THE EFFECTS OF TEMPERATURE AND HUMIDITY ON THE EGGS OF *AEDES AEGYPTI* (L.) AND *AEDES ALBOPICTUS* (SKUSE) IN TEXAS

A Dissertation

by

CATHERINE ZINDLER DICKERSON

Submitted to the Office of Graduate Studies of
Texas A&M University
in partial fulfillment of the requirements for the degree of

DOCTOR OF PHILOSOPHY

December 2007

Major Subject: Entomology

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Approved by:

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ABSTRACT

The Effects of Temperature and Humidity on the Eggs of *Aedes aegypti* (L.) and *Aedes albopictus* (Skuse) in Texas. (December 2007)

Catherine Zindler Dickerson, B.S., Texas A&M University

Chair of Advisory Committee: Dr. Jimmy K. Olson

Causative influences that impact the separation of *Ae. aegypti* and *Ae. albopictus* populations in different geographic areas were determined, as well as how they are affected by the abiotic conditions as seen in the habitats they frequent in Texas. The eggs of *Ae. albopictus* and *Ae. aegypti* collected from McAllen and Brownsville, Texas, and laboratory populations of these two species were subjected to 25 different temperature and relative humidity conditions for up to three months. In most treatments, *Ae. aegypti* eggs had a greater percent hatch than *Ae. albopictus*, regardless of temperature or relative humidity. With an increase in relative humidity, the percent hatch for both species increased, but at the higher temperatures of 32˚ and 35˚C the amount of time the eggs were exposed to those temperatures had a greater negative effect on the percent hatch than did the positive effect of increase in relative humidity.

The surface area, volume and surface-area-to-volume ratio of *Ae. aegypti* and *Ae. albopictus* eggs with and without the chorionic egg pad, and the size of the chorionic egg pad were calculated for fifty eggs of each species of mosquito from populations collected in McAllen and Brownsville and from the laboratory populations. *Ae. aegypti* had a larger egg volume, and a larger surface area; but, it is likely their larger egg pad

compensates for this high surface-area-to-volume ratio by holding moisture along the egg's surface and that the egg pad is associated with the high desiccation resistance seen in *Ae. aegypti* eggs.

Development rates for both species of mosquitoes from populations collected in Galveston and Brownsville, Texas, and laboratory populations were produced by measuring the development time from a hatched egg to the adult at seven temperatures. The temperature optima (28˚-33˚C) were similar for all populations; however, the rate of development for *Ae. aegypti* was significantly faster at the temperature optima. It is likely that this faster development rate in the *Ae. aegypti* population helps to maintain a population in climates that have this range of temperatures given that *Ae. albopictus* is a superior competitor in the larval and adult stages.

DEDICATION

I dedicate this dissertation to Jacqueline Fisher, the wisest woman I have ever known. I am privileged to have her friendship and blessed to be able to call her mom.

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I would like to thank my committee chair, Dr. Jimmy K. Olson without whom none of this would have been possible. I would also like to thank my committee members, Dr. Leon Russell, Dr. Pete Teel and Dr. Kirk Winemiller, for the time and energy that they put forth into guiding me throughout the course of this research. I am especially thankful to Dr. Jim Woolley, who allowed me the use of his imaging equipment which was essential to the completion of the chorionic egg pad study. I also thank Dr. Mark Wright for all his knowledge and patience, and Dr. Bobb Gorena and Dr. James Austin for their guidance in statistical matters.

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always had a smile for me, and my husband who sacrificed his own wants many times in order to support me, and did so with a kind heart.

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CHAPTER I

INTRODUCTION

The yellow fever mosquito, *Aedes aegypti* (L.), is a floodwater species that, in the U.S., lays eggs and completes its life cycle primarily in artificial containers. They are warm weather mosquitoes and often occur in high numbers after floods. Accounts of *Ae. aegypti* in the Americas date back before the 1650's (Spielman and D'Antonio 2001). *Ae. aegypti* is a primary vector for such anthroponoses as Yellow fever and Dengue fever.

In August of 1985, *Aedes albopictus* (Skuse), the Asian tiger mosquito, was discovered in a tire dump in Houston, Texas (Sprenger and Wuithiranygool 1986). *Ae. albopictus* is also a floodwater species and a secondary vector of a multitude of diseases including West Nile and Dengue fever. *Ae. albopictus* may thus amplify a disease outbreak. Since 1985, *Ae. albopictus* has become established throughout the United States as far north as Nebraska and into southern Florida (Moore 1999). *Ae. albopictus* has been found but is not considered to be established in California, New Mexico and Washington (Moore 1999). As *Ae. albopictus* extended its distribution in the southern United States, it appeared to displace *Ae. aegypti* (Francy et al. 1990, Hobbs et al. 1991). This is in contrast to observations in Asia where *Ae. albopictus* was endogenous and *Ae. aegypti* was the "invader". Stanton (1920) first reported the presence of *Ae. aegypti* in Asia. Subsequently, *Ae. albopictus* populations seemed to be displaced by *Ae. aegypti* (MacDonald 1956, Chan et al. 1971).

This dissertation follows the style of the *Journal of the American Mosquito Control Association*.

Even though the specific circumstances in Asia and the United States were different, there are many situational similarities. In both instances, *Ae. albopictus* was found predominantly in areas with dense vegetation and low housing density, while *Ae. aegypti* was predominantly found in urban areas with little vegetation and numerous artificial containers that could hold water.

It has become increasingly important to know with more precision, which geographic locations in Texas can support which species. This is especially true along the Texas-Mexico border due to the threat of Dengue Fever, which is prevalent in mosquito populations in Mexico and has recently spread into the Texas Rio Grande Valley (CDC 2007). There are four serotypes of Dengue, and following the exposure of one serotype with another serotype increases the likelihood of the most severe presentation of this virus, which is Dengue Hemorrhagic Fever (DHF). The CDC recently announced that after a serosurvey on the Dengue virus in the Brownsville, Texas human population, 38% of the Brownsville residents tested were positive for antibodies in response to previous exposure to the Dengue virus. Similar results were found in other border towns, and have led the CDC to announce the possibility that the residents along the Texas-Mexico border may now be more susceptible to DHF (CDC 2007).

Prevention of a disease outbreak such as Dengue requires one or more of the components of the disease cycle must be removed, i.e. the pathogen, vector, reservoir and/or susceptible host. Along the Texas-Mexico border, people from Texas travel into Mexico regularly (personal contact: Public Health Inspector, Carlos Perez, McAllen

Health department) and thus pose a threat as carriers (reservoirs) of Dengue when they re-enter Texas. In this situation, it is unrealistic to rely on breaking the disease cycle by targeting the reservoir, because people infected with dengue may not exhibit symptoms for over a week, at which point they could have already re-entered Texas and begun to infect mosquitoes. According to the CDC, approximately 100 to 200 suspected cases of Dengue are introduced into the United States each year by travelers. There is currently no vaccine for this virus (pathogen), and it is unrealistic to remove Texans (susceptible hosts) from the locations in the state where the virus is introduced. Thus, the most practical way to prevent this disease from spreading further into Texas is to target the vectors, i.e. *Ae. aegypti* and *Ae. albopictus*.

It is relevant which mosquito species is in which area because their vector potentials for Dengue virus transmission are different. *Ae. aegypti* (primary vector), when present, can cause an epidemic of Dengue Fever, which could be made worse by the addition of *Ae. albopictus* (secondary vector) (Mullen and Durden 2002). *Ae. albopictus* alone cannot readily perpetuate the disease cycle. Knowledge of the species present in an area threatened by Dengue, will better equip health officials to combat the disease.

Several experiments suggest that the abiotic conditions in the different habitats frequented by *Ae. aegypti* and *Ae. albopictus* determine which species dominates which habitat (Fontenille and Rodhain 1989, Sota and Mogi 1992, and Juliano et al. 2002**).** According to Mogi et al. (1996), comparative studies describing environments that favor each species are essential to predicting population trends in changing environments. The

larval and adult stages of *Ae. albopictus* and *Ae. aegypti* have been extensively studied with regards to their climatic requirements; so, the research described herein focused primarily on the egg stage of these two species. The egg stage is important because it is the stage in which these floodwater mosquitoes wait out unfavorable climatic conditions (Sota and Mogi 1992). Once climatic requirements for the eggs of each species have been determined, it may be possible to determine where each species will live depending upon the seasonal climate of a given location.

Thomson (1938) noted that "Under natural conditions in the field, the interpretation of behavior is extremely difficult and necessarily inconclusive because so many factors vary at the same time". For this reason, the variables for this study have been restricted to temperature, relative humidity (RH) and time. Due to small size and large surface-area-to-volume ratio, eggs are at high risk of desiccation. Thus, specific aspects of climate that should most influence the eggs are temperature and humidity. The eggs must wait for rainfall to stimulate hatching, so the length of time that eggs can tolerate various temperature and humidity conditions is also a factor.

Mosquitoes of the same species from different regions of Texas could respond very differently to environmental tests. Insects evolve or acclimate to their specific habitats resulting in insects of the same species from different regions performing differently under the same conditions (Campbell et al. 1974 and Mogi et al. 1996). To compensate and account for this variation, this research was conducted with three different strains of each mosquito species for a total of six different mosquito populations.

The objectives of this research were to determine what causative influences on the eggs of *Ae. aegypti* and Ae. *albopictus* impact their separation into different geographic areas, and how these influences are affected by different origins of populations specifically from South Texas due to the increasing threat of Dengue fever for which these two species are vectors.

CHAPTER II

LITERATURE REVIEW

Interactions between *Ae. albopictus* and *Ae. aegypti* were first observed in Asia. *Ae. albopictus* mosquitoes are thought to have originated from northern Asia (Hawley et al. 1987). *Ae. aegypti*, which is endogenous to Africa, was first reported in Asia in 1920 (Stanton 1920). As time passed, *Ae. aegypti* appeared to move into the urban parts of Asia, displacing *Ae. albopictus* (MacDonald 1956, Chan et al. 1971). The two species separated into distinctly different habitats: *Ae. albopictus* was found primarily in rural and suburban-forested habitats with trees and dense vegetation, while *Ae. aegypti* was found in urban areas with few trees or other vegetation. MacDonald (1956) attributed this separation mainly to available water sources. The Asian people often had water-filled ant traps around their houses where *Ae. aegypti* larvae could be found. MacDonald (1956) noted that the native Malayans were not in the practice of storing water around their houses, and they had fewer problems with *Ae. aegypti* than others who did. Similarly, in Venezuela *Ae. aegypti* is most prevalent in the areas of poor housing without indoor plumbing or piped water, which necessitates water being stored in containers (Barrera et al. 1993). Similarly, when *Ae. aegypti* invaded Asia, most of the people lived in slum houses or shop houses (MacDonald 1956) that did not have good piped water supplies. In contrast, when *Ae. albopictus* entered the United States, areas initially infested had houses with indoor plumbing, and few people used water-filled ant traps. Many people in urban areas had planted trees and other vegetation

around their houses, thus diminishing the habitat favorable for *Ae. aegypti* and creating an ideal habitat for *Ae. albopictus***.**

Many experiments have been conducted with these two species to examine the mechanisms of displacement. In experiments testing adult survivorship, *Ae. aegypti* lived longer then *Ae. albopictus* using Indian strains of both species (Bhattacharya and Dey 1969) and Vietnamese strains (Hein 1976). Conflicting results were shown by Galliard (1962) with a Vietnamese strain of both species. MacDonald (1956) compared emergence rates of *Ae. albopictus* and *Ae. aegypti* adults from open jars and tree holes and found that *Ae. aegypti* emerged at a significantly higher rate than *Ae. albopictus* in both experiments.

Studies comparing fecundity of the two species have produced conflicting results. Hein (1976), Sucharit and Tumrasvin (1981) and Black et al. (1989) compared total lifetime fecundity of the two species, and they reported *Ae. aegypti* to be more fecund. Galliard (1962) found *Ae. albopictus* to be more fecund. Soekiman et al. (1984) observed that *Ae. aegypti* laid more eggs per batch than *Ae. albopictus* with a Java strain of both. In Hein's (1976) experiments, *Ae. albopictus* laid more eggs per milligram of blood ingested than did *Ae. aegypti*. Sames (1999) found egg production of *Ae. albopictus* dropped significantly at high temperatures, whereas *Ae. aegypti* had only a slight decrease. Black et al. (1989) concluded that *Ae. aegypti* males are more sexually aggressive than *Ae. albopictus* males, but when harassed by *Ae. aegypti* males, there was no effect on oviposition rates of *Ae. albopictus* females. In direct contrast, laboratory

studies with Louisiana strains of each species found male *Ae. albopictus* more sexually aggressive in attempting to mate with female *Ae. aegypti* as compared to *Ae. aegypti* males in their attempt to mate with female *Ae. albopictus* (Nasci et al 1989). The time required for both species to take a blood meal was observed to be the same by Soekiman et al. (1984), but Hein (1976) reported that *Ae. albopictus* takes longer to feed than *Ae. aegypti*.

Chan et al. (1971) tested larval competition in tires and jars containing a sub-optimal amount of food. They observed a slightly higher emergence rate for *Ae. aegypti* in the jars, but no difference in tires. Sucharit et al. (1978) found that *Ae. albopictus* larvae developed faster when reared with *Ae. aegypti* with optimal food available, but *Ae. aegypti* out-competed *Ae. albopictus* under conditions of sub-optimal food in mixed populations. Sames (1999) found that *Ae. albopictus* larvae developed faster than *Ae. aegypti* at low temperatures, such as 16 °C, but Lounibos et al. (2002) found no significant effect of larval competition at temperatures between 24 and 30°C. Sames (1999) also observed that development time is not effected by blood meal source, i.e., chicken or human. An increase in the survival rate of *Ae. aegypti* larvae was observed when they were reared in mixed populations with *Ae. albopictus* under both optimal and sub-optimal diets (Black et al. 1989) and resulted in only a slight reduction in survival rates of *Ae. albopictus*. In South Florida, Juliano (1998) found that *Ae. albopictus* larvae experienced positive population growth as compared to *Ae. aegypti* in tires with low resources and dense populations. Juliano et al. (2004) demonstrated that inter-specific

competition with *Ae. albopictus* larvae observed in field experiments using cemetery vases significantly reduced survival in *Ae. aegypti* under naturally occurring density levels.

It has been shown that the protozoan parasite, *Ascogregarina taiwanesis*, typically found in *Ae. albopictus* larvae can infect the larvae of *Ae. aegypti* and in some cases result in a high rate of mortality (Blackmore et al 1995). However, the protozoan parasite of *Ae. aegypti* larvae, *Ascogregarina culicis*, does not infect *Ae. albopictus*. Garcia et al. (1994) has determined that *Ascogregarina taiwanesis* is very rare in nature and actually infects both species equally.

