body mass was primarily due to load variation rather than variation in size, as abdominal diameter was linearly related to body mass (Fig. 3), whereas neither wing length or wing area were related to body mass (Fig. 3). Similarly, for the experiment examining the effect of body mass on  $V_{CO_2}$  at 29°C, abdominal diameter was significantly correlated with body mass but not wing length or thorax diameter (Table 1).

### **Effects of load and temperature on *Aedes aegypti* flight $V_{CO_2}$ :**

For mosquitos flown at 29°C,  $\log V_{CO_2}$  increased linearly with  $\log$  body mass, with a slope not significantly different from 1 on a log-log plot (Fig. 4,  $p < 0.05$ ,  $R^2 = 0.1162$ ).

Over the entire data set, flight  $V_{CO_2}$  increased significantly with temperature (Fig. 5,  $F_{1,53}$

= 48.81,  $p < 0.0001$ ). There was a significant interaction between temperature and load status, as well as significant independent effects of temperature and load (GLM, Table 2,  $P < .05$ ). To further explore this interaction I tested the effect of temperature and mass category (highest and lowest 30% of mosquitos by mass). Flight  $V_{CO_2}$  increased much more strongly with air temperature in the heavier, loaded mosquitos (Fig. 6), and there was a highly significant temperature\*body mass category effect (Table 3).

### **Discussion:**

*Aedes aegypti* mosquitos showed a classic thermal performance curve in their tendency to fly, with most females able to fly between 18.5°C and 29°C, but higher or lower temperatures inhibiting flight for many, with temperatures below 10°C or above 40°C precluding flight (Fig. 1). For flying mosquitos, flight  $V_{CO_2}$  increased strongly with air temperature, and loading causes a further dramatic increase in flight  $V_{CO_2}$ . Combined, temperature and load cause up to a 6x variation in flight  $V_{CO_2}$ , and likely the energetic cost of flight.

Low and high-temperature limits on flight can provide an important bottleneck on adult mobility, and these thermal limits on flight likely contribute to the seasonal variation in mosquito populations in Maricopa County and elsewhere. *Aedes aegypti* mosquitos need to fly to feed on nectar or blood feed. Without either, the life cycle of the mosquitos is halted. In Maricopa County, where this study was conducted, daily average air temperatures during winter (December to March) are generally below 15°C, likely limiting flight except during occasional sunny mid-afternoon hours. Thus my data suggest that both cold-suppression of adult flight capabilities and larval growth likely

contribute to the suppression of *Aedes aegypti* populations between December and March in Maricopa County (Chapter 1). Conversely during the summer and fall, Maricopa County daytime high temperatures often exceed 40°C in the shade, likely completely excluding flight. However, in the summer months, early morning, night and evening temperatures permit flight (Chapter 1), as well as larval development (Chapter 2) and this corresponds with periods of rising *Aedes aegypti* populations (Chapter 2).

I did not measure respiratory quotient, so I cannot with confidence convert my  $V_{CO_2}$  values to metabolic rates. However, previous studies have suggested that carbohydrates in the form of glycogen and simple sugars from nectars are likely the primary fuel source for flight for sugar-water-fed mosquitos (Clements, 1955; Nayar & Van Handel, 1971). Mosquitos who were exhausted from flight activities were able to be flown again shortly after a nectar meal, and glycogen stores were not depleted when monosaccharides were available, suggesting they are preferred for flight (Nayar & Van Handel, 1971). Outside of Culicidae, other Dipterans such as *Drosophila*, have been shown to primarily metabolize carbohydrates during flight, strengthening my belief that the respiratory quotients of my animals were likely near one (Chadwick, 1947). Assuming flight is fueled by carbohydrates, my data suggest that the average flight metabolic rate ranges from a low of x mW for unloaded mosquitos at 18°C to a high of x mW for loaded mosquitos flying at 38°C.

There was a wide variation in body mass among the mosquitos, which could in theory be due to variation in size (body dimensions), condition (e.g. fat content) or loading (amount of sugar water recently consumed). I found no significant correlation

between thorax diameter or wing length with body mass, but a strong correlation between abdominal diameter and body mass (Table 1), suggesting that the majority of variation in body size was due to variation in loading. Additionally,  $V_{CO_2}$  increased strongly with body mass, with a slope on a log-log plot of 1 for the mosquitos flown at 29°C. In general, flight  $V_{CO_2}$  scales with mass with a slope of 1 or less on a log-log plot for intra- or across species size variation (Chown et al., 2007; Darveau, 2005; Kleiber, 1947), supporting the hypothesis that body mass variation is due to loading. As noted above, theoretical studies suggest that load carriage should require a similar mechanical power output as body weight.

The mechanisms causing the significant interaction between temperature and loading (Tables 2,3; Fig. 6) remain somewhat unclear. Higher body temperatures should allow greater aerodynamic power output (Lehmann, 1999), and greater load carriage. However, body masses did not vary across air temperatures for flying mosquitos (Fig. 2), suggesting that at all temperatures, in this study, mosquitos carried similar loads. Plausibly the mosquitos had greater acceleration and mobility during flight at higher air temperatures, but I did not assess this possibility. Future experiments should test how body temperature affects load carriage capacity and flight capacities in mosquitos. In any case, the higher cost associated with flight and load carriage at higher air temperatures suggests that mosquitos will require considerably more fuel to fly similar distances at higher air temperatures. I can calculate the theoretical maximum flight time (using food reserves) for an adult *Aedes aegypti* mosquito based on an assumed RQ of one, and a load of one microliter of 40% nectar (sugar/water weight). Doing so, I find that an adult *Aedes*

*aegypti* mosquito can sustain flight for 4.1 hours at 37°C, and for 39 hours at 15°C.

Examining the documented *Aedes aegypti* flight speed literature, sugar fed mosquitos have been recorded flying at an average of 0.5 km/hour (Rowley & Graham, 1968a), suggesting that at high temperatures aedes mosquitos may be limited to distances as short as 7.1 km total flight range, or as high as 66.3 km during cooler seasons. This suggests that the typically low dispersal range of *Aedes aegypti* mosquitos (0-800m) is not a fuel range limitation, but is behavioral or tied to some other physiological factor (Harrington et al., 2005; Honório et al., 2003; Juarez et al., 2020; Valerio et al., 2012).

Figures:

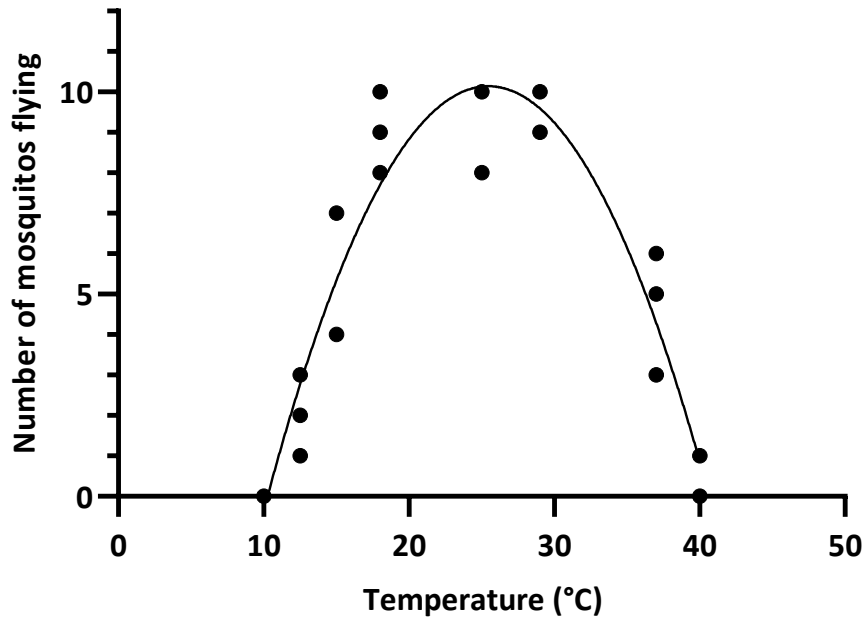
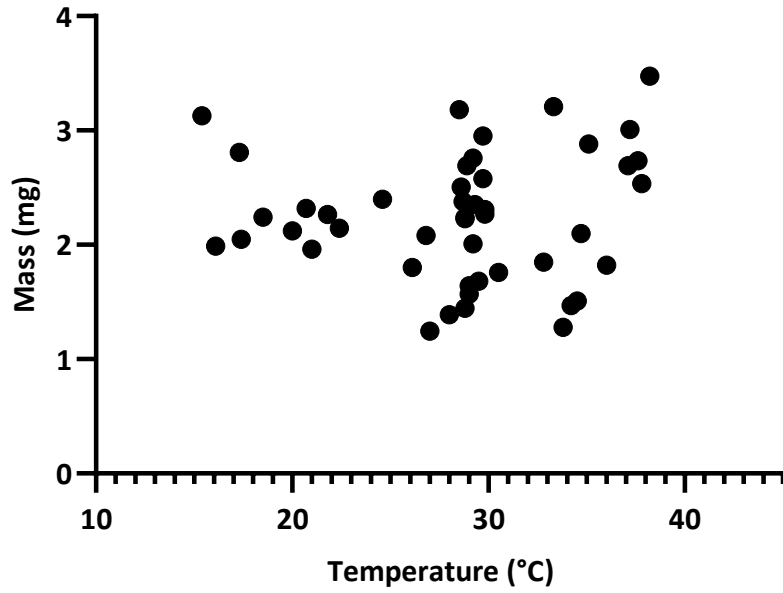


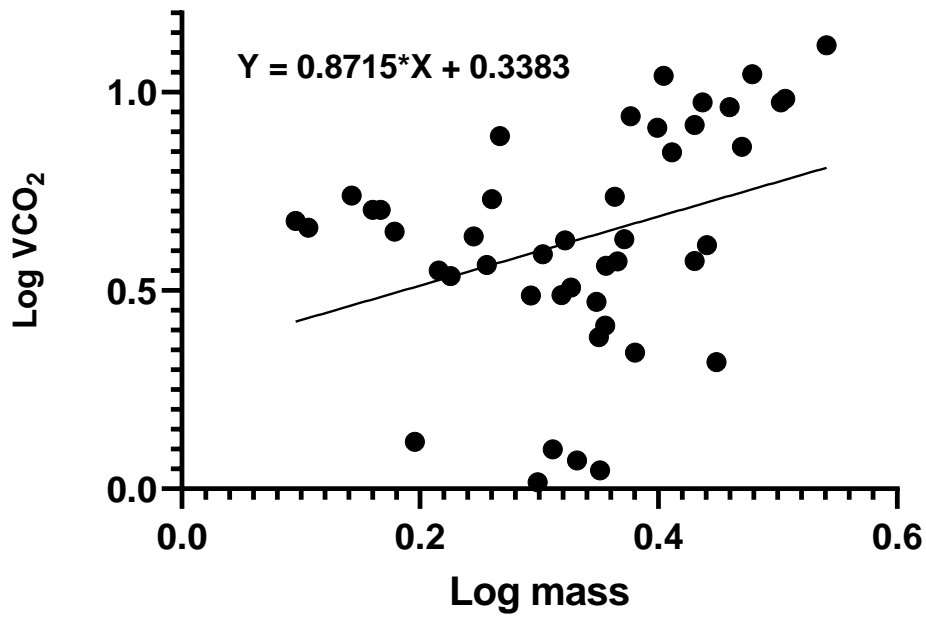
Figure 1:

Tendency for voluntary flight after stimulation as a function of air temperature. Three replicates of 10 female mosquitos were tested at each temperature  $y = -.0439X^2 + 2.236X + (-18.33)$ .



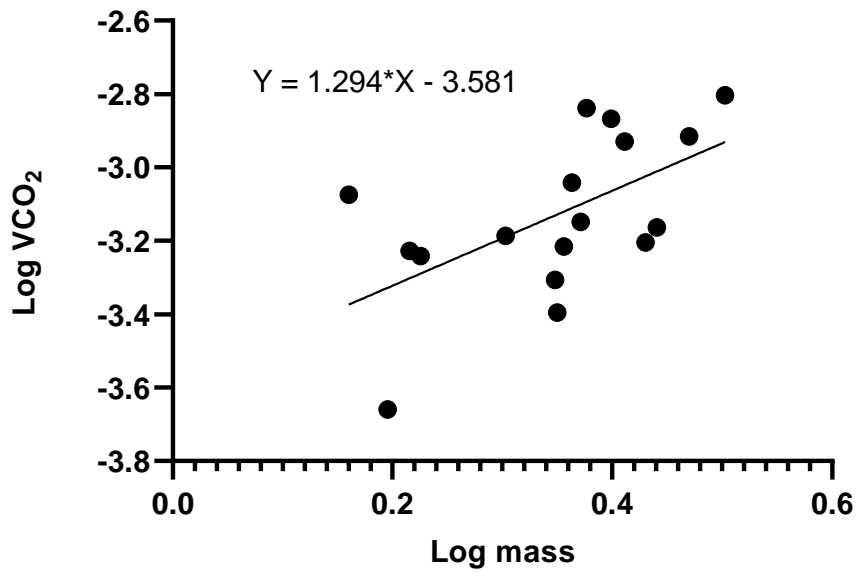


**Figure 2:** There was no significant effect of temperature on mass, indicating the animals were sampled evenly along the full temperature range.



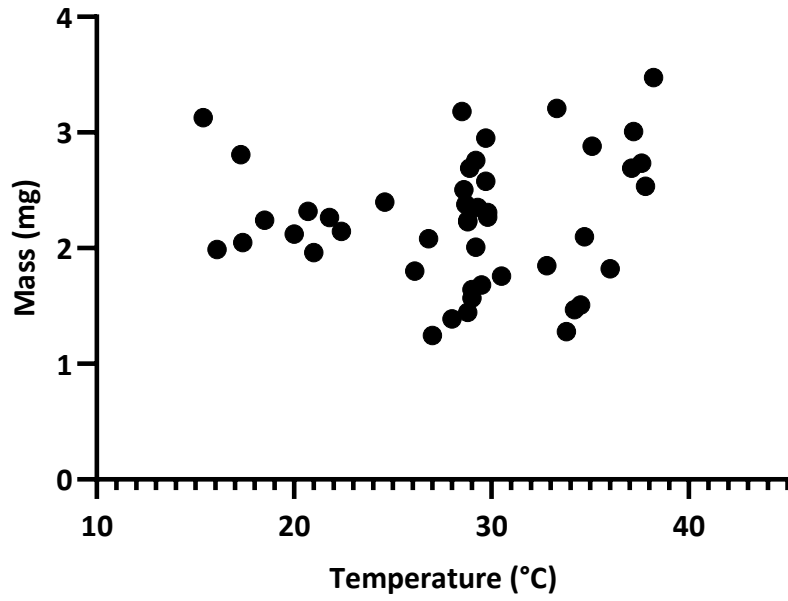
**Figure 3**

LOG VCO<sub>2</sub> (ul/hr) scaled significantly with LOG mass (mg) (pooling all temperatures, linear regression,  $p < 0.05$ ,  $R^2 = 0.1162$ ).