The geographic locations where *Ae. albopictus* and *Ae. aegypti* live may depend much more on differing climatic requirements than on interactive competition. In a study on the island of Madagascar that compared temperature, number of dry months and millimeters of rainfall to abundance of *Ae. aegypti* and *Ae. albopictus,* Fontenille and Rodhain (1989) found that *Ae. albopictus* dominated places with more than 1,000mm of rainfall annually and no more than six dry months. *Ae. aegypti* was the predominate species in areas that received less than 2,000mm of annual rainfall and experienced up to nine dry months a year. Other studies have reported a correlation between rainfall and adult *Ae. albopictus* abundance (Khan 1980), oviposition rates (Ho et al. 1971), and biting rates (Gould et al. 1970). At the end of the dry season in Chiang Mai, Thailand, only the eggs of *Ae. aegypti* were found in rural ovitraps, and the proportions of *Ae. albopictus* eggs increased during the rainy seasons (Mogi et al. 1988). Similar results were found in urban ovitraps (Mogi et al. 1988), in cemetery vases and in

tires (Juliano et al. 2002). In contrast, studies in Malaysia (Sulaiman and Jeffrey 1986) and in Japan (Mori and Wada 1978) showed no correlation between *Ae. albopictus* population size and rainfall. In Houston, Texas, an explosion in the population of *Ae. aegypti* occurred after a severe flood in the summer of 2000 following a couple of years of record dry spells (personal observation and communication with Harris County Mosquito Control district personnel). The district reported very low to non-existent numbers of *Ae. aegypti* before the flood.

The effects of temperature and humidity on the adults of these two species, as well as many others have been extensively studied. Mogi et al. (1996) compared desiccation survival times of adult *Ae. albopictus* and *Ae. aegypti* under 90% and 70% relative humidity (RH) and 25˚C. *Ae. aegypti* was found to be more resistant to desiccation than *Ae. albopictus*. In experiments involving mixed populations of *Ae. albopictus* and *Ae. aegypti*, Costanzo et al. (2005) found *Ae. albopictus* was negatively impacted by interspecific competition under drying conditions and inversely impacted under fluctuations between wet and dry conditions. The reverse was true for *Ae. aegypti*. Costanzo et a. (2005) attributed this effect which most greatly impacted the adult stage, on the effects of drying during the egg stage. Research by Thomson (1938) on the reactions of the mosquito, *Culex fatigans* to temperature and humidity indicates that adult females are very sensitive to changes in humidity and temperature. At 29˚C, *C. fatigans* could detect a difference of 1˚C. All females avoided very high and very low levels of temperature and humidity, with blood fed females showing the strongest reactions and hungry females showing the weakest.

Research has been conducted on temperature-dependent development rates for *Ae. aegypti* by Bar-Zeeve (1958), Kasule (1986), Rueda et al. (1990), Sames (1999) and Southwood et al. (1972) and for *Ae. albopictus* by Sames (1999). In each case the results varied with the origin of the mosquito. A study on physiological time by Taylor (1981) that pooled development rates for 54 species of insects and seasonal trends, suggests that very similar species with differing temperature optimums might experience species replacement seasonally in areas that have high summer temperatures.

There has been minimal research on the effects of temperature and humidity on the eggs of *Ae. albopictus* and *Ae. aegypti*. In Japan, Sota and Mogi (1992) measured survival times of eggs from several *Aedes* species including *Ae. aegypti* and *Ae. albopictus* under three different humidity conditions (42%, 68% and 88% RH) at 25˚C. *Ae. aegypti* survived longer then *Ae. albopictus* at all humidity conditions. Sota and Mogi (1992) attributed this to egg volume, with *Ae. aegypti* having the greatest egg volume and thus the greatest ability to resist desiccation. In Florida, Juliano et al. (2002) compared egg mortality rates of *Ae. aegypti* and *Ae. albopictus* under different temperature (22˚, 24˚ and 26˚C) and humidity (25%, 55%, 75%, and 95% RH) combinations. Juliano et al. (2002) found the effects of temperature and humidity on egg mortality significantly different between the two species, with *Ae. albopictus* experiencing much higher mortality at all combinations except at the highest humidity. Over a three-month period, they did not find a significant interaction between temperature and/or humidity and egg mortality of *Ae. aegypti* until the third month (90

days). This indicates the effects of temperature and humidity increase with time. *Ae. aegypti* eggs had a lower rate of mortality with a higher level of humidity.

Hien (1975) compared the resistance of *Ae. albopictus* and *Ae. aegypti* eggs to low humidity (60-70%RH) at 25˚C over a four-month period. At all time intervals *Ae. albopictus* was found to be more resistant to desiccation, resulting in a higher percentage of hatched larvae. In addition, the percentage of hatching larvae increased to the first month for *Ae. aegypti* and to the second month for *Ae. albopictus* and then gradually dropped.

All of this research has broadened our knowledge, but the climate combinations evaluated span a very narrow range and cannot be reliably applied to Texas strains. Research by Chesson and Huntly (1997) suggest fluctuating harsh or stressful conditions in addition to temporal niche opportunities may play a role in species replacement. Thus, the purpose of the research herein was to assess how immature embryos (eggs) of *Ae. albopictus* and *Ae. aegypti* from Texas will be affected by humidity and temperature over a broad range of combinations over time. It is hypothesized that the eggs of *Ae. aegypti* and *Ae. albopictus* will have different percentages of eggs hatch at different levels of relative humidity and temperature, and that these differences will not be the same between the two species.

Sota and Mogi (1992) have found that eggs with large volume were more resistant to desiccation, and that *Ae. aegypti* eggs were significantly larger then the eggs of *Ae. albopictus*. Christophers (1960) mentioned the chorionic egg pad on the *Ae. aegypti* egg that is produced by the epichorion and is part of the exochorion. The

exochorion functions in desiccation resistance and the chorionic egg pad functions to anchor the eggs ventral side up. Christophers (1960) described the chorionic egg pad as a gelatinous pad formed by the swelling of the epichorion in water. An effort was made to compare egg volume and the size of the chorionic egg pad of each species and to verify if there are any differences between species in this regard.

CHAPTER III

EGG DESICCATION RESISTANCE STUDY

Introduction

Much research has been devoted to examining the mechanisms of displacement between *Ae. aegypti* and *Ae. albopictus*. However, there has been minimal research on the effects of temperature and humidity on the eggs of *Ae. albopictus* and *Ae. aegypti*. The egg stage is important because it is the stage in which these mosquitoes wait out unfavorable climatic conditions (Sota and Mogi 1992). Due to small size and large surface-area-to-volume ratio, eggs are at high risk of desiccation. Thus, specific aspects of climate that should most influence egg mortality are temperature and humidity. The eggs must wait for rainfall to stimulate hatching, so the length of time that eggs can tolerate various temperature and humidity conditions is also a factor.

Studies by Sota and Mogi (1992) and Juliano et al. (2002) have found *Ae. aegypti* eggs to be more desiccation resistant as compared to *Ae. albopictus* eggs subjected to various temperature and relative humidity combinations. Egg mortality as an effect of temperature and humidity on the eggs was found to be amplified with an increase in time of exposure to the climatic conditions. In contrast, Hien (1975) found *Ae. albopictus* eggs to be more resistant to desiccation as compared to the eggs of *Ae. aegypti*. These studies, although informative, were conducted using a very narrow range of temperature and humidity combinations and cannot be reliably related to Texas populations. Thus, the purpose of this study was to assess how immature embryos

(eggs) of *Ae. albopictus* and *Ae. aegypti* from Texas are affected by humidity and temperature over a broad range of combinations ranging from 15-95% RH and 15-35˚C for up to three months.

The previously mentioned experiments (Sota and Mogi 1992, Juliano et al. 2002 and Hien 1975) were conducted using various saturated salt solutions to maintain humidity. This limited the humidities the researchers could maintain and the temperatures, because a different salt is required to maintain different humidities at a certain temperature and only a small range of humidities can be achieved over a small range of temperatures. Additionally, an assumption that the different salts did not affect the percent hatch had to also be made. In this study, a methodology was created for using glycerol instead of saturated salts to maintain humidity based on the research done on the properties of glycerol by Newman (1968).

In general, insects evolve or acclimate to their specific habitats through selection pressure, resulting in insects of the same species from different regions performing differently under the same conditions as seen in studies by Campbell et al. (1974) and Mogi et al. (1996). For this reason, this study was performed with mosquitoes of each species collected from McAllen and Brownsville, TX, where they are sympatric. In addition, laboratory populations of each species were also included.

Materials and Methods

Eggs produced by the six different mosquito populations were subjected to 25 different temperature and humidity combinations for a time period of one, two, three,

four, eight and twelve weeks, after which time they were hatched and their hatching percentages were compared using a Repeated Measures ANOVA.

Aedes larvae were collected from Brownsville and McAllen, TX, from vases in cemeteries and from tires. *Ae. albopictus* and *Ae. aegypti* are sympatric in these areas so both mosquitoes species were collected and used to established new colonies in the laboratory. The current study was conducted using these four populations as well as a laboratory population of each species. The *Ae. aegypti* population maintained in the lab was collected in 1955 from Galveston, TX. A new laboratory population of *Ae. albopictus* was established by collecting mosquito eggs on Little Black Jars (LBJs) in the twin cities of Bryan and College Station, TX. The eggs were hatched and reared in the laboratory at 27˚C and 75% RH with a 14:10 L:D photoperiod. The adults were blood fed on chickens and produced eggs which were subsequently hatched and reared to produce another generation. This process was repeated until the seventh generation, at which point they were considered a true laboratory population based on the number of generations it takes a pesticide resistant mosquito strain to revert back to being susceptible (personal communication with Dr. Jimmy K. Olson, Texas A&M University). Voucher specimens for each population have been deposited in the Texas A&M University museum collection (voucher #670). All mosquito populations were maintained at 27˚C and 75% RH with a 14:10 L:D photoperiod. The adults were fed on chickens as their blood-meal source and given sugar water (10% sucrose). Larvae were given larval food consisting of ground Tetramin® Fish food.

Eggs were collected in Little Black Jars (LBJs) lined with paper towels in 24-hour increments for three days starting on the third day after the females had taken a blood meal. Each paper towel with eggs was kept at 27˚C and 100% RH for 24 hours to allow the eggs to embryonate. The paper towels were then divided into two portions. A small portion of eggs was kept an additional six days at 27˚C and 100% RH and then hatched by active vacuum in order to provide a control. The remaining portion of the paper towel with eggs was cut into squares containing 20-50 eggs. Eggs were not taken off the paper towel to ensure the exochorion and chorionic egg pad stayed intact. As previously noted, the exochorion functions in desiccation resistance (Christophers 1960) and it is possible the chorionic egg pad does as well. The eggs were tested under 25 different temperature and relative humidity conditions: 15˚, 21˚, 27˚, 32˚ and 35˚C in combination with 15%, 35%, 55%, 75% and 95% RH. For every temperature and humidity combination, 30 squares of paper with eggs were placed on a Petri dish and set in a 19.1 x 25.4 cm sealed plastic box. Humidity within the box was maintained using various proportions of glycerol and deionized water. The appropriate proportions of glycerol and water were chosen according to Figure 1, and placed in a Petri dish. The correct proportions were determined by a modified version of directions found in Newman's (1968) paper on the properties of glycerol and by a methodology determined particularly for this study (Table 1).

Hot Pac® upright incubator cabinets housed the plastic boxes, maintained temperature and a 14:10 L:D photoperiod. The temperature fluctuated between ±3˚C in the chambers maintaining 32˚C and 35˚C, and ±1˚C in the cabinets maintaining 15˚C,

21˚C and 27˚C. After one, two, three, four, eight and twelve weeks, five sets of eggs were removed, counted, and each set was placed in a vial with 0.1 - 0.5 ml of de-ionized water and stimulated to hatch by being submerged and placed under active vacuum (13.3 psi) for 15 minutes.

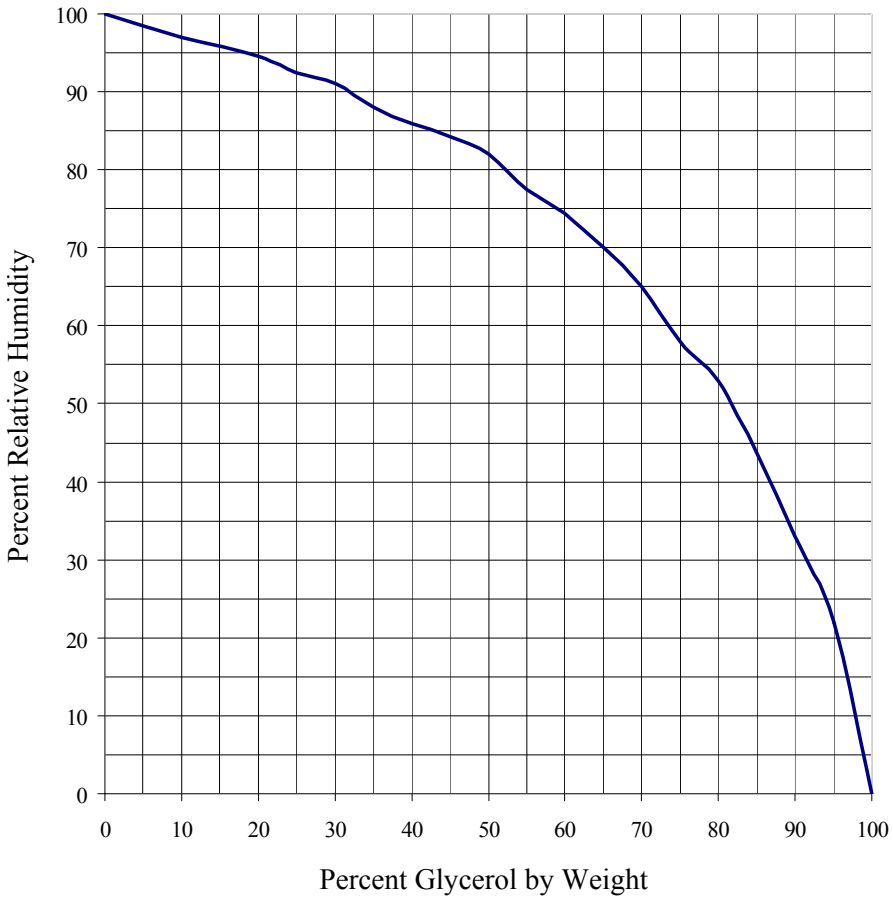

Figure 1. Percent aqueous glycerol needed to achieve the corresponding relative humidity for temperatures 0-70˚C (modified and adapted from Newman 1968).

Table 1. Glycerol solutions used for controlling relative humidity in experiments involving eggs of *Ae. albopictus* and *Ae. aegypti*.

%RH	% Glycerol	Water (gm)*	Glycerol (gm)*	Variation**
15%	98.5	19.52	0.48	+-0.5% RH
35%	89.5	17.90	2.10	+-0.5% RH
55%	77.5	15.50	4.50	+-0.5% RH
75%	58	11.70	8.30	+-0.5% RH
95%	19.3	3.87	16.13	+-1.0% RH

*Due to the viscous nature of glycerol, it was measured by weight resulting in a final solution of 20gms in each Petri dish.
**This is the amount of fluctuation of relative humidity within the box. As determined by preliminary data, the glycerol solutions were replaced every two weeks in order to maintain a steady relative humidity.