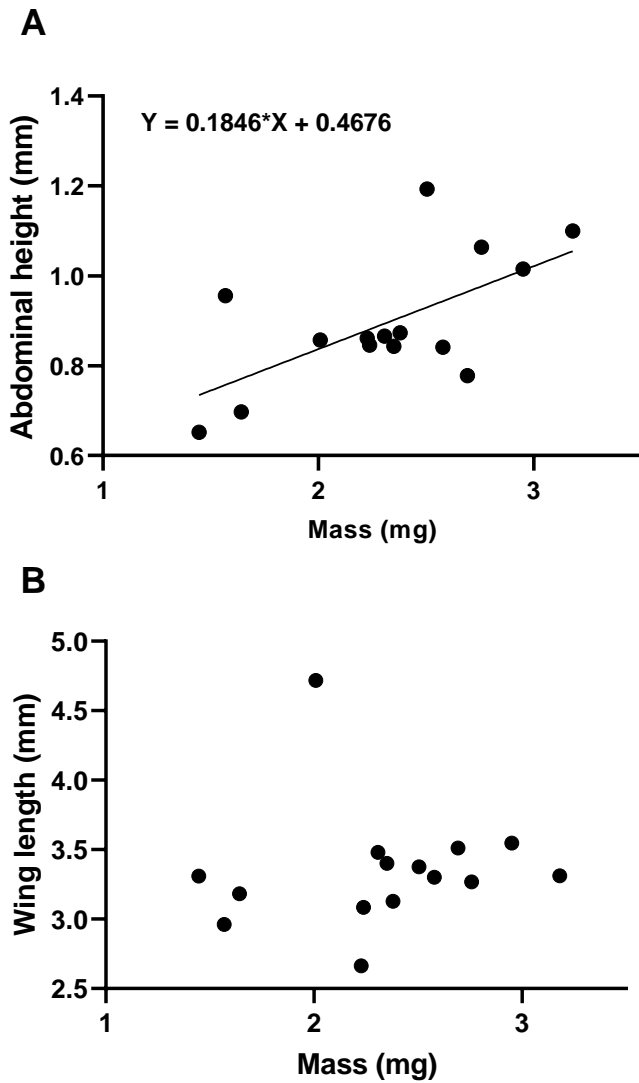
**Figure 4**

At 29°C, log VCO<sub>2</sub> increased significantly with log mass ( $r^2 = 0.333$ ,  $P < 0.05$ , 95% CI = 0.2872 to 2.30)



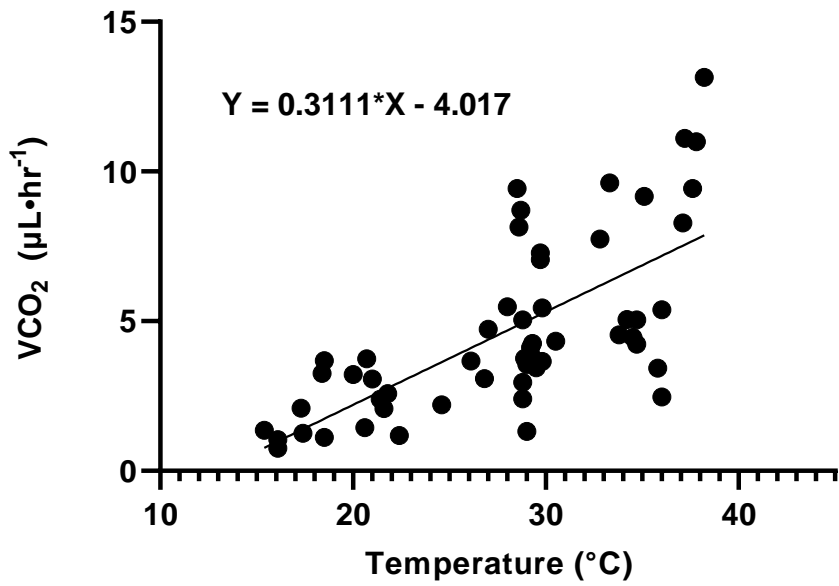
**Figure 5**

Body masses of mosquitos flown did not vary with air temperature ( $p > 0.05$ ).

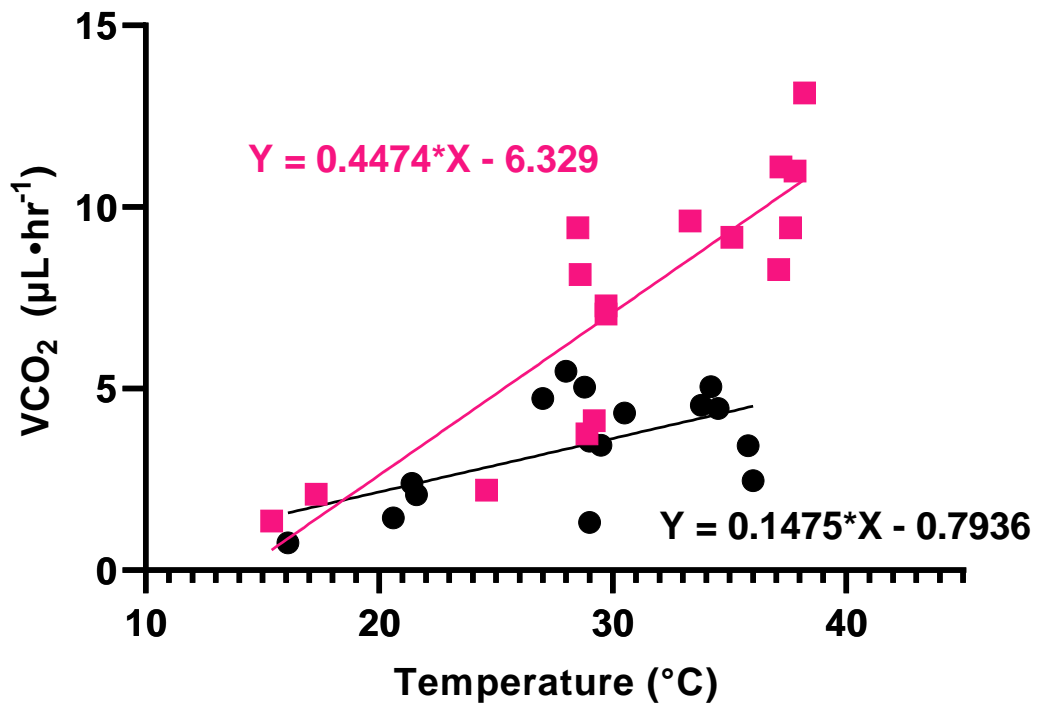


**Figure 6**

For all animals flown, abdominal height was linearly related to body mass ( $p < .05$ ), but unrelated to wing length or thorax diameter (data not shown), suggesting that the variation in mass between animals was primarily due to loading, and not a difference in body size.



**Figure 7** VCO<sub>2</sub> increased with air temperature (pooling all body masses, linear regression,  $p < .0005$ ,  $r^2 = 0.4794$ ).



**Figure 8**

(A) VCO<sub>2</sub> vs air temperature for the lightest 30% of mosquitos, (black circles, linear regression,  $p < 0.05$ ,  $r^2=0.341$ ) and the heaviest 30% of mosquitos (red squares, linear regression,  $p < .0001$ ,  $r^2 = 0.757$ ).