Note: Calculation used:
Amt. sol. by wt. x % glycerol (from Fig. 1) = $\frac{\text{Amt of glycerol (g)}}{1.2595\text{g/ml}}$ = ml of glycerol
Amt. sol. by wt – ml of glycerol = amount of water
1.2595g= Density of glycerol, Density of water = 1g

Larval food (ground Tetramin®) was added to each vial of eggs and they were returned to their temperature cabinets. Eggs were checked for hatching after several weeks and the unhatched eggs were bleached using sodium hypochlorite (Mortenson 1950). Bleaching the eggs made it possible to see inside the chorion to the egg tooth on the larvae and determine if embryonization took place. Eggs containing pharate larvae were considered dead. The percent hatch for each replication was the proportion of eggs that hatched per total number of embryonated eggs. For statistical analysis, the proportion for each replicate was arc sin transformed (Ott 1984), making it possible to run a parametric test on data that is not normally distributed. The following equation was utilized for the arc sine transformation,

$$\text{Percent hatch} = \sin^{-1} \sqrt{\pi}$$

The observed proportion of hatched eggs to embryonated eggs (π) is the number of hatched eggs divided by the number of viable eggs. Using SPSS (SPSS 1999), a repeated measures multivariate ANOVA was performed with between-subject factors of species, population, temperature and relative humidity with the arc sin transformed value as the within-subject factor with 6 levels corresponding to weeks 1, 2, 3, 4, 8 and 12. All main effects were accessed as well as all interactions. Significant interactions were further accessed using the sequential Bonferroni adjustment with experimentwise $\alpha = 0.05$.

The control eggs were given four weeks to hatch. The proportion of embryonated eggs that hatched was assessed by bleaching and the number was arc sin transformed (Ott 1993). Using SPSS, an ANOVA was performed to see if the controls

were significantly different from other controls of the same species and population. Eggs used in this study from the wild populations were of the F2, F3 or F4 progeny. The large number of eggs needed for this study, and the subsequent demands on the colonies made it necessary to use multiple generations. However, this effect is assumed to be null and was verified by the previously mentioned control method.

Results and Discussion

There was no significant difference between the different batches of eggs set aside for control purposes (ANOVA, $p > 0.05$). For this reason, all the significant differences among the six populations can be attributed to the testing variables and not egg batch differences.

Mauchly's test for Sphericity verified the homogeneity of variance assumption, which indicated that the standard deviations of the populations for all the dependent variables, i.e., the six measures of percent hatch, are equal. The multivariate test for within-subject effects was significant for all main effects: Temperature, Species, Relative Humidity, Weeks and Origin of Population, as well as all 2-way, 3-way and 4-way interactions and the 5-way interaction ($F = 4.10$, $p < .0001$), indicating that they all have a significant effect on the percent hatch. Multiple comparisons were performed on all main effects, however, to determine how the multiple interactions effected the mean percent hatch, multiple ANOVA tests were performed on all the effects, with the data split by population, species, temperature, relative humidity and weeks and combinations thereof.

The 5-way interaction of all the variables was significant, which generally indicates that, by themselves, their effects cannot be assessed. However, researchers, such as those with mosquito control districts, control for mosquitoes in the field where it is unlikely they will know all of these variables when making mosquito management decisions. For this reason, the 3-way interactions of "species-population-temperature", "species-population-relative humidity" and "species-population-time" are discussed as to how they influence percent hatch, as well as how their interactions change or affect that influence. The 4-way interaction of all the main effects not including the effect of population, i.e. species-time-temperature-relative humidity is also presented here. The means from all treatments appear in the Appendix.

Temperature. For every population, at most temperatures, the two species were significantly different from each other, except for the McAllen population at 21˚C (Table 2, Fig. 2), which is likely due to *Ae. albopictus* experiencing its maximum percentage of hatch at this temperature. Eggs of *Ae. albopictus* from Brownsville did not have a significantly different percent hatch from those of *Ae. aegypti* at 15˚C, 21˚C or 35˚C. At all other temperatures, the eggs of *Ae. albopictus* had a significantly lower hatching percentage than did those of *Ae. aegypti*, which experienced their highest percent of hatch at 27˚C. In most cases, eggs of *Ae. albopictus* from Brownsville had a significantly higher percent hatch than those of the other two populations (Fig. 2B); and unlike the eggs from other *Ae. albopicutus* populations, the eggs of *Ae. albopictus* from

Brownsville had their highest percent hatch at 27˚C similar to the *Ae. aegypti* populations.

Table 2. Mean percent hatch (and standard error) for each temperature for experimental egg populations of *Ae. albopictus* and *Ae. aegypti* held at different temperatures.

Temp	*Species*	BWN[2]	McAllen[3]	Lab[4]	Total
15˚C	Albo[1]	60.02 (1.07)x[5]	52.56 (1.00)y*	53.68 (0.92)y*	55.42 (0.58)a[6]*
	Aeg	60.59 (0.76)X	62.06 (0.79)X	56.81 (0.75)X	59.66 (0.44)A
21˚C	Albo	76.74 (1.07)x	76.91 (1.00)x	71.89 (0.92)y*	75.18 (0.58)b*
	Aeg	75.02 (0.76)X	77.62 (0.71)Y	84.20 (0.75)Z	78.95 (0.43)B
27˚C	Albo	81.88 (1.07)x*	69.50 (1.00)y*	70.11 (0.92)y*	73.83 (0.58)b*
	Aeg	87.34 (0.76)X	90.45 (0.77)Y	83.70 (0.75)Z	85.10 (0.44)C
32˚C	Albo	58.47 (1.07)x*	50.63 (1.00)y*	48.69 (0.92)y*	52.60 (0.58)c*
	Aeg	68.66 (0.76)X	64.61 (0.71)X	66.93 (0.75)X	66.74 (0.43)E
35˚C	Albo	59.89 (1.07)x	55.02 (1.00)x*	41.27 (0.92)y*	52.06 (0.58)c*
	Aeg	60.23 (0.76)XY	58.36 (0.71)X	65.03 (0.75)Y	61.21 (0.43)A

[1]Albo = *Ae. albopictus* and Aeg = *Ae. aegypti*
[2]Mosquitoes from the population collected in Brownsville, TX.
[3]Mosquitoes from the population collected in McAllen, TX.
[4]Mosquitoes from the laboratory population.
[5] Means with the same letter are not significantly different according to the Tukey's test (α = 0.05). For each temperature, each population is compared intra-specifically and designated' x', 'y' or 'z '(*Ae. albopictus* is in lower case and *Ae. aegypti* is in upper case.
[6]The mean value for each species is compared to that species among the different temperatures with a, b, c, d or e.
*Indicates the two species within the population are significantly different (Tukey α = .05).

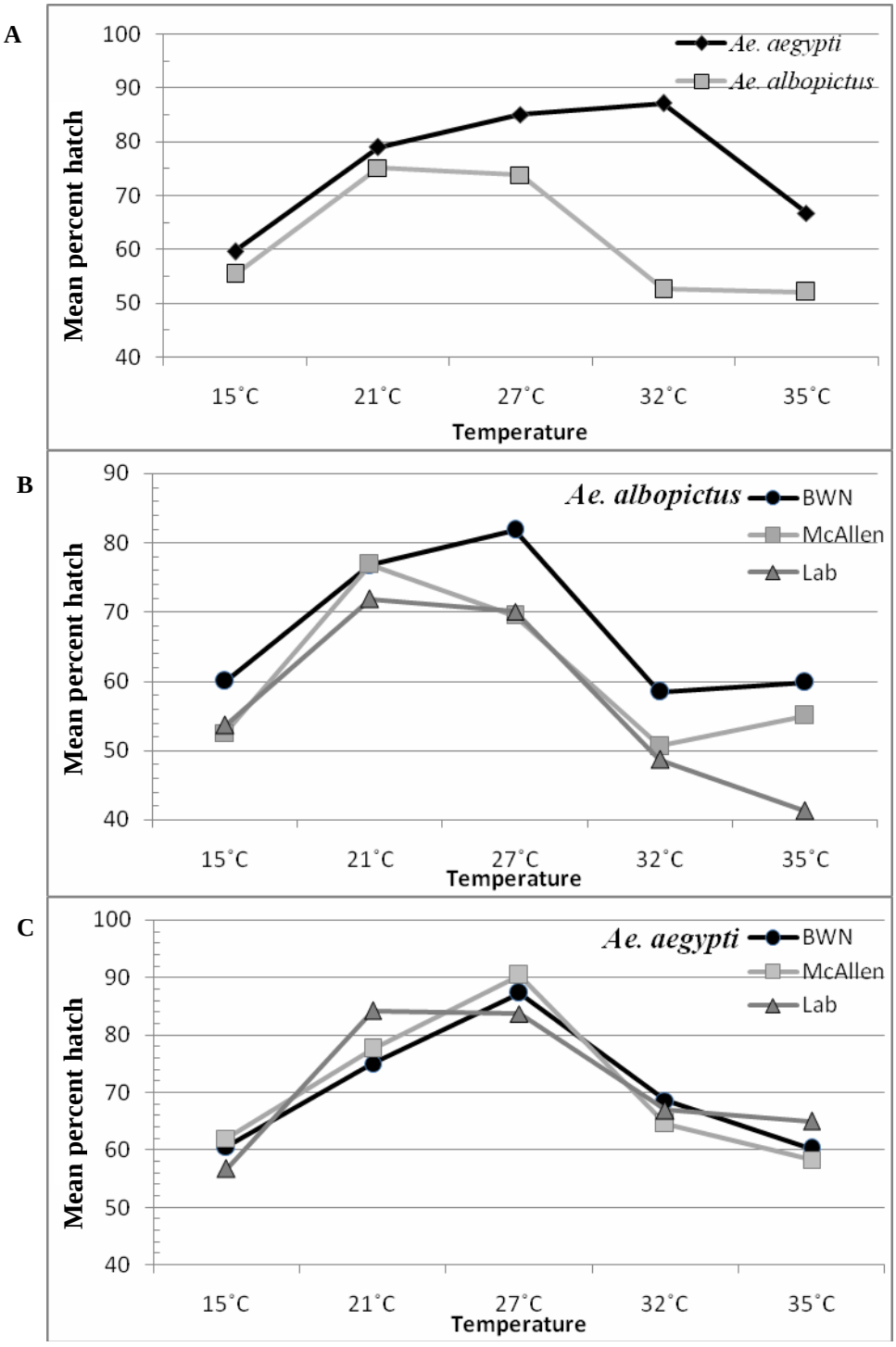

Figure 2. Graphical representation of the effect of temperature on the eggs of *Ae. albopictus* and *Ae. aegypti* (A), the different populations of *Ae. albopictus* (B) and the different populations of *Ae. aegypti* (C).

Percent egg hatch of the *Ae. aegypti* population (Fig. 2C) was not significantly different at the lowest temperature of 15˚C, but they were all significantly different at 21˚C and 27˚C. The egg hatching percentages of the laboratory population of *Ae. aegypti* increased the most from 15˚C to 21˚C at which they had the highest percent for all populations. However, the eggs of the wild populations reached their highest percent hatch at 27˚C, with the population from McAllen experiencing the highest percent out of the three populations and the laboratory population the lowest. At 32˚C the percent hatch for all *Ae. aegypti* eggs decreased, and none were significantly different from each other. However, as the temperature increased to 35˚C, percent hatch for eggs from the wild populations decreased significantly more than did those from the laboratory population, but hatching percentages for eggs from the McAllen population of *Ae. aegypti* decreased the most.

Ae. albopictus eggs were the most affected by temperature, such that they experienced the lowest percent egg hatch at the highest temperatures (Fig. 2A). As the temperature decreased to 27˚C and 21˚C, they reached their highest percentage of hatch followed by a significant decrease at 15˚C that resulted in a hatching percentage that was still significantly higher than that at the highest temperatures.

Ae. aegypti had the lowest percentages of hatch at the lowest and highest temperatures. The percent hatch increased significantly from 35˚C to 32˚C and even more so from 15˚C to 21˚C before it peaked at 27˚C (Fig. A).

Relative Humidity. For every population at every relative humidity (RH) the two species were significantly different from each other in regards to the effects of humidity on their eggs, with only one exception in the Brownsville population at the highest relative humidity (Table 3 and Fig. 3). At the lower levels of humidity (15-55%), the percent egg hatch for the different populations of *Ae. albopictus* were all significantly different from each other (Fig. 3B). At 75% RH, the hatching percentages for eggs from wild populations were significantly different from each other, but neither was significantly different from those for eggs from the laboratory population. At the highest relative humidity, eggs from all populations of *Ae. albopictus* had their highest percent hatch, with those from the population from Brownsville being significantly greater than those from the other two.

Ae. albopictus eggs from the Brownsville population had a significantly higher percent hatch at the lowest level of humidity than those from the other populations, and the percent hatch increased as the relative humidity increased (Fig. 3C). However, eggs from the McAllen population of *Ae. aegypti* experienced the greatest increase in percent hatch with the increase in humidity, having the highest percent of them all at 95% RH. The eggs from laboratory populations experienced the most rapid increase from 15% RH to 35% and 55% RH.

Percent hatch of the two species significantly increased in the exact same manner from 15% RH to a maximum percent hatch at 95% RH (Fig. 3A). Neither species experienced a significantly different percent hatch at 55% or 75% RH,

Table 3. Mean percent hatch (and standard error) for eggs of *Ae. albopictus* and *Ae. aegypti* held at different relative humidity levels.

RH%	*Species*	BWN[2]	McAllen[3]	Lab[4]	Total
15%	Albo[1]	61.28 (1.07)x[5]*	57.81 (1.00)y*	45.15 (0.92)z*	54.75 (0.58)a[6]*
	Aeg	65.01 (0.76)X	61.16 (0.85)Y	62.00 (0.75)Y	62.84 (0.45)A
35%	Albo	65.66 (1.07)x*	56.53 (1.00)y*	51.91 (0.92)z*	58.03 (0.58)b*
	Aeg	68.45 (0.76)XY	66.19 (0.71)X	70.03 (0.75)Y	68.22 (0.43)B
55%	Albo	68.74 (1.07)x*	61.84 (1.00)y*	57.88 (0.92)z*	62.82 (0.58)c*
	Aeg	73.24 (0.76)XY	70.75 (0.72)X	74.49 (0.75)Y	72.83 (0.43)C
75%	Albo	66.51 (1.07)x*	61.07 (1.00)y*	64.37 (0.92)xy*	63.98 (0.58)c*
	Aeg	69.14 (0.76)X	75.44 (0.71)Y	73.30 (0.75)Y	72.63 (0.43)C
95%	Albo	74.83 (1.07)x	67.37 (1.00)y*	66.34 (0.92)y*	69.51 (0.58)d*
	Aeg	76.01 (0.76)X	79.38 (0.71)Y	76.83 (0.75)x	77.41 (0.43)D

[1]Albo = *Ae. albopictus* and Aeg = *Ae. aegypti*
[2]Mosquitoes from the population collected in Brownsville, TX.
[3]Mosquitoes from the population collected in McAllen, TX.
[4]Mosquitoes from the laboratory population.
[5] Means with the same letter are not significantly different according to the Tukey's test ($\alpha = 0.05$). For each humidity, each population is compared intra-specifically and designated' x', 'y' or 'z '(*Ae. albopictus* is in lower case and *Ae. aegypti* is in upper case.
[6]The mean value for each species is compared to that species among the different humidities with a, b, c, d or e.
*Indicates the two species within the population are significantly different (Tukey $\alpha = .05$).