**Tables:**

**Table 1**

**General linear model table for effect air temperature and mass on VCO<sub>2</sub>. Asterisks indicate significant results.**

GLM table

	Estimate	Std. Error	t value	Pr(> t )
(Intercept)	3.99667	3.26177	1.225	0.226088
Air temperature	-0.14114	0.11252	-1.254	0.215422
Mass	-3.20992	1.45904	-2.2	0.032362*
Air temperature: mass	0.19043	0.04911	3.878	0.000303*



**Table 2**

**General linear model table examining the effect of temperature and mass category (top and bottom 30% of mass) on VCO<sub>2</sub>. Asterisks indicate significant results.**

GLM Table	Estimate	Std. Error	t value	Pr(> t )	
(Intercept)	-0.7936	1.98606	-0.4	0.6925	
Temperature	0.14752	0.06834	2.159	0.0396	*
Mass category	-5.53535	2.69134	-2.057	0.04914	*
Temperature: Mass category	0.29991	0.08971	3.343	0.00236	**

**Table 3**

**Pearson correlation matrix for body dimensions and mass for the animals flown at 29°C.**

	Mass	Abdominal height	thorax width	wing length
Mass	1.000	<b>0.627*</b>	0.454	0.063
Abdominal height	<b>0.627*</b>	1.000	0.381	-0.005
thorax width	0.454	0.381	1.000	-0.072
wing length	0.063	-0.005	-0.072	1.000

## Discussion

### Winter cold limits larvae and adults, so mosquitos must overwinter as eggs in Maricopa County

My results show conclusively that the adult and larva life stages are limited during Maricopa County's winter months. Without these seasonal abiotic limitations on the larvae, it's likely that there would be no seasonal die offs, resulting in the year-round continued success of *Aedes aegypti* mosquitos. Without the seasonal die offs, it is probable that peak populations will reach higher levels than I have seen historically, unless increased temperatures during the summer months constrains fitness.

While the egg stage remains the probable source of overwintering survival, eggs still face high mortality in the relatively dry desert winter. This high mortality during the winter season is the likely culprit for the heavily suppressed spring populations which take up to several months to reach the previous year's peak population totals. It is likely that the amount of egg mortality in the overwintering period dictates the rate and peak levels of trapped adults the following season, however a more detailed analysis estimating the actual number of surviving eggs vs peak population levels is needed to confirm these suspicions. If true, wetter than average winter months should be associated with earlier and higher peak populations the following year.

The rapid urbanization of the desert southwest has created an environment that allows these mosquitos to flourish. *Aedes aegypti* mosquitos are well adapted to human hosts (Braks et al., 2006; Crawford et al., 2017), and are very capable of taking advantage

of both our indoor and outdoor infrastructure. Evidence for this has been found in Mexico, where adult *Aedes* are frequently documented hiding in bedrooms, low to the ground, feeding on house pets and human hosts (Barrera et al., 2012; Ponlawat & Harrington, 2005). Populations in Washington, D.C. have been found to make it through the winter months using storm drain systems to survive for at least four years. Similar situations exist in temperate regions of Asia, where multiple life stages take shelter in exposed rooftop water tanks.

Maricopa County Vector Control has documented mosquitoes are utilizing Phoenix's flood irrigation systems as summer shelters, and I have personally found adults exhibiting shade seeking behavior in dense foliage and large runoff culverts, seeking shelter from the Phoenix sun.

If such humid microhabitats could be reduced or eliminated (Healy et al., 2014; Seidahmed & Eltahir, 2016b), desert regions stand a realistic chance of greatly reducing *Aedes aegypti* (and disease prevalence) populations without resorting to non-specific insecticides which place native pollinators at risk. This research highlights the great opportunity that we have as stewards of our desert ecosystems to fund public outreach campaigns that could serve to limit the available breeding habitat of the *Aedes aegypti* mosquito. Actions such as maintaining derelict swimming pools, draining water-bearing refuse, and removing stowaway mosquito eggs from potted plants (Walker et al., 2018) are all activities that can be outsourced to the public, alleviating the financial burden of mosquito abatement from the county and state institutions. Citizens can also choose to utilize xeriscaping, or water-conscious desert landscaping, which would further reduce

the required water usage for urban areas, mitigating the number of humid and aquatic microhabitats for overwintering eggs and larva.

## REFERENCES

- Abramoff, M. D., Magalhães, P. J., & Ram, S. J. (2004). Image processing with ImageJ. *Biophotonics International*, *11*(7), 36–42.
- Angilletta, M. J. (2009). *Thermal Adaptation: A Theoretical and Empirical Synthesis*. Oxford University Press.  
<https://doi.org/10.1093/acprof:oso/9780198570875.001.1>
- Arana-Guardia, R., Baak-Baak, C. M., Loroño-Pino, M. A., Machain-Williams, C., Beaty, B. J., Eisen, L., & García-Rejón, J. E. (2014). Stormwater drains and catch basins as sources for production of *Aedes aegypti* and *Culex quinquefasciatus*. *Acta Tropica*, *134*, 33–42. <https://doi.org/10.1016/j.actatropica.2014.01.011>
- Arrivillaga, J., & Barrera, R. (2004). Food as a limiting factor for *Aedes aegypti* in water-storage containers. *Journal of Vector Ecology*, *11*.
- Au, T. F., & Bonebrake, T. C. (2019). Increased Suitability of Poleward Climate for a Tropical Butterfly (*Euripus nycetelius*) (Lepidoptera: Nymphalidae) Accompanies its Successful Range Expansion. *Journal of Insect Science*, *19*(2).  
<https://doi.org/10.1093/jisesa/iez105>
- Baker, L. A., Brazel, A. J., Selover, N., Martin, C., McIntyre, N., Steiner, F. R., Nelson, A., & Musacchio, L. (2002). Urbanization and warming of Phoenix (Arizona, USA): Impacts, feedbacks and mitigation. *Urban Ecosystems*, *6*(3), 183–203.  
<https://doi.org/10.1023/A:1026101528700>
- Bale, J. S. (2002). Insects and low temperatures: From molecular biology to distributions and abundance. *Philosophical Transactions of the Royal Society of London. Series B: Biological Sciences*, *357*(1423), 849–862.
- Barrera, R., Amador, M., & Clark, G. G. (2006). Ecological factors influencing *Aedes aegypti* (Diptera: Culicidae) productivity in artificial containers in Salinas, Puerto Rico. *Journal of Medical Entomology*, *43*(3), 484–492.  
[https://doi.org/10.1603/0022-2585\(2006\)43\[484:efiaad\]2.0.co;2](https://doi.org/10.1603/0022-2585(2006)43[484:efiaad]2.0.co;2)
- Barrera, R., Bingham, A. M., Hassan, H. K., Amador, M., Mackay, A. J., & Unnasch, T. R. (2012). Vertebrate Hosts of *Aedes aegypti* and *Aedes mediovittatus* (Diptera:

- Culicidae) in Rural Puerto Rico. *Journal of Medical Entomology*, 49(4), 917–921. <https://doi.org/10.1603/ME12046>
- Bar-Zeev, M. (1958). The Effect of Temperature on the Growth Rate and Survival of the Immature Stages of *Aedes aegypti* (L.). *Bulletin of Entomological Research*, 49(1), 157–163. <https://doi.org/10.1017/S0007485300053499>
- Bates, D. (2007). *The lme4 package*.
- Bermudi, P. M. M., Kowalski, F., Menzato, M. M., Ferreira, M. da C., dos Passos, W. B. S., Oku, V. J. A., Kumow, A., Lucio, T. V. F. M., Lima-Camara, T. N., Urbinatti, P. R., & Chiaravalloti, F. (2017). *Aedes aegypti* breeding site in an underground rainwater reservoir: A warning. *Revista de Saúde Pública*, 51. <https://doi.org/10.11606/S1518-8787.2017051000087>
- Bhat, M. A., & Krishnamoorthy, K. (2014). Entomological investigation and distribution of *Aedes* mosquitoes in Tirunelveli, Tamil Nadu, India. *International Journal of Current Microbiology Application Sciences*, 3(10), 253–260.
- Bonebrake, T. C., Boggs, C. L., Stamberger, J. A., Deutsch, C. A., & Ehrlich, P. R. (2014). From global change to a butterfly flapping: Biophysics and behaviour affect tropical climate change impacts. *Proceedings of the Royal Society B: Biological Sciences*, 281(1793), 20141264.
- Brakefield, P., & Mazzotta, V. (2002). Matching field and laboratory environments: Effects of neglecting daily temperature variation on insect reaction norms. *Journal of Evolutionary Biology*, 8, 559–573. <https://doi.org/10.1046/j.1420-9101.1995.8050559.x>
- Braks, M. a. H., Juliano, S. A., & Lounibos, L. P. (2006). Superior reproductive success on human blood without sugar is not limited to highly anthropophilic mosquito species. *Medical and Veterinary Entomology*, 20(1), 53–59. <https://doi.org/10.1111/j.1365-2915.2006.00612.x>
- Buckley, L. B., & Huey, R. B. (2016). How Extreme Temperatures Impact Organisms and the Evolution of their Thermal Tolerance. *Integrative and Comparative Biology*, 56(1), 98–109. <https://doi.org/10.1093/icb/icw004>
- Byttebier, B., De Majo, M., & Fischer, S. (2014). Hatching Response of *Aedes aegypti* (Diptera: Culicidae) Eggs at Low Temperatures: Effects of Hatching Media and

- Storage Conditions. *J Med Entomol*, 51, 97–103.  
<https://doi.org/10.1603/ME13066>
- Campbell, J. B., Andersen, M. K., Overgaard, J., & Harrison, J. F. (2018). Paralytic hypo-energetic state facilitates anoxia tolerance despite ionic imbalance in adult *Drosophila melanogaster*. *Journal of Experimental Biology*, 221(12).  
<https://doi.org/10.1242/jeb.177147>
- Capinha, C., Rocha, J., & Sousa, C. A. (2014). Macroclimate Determines the Global Range Limit of *Aedes aegypti*. *EcoHealth*, 11(3), 420–428.  
<https://doi.org/10.1007/s10393-014-0918-y>
- Carrington, L. B., Armijos, M. V., Lambrechts, L., Barker, C. M., & Scott, T. W. (2013). Effects of Fluctuating Daily Temperatures at Critical Thermal Extremes on *Aedes aegypti* Life-History Traits. *PLOS ONE*, 8(3), e58824.  
<https://doi.org/10.1371/journal.pone.0058824>
- Chadee, D. D., & Martinez, R. (2016). *Aedes aegypti* (L.) in Latin American and Caribbean region: With growing evidence for vector adaptation to climate change? *Acta Tropica*, 156, 137–143.  
<https://doi.org/10.1016/j.actatropica.2015.12.022>
- Chadwick, L. E. (1947). The respiratory quotient of *drosophila* in flight. *The Biological Bulletin*, 93(3), 229–239. <https://doi.org/10.2307/1537971>
- Chang, L.-H., Hsu, E.-L., Teng, H.-J., & Ho, C.-M. (2007). Differential Survival of *Aedes aegypti* and *Aedes albopictus* (Diptera: Culicidae) Larvae Exposed to Low Temperatures in Taiwan. *Journal of Medical Entomology*, 44(2), 205–210.  
<https://doi.org/10.1093/jmedent/44.2.205>
- Chown, S. L., Marais, E., Terblanche, J. S., Klok, C. J., Lighton, J. R. B., & Blackburn, T. M. (2007). Scaling of insect metabolic rate is inconsistent with the nutrient supply network model. *Functional Ecology*, 21(2), 282–290.
- Christophers, S. (1960). *Aedes aegypti* (L.) the yellow fever mosquito: Its life history, bionomics and structure. *Aedes Aegypti (L.) the Yellow Fever Mosquito: Its Life History, Bionomics and Structure*.



- Clements, A. N. (1955). The Sources of Energy for Flight in Mosquitoes. *Journal of Experimental Biology*, 32(3), 547–554.
- Crawford, J. E., Alves, J. M., Palmer, W. J., Day, J. P., Sylla, M., Ramasamy, R., Surendran, S. N., Black, W. C., Pain, A., & Jiggins, F. M. (2017). Population genomics reveals that an anthropophilic population of *Aedes aegypti* mosquitoes in West Africa recently gave rise to American and Asian populations of this major disease vector. *BMC Biology*, 15(1), 16. <https://doi.org/10.1186/s12915-017-0351-0>
- Crozier, L. (2003). Winter warming facilitates range expansion: Cold tolerance of the butterfly *Atalopedes campestris*. *Oecologia*, 135(4), 648–656. <https://doi.org/10.1007/s00442-003-1219-2>
- Curtsinger, J. W., & Laurie-Ahlberg, C. C. (1981). Genetic Variability of Flight Metabolism in *Drosophila Melanogaster*. I. Characterization of Power Output During Tethered Flight. *Genetics*, 98(3), 549–564.
- Darriet, F., & Chandre, F. (2013). Efficacy of six neonicotinoid insecticides alone and in combination with deltamethrin and piperonyl butoxide against pyrethroid-resistant *Aedes aegypti* and *Anopheles gambiae* (Diptera: Culicidae). *Pest Management Science*, 69(8), 905–910. <https://doi.org/10.1002/ps.3446>
- Darveau, C.-A. (2005). Allometric scaling of flight energetics in Panamanian orchid bees: A comparative phylogenetic approach. *Journal of Experimental Biology*, 208(18), 3581–3591. <https://doi.org/10.1242/jeb.01776>
- Day, J. F. (2016). Mosquito Oviposition Behavior and Vector Control. *Insects*, 7(4). <https://doi.org/10.3390/insects7040065>
- De Majo, M. S., Montini, P., & Fischer, S. (2017). Egg Hatching and Survival of Immature Stages of *Aedes aegypti* (Diptera: Culicidae) Under Natural Temperature Conditions During the Cold Season in Buenos Aires, Argentina. *Journal of Medical Entomology*, 54(1), 106–113. <https://doi.org/10.1093/jme/tjw131>
- Dillon, M. E., & Frazier, M. R. (2006). *Drosophila melanogaster* locomotion in cold thin air. *Journal of Experimental Biology*, 209(2), 364–371.

- Dzul-Manzanilla, F., Ibarra-López, J., Bibiano Marín, W., Martini-Jaimes, A., Leyva, J. T., Correa-Morales, F., Huerta, H., Manrique-Saide, P., & Vazquez-Prokopec, G. M. (2017). Indoor Resting Behavior of *Aedes aegypti* (Diptera: Culicidae) in Acapulco, Mexico. *Journal of Medical Entomology*, *54*(2), 501–504. <https://doi.org/10.1093/jme/tjw203>
- Ellington, C. P., & Lighthill, M. J. (1984). The aerodynamics of hovering insect flight. VI. Lift and power requirements. *Philosophical Transactions of the Royal Society of London. B, Biological Sciences*, *305*(1122), 145–181. <https://doi.org/10.1098/rstb.1984.0054>
- Farnesi, L. C., Menna-Barreto, R. F. S., Martins, A. J., Valle, D., & Rezende, G. L. (2015). Physical features and chitin content of eggs from the mosquito vectors *Aedes aegypti*, *Anopheles aquasalis* and *Culex quinquefasciatus*: Connection with distinct levels of resistance to desiccation. *Journal of Insect Physiology*, *83*, 43–52. <https://doi.org/10.1016/j.jinsphys.2015.10.006>
- Feuerbacher, E., Fewell, J. H., Roberts, S. P., Smith, E. F., & Harrison, J. F. (2003). Effects of load type (pollen or nectar) and load mass on hovering metabolic rate and mechanical power output in the honey bee *Apis mellifera*. *Journal of Experimental Biology*, *206*(11), 1855–1865. <https://doi.org/10.1242/jeb.00347>
- Gage, K. L., Burkot, T. R., Eisen, R. J., & Hayes, E. B. (2008). Climate and Vectorborne Diseases. *American Journal of Preventive Medicine*, *35*(5), 436–450. <https://doi.org/10.1016/j.amepre.2008.08.030>
- Giordano, B. V., Gasparotto, A., Liang, P., Nelder, M. P., Russell, C., & Hunter, F. F. (2020). Discovery of an *Aedes* (*Stegomyia*) *albopictus* population and first records of *Aedes* (*Stegomyia*) *aegypti* in Canada. *Medical and Veterinary Entomology*, *34*(1), 10–16. <https://doi.org/10.1111/mve.12408>
- Githeko, A. K., Lindsay, S. W., Confalonieri, U. E., & Patz, J. A. (2000). Climate change and vector-borne diseases: A regional analysis. *Bulletin of the World Health Organization*, *78*(9), 1136–1147.
- Gubler, D. J., & Meltzer, M. (1999). Impact of Dengue/Dengue Hemorrhagic Fever on The Developing World. In K. Margniorosch, F. A. Murphy, & A. J. Shatkin (Eds.), *Advances in Virus Research* (Vol. 53, pp. 35–70). Academic Press. [https://doi.org/10.1016/S0065-3527\(08\)60342-5](https://doi.org/10.1016/S0065-3527(08)60342-5)

- Hanson, S., & Craig, G. (1995). Relationship between cold hardiness and supercooling point in *Aedes albopictus* eggs. *Journal of the American Mosquito Control Association*, 11(1), 35–38. PubMed.
- Harrington, L. C., Scott, T. W., Lerdthusnee, K., Coleman, R. C., Costero, A., Clark, G. G., Jones, J. J., Kitthawee, S., Kittayapong, P., Sithiprasasna, R., & Edman, J. D. (2005). Dispersal of the dengue vector *Aedes aegypti* within and between rural communities. *The American Journal of Tropical Medicine and Hygiene*, 72(2), 209–220.
- Hatchett, S. P. (1946). Winter survival of *Aedes aegypti* in Houston, Texas. *Public Health Reports (Washington, D.C.: 1896)*, 61, 1234–1244.
- Healy, K., Hamilton, G., Crepeau, T., Healy, S., Unlu, I., Farajollahi, A., & Fonseca, D. M. (2014). Integrating the Public in Mosquito Management: Active Education by Community Peers Can Lead to Significant Reduction in Peridomestic Container Mosquito Habitats. *PLOS ONE*, 9(9), e108504. <https://doi.org/10.1371/journal.pone.0108504>
- Heinrich, B. (2013). *The Hot-Blooded Insects: Strategies and Mechanisms of Thermoregulation*. Springer Science & Business Media.
- Honório, N. A., Silva, W. da C., Leite, P. J., Gonçalves, J. M., Lounibos, L. P., & Lourenço-de-Oliveira, R. (2003). Dispersal of *Aedes aegypti* and *Aedes albopictus* (Diptera: Culicidae) in an urban endemic dengue area in the State of Rio de Janeiro, Brazil. *Memórias Do Instituto Oswaldo Cruz*, 98(2), 191–198. <https://doi.org/10.1590/S0074-02762003000200005>
- Horel, J., Splitt, M., Dunn, L., Pechmann, J., White, B., Ciliberti, C., Lazarus, S., Slemmer, J., Zaff, D., & Burks, J. (2002). MESOWEST: COOPERATIVE MESONETS IN THE WESTERN UNITED STATES. *Bulletin of the American Meteorological Society*, 83(2), 211–226. [https://doi.org/10.1175/1520-0477\(2002\)083<0211:MCMITW>2.3.CO;2](https://doi.org/10.1175/1520-0477(2002)083<0211:MCMITW>2.3.CO;2)
- Huey, R. B., & Berrigan, D. (2001). Temperature, Demography, and Ectotherm Fitness. *The American Naturalist*, 158(2), 204–210. <https://doi.org/10.1086/321314>
- Jass, A., Yerushalmi, G. Y., Davis, H. E., Donini, A., & MacMillan, H. A. (2019). An impressive capacity for cold tolerance plasticity protects against ionoregulatory

- collapse in the disease vector *Aedes aegypti*. *Journal of Experimental Biology*, 222(24). <https://doi.org/10.1242/jeb.214056>
- Josephson, R. K. (1981). *Temperature and the mechanical performance of insect muscle*.
- Juarez, J. G., Garcia-Luna, S., Chaves, L. F., Carbajal, E., Valdez, E., Avila, C., Tang, W., Martin, E., Barrera, R., Hemme, R. R., Mutebi, J.-P., Vuong, N., Roark, E. B., Maupin, C. R., Badillo-Vargas, I. E., & Hamer, G. L. (2020). Dispersal of female and male *Aedes aegypti* from discarded container habitats using a stable isotope mark-capture study design in South Texas. *Scientific Reports*, 10(1), 6803. <https://doi.org/10.1038/s41598-020-63670-9>
- Kalnay, E., & Cai, M. (2003). Impact of urbanization and land-use change on climate. *Nature*, 423(6939), 528–531. <https://doi.org/10.1038/nature01675>
- Kampen, H., Jansen, S., Schmidt-Chanasit, J., & Walther, D. (2016). Indoor development of *Aedes aegypti* in Germany, 2016. *Eurosurveillance*, 21(47). <https://doi.org/10.2807/1560-7917.ES.2016.21.47.30407>
- Kearney, M., Porter, W. P., Williams, C., Ritchie, S., & Hoffmann, A. A. (2009). Integrating biophysical models and evolutionary theory to predict climatic impacts on species' ranges: The dengue mosquito *Aedes aegypti* in Australia. *Functional Ecology*, 23(3), 528–538. <https://doi.org/10.1111/j.1365-2435.2008.01538.x>
- Khan Salah Uddin, Ogden Nicholas H., Fazil Aamir A., Gachon Philippe H., Dueymes Guillaume U., Greer Amy L., & Ng Victoria. (n.d.). Current and Projected Distributions of *Aedes aegypti* and *Ae. Albopictus* in Canada and the U.S. *Environmental Health Perspectives*, 128(5), 057007. <https://doi.org/10.1289/EHP5899>
- Khan Salah Uddin, Ogden Nicholas H., Fazil Aamir A., Gachon Philippe H., Dueymes Guillaume U., Greer Amy L., & Ng Victoria. (2020). Current and Projected Distributions of *Aedes aegypti* and *Ae. Albopictus* in Canada and the U.S. *Environmental Health Perspectives*, 128(5), 057007. <https://doi.org/10.1289/EHP5899>