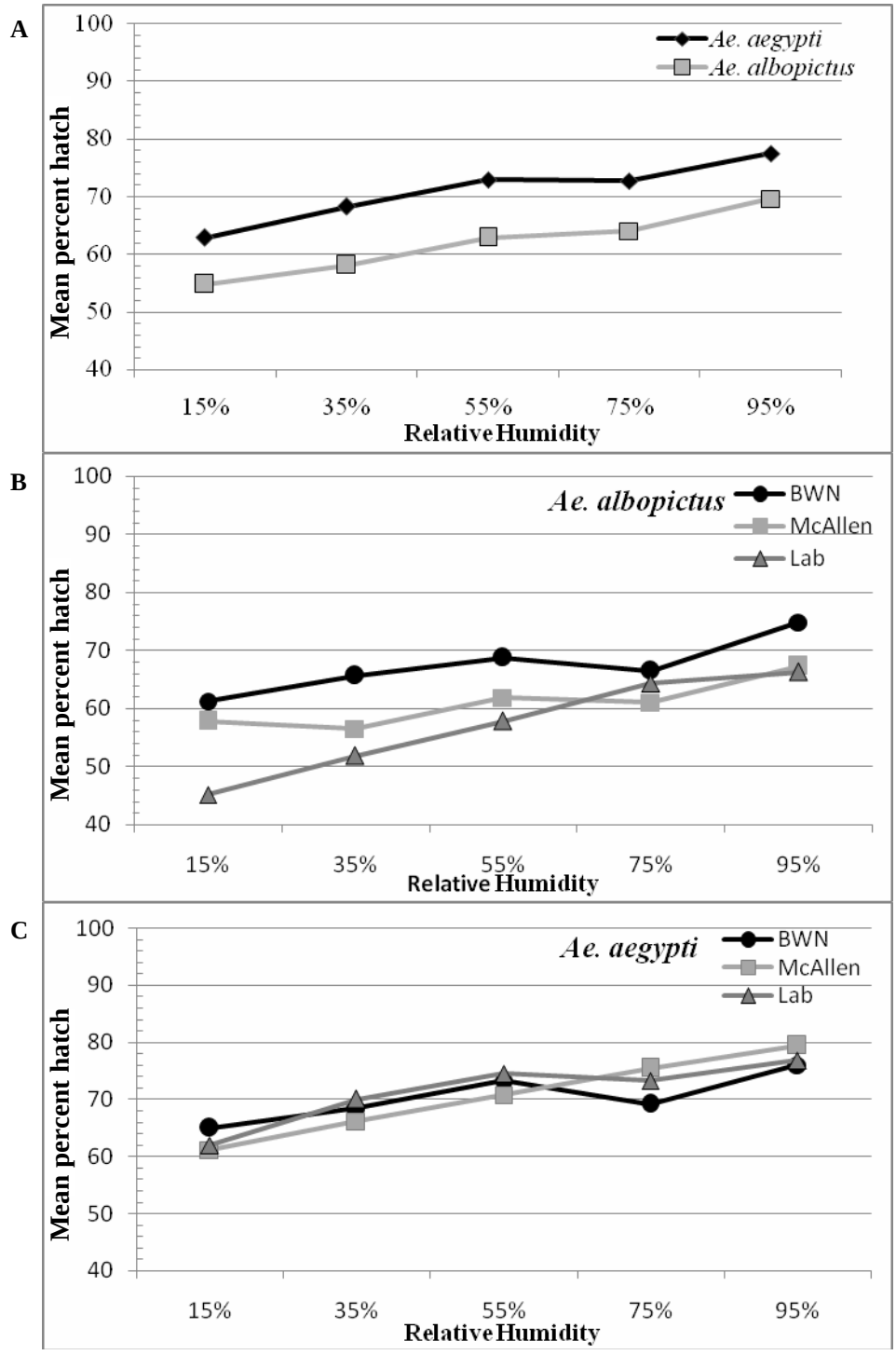

Figure 3. Graphical representation of the effect of humidity on the eggs of *Ae. albopictus* and *Ae. aegypti* (A), the different populations of *Ae. albopictus* (B) and the different populations of *Ae. aegypti* (C).

indicating little variation at these moderate levels of humidity. At every level of humidity, eggs of *Ae. aegypti* had a significantly higher percentage of hatch than did those of *Ae. albopictus*.

Time. The mean percent hatch for the eggs of each species' population over the six different time intervals (1, 2, 3, 4, 8 and 12 weeks) decreased after an initial peak at the first or second week period to an overall low in eggs hatched after twelve weeks (Fig. 4A) [eggs of *Ae. aegypti* from the wild populations were the exception]. Their hatching percentages decreased at the third week, but unlike the other populations, they had another peak in percent hatch at the fourth week before decreasing again. In all populations separately and combined, the percent hatch for *Ae. aegypti* eggs was significantly higher than that for *Ae. albopictus* eggs, except for the Brownsville population in which the difference was only significant for weeks four and eight (Table 4).

The eggs from the Brownsville population of *Ae. albopictus* had a significantly higher percent hatch than did eggs from the other two populations for all time periods (Fig. 4B). For eggs hatched in the first three weeks, neither of the other two populations were significantly different from each other; however, from the fourth week on, eggs from the laboratory population of *Ae. albopictus* had a significantly lower percentage of hatch than did those from the McAllen population.

The eggs from the two wild populations of *Ae. aegypti* did not have significantly different percentages of hatch at any time period (Table 4). This may stem from the fact

Table 4. Mean percent hatch (and standard error) for *Ae. aegypti* and *Ae. albopictus* eggs for each population per week.

Week	*Species*	BWN[2]	McAllen[3]	Lab[4]	Total
1	Albo[1]	73.05 (1.09)x[5]	66.41 (1.09)y*	69.01 (1.09)y*	69.49 (0.63)a[6]*
	Aeg	74.08 (0.79)XY	77.30 (0.82)X	72.22 (0.79)Y	74.50 (1.09)A
2	Albo	74.89 (1.16)x	65.04 (1.16)y*	61.99 (1.16)y*	67.31 (0.67)a*
	Aeg	76.95 (0.75)X	77.62 (0.78)X	76.95 (0.75)X	77.16 (1.09)B
3	Albo	67.92 (0.91)x	61.51 (0.91)y*	58.15 (0.91)y*	62.53 (0.52)b*
	Aeg	69.49 (0.88)X	71.78 (0.92)X	73.45 (0.88)Y	71.57 (1.09)C
4	Albo	68.63 (1.13)x*	63.68 (1.13)y*	58.36 (1.13)z*	63.55(0.65) b*
	Aeg	74.90 (0.74)X	74.50 (0.77)X	70.87 (0.74)Y	73.41 (1.09)AC
8	Albo	59.93 (1.06)x*	56.83 (1.06)y*	49.74 (1.06)z*	55.50 (0.61)c*
	Aeg	64.45 (0.85)X	63.28 (0.88)X	69.39 (0.85)Y	65.74 (1.09)D
12	Albo	59.99 (0.99)x	52.08 (0.99)y*	45.52 (0.99)z*	52.53 (0.57)d*
	Aeg	62.36 (0.76)X	61.37 (0.80)X	65.11 (0.76)Y	62.97 (1.09)E

[1]Albo = *Ae. albopictus* and Aeg = *Ae. aegypti*
[2]Mosquitoes from the population collected in Brownsville, TX.
[3]Mosquitoes from the population collected in McAllen, TX.
[4]Mosquitoes from the laboratory population.
[5] Means with the same letter are not significantly different according to Tukey's test ($\alpha = 0.05$). For each temperature, each population is compared intra-specifically and designated' x', 'y' or 'z '(*Ae. albopictus* is in lower case and *Ae. aegypti* is in upper case.
[6]The mean value for each species is compared to that species among the different weeks with a, b, c, d or e.
*Indicates the two species within the population are significantly different (Tukey $\alpha = .05$).

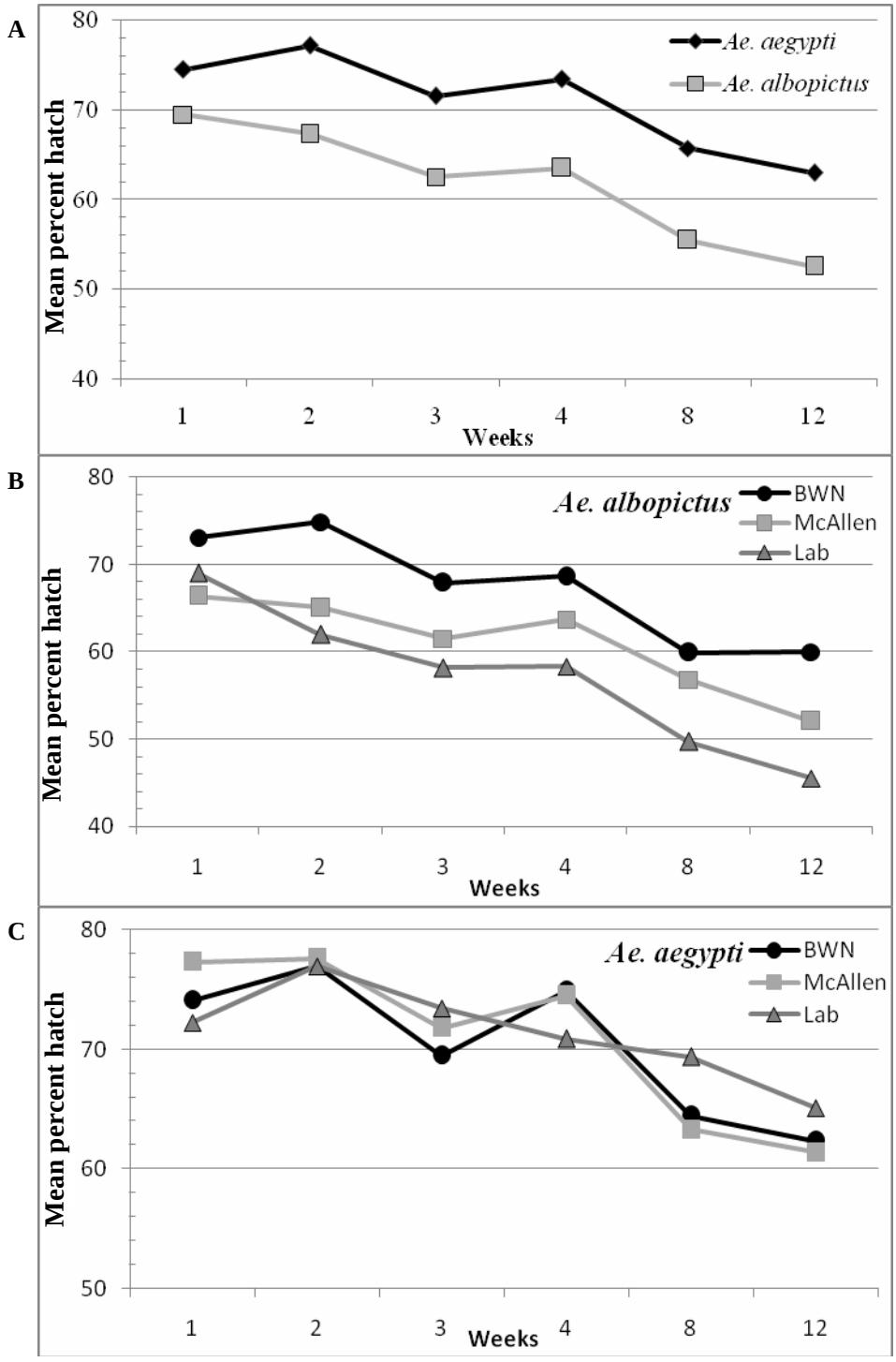

Figure 4. Graphical representation of the effect of time on the eggs of *Ae. albopictus* and *Ae. aegypti* (A), the different populations of *Ae. albopictus* (B) and the different populations of *Ae. aegypti* (C).

that the two populations originated only fifty miles from each other. The eggs from the laboratory population of *Ae. aegypti* had a significantly lower percent hatch than eggs from the other two populations for eggs hatched after the first week, but had an increase at the second week so that there was no significant difference among the populations in the second week. The eggs of *Ae. aegypti* from all populations had the highest percentage of hatch in the second week (Fig. 4C), unlike those of *Ae. albopictus*, which had their highest percent hatch in the first week (Fig. 4B). After the second week, the percent hatch for *Ae. aegypti* eggs from the laboratory population decreased, but not at the same rate as eggs of the wild populations of *Ae. aegypti*, nor did they reach a percent hatch as low as eggs of the wild populations in the twelfth week.

Eggs from all populations of *Ae. albopictus* had a significantly higher percent hatch for eggs hatched after the first and second week (Fig. 4A). This was followed by a significant decrease in the third and fourth week, and subsequent significant differences in the eighth and twelfth weeks.

As previously mentioned, *Ae. aegypti* eggs had a significant increase in hatching percentage in eggs hatched after the first week to the second week. This percentage significantly decreased in the third and fourth weeks, but in the fourth week the percent hatch was similar to that of the first week. After eight weeks, the eggs had a significant decrease in percent hatch, and again after twelve weeks.

It is clear that the amount of time elapsed before *Aedes* eggs hatch influences their hatching percentage, and that the degree that time affects the species is influence by the origin of the population, especially in the case of *Ae. albopictus*.

Table 5. Mean percent hatch (and standard error) of *Ae. aegypti* and *Ae. albopictus* eggs for each species and each population thereof.

Species	BWN[1]	McAllen[2]	Lab[3]	Total
Ae. albopictus	67.40 (0.45) x[4]*	60.93 (0.45)y*	57.13 (0.45)z*	61.82(0.26)*
Ae. aegypti	70.37 (0.33)X	70.98 (0.34)X	71.33 (0.33)X	70.89(0.19)

[1]Mosquitoes from the population collected in Brownsville, TX.
[2]Mosquitoes from the population collected in McAllen, TX.
[3]Mosquitoes from the laboratory population.
[4]Means with the same letter within the same species are not significantly different according to Tukey's test (α = 0.05). (*Ae. albopictus* is in lower case and *Ae. aegypti* is in upper case.)