- Kingsolver, J. G. (1983). Ecological Significance of Flight Activity in Colias Butterflies: Implications for Reproductive Strategy and Population Structure. *Ecology*, 64(3), 546–551. <https://doi.org/10.2307/1939974>
- Kingsolver, J. G., Arthur Woods, H., Buckley, L. B., Potter, K. A., MacLean, H. J., & Higgins, J. K. (2011). Complex Life Cycles and the Responses of Insects to Climate Change. *Integrative and Comparative Biology*, 51(5), 719–732. <https://doi.org/10.1093/icb/icr015>
- Kleiber, M. (1947). Body size and metabolic rate. *Physiological Reviews*, 27(4), 511–541.
- Košťál, V., Vambera, J., & Bastl, J. (2004). On the nature of pre-freeze mortality in insects: Water balance, ion homeostasis and energy charge in the adults of *Pyrrhocoris apterus*. *Journal of Experimental Biology*, 207(9), 1509–1521. <https://doi.org/10.1242/jeb.00923>
- Lehmann, F. O. (1999). Ambient temperature affects free-flight performance in the fruit fly *Drosophila melanogaster*. *Journal of Comparative Physiology. B, Biochemical, Systemic, and Environmental Physiology*, 169(3), 165–171. <https://doi.org/10.1007/s003600050207>
- Lima, A., Lovin, D. D., Hickner, P. V., & Severson, D. W. (2016). Evidence for an Overwintering Population of *Aedes aegypti* in Capitol Hill Neighborhood, Washington, DC. *The American Journal of Tropical Medicine and Hygiene*, 94(1), 231–235. <https://doi.org/10.4269/ajtmh.15-0351>
- Liu-Helmersson, J., Rocklöv, J., Sewe, M., & Brännström, Å. (2019). Climate change may enable *Aedes aegypti* infestation in major European cities by 2100. *Environmental Research*, 172, 693–699. <https://doi.org/10.1016/j.envres.2019.02.026>
- Lubawy, J., Urbański, A., Colinet, H., Pflüger, H.-J., & Marciniak, P. (2020). Role of the Insect Neuroendocrine System in the Response to Cold Stress. *Frontiers in Physiology*, 11. <https://doi.org/10.3389/fphys.2020.00376>
- MacDonald, G. M. (2010). Water, climate change, and sustainability in the southwest. *Proceedings of the National Academy of Sciences*, 107(50), 21256–21262. <https://doi.org/10.1073/pnas.0909651107>

- MacMillan, H. A., & Sinclair, B. J. (2011). The role of the gut in insect chilling injury: Cold-induced disruption of osmoregulation in the fall field cricket, *Gryllus pennsylvanicus*. *Journal of Experimental Biology*, *214*(5), 726–734. <https://doi.org/10.1242/jeb.051540>
- Marden, J. H. (1989). Effects of Load-Lifting Constraints on the Mating System of a Dance Fly. *Ecology*, *70*(2), 496–502. <https://doi.org/10.2307/1937553>
- Marden, J. H. (1994). From damselflies to pterosaurs: How burst and sustainable flight performance scale with size. *American Journal of Physiology-Regulatory, Integrative and Comparative Physiology*, *266*(4), R1077–R1084.
- Marinho, R. A., Beserra, E. B., Bezerra-Gusmão, M. A., Porto, V. de S., Olinda, R. A., & Dos Santos, C. A. (2016). Effects of temperature on the life cycle, expansion, and dispersion of *Aedes aegypti* (Diptera: Culicidae) in three cities in Paraiba, Brazil. *Journal of Vector Ecology*, *41*(1), 1–10.
- Marshall, K. E., & Sinclair, B. J. (2012). The impacts of repeated cold exposure on insects. *Journal of Experimental Biology*, *215*(10), 1607–1613. <https://doi.org/10.1242/jeb.059956>
- Metzger, M., Yoshimizu, M., Padgett, K., Hu, R., & Kramer, V. (2017). Detection and Establishment of *Aedes aegypti* and *Aedes albopictus* (Diptera: Culicidae) Mosquitoes in California, 2011-2015. *Journal of Medical Entomology*, *54*. <https://doi.org/10.1093/jme/tjw237>
- Mronz, M., & Lehmann, F.-O. (2008). The free-flight response of *Drosophila* to motion of the visual environment. *Journal of Experimental Biology*, *211*(13), 2026–2045. <https://doi.org/10.1242/jeb.008268>
- Nayar, J. K., & Van Handel, E. (1971). The fuel for sustained mosquito flight. *Journal of Insect Physiology*, *17*(3), 471–481. [https://doi.org/10.1016/0022-1910\(71\)90026-6](https://doi.org/10.1016/0022-1910(71)90026-6)
- Overgaard, J., & MacMillan, H. A. (2017). The Integrative Physiology of Insect Chill Tolerance. *Annual Review of Physiology*, *79*(1), 187–208. <https://doi.org/10.1146/annurev-physiol-022516-034142>
- Parmesan, C., Ryrholm, N., Stefanescu, C., Hill, J. K., Thomas, C. D., Descimon, H., Huntley, B., Kaila, L., Kullberg, J., Tammaru, T., Tennent, W. J., Thomas, J. A.,

- & Warren, M. (1999). Poleward shifts in geographical ranges of butterfly species associated with regional warming. *Nature*, 399(6736), 579–583. <https://doi.org/10.1038/21181>
- Pless, E., & Raman, V. (2018). Origin of *Aedes aegypti* In Clark County, Nevada. *Journal of the American Mosquito Control Association*, 34(4), 302–305. <https://doi.org/10.2987/18-6776.1>
- Ponlawat, A., & Harrington, L. C. (2005). Blood Feeding Patterns of *Aedes aegypti* and *Aedes albopictus* in Thailand. *Journal of Medical Entomology*, 42(5), 844–849. <https://doi.org/10.1093/jmedent/42.5.844>
- R Core Team, R. C. T. (2013). *R: A language and environment for statistical computing*.
- Rinehart, J. P., Li, A., Yocum, G. D., Robich, R. M., Hayward, S. A. L., & Denlinger, D. L. (2007). Up-regulation of heat shock proteins is essential for cold survival during insect diapause. *Proceedings of the National Academy of Sciences*, 104(27), 11130–11137. <https://doi.org/10.1073/pnas.0703538104>
- Rivas, M. L., Esteban, N., & Marco, A. (2019). Potential male leatherback hatchlings exhibit higher fitness which might balance sea turtle sex ratios in the face of climate change. *Climatic Change*, 156(1), 1–14. <https://doi.org/10.1007/s10584-019-02462-1>
- Roberts, S. P., & Harrison, J. F. (1998). Mechanisms of Thermoregulation in Flying Bees1. *American Zoologist*, 38(3), 492–502. <https://doi.org/10.1093/icb/38.3.492>
- Rochlin, I., Ninivaggi, D. V., Hutchinson, M. L., & Farajollahi, A. (2013). Climate change and range expansion of the Asian tiger mosquito (*Aedes albopictus*) in Northeastern USA: Implications for public health practitioners. *PloS One*, 8(4), e60874. <https://doi.org/10.1371/journal.pone.0060874>
- Rowley, W. A., & Graham, C. L. (1968a). The effect of age on the flight performance of female *Aedes aegypti* mosquitoes. *Journal of Insect Physiology*, 14(5), 719–728. [https://doi.org/10.1016/0022-1910\(68\)90230-8](https://doi.org/10.1016/0022-1910(68)90230-8)
- Rowley, W. A., & Graham, C. L. (1968b). The effect of temperature and relative humidity on the flight performance of female *Aedes aegypti*. *Journal of Insect Physiology*, 14(9), 1251–1257. [https://doi.org/10.1016/0022-1910\(68\)90018-8](https://doi.org/10.1016/0022-1910(68)90018-8)