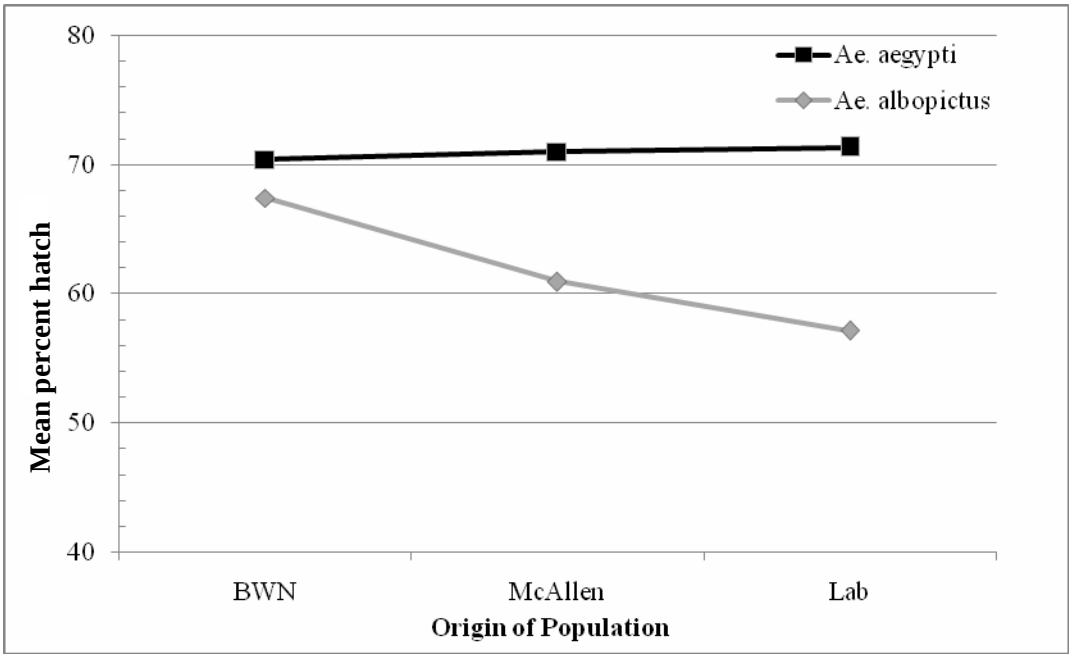

Figure 5. Graphical representation of the overall mean percent hatch of the eggs of *Ae. albopictus* and *Ae. aegypti* divided by population origin.

Population. The mean percent hatch for eggs of all populations of both species were compared (Table 5). Eggs from every population of *Ae. albopictus* were significantly different from one another, with the eggs from the population from Brownsville experiencing the highest percent of hatching, the laboratory population the lowest, and the McAllen population falling in the middle (Fig. 5). In contrast, none of the eggs from the different populations of *Ae. aegypti* were significantly different from one another. Nonetheless, for every population the two species', *Ae. aegypti* and *Ae. albopictus*, percent egg hatch was significantly different from each other. Analyzing the previous main effects, it was revealed that the location from which the species were collected significantly influenced the way their eggs respond to climatic variables. Thus, before assumptions are made on how a species or population in a specific region will respond to climatic variables, it is import to perform tests on the specific population in question.

Species-Relative Humidity-Temperature-Time. The mean percent hatch for eggs at all levels of humidity and for each species at each time interval that occurred at the five temperatures were charted and their totals compared. With this many variables to consider, only the most prominent trends are mentioned herein.

15˚C. The eggs of *Ae. aegypti* had a higher percent hatch than *Ae. albopictus* eggs at most combinations of relative humidity and time at this temperature (Table 6). However, there were some combinations for which *Ae. albopictus* had a higher percent hatch. After one week of exposure to 15˚C, the eggs of *Ae. albopictus* had a significantly higher percent hatch than those of *Ae. aegypti* at 15%, 55% and 95% RH.

Table 6. Mean percent hatch (and standard error) for eggs of *Ae. albopictus* and *Ae. aegypti* at each experimental relative humidity and week of total exposure at 15˚C.

		Weeks						
RH%	*Species*	1	2	3	4	8	12	*Total*
15%	Albo[1]	47.89 (3.13)*	42.01(3.36)*	50.82(2.62)*	60.07(3.25)	47.21(3.07)	46.39(2.85)*	49.06(1.14)a[3]
	Aeg	32.34 (2.79)	62.76(2.64)	39.99(3.12)	54.39(2.63)	51.56(3.00)	57.90(2.70)	49.82(1.39)A
35%	Albo	51.92 (3.13)	37.93(3.36) *	46.53(2.62)*	83.03(3.25)*	72.74(3.07)*	50.50(2.85)*	46.81(1.14)a*
	Aeg	46.12 (2.28)	50.15(2.16)	54.54(2.54)	63.49(2.15)	53.26(2.45)	63.60(2.21)	55.19(1.14)B
55%	Albo	56.10 (3.13)*	57.20(3.36)	50.73(2.62)	49.96(3.25)*	54.78(3.07)*	59.87(2.85)	54.77(1.14)b*
	Aeg	42.26 (2.28)	60.06(2.16)	49.76(2.54)	71.44(2.15)	72.79(2.45)	61.73(2.21)	59.67(1.14)C
75%	Albo	62.38 (3.13)	69.25(3.36)*	50.06(2.62)	59.46(3.25)*	63.43(3.07)	66.95(2.85)*	61.92(1.14)c
	Aeg	61.73 (2.21)	52.28(2.16)	52.72(2.54)	69.27(2.15)	64.96(2.45)	78.52(2.21)	61.72(1.14)C
95%	Albo	66.03 (3.13)*	60.43(3.36)*	59.45(2.62)	67.74(3.25)	59.63(3.07)*	73.95(2.85)	64.54(1.14)c*
	Aeg	55.64 (2.28)	71.52(2.16)	62.29(2.54)	73.22(2.15)	69.35(2.45)	79.58(2.21)	68.60(1.14)D
Total	Albo	56.86 (1.23) * vx[2]	53.37(1.27) * vw	51.52(1.15) w	58.82(1.23) * x	55.01(1.24) * vwx	56.95(1.14) * vwx	55.42(0.51)*
	Aeg	46.75 (1.27) V	59.11(1.31) W	52.71(1.20) V	67.22(1.28) X	63.16(1.29) WX	69.01(1.18) X	59.66(0.53)

[1]Albo = *Ae. albopictus* and Aeg = *Ae. aegypti*

[2] Means with the same letter are not significantly different according to Tukey's test (α = 0.05). For each temperature, each population is compared intra-specifically and designated' x', 'y' or 'z '(*Ae. albopictus* is in lower case and *Ae. aegypti* is in upper case.

[3]The mean value for each species is compared to that species among the different weeks with a, b, c, d or e.

*Indicates the two species are significantly different (Tukey α = .05).

This also occurred after the second week at 75% RH, after the third week at 15% RH and after the third and fourth week at 35% RH.

With the means for each time interval combined, the overall percent hatch for 35%, 55% and 95% RH was higher for *Ae. aegypti* than *Ae. albopictus*. There was no significant difference in percent hatch at 15% or 75% RH between the two species.

An interesting phenomenon can be seen when comparing percent egg hatch at 15˚C over various time intervals. For both species, the percent egg hatch actually increased with the increase of the length of time to hatching. This may be attributed to development being slowed at low temperatures, resulting in more time needed to properly develop.

21˚C. When comparing the percent hatch of *Ae. aegypti* and *Ae. albopictus* eggs across the different levels of humidity and time periods at 21˚C, there are very few instances in which there was a significant difference in mean percent hatch (Table 7). There was only one instance in which *Ae. albopictus* eggs had a higher percent hatch for a treatment than *Ae. aegypti* eggs and it occurred at 55% RH at the third week. *Ae. aegypti* eggs appeared to have a significantly higher percentage of eggs hatching at the mid-levels of humidity in combination with a later time period. This is probably because 21˚C is the temperature at which the eggs of *Ae. albopictus* experience the greatest percent of hatch. The highest percent hatch at each humidity over the different time periods for each species varied greatly. *Ae. albopictus* eggs had their highest percentage of hatch with eggs held for two to four weeks. *Ae. aegypti* eggs were was similar, except that, at 75% RH, the highest percent was after the first week.

Table 7. Mean percent hatch (and standard error) for eggs of *Ae. albopictus* and *Ae. aegypti* at each experimental relative humidity and week of total exposure at 21˚C.

		Weeks						
RH%	Species	1	2	3	4	8	12	Total
15%	Albo[1]	58.62 (3.13)	67.07(3.36)	66.95(2.62)*	63.85(3.25)	49.24(3.07)	46.04(2.85)*	58.63 (1.14)a[3]*
	Aeg	59.62 (2.28)	71.38(2.16)	75.69(2.54)	70.32(2.15)	57.77(2.45)	56.29(2.21)	65.18(1.14)A
35%	Albo	79.48(3.13)	78.53(3.36)*	78.37(2.62)	83.03(3.25)	72.74(3.07)	50.50(2.85)*	73.78(1.14)b*
	Aeg	82.07(2.28)	85.70(2.16)	75.69(2.54)	79.21(2.15)	77.99(2.45)	71.21(2.21)	78.65(1.14)B
55%	Albo	86.60(3.13)	78.15(3.36)	87.86(2.62)*	77.66(3.25)*	67.42(3.07)*	70.54(2.85)	78.04(1.14)b
	Aeg	81.77(2.28)	73.10(2.16)	76.78(2.54)	85.19(2.15)	83.99(2.45)	75.92(2.21)	79.46(1.14)B
75%	Albo	75.58(3.13)*	83.64(3.36)	81.07(2.62)*	77.62(3.25)	74.39(3.07)	75.88(2.85)	78.03(1.14)b*
	Aeg	90.68(2.28)	85.52(2.16)	87.86(2.54)	78.49(2.15)	76.64(2.45)	75.89(2.21)	82.51(1.14)B
95%	Albo	93.30(3.13)	88.56(3.36)	93.18(2.62)	85.07(3.25)	83.45(3.07)	81.01(2.85)	87.43(1.14)c
	Aeg	91.38(2.28)	91.77(2.16)	93.75(2.54)	89.34(2.15)	84.95(2.45)	82.46(2.21)	88.94(1.14)C
Total	Albo	78.72(1.23) v[2]	79.19(1.27) v	81.49(1.15) v	77.45(1.23) v	69.45(1.24)* w	64.79(1.14) * w	75.18(0.51)*
	Aeg	81.11(1.23) V	81.49(1.27) V	81.95(1.15) V	80.51(1.23) VW	76.27(1.24) WX	72.35(1.14) X	78.95(0.51)

[1]Albo = *Ae. albopictus* and Aeg = *Ae. aegypti*

[2] Means with the same letter are not significantly different according to Tukey's test (α = 0.05). For each temperature, each population is compared intra-specifically and designated' x', 'y' or 'z '(*Ae. albopictus* is in lower case and *Ae. aegypti* is in upper case.

[3]The mean value for each species is compared to that species among the different weeks with a, b, c, d or e.

*Indicates the two species within are significantly different (Tukey α = .05).

Overall, the eggs of *Ae. albopictus* and *Ae. aegypti* were significantly affected by humidity at 21˚C in the same manner. They both had the significantly lowest percent hatch at the lowest level of humidity and significantly highest hatching percentage at the highest humidity, with no significant difference at the other humidity levels. Additionally, *Ae. aegypti* eggs did not have a significantly higher percent hatch at 55% or 95% RH. This is likely attributed to there being no significant increase in percent hatch for *Ae. aegypti* from 35% to 55%RH.

Over the different time periods, there was little difference in hatching percentages within each species and among the separate species. There was a significant decrease in percent hatch for eggs held for eight and twelve weeks only. Only at these two time periods was the percent hatch for *Ae. aegypti* eggs significantly higher than the percent hatch of *Ae. albopictus* eggs.

These results indicate that, although there is a decreasing effect on the percent hatch by the increase of time before being hatched and lower levels of humidity, at 21˚C these effects are minimal and do not lead to any great increase or decrease in either species.

27˚C. Percent egg hatch at all humidity levels and across the different time periods at this temperature were mostly different (Table 8), with *Ae. aegypti* eggs having a significantly higher percent hatch than *Ae. albopictus*. However, there was no significant difference in percent hatch for eggs hatched after the first week that were held at 35%, 55% or 75% RH, or eggs held at 95% RH and hatched after weeks 2, 3, 4 and 8.

Table 8. Mean percent hatch (and standard error) for eggs of *Ae. albopictus* and *Ae. aegypti* at each experimental relative humidity and week of total exposure at 27˚C.

		Weeks						
RH%	Species	1	2	3	4	8	12	Total
15%	Albo[1]	74.69(3.13)*	74.13(3.36)*	68.01(2.62)*	68.59(3.25)*	47.69(3.07)*	39.91(2.85)*	62.17(1.14)a[3]*
	Aeg	86.85(2.52)	84.02(2.39)	82.26(2.81)	85.86(2.37)	74.30(2.71)	59.76(2.44)	78.84(1.26)A
35%	Albo	86.66(3.13)	80.47(3.36)*	77.90(2.62)*	73.55(3.25)*	47.57(3.07)*	49.26(2.85)*	69.24(1.14)b*
	Aeg	91.54(2.28)	92.55(2.16)	92.88(2.54)	92.39(2.15)	88.03(2.45)	73.31(2.21)	88.45(1.14)B
55%	Albo	88.42(3.13)	86.36(3.36)	87.48(2.62)*	80.85(3.25)*	78.75(3.07)*	55.87(2.85)*	79.62(1.14)c*
	Aeg	90.52(2.37)	90.89(2.25)	92.64(2.65)	96.14(2.23)	90.55(2.55)	85.29(2.30)	91.01(1.18)BC
75%	Albo	86.57(3.13)	86.13(3.36)*	88.33(2.62)*	81.77(3.25)*	77.97(3.07)*	60.50(2.85)*	80.21(1.14)c*
	Aeg	92.47(2.28)	95.42(2.16)	94.43(2.54)	93.36(2.15)	90.99(2.45)	87.74(2.21)	92.40(1.14)C
95%	Albo	77.66(3.13)*	83.15(3.36)	83.46(2.62)	75.89(3.25)*	77.37(3.07)	70.03(2.85)*	77.93(1.14)c*
	Aeg	90.86(2.28)	86.30(2.16)	84.37(2.54)	85.74(2.15)	82.52(2.45)	80.86(2.21)	85.11(1.14)D
Total	Albo	82.80(1.23) *	82.05(1.27) *	81.04(1.15) *	76.13(1.23) *	65.87(1.24) *	55.11(1.14) *	73.83(0.51)*
		v[2]	v	v	w	x	y	
	Aeg	90.45(1.26)	89.84(1.30)	89.32(1.19)	90.70(1.27)	85.28(1.28)	77.39(1.18)	87.16(0.52)
		V	V	VW	V	W	X	

[1]Albo = *Ae. albopictus* and Aeg = *Ae. aegypti*

[2] Means with the same letter are not significantly different according to Tukey's test (α = 0.05). For each temperature, each population is compared intra-specifically and designated' x', 'y' or 'z '(*Ae. albopictus* is in lower case and *Ae. aegypti* is in upper case.

[3]The mean value for each species is compared to that species among the different weeks with a, b, c, d or e.

*Indicates the two species within are significantly different (Tukey α = .05).

Overall, *Ae. albopictus* eggs had a significant increase in percent hatch from 15% to 55% RH, but there was no additional increase in percent hatch with the additional increase in humidity. Similarly, *Ae. aegypti* eggs had an increase in percent hatch from 15% to 55% RH, but actually had a decrease in percent hatch at the highest humidity. This may be because of the presence of mites found at the highest humidity at this temperature. 27˚C may be an optimal temperature for the mosquitoes, but it is also favorable to other organisms, such as mites, bacteria and fungus, especially in combination with high levels of humidity such as was seen here.

There was a trend in both species of decreasing percent egg hatch with an increase in time spent under the treatment conditions beginning at the fourth week. This resulted in the lowest percent hatch occurring at the longest time periods and was confounded by the effect of the low humidity of 15% RH for which both species experienced their lowest percent hatch.

32˚C. At this temperature, *Ae. albopictus* eggs had irregular patterns of increases and decreases in percent hatch at all combinations of humidity and periods of time (Table 9). There was a significant difference between the two species at most combinations of humidity and time, such that *Ae. aegypti* eggs had a significantly higher percent hatch. However, at 15% RH the percent hatch at the fourth week was significantly higher for *Ae. albopictus* eggs than for *Ae. aegypti* eggs. *Ae. albopictus* eggs consistently had a higher percent hatch at the first or second week for all levels of humidity. At all other humidity levels and time periods, the resulting percent hatch was always inconsistent for *Ae. albopictus* eggs. Except for the highest humidity, the

Table 9. Mean percent hatch (and standard error) for eggs of *Ae. albopictus* and *Ae. aegypti* at each experimental relative humidity and week of total exposure at 32˚C.

		Weeks						
RH%	*Species*	*1*	2	*3*	*4*	8	*12*	*Total*
15%	Albo[1]	43.80(3.13)*	59.67(3.36)	36.51(2.62)*	63.48(3.25)*	45.82(3.07)	49.87(2.85)	49.86(1.14)a[3]*
	Aeg	68.04(2.28)	65.41(2.16)	61.55(2.54)	49.24(2.15)	46.03(2.45)	48.04(2.21)	56.38(1.14)A
35%	Albo	50.66(3.13)*	61.84(3.36)	38.93(2.62)*	49.47(3.25)*	39.91(3.07)	58.42(2.85)	49.87(1.14)a*
	Aeg	75.67(2.28)	68.73(2.16)	64.01(2.54)	60.19(2.15)	43.64(2.45)	55.31(2.21)	61.26(1.14)B
55%	Albo	68.17(3.13)*	59.68(3.36)*	37.05(2.62)*	46.98(3.25)*	45.65(3.07)*	41.83(2.85)*	49.89(1.14)a*
	Aeg	93.16(2.28)	88.69(2.16)	77.70(2.54)	67.97(2.15)	52.98(2.45)	56.72(2.21)	72.87(1.14)C
75%	Albo	71.22(3.13)*	58.21(3.36)*	42.86(2.62)*	51.73(3.25)*	35.05(3.07)*	43.33(2.85)	50.40(1.14)a*
	Aeg	81.04(2.28)	79.41(2.16)	67.43(2.54)	69.23(2.15)	44.65(2.45)	46.05(2.21)	64.64(1.14)B
95%	Albo	83.86(3.13)*	78.86(3.36)*	73.42(2.62)*	70.43(3.25)*	37.17(3.07)*	34.10(2.85)*	62.97(1.14)b*
	Aeg	94.45(2.28)	91.66(2.16)	84.43(2.54)	86.30(2.15)	69.61(2.45)	44.70(2.21)	78.53(1.14)D
Total	Albo	63.54(1.23)* v[2]	63.65(1.27)* v	45.76(1.15)* w	56.42(1.23)* x*	40.72(1.24)* w	45.51(1.14)* w	52.60(0.51)*
	Aeg	82.47(1.23) V	78.78(1.27) V	71.02(1.15) W	66.59(1.23) W	51.38(1.24) X	50.17(1.14) X	66.74(0.51)

[1]Albo = *Ae. albopictus* and Aeg = *Ae. aegypti*

[2] Means with the same letter are not significantly different according to Tukey's test ($\alpha = 0.05$). For each temperature, each population is compared intra-specifically and designated' x', 'y' or 'z '(*Ae. albopictus* is in lower case and *Ae. aegypti* is in upper case.

[3]The mean value for each species is compared to that species among the different weeks with a, b, c, d or e.

*Indicates the two species within are significantly different (Tukey $\alpha = .05$).

increase in humidity did not significantly increase the hatching percentage. The inconsistencies that *Ae. albopictus* eggs demonstrated at this temperature may be indicative of the species' poor performance at this high temperature. This may be a temperature in which some individuals may have the ability to cope, but not as a collective group or population.

Ae. aegypti eggs hatched at a higher percentage at all humidity levels after the first or second week, with the highest percentages occurring under the highest levels of humidity. In general, hatching percentages significantly increased as humidity increased, with the exception of the 75% RH level. At this level, *Ae. aegypti* eggs experienced a significant decrease in percent hatch before increasing again at the 95% RH level.

The effect of time seemed especially potent at 32˚C, with *Ae. albopictus* eggs experiencing a significant decrease in percent hatch after only two weeks of exposure. *Ae. aegypti* had a drop to approximately 50 percent after eight weeks of exposure. After the initial drop in percent hatch for the eggs of each species, there was not another drop with the addition of four more weeks of exposure.

35˚C. Percent egg hatch was not significantly different for either *Ae. albopictus* or *Ae. aegypti* at any humidity after one week, nor at the lowest levels of humidity at the eighth and twelfth weeks. A significant difference was found between the eggs of the two species at most other combinations of humidity and time at this temperature (Table 10) such that *Ae. aegypti* eggs hatched at a significantly higher percent than *Ae. albopictus* eggs. At this temperature, *Ae. albopictus* did not exhibit any of their previous

Table 10. Mean percent hatch (and standard error) for eggs of *Ae. albopictus* and *Ae. aegypti* at each experimental relative humidity and week of total exposure at 35˚C.

		Weeks						
RH%	Species	1	2	3	4	8	12	Total
15	Albo[1]	55.61(3.13)	62.11(3.36)*	58.33(2.62)	49.18(3.25)*	48.06(3.07)	50.75(2.85)	54.01(1.14)a[3]*
	Aeg	58.28(2.28)	73.27(2.16)	54.72(2.54)	67.58(2.15)	47.71(2.45)	56.13(2.21)	59.61(1.14)AB
35	Albo	56.59(3.13)	57.23(3.36)*	46.04(2.62)*	46.70(3.25)*	51.91(3.07)	44.35(2.85)	50.47(1.14)a*
	Aeg	59.42(2.28)	73.56(2.16)	53.40(2.54)	64.57(2.15)	51.75(2.45)	42.69(2.21)	57.56(1.14)a
55	Albo	62.22(3.13)	58.98(3.36)*	43.20(2.62)*	53.02(3.25)	47.40(3.07)*	45.81(2.85)	51.77(1.14)a*
	Aeg	69.58(2.28)	72.59(2.16)	61.14(2.54)	55.70(2.15)	62.25(2.45)	45.50(2.21)	61.13(1.14)AB
75	Albo	73.49(3.13)	48.34(3.36)*	49.41(2.62)*	45.81(3.25)*	42.00(3.07)*	36.98(2.85)*	49.34(1.14)a*
	Aeg	79.73(2.28)	70.81(2.16)	65.65(2.54)	53.83(2.15)	49.95(2.45)	51.18(2.21)	61.86(1.14)BC
95	Albo	79.81(3.13)	64.79(3.36)*	67.23(2.62)	50.06(3.25)*	42.85(3.07)*	23.50(2.85)*	54.71(1.14)a*
	Aeg	82.24(2.28)	86.75(2.16)	73.04(2.54)	66.46(2.15)	50.55(2.45)	36.14(2.21)	65.86(1.14)C
Total	Albo	65.54(1.23)*	58.29(1.27)*	52.84(1.15)*	48.96(1.23)*	46.44(1.24)*	40.28(1.14)*	52.06(0.51)*
		v[2]	vw	wx	x	x	y	
	Aeg	69.85(1.23)	75.40(1.27)	61.59(1.07)	61.59(1.15)	52.44(1.24)	46.33(1.14)	61.21(0.51)
		V	W	X	X	Y	Z	

[1]Albo = *Ae. albopictus* and Aeg = *Ae. aegypti*

[2] Means with the same letter are not significantly different according to Tukey's test (α = 0.05). For each temperature, each population is compared intra-specifically and designated' x', 'y' or 'z '(*Ae. albopictus* is in lower case and *Ae. aegypti* is in upper case.

[3]The mean value for each species is compared to that species among the different weeks with a, b, c, d or e.

*Indicates the two species within are significantly different (Tukey α = .05).

inconsistencies. After the first or second week, they had their highest percent hatch for all levels of humidity. *Ae. albopictus* eggs had a significant decrease in percent hatch with an increase in the time of exposure to the temperature. When all of the mean hatching percentages at the different time periods were combined, there was no significant difference in percent hatch at the different humidity combinations. This indicates that the amount of time the eggs are exposure to this high temperature has a greater impact on the percent hatch than the humidity level. Thus, *Ae. albopictus* may be able to populate areas that experience this high of a temperature, in combination with frequent rains, or frequent watering such as the conditions in a well-maintained cemetery in the Texas Rio Grande Valley. As noted earlier, that is where most of the wild populations were collected.

Ae. aegypti eggs experienced the highest percent hatch after two weeks at all humidity levels except 75% RH, for which the first week had the highest percentage of hatch. Overall, *Ae. aegypti* eggs had very subtle differences in percent hatch when all the means for each week were combined. Although, the significantly highest percent hatch did occur at the highest humidity. Similar to the eggs of *Ae. albopictus*, those of *Ae. aegypti* seemed more affected by the length of exposure to this temperature than the levels of humidity. Overall, after the significant increase in percent hatch at the second week, there was a significant decrease at week three, eight and twelve. Even though *Ae. aegypti* eggs experienced a higher percent hatch at this temperature than did those of *Ae. albopictus*, it appears that they would also benefit by reducing the amount of time exposed to it, such as frequent rains or frequent watering as described above.

Findings reported here support the idea that *Ae. albopictus* populations out-compete *Ae. aegypti* populations at temperatures near 21˚C. *Ae. albopictus* has a disadvantage at higher temperatures. These results support the idea that reducing watering of yards and cemeteries in regions that experience high temperatures (32˚C and 35˚C) would help control populations of *Aedes* mosquitoes.

CHAPTER IV

CHORIONIC EGG PAD STUDY

Introduction

The eggs of floodwater mosquitoes are especially relevant in determining the species' climatic boundaries, because it is in this stage that they endure unfavorable climatic conditions (Sota and Mogi 1992). In several studies (Juliano et al. 2002, Sota and Mogi 1992, and Hien 1975) it has been determined that there is a significant difference in the mortality rates of *Ae. aegypti* and *Ae. albopictus* eggs that have been subjected to different relative humidity and temperature regimes. Juliano et al. (2002) found the effects of temperature and humidity on egg mortality significantly different between the two species, with *Ae. albopictus* experiencing higher mortality at all combinations except at the highest humidity. Sota and Mogi (1992) found that *Ae. aegypti* survived longer than *Ae. albopictus* at all humidity levels they tested at their experimental temperature of 25˚C and attributed this to egg volume. *Ae. aegypti* had the greatest egg volume and thus the greatest ability to resist desiccation. Sota and Mogi (1992) conducted their study in Japan, and due to the huge geographic difference between Japan and Texas, it is important to verify if this is the case with Texas mosquito populations.

In Christophers' book on *Ae. aegypti* (1960), it mentioned the chorionic egg pad on the *Ae. aegypti* egg that is produced by the epichorion and part of the exochorion. He stated that the exochorion functions in desiccation resistance and the chorionic egg pad functions to anchor the eggs dorsal side up. Christophers (1960) described the chorionic

egg pad as a gelatinous pad formed by the swelling of the epichorion in water. It was proposed in the current study that there may be a correlation between desiccation resistance and the size of the egg pad, due to its moisture-rich nature. It is possible that the size of the egg pad differs between the two species in relation to the differences in the desiccation tolerance of *Ae. albopictus* and *Ae. aegypti* eggs. This leads to the purpose of this study, which was to determine if there are any differences in egg pad size between *Ae. aegypti* and *Ae. albopictus* and to verify if Texas populations differ in this regard and/or in their egg volume as well. It is hypothesized that the egg volume and size of the egg pad on the eggs of *Ae. aegypti* and *Ae. albopictus* are different between the two species and for populations of different origins.

Chrisophers (1960) viewed the gelatinous pad under the microscope by allowing tiny particles to attach themselves to it. In an effort to use more modern and reliable methods to view the egg pad, freshly laid eggs were viewed using an Environmental Scanning Electron Microscope (ESEM), which can scan wet specimens. Although this was successful, it was labor intensive and expensive. Through trial and error, a stain was found that would adhere to the egg pad and render it easily seen under a dissecting scope which facilitated the current study.

Materials and Methods

This study was conducted on the same six populations as the previous study, and the materials and methods for rearing the mosquitoes was the same as described in

chapter 3. The F3 progeny of the *Ae. albopictus* and *Ae. aegypti* populations from Brownsville and McAllen, TX were specifically used in this study.

Fifty eggs of each species from each population were measured, so that 150 eggs of each species were assessed. Eggs were collected on paper towels inside water-filled LBJs three days after the females received a blood meal. They were collected in four hour time periods to reduce the possibility of the eggs crowding and touching each other. The wet paper towels were removed from the LBJs and sealed inside a 9.1 x 25.4 cm box for 48 hours (Fig. 6). The sealed boxes were housed in an environmental chamber set at 27˚C, and maintained a 14:10 L:D photoperiod. After 48 hours, a small strip of the paper towel with eggs was cut off and placed in a Petri dish for staining. A giemsa stain was applied with a dropper to the eggs on the paper towel so as to cover them completely. The stain was allowed to sit for five minutes before distilled water was squirted into the dish directly at the eggs in an effort to gently dislodge the eggs from the paper towel. The paper towel was removed after the remaining eggs were squirted off. The egg pad on the egg was then able to be viewed under the optics of a microscope. In this case a Leica MZ APO dissecting scope fitted with an AxioCam MRc 5 camera (Zeiss, Germany) was used to capture digital images of every egg using the imaging program, AxioVision Rel 4.5 (Ziess, Germany). The images were then imported into Optimus 6.5 (Media Cybernetics 1999) for measurement.

A

B

Figure 6. Little Black Jar (LBJ) with paper towel for collecting eggs (A), plastic box mosquito eggs were housed for forty-eight hours (B).

The shape of a mosquito egg is that of a prolate spheroid (Hawley 1985), whose volume is described by:

$$V = 4/3\ \pi\ ab^2$$

where *a* is the semi-minor axis and *b* is the semi-major axis **(**Fig. 7). For each egg, the major axis was measured and two measurements were taken for the semi-minor axis. The chorionic egg pad is a swelling of the epichorion that is produced from the ventral half of the egg. Thus, the volume of the egg with the egg pad is derived from the dorsal side of the egg which is a function of *a* the semi-major axis and *b* the semi- minor axis *[f(a, b)*] and the ventral side of the egg which is a function of *a* the semi-major axis and *c* the semi-minor axis with the egg pad [*f*(*a, c*)].

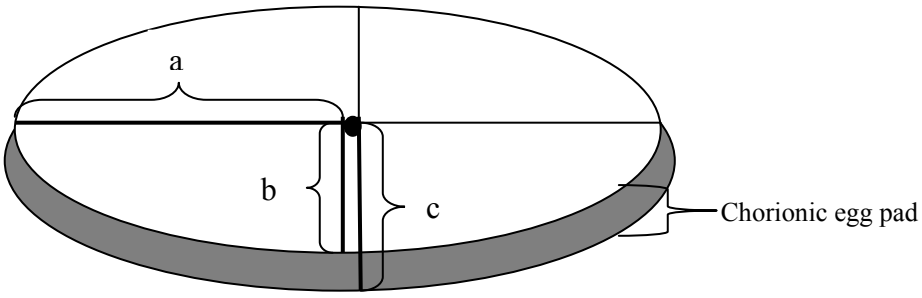

Figure 7. Graphical representation of the prolate spheroid egg and chorionic egg pad. (a) semi-major axis, (b) semi-minor axis, (c) semi-minor axis including the egg pad.