- Ryan, S. J., Carlson, C. J., Mordecai, E. A., & Johnson, L. R. (2019). Global expansion and redistribution of Aedes-borne virus transmission risk with climate change. *PLOS Neglected Tropical Diseases*, *13*(3), e0007213. <https://doi.org/10.1371/journal.pntd.0007213>
- Saleeza, S. N. R., Norma-Rashid, Y., & Sofian-Azirun, M. (2011). Mosquitoes larval breeding habitat in urban and suburban areas, Peninsular Malaysia. *World Acad Sci Eng Technol*, *58*(58), 569–573.
- Seidahmed, O. M. E., & Eltahir, E. A. B. (2016a). A Sequence of Flushing and Drying of Breeding Habitats of *Aedes aegypti* (L.) Prior to the Low Dengue Season in Singapore. *PLOS Neglected Tropical Diseases*, *10*(7), e0004842. <https://doi.org/10.1371/journal.pntd.0004842>
- Seidahmed, O. M. E., & Eltahir, E. A. B. (2016b). A Sequence of Flushing and Drying of Breeding Habitats of *Aedes aegypti* (L.) Prior to the Low Dengue Season in Singapore. *PLOS Neglected Tropical Diseases*, *10*(7), e0004842. <https://doi.org/10.1371/journal.pntd.0004842>
- Shipley, J. R., Twining, C. W., Taff, C. C., Vitousek, M. N., Flack, A., & Winkler, D. W. (2020). Birds advancing lay dates with warming springs face greater risk of chick mortality. *Proceedings of the National Academy of Sciences*, *117*(41), 25590–25594. <https://doi.org/10.1073/pnas.2009864117>
- Shope R. (1991). Global climate change and infectious diseases. *Environmental Health Perspectives*, *96*, 171–174. <https://doi.org/10.1289/ehp.9196171>
- Sota, T., & Mogi, M. (1992a). Survival time and resistance to desiccation of diapause and non-diapause eggs of temperate *Aedes* (*Stegomyia*) mosquitoes. *Entomologia Experimentalis et Applicata*, *63*(2), 155–161. <https://doi.org/10.1111/j.1570-7458.1992.tb01570.x>
- Sota, T., & Mogi, M. (1992b). Interspecific variation in desiccation survival time of *Aedes* (*Stegomyia*) mosquito eggs is correlated with habitat and egg size. *Oecologia*, *90*(3), 353–358. <https://doi.org/10.1007/BF00317691>
- Souza, R. L., Mugabe, V. A., Paploski, I. A. D., Rodrigues, M. S., Moreira, P. S. dos S., Nascimento, L. C. J., Roundy, C. M., Weaver, S. C., Reis, M. G., Kitron, U., & Ribeiro, G. S. (2017). Effect of an intervention in storm drains to prevent *Aedes aegypti* reproduction in Salvador, Brazil. *Parasites & Vectors*, *10*(1), 328. <https://doi.org/10.1186/s13071-017-2266-6>



- Sprigg, W. A., & Hinkley, T. K. (2000). *Preparing for a changing climate: The potential consequences of climate variability and change: Southwest* [Report]. USGS Publications Warehouse. <http://pubs.er.usgs.gov/publication/70178471>
- Stevenson, R. D. (1985a). Body size and limits to the daily range of body temperature in terrestrial ectotherms. *The American Naturalist*, *125*(1), 102–117.
- Stevenson, R. D. (1985b). The relative importance of behavioral and physiological adjustments controlling body temperature in terrestrial ectotherms. *The American Naturalist*, *126*(3), 362–386.
- Strohm, null, & Linsenmair, null. (1997). Female size affects provisioning and sex allocation in a digger wasp. *Animal Behaviour*, *54*(1), 23–34.  
<https://doi.org/10.1006/anbe.1996.0431>
- Travis, J. M. J. (2003). Climate change and habitat destruction: A deadly anthropogenic cocktail. *Proceedings of the Royal Society of London. Series B: Biological Sciences*, *270*(1514), 467–473. <https://doi.org/10.1098/rspb.2002.2246>
- Tsuda, Y., & Takagi, M. (2001). Survival and Development of *Aedes aegypti* and *Aedes albopictus* (Diptera: Culicidae) Larvae Under a Seasonally Changing Environment in Nagasaki, Japan. *Environmental Entomology*, *30*(5), 855–860.  
<https://doi.org/10.1603/0046-225X-30.5.855>
- Tsuji, J. S., Kingsolver, J. G., & Watt, W. B. (1986). Thermal physiological ecology of *Colias* butterflies in flight. *Oecologia*, *69*(2), 161–170.  
<https://doi.org/10.1007/BF00377616>
- Tun-Lin, W., Burkot, T. R., & Kay, B. H. (2000). Effects of temperature and larval diet on development rates and survival of the dengue vector *Aedes aegypti* in north Queensland, Australia. *Medical and Veterinary Entomology*, *14*(1), 31–37.  
<https://doi.org/10.1046/j.1365-2915.2000.00207.x>
- Valerio, L., Facchinelli, L., Ramsey, J. M., & Scott, T. W. (2012). Dispersal of Male *Aedes aegypti* in a Coastal Village in Southern Mexico. *The American Journal of Tropical Medicine and Hygiene*, *86*(4), 665–676.  
<https://doi.org/10.4269/ajtmh.2012.11-0513>

- Vontas, J., Kioulos, E., Pavlidi, N., Morou, E., della Torre, A., & Ranson, H. (2012). Insecticide resistance in the major dengue vectors *Aedes albopictus* and *Aedes aegypti*. *Pesticide Biochemistry and Physiology*, *104*, 126–131. <https://doi.org/10.1016/j.pestbp.2012.05.008>
- Wagner, D. L., & Van Driesche, R. G. (2010). Threats posed to rare or endangered insects by invasions of nonnative species. *Annual Review of Entomology*, *55*, 547–568. <https://doi.org/10.1146/annurev-ento-112408-085516>
- Walck, J. L., Hidayati, S. N., Dixon, K. W., Thompson, K., & Poschlod, P. (2011). Climate change and plant regeneration from seed. *Global Change Biology*, *17*(6), 2145–2161. <https://doi.org/10.1111/j.1365-2486.2010.02368.x>
- Walker, K. R., Williamson, D., Carrière, Y., Reyes-Castro, P. A., Haenchen, S., Hayden, M. H., Jeffrey Gutierrez, E., & Ernst, K. C. (2018). Socioeconomic and Human Behavioral Factors Associated With *Aedes aegypti* (Diptera: Culicidae) Immature Habitat in Tucson, AZ. *Journal of Medical Entomology*, *55*(4), 955–963. <https://doi.org/10.1093/jme/tjy011>
- Wang, H.-S., Zhou, C.-S., Guo, W., & Kang, L. (2006). Thermoperiodic acclimations enhance cold hardiness of the eggs of the migratory locust. *Cryobiology*, *53*(2), 206–217. <https://doi.org/10.1016/j.cryobiol.2006.06.003>
- Wolf, Th. J., Schmid-Hempel, P., Ellington, C. P., & Stevenson, R. D. (1989). Physiological Correlates of Foraging Efforts in Honey-Bees: Oxygen Consumption and Nectar Load. *Functional Ecology*, *3*(4), 417–424. <https://doi.org/10.2307/2389615>
- Wong, J., Stoddard, S. T., Astete, H., Morrison, A. C., & Scott, T. W. (2011). Oviposition Site Selection by the Dengue Vector *Aedes aegypti* and Its Implications for Dengue Control. *PLOS Neglected Tropical Diseases*, *5*(4), e1015. <https://doi.org/10.1371/journal.pntd.0001015>
- Young, J. F. (1967). Humidity control in the laboratory using salt solutions—A review. *Journal of Applied Chemistry*, *17*(9), 241–245. <https://doi.org/10.1002/jctb.5010170901>
- Yuan, F., & Bauer, M. E. (2007). Comparison of impervious surface area and normalized difference vegetation index as indicators of surface urban heat island effects in

Landsat imagery. *Remote Sensing of Environment*, 106(3), 375–386.  
<https://doi.org/10.1016/j.rse.2006.09.003>