The volume of the egg with the egg pad included was derived by calculating the volume of the egg and the volume of the egg with the egg pad included and adding half of those values together to form the entire volume of the egg. The equation for the volume of the egg with the egg pad (V^p) is:

$$V^p = 1/2\ (4/3\ \pi\ ab^2) + 1/2(4/3\ \pi\ ac^2)$$

where *a* is the semi-major axis, *b* is the semi-minor axis and *c* is the semi-minor axis including the egg pad.

The surface area of the egg was also calculated due to the impact of the surface area to volume ratio on desiccation. The surface area (SA) of a prolate spheroid is derived from the following equation:

$$SA = 2\pi b^2 + 2\pi\ (ab/e)\ \sin^{-1} e$$

where *b* is the semi-minor axis, *a* is the semi-major axis and *e* is the eccentricity of the prolate spheroid derived from the following equation:

$$e = \sqrt{1 - b^2/a^2}$$

The surface area of the egg with the addition of the egg pad is a function of the surface area of the egg [$f(a, b)$] and the surface area of the egg with the egg pad [$f(a, c)$]. However, the egg pad only influences the ventral half of the egg, so the surface area of the egg with the egg pad (SA^p) takes on the following form:

$$SA^p = 1/2\ f(a, b) + 1/2\ f(a, c)$$

The above form is translated into the equation such that:

$$SA^p = 1/2(2\pi b^2 + 2\pi\ (ab/e)\ \sin^{-1} e) + 1/2(2\pi c^2 + 2\pi\ (ac/e^p)\ \sin^{-1} e^p)$$

where *b* is the semi-minor axis, *e* is the eccentricity derived from the previously mentioned equation, *a* is the semi-major axis, *c* is the semi-minor axis including the egg pad and (e^p) is the eccentricity of the egg with the egg pad, derived from the following equation:

$$e^p = \sqrt{1 - c^2/a^2}$$

A high surface-area-to-volume ratio translates into a large area for moisture loss with a small amount of moisture contained within. This is a common problem in small animals, such as insects, and was addressed in this study by calculating the surface-area to-volume ratio of the eggs with and with considering the egg pad. The surface-area-to-volume ratio (SA/V) was calculated by dividing the surface area (SA) by the volume (V), and the surface-area-to-volume-ratio including the egg pad (SA^P/V^P) was calculated by dividing the surface area of the egg including the pad (SA^P) by the volume of the egg including the pad (V^P).

In addition to the volume, surface area and surface-area-to-volume ratios of the eggs, the surface area of the chorionic egg pad was also calculated. The amount of area on the egg that the egg pad covers is relevant, because the egg pad is like a wet sponge on the egg. The larger the area covered by the egg pad, the more moisture can be applied to the egg. This may thus reduce or counteract the amount of moisture that is lost due to the high surface-area-to-volume ratios these eggs experience. The surface area of the egg pad was derived by subtracting the surface area of the egg (SA) by the surface area of the egg including the egg pad (SA^P) (Fig. 8).

Once all the calculations were made and the values recorded for all 300 eggs, a multivariate analysis of variance (MANOVA) was performed using the multivariate option of the general linear model (GLM) procedure in (SPSS 1999) species, "population" and their interaction were used as fixed effects and the six variables analyzed were: volume (V), volume including egg pad (V^P), surface area (SA), surface area including egg pad (SA^P) and the surface area of the egg pad (EggPad). Following the significant multivariate effect of the factors on the variables, a univariate analysis of variance (ANOVA) test was conducted using the GLM function in SPSS. For significant effects, Dunnets's T3 multiple comparison tests were performed for means comparisons due to its ability to assess data for which equal variances are not assumed (Sheskin 2007).

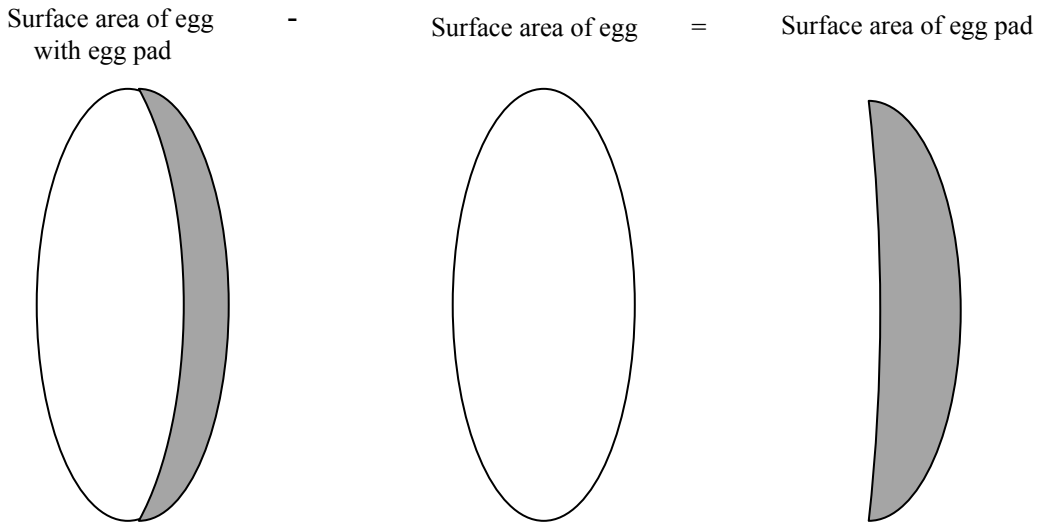

Figure 8. Surface area of egg pad derived by subtracting the surface area of the egg from the surface area of the egg plus the egg pad.

Results and Discussion

The multivariate test, Wilk's Lambda was significant ($p<.0001$) for all effects included in this study; i.e., Population, Species and Species-Population, indicating that the dependant variables, volume (V), volume including egg pad ($V^{P)}$, surface area (SA), surface area including egg pad (SA^P) and the surface area of the egg pad (EggPad) were significantly affected by Population, Species and Species*Population. The tests of the between-subject effects, revealed a significant effect for each of the dependant variables ($p<0.05$) which indicated a need to perform a univariate ANOVA on all dependant variables with regards to all effects. All of the variables failed the Levene's test of Equality of Error Variances with the exception of the surface area (SA) and the surface area including egg pad (SA^P) variables. Multiple comparisons of the dependent variables (Table 11) were performed with Dunnett's T3 adjustment which corrects for unequal standard deviations of the variables among the populations.

Volume. The volume (V) of *Ae. albopictus* eggs was significantly different from those of the *Ae. aegypti* populations. The mean egg volume of the Ae. *albopictus* eggs was significantly smaller than *Ae. aegypti* in the wild populations, but the opposite was true for the lab populations. There is likely a selection pressure in nature for a larger egg volume that does not exist in the optimal conditions of high humidity and 27˚C that the *Ae. aegypti* eggs from the lab population have been subjected to for over fifty years. The laboratory population of *Ae. albopictus*, however has not been exposed to these conditions nearly as long. Regardless of this difference, the total mean volume of all *Ae.*

Table 11. Mean value and standard deviation of each response variable for *Ae. aegypti* and *Ae. albopictus* eggs by species and population, (significant correlations and standard deviations (in parentheses) are depicted).

	(N)[1]	BWN (100)[1]	McAllen (100)	Lab (100)	Total (300)
Volume (mm^3)	Albo[4] (150)[1]	12.31(2.8)a[3]x[4]	13.79(2.6)ay	15.22(3.0)az	13.77(3.0)a
	Aeg (150)	15.93(4.4)bx	16.55(4.7)bx	11.61(2.7)by	14.70(4.5)b
	Total (300)	14.12(4.1)xy	15.17(4.0)x	13.41(3.4)y	14.24(3.9)
Vol^P (mm^3)	Albo (150)	18.59(4.7)ax	18.61(4.0)ax	20.98(4.4)ay	19.39(4.5)a
	Aeg (150)	25.25(6.2)bx	26.84(7.3)bx	18.00(3.9)by	23.36(7.1)b
	Total (300)	21.92(6.4)x	22.73(7.2)x	19.49(4.4)y	21.38(6.2)
SA (mm^2)	Albo (150)	1268.31(166.4)ax	1496.87(152.7)ay	1612.22(159.1)az	1459.13(213.7)a
	Aeg (150)	1784.72(253.4)bx	1813.42(249.6)bx	1420.09(184.3)by	1672.74(291.6)b
	Total (300)	1526.52(335.9)x	1655.15(260.1)y	1516.15(196.7)x	1565.94(276.7)
SA^P (mm^2)	Albo (150)	1450.49(183.53)ax	1669.68(156.18)ay	1819.26(174.11)az	1646.47(228.38)a
	Aeg (150)	2129.95(278.81)bx	2176.09(277.45)bx	1678.01(203.29)by	1994.68(339.57)b
	Total (300)	1790.22(414.40)x	1922.88(339.02)y	1748.63(201.24)x	1820.58(337.44)
Egg pad (mm^2)	Albo (150)	182.17(64.66)axy	172.80(64.27)ax	207.03(70.38)ay	187.33(67.62)a
	Aeg (150)	345.22(95.40)bx	362.66(86.78)bx	257.91(80.15)by	321.93(98.47)b
	Total (300)	263.69(115.27)x	267.73(121.96)x	232.47(79.28)y	254.63(107.95)
S/V (mm^2/ mm^3)	Albo (150)	106.89(19.18)ax	110.99(15.62)ax	108.25(12.46)ax	108.71(15.97)a
	Aeg (150)	115.85(15.84)bx	114.16(17.70)ax	125.73(15.97)by	118.58(17.19)b
	Total (300)	111.37(18.07)x	112.57(16.69)x	116.99(16.74)x	113.65(17.29)
S^P/V^P (mm^2/ mm^3)	Albo (150)	81.55(15.38)ax	92.55(14.96)ay	88.815(10.67)ay	87.64(14.49)a
	Aeg (150)	86.76(11.11)ax	84.46(13.28)bx	95.45(11.38)by	88.89(12.79)a
	Total (300)	84.16(13.60)x	88.50(14.65)xy	92.13(11.47)y	88.89(13.66)

[1]sample size, (Vol^P) volume of egg including the egg pad, (SA) surface area of egg, (SA^P) surface area of egg including the egg pad, (S/V) surface area to volume ratio and (S^P/V^P) surface area to volume ratio of egg including the egg pad.

[3]Means in rows that are not significantly different (ANOVA with Dunnet's T3, $p > 0.05$) are followed by the same letter; x, y or z.

[4]Means in columns that are not significantly different (ANOVA with Dunnet's T3, $P > 0.05$) are followed by the same letter; a, b or c.

albopictus eggs measured, was significantly smaller than the total of all *Ae. aegypti* eggs measured which is consistent with what Sota and Mogi (1992) found. Among the populations, the mean volumes of the *Ae. albopictus* eggs were significantly different from each other, with the population from Brownsville being the smallest and the laboratory colony the largest. The *Ae. aegypti* eggs from Brownsville and McAllen were not significantly different from each other, but they were both significantly larger than those from the laboratory population Overall, the mean value of both species of eggs from McAllen were significantly larger than that of the combined mean value of the lab population, but neither set was significantly different from that of the Brownsville population.

Volume^{P}. With the addition of the egg pad in the calculation of egg volume, the different species within each population were still significantly different from each other. The mean volumes (V^{P}) of *Ae. albopictus* for the wild populations were still significantly less than those of *Ae. aegypti*, whereas the opposite was true for the lab populations. The volume of the eggs from the wild populations of *Ae. albopictus* were no longer significantly different from each other when including the egg pad, but they were both significantly less than that of the lab population. All of the *Ae. albopictus* eggs measured had a significantly smaller mean egg volume compared to *Ae. aegypti* eggs. The *Ae. aegypti* eggs from the wild populations were not significantly different from each other, but were significantly larger than the lab population. In contrast to the mean egg volume calculated without including the egg pad, the values of the mean egg

volume of the wild population of both species combined were significantly different from that of the laboratory population, but not from each other. In this case, the mean egg volume was significantly larger in the wild populations than in the lab population.

Surface Area. For every population, the mean surface area (SA) of the *Ae. albopictus* eggs was significantly different from that of the *Ae. aegypti* within the same population. The mean surface area of the *Ae. albopictus* eggs was significantly smaller than those of the wild populations of *Ae. aegypti*, but the opposite was the case for the lab populations. Despite this difference, the total mean surface area of all *Ae. albopictus* eggs measured was significantly less than the mean total of all *Ae. aegypti* eggs measured, which would generally indicate less moisture loss and a greater ability to resist desiccation. Among the populations, the mean surface area of the *Ae. albopictus* eggs were all significantly different from each other, with the population from Brownsville being the smallest and the laboratory population the largest. The mean surface area of the *Ae. aegypti* eggs from the lab population was significantly less than either wild population, but the wild populations of *Ae. aegypti* were not significantly different from each other. The mean surface area of all the eggs from McAllen, regardless of species, was significantly larger than those of the Brownsville population or the lab population. There was no significant difference between the combined eggs of the Brownsville population and that of the lab.

Surface Area^{P}. Although the mean surface area (SA^{P}) for all species and population combinations increased by including the egg pad in calculating the surface area, there

were no significance changes from what was seen in the mean values of the surface area calculated without including the egg pad.

Egg Pad Size. For every population, the mean size of the egg pad for the eggs of *Ae. albopictus* was significantly different from those of the *Ae. aegypti* populations. In this instance the mean egg pad size was larger for all populations of *Ae. aegypti* when comparing the two species within their respective populations and when comparing the sum of all *Ae. aegypti* to all *Ae. albopictus*. Among the populations, the mean egg pad size of the *Ae. albopictus* eggs from McAllen were significantly less than those of the lab population, but neither was significantly different from the Brownsville population. The mean egg pad size of the *Ae. aegypti* eggs from Brownsville and McAllen were not significantly different from each other, but they were both significantly larger than those from the laboratory population. Overall, the combined mean egg pad size of both species of eggs from Brownsville and those from McAllen were significantly larger than that of the combined mean value of the lab population.

Surface-Area-to-Volume Ratio. The mean value of surface-area-to-volume ratio for the Brownsville and lab populations of *Ae. albopictus* eggs was significantly smaller than the *Ae. aegypti* from the same population. The two species from McAllen were not significantly different in this regard; however, the sum of all *Ae. albopictus* eggs measured had a significantly smaller surface-area-to-volume-ratio as compared to the sum total of the *Ae. aegypti* eggs measured. There was no significant difference among

the populations of *Ae. albopictus*. The *Ae. aegypti* eggs from the wild populations were not significantly different from each other either , but the lab population was significantly greater than both of them. Overall, there was no significant difference between the means of the different populations when combining both species together.

Surface-Area^{P}-to-Volume^{P} Ratio. For every combination of species and population, the mean value of the-surface-area-to-volume ratio (SA^{P}/V^{P}) decreased from the above mentioned ratio with the addition of the egg pad factored into the calculation. The mean surface-area-to-volume ratio with the addition of the egg pad was significantly different between the *Ae. albopictus* and *Ae. aegypti* species within the McAllen population and the lab population. The mean ratio was no longer significantly different among the two species in the Brownsville population, nor was there a significant difference among all of the *Ae. albopictus* eggs combined when compared to all of the *Ae. aegypti* eggs combined. This is the only dependent variable for which there was no significant difference between the means of all the *Ae. albopictus* eggs as compared to the mean value of all the *Ae. aegypti* eggs. The mean surface-area-to-volume ratio including the egg pad was significantly less for the *Ae. albopictus* Brownsville population as compared to the other populations, although there is no significant difference among the McAllen and lab populations of *Ae. albopictus*. The wild population's surface-area-to volume-ratio was significantly less than that of the lab population with the addition of the egg pad. However, when including the egg pad in the calculation, the mean ratio of all of the eggs from the Brownsville population combined is significantly less than that

of the lab population, but neither is significantly different from the combined mean value of the McAllen population.

Egg volume was greater in the *Ae. aegypti* eggs than *Ae. albopictus*; however, the surface area was larger, as well as the surface-area-to-volume ratio. This indicates a greater surface of cuticle for which water could be lost, and a higher susceptibility to desiccation. However, *Ae. aegypti* eggs were more desiccation resistant, which indicates another factor may be involved. Several studies have described the capacity for active water vapor absorption in insects and other various arthropods, a list of which can be found in Wright and Machin (1993). The physiological process for active water vapor absorption is not fully known, however there are a few elements all systems include. There must be a surface that collects water in contact with the cuticular surface of the arthropod, and there must be a mechanism that moves the water vapor into the arthropod (Wright and Machin 1993). The chorionic egg pad is a moisture rich structure in contact with the egg's cuticle, perhaps providing one of the necessary elements for active water vapor absorption. There would be more moisture in a larger egg pad, so that eggs with a larger egg pad would have access to a larger source of water vapor. Further research is required in order to determine if the necessary mechanism for moving the water vapor internally is present. However, in the event that this mechanism does not exist, the gelatinous egg pad on the side of the egg could contribute to desiccation resistance by reducing the direct contact of the air to that portion of the egg cuticle and the subsequent water loss.

The Giemsa stain worked exceptionally well in staining the egg pad. Exactly what the Giemsa stain was binding to on the egg pad was not addressed in this study, but this researcher thought it was important to note that the egg pad did indeed stain a pinkish-purple color. The Giemsa stain is a "Romanowsky-type" stain made from a mixture of methylene blue, azure and eosin compounds (Lyon et al. 1994). It is commonly used for blood smears due to its metachromasia properties, meaning some tissues will stain a different color than the dye itself. The Giemsa stain is blue, but the chorionic egg pad stained purple, which is common in staining of mast cell granules, mucin, cartilage and amyloid (Lyon et al. 1994).

CHAPTER V

PHYSIOLOGICAL TIME STUDY

Introduction

Temperature-dependent development rates have been determined for *Ae. aegypti* (Bar-Zeeve 1958, Kasule 1986, Rueda et al. 1990, Sames 1999, Southwood et al. 1972) and *Ae. albopictus* (Sames 1999), although the results varied with the strain's origin. This indicates that perhaps the temperature-dependant development rate curve for a given species might be different for populations of that species from different locations. However, there has not been a comparative study on the development rates of *Ae. aegypti* and *Ae. albopictus* on a region-by-region basis to explain the shifting of dominance and/or prevalence of the two species.

In order to know which mosquito species dominates where, geographically speaking, it might be important to know how the different species are affected by temperature, especially in the summer. According to a study on physiological time by Taylor (1981) that pooled development rates for 54 species of insects, several of which were mosquitoes, very similar species with differing temperature optimums (the temperature at which development is fastest) might experience species replacement seasonally in areas that have high summer temperatures. For these reasons, a study on physiological time as it pertains to *Ae. aegypti* and *Ae. albopictus* with the consideration of their region of origin, was added to this dissertation, resulting in temperature-dependent growth curves for several mosquito populations collected in Southern Texas.

In the Texas cities of Galveston and Brownsville, *Ae. albopictus* and *Ae. aegypti* are sympatric; so, mosquito eggs and larvae of each species were collected from these cities to established new colonies in the laboratory. According to The Weather Channel archives (theweatherchanel.com), Brownsville's average temperature is about 5˚C warmer than that of Galveston. Development rate curves were made from these four mosquito populations as well as from laboratory populations that have been established at the Texas A&M mosquito research facility. The *Ae. aegypti* UTMB (University of Texas Medical Branch) strain has been established since 1955 from a colony collected in Galveston, Texas, and the *Ae. albopictus* TAMU (Texas A&M University) strain has been established since 1987 collected in College Station, Texas. Both laboratory strains have since been maintained at 27˚C and 75% RH with a 14:10 L:D photoperiod.

Materials and Methods

Mosquitoes were reared as described in chapter 3. Eggs were collected for three days in Little Black Jars (LBJs) lined with paper towels. The paper towels with eggs were removed from the LBJs and placed inside a 19.1 x 25.4 cm sealed plastic box (Fig. 9A and 9B) and placed in an environmental chamber for one week at optimal conditions of 27˚C, 100% RH and a 14:10 L:D photoperiod. After one week, the paper towels containing eggs were cut into strips containing 50 eggs each. The strips were placed individually into 5 dram vials containing distilled water and the eggs were hatched by active vacuum. The contents of the vials were then poured into the bottom half of a mosquito breeder fashioned out of two two-litter plastic bottles with the top third cut off

A

B

C

Figure 9. Plastic box eggs were housed in for one week (A), Little Black Jar (LBJ) with paper towel for collecting eggs (B), mosquito breeder made from 2 two-liter plastic bottles (C).

and the bottoms joined together (Fig. 9C). 250ml of distilled water was added to the mosquito breeder as well as an optimal amount of larval food (ground Tetramin®), (about 3g in suspension). The mosquito breeders were then placed into environmental chambers that maintained a 14:10 L:D photoperiod and one of seven temperatures: 14˚, 17˚, 21˚, 27˚, 30˚, 33˚ and 36˚C. The total time for development was considered the length of time it took from when the eggs were hatched by vacuum to the first adult seen. The mosquito breeders were checked for emerging adults every 12 hours after pupae were observed.

For each of the six mosquito populations at each of the seven temperatures, five sets of 50 eggs were evaluated. The F2 progeny of the wild populations were evaluated for most treatments, but due to the inability to produce more individuals of that generation, some treatments were done with the F4 generation. For treatments conducted with the F4 generation, a duplicate of a temperature treatment previously conducted with an F2 was done for that population to control for potential differences. The mean development times for each of the six mosquito populations were calculated by the descriptive statistics option in SPSS (SPSS 1999). The percent development per day at a given temperature was calculated by determining the development rate (R) at each temperature *t* using the mean time in hours it takes to develop from an egg hatched by active vacuum to an adult *d* divided by the number of hours in a day (24), which returns the number of days it takes to complete development. 100 divided by the number of days needed for development equals the percent development that takes place

each day, and follows the following formula:

$R(t) = 100/ (d/24)$.

The percent development per day at a given temperature was plotted at each temperature for each of the six species and population combinations separately via a scatter plot of the values using Excel (Microsoft®, Office Excel® 2007) and forming a development rate curve. The temperature at which the mosquitoes develop the fastest, the optimal temperature (Tm), was determined, in addition to the maximum rate of development (Rm). These variables correspond to those analyzed by Taylor (1981) in his study on physiological time in insects.

The development times were analyzed by analysis of variance (ANOVA) using the general linear model (GLM) option in SPSS (SPSS 1999). All main effects of temperature, species and population and all 2-way and 3-way interactions were included in the model. In order to compare the populations and species to one another, post hoc tests on the means were performed with the data split by temperature and using Dunnett's T3 test for data of unequal variance and small sample size in SPSS (SPSS 1999). Using the Proc GLM procedure in SAS (SAS Institute Inc. 2002), all treatments were compared to one another by least squared means.

Results and Discussion

The 3-way interaction of temperature, population and time on the main effect of developmental time, resulted in an F value of 10.876 ($p < 0.0001$).

Mean Development Times. The mean development times (Table 12) and standard error (Table 13) at each temperature were calculated for each species and population combination. *Ae. albopictus* and *Ae. aegypti* from the laboratory populations were the only ones to reach the adult stage at 14˚C. A few *Ae. aegypti* from the Galveston populations lasted about three weeks, but died as first and 2nd instar larvae. Several *Ae. albopictus* from the Valley population as well as the Galveston population died as 3rd and 4th instar larvae after about two and a half months inside the incubation chamber with the appropriate amount of food added. *Ae. aegypti* from the Valley experienced little to no growth at 14˚C. It appears that 14˚C is a stressful or limiting temperature for *Ae. albopictus* and *Ae. aegypti* from these south Texas locations.

Ae. albopictus from the Valley and from the laboratory population experienced death as 2nd, 3rd and 4th instar larvae at 36˚C, which suggests that this is another stressful or limiting temperature especially for *Ae. albopictus*. However, in the *Ae. aegypti* laboratory population, only two of the five sets of mosquito breeders had mosquitoes that reached the adult stage and only one adult in each set.

The results of the least squared means tests (Table 14) show that, at every temperature the development rates for all of the *Ae. aegypti* studied and all of the *Ae. albopictus* studied are significantly different from each other and among themselves except for three instances. *Ae. albopictus* and *Ae. aegypti* at 27˚, 30˚ and 33˚C are not significantly different intraspecifically, indicating that the development rates do not vary greatly at these temperatures for either species. At 36˚C, the mean development time for *Ae. albopictus* was not significantly different from that of *Ae. albopictus* or *Ae. aegypti*

Table 12. Mean development rate in hours and number[1] (in parentheses) of mosquito breeders in which *Ae. albopictus* and *Ae. aegypti* adults were produced.

Pop	Species	14˚C	17˚C	21˚C	27˚C	30˚C	33˚C	36˚C	Total
Gal[2]	Albo[6]	death	467.2(5)	394.4(5)	195.2(5)	188.6(5)	179(5)	256.6(5)	280.1 (30)
	Aeg[7]	death	487(5)	301(5)	149.5(10)[9]	164.8(5)[8]	148.6(5)[8]	153.4(5)	221.9 (35)
	Total	death	477.1(10)	347.7(10)	164.7(15)	176.7(10)	163.8(10)	205(10)	280.1(65)
Val[3]	Albo	death	527.6(5)	268.4(5)	196.3(10)[9]	159.4(5)	166.6(5)	death	252.4 (30)
	Aeg	death	452.6(5)	281.8(5)	170.8(5)	120.4(5)	131.8(5)	220(5)	227.6 (30)
	Total	death	490(10)	275.1(10)	186.75(15)	139.9(10)	149.2(10)	220(5)	252.4(60)
Lab[4]	Albo	1057.2(5)	521.4(5)	275.1(10)	146.4(5)	221.4(5)	197.2(4)	death	390.3 (34)
	Aeg	983.6(5)	503.6(5)	238(10)	144.2(5)	148.2(5)	152.4(5)	217.5(2)	337.1 (37)
	Total	1057.2(10)	512.5(10)	256.5(20)	145.3(10)	184.8(10)	172.3(9)	217.5(2)	390.3(71)
All[5]	Albo	1057.2(5)	505.4(15)	303.2(20)	183.5(20)	189.8(15)	179.8(14)	256.6(5)	311.1 (94)
	Aeg	983.6(5)	481.1(15)	264.7(20)	154.3(20)	144.4(15)	144.2(15)	191.8(12)	265.1 (103)
	Total	1020.4(10)	493.2(30)	283.9(40)	168.6(40)	167.1(30)	179.8(29)	210.9(17)	287.1 (197)

[1]For each treatment, five sets of mosquito breeders containing 50 eggs were processed, except in the case of *Ae. aegypti* and *Ae. albopictus* Lab at 21˚C, *Ae. aegypti* Galveston at 27˚C and *Ae. albopictus* at 27˚C in which there were ten sets.
[2]Populations collected in Galveston County, TX.
[3]Populations collected in the Texas Rio Grande Valley.
[4]Laboratory population (*Ae. albopictus* TAMU and *Ae. aegypti* UTMB).
[5]All population's means together.
[6]Albo, *Ae. albopictus*
[7]Aeg, *Ae. aegypti*.
[8]F4 progeny
[9]F2 and F4 progeny (five sets of each)

Table 13. Standard error for mean development rates of laboratory and experimental populations of *Ae. albopictus* and *Ae. aegypti* as determined by ANOVA.

Pop	Species	14˚C	17˚C	21˚C	27˚C	30˚C	33˚C	36˚C
Gal[1]	Albo[5]	death	7.0	10.3	5.4	9.4	5.6	5.4
	Aeg[6]	death	4.3	3.0	2.4	5.3	7.7	13.7
Val[2]	Albo	death	7.0	10.3	3.8	9.4	5.6	death
	Aeg	death	4.3	3.0	3.1	5.3	7.7	13.7
Lab[3]	Albo	28.1	7.0	7.3	5.4	9.4	6.3	death
	Aeg	14.6	4.3	2.1	3.4	5.3	7.7	21.7
All[4]	Albo	28.1	4.1	5.4	2.8	5.4	3.4	5.4
	Aeg	14.6	2.5	1.6	1.7	3.0	4.5	9.7

[1]Populations collected in Galveston County, TX.
[2]Populations collected in the Texas Rio Grande Valley.
[3]Laboratory population (*Ae. albopictus* TAMU and *Ae. aegypti* UTMB).
[4]All population's means together.
[5]Albo, *Ae. albopictus*
[6]Aeg, *Ae. aegypti*

Table 14. Resulting p-values for least squared means test on the development times for *Ae. albopictus* and *Ae. aegypti* treated as separate species.

		17˚C		21˚C		27˚C		30˚C		33˚C		36˚C	
		Albo[1]	Aeg[2]	Albo	Aeg	Albo	Aeg	Albo	Aeg	Albo	Aeg	Albo	Aeg
17˚C	Albo		.0045*	*	*	*	*	*	*	*	*	*	*
	Aeg	0.0045*		*	*	*	*	*	*	*	*	*	*
21˚C	Albo	*[3]	*		*	*	*	*	*	*	*	0.387	*
	Aeg	*	*	*		*	*	*	*	*	*	0.1197	*
27˚C	Albo	*	*	*	*		0.0005*	0.1973	*	0.88	*	*	0.0546
	Aeg	*	*	*	*	0.0005*		*	0.2906	0.0008*	0.2793	*	*
30˚C	Albo	*	*	*	*	0.1973	*		*	0.2894	*	*	0.4491
	Aeg	*	*	*	*	*	0.2906	*		*	0.9811	*	*
33˚C	Albo	*	*	*	*	0.88	0.0008*	0.2894	*		*	*	0.0908
	Aeg	*	*	*	*	*	0.2793	*	0.9811	*		*	*
36˚C	Albo	*	*	0.387	0.1197	*	*	*	*	*	*		*
	Aeg	*	*	*	*	0.0546	*	0.4491	*	0.0908	*	*	

[1]Albo, *Ae. albopictus*
[2]Aeg, *Ae. aegypti*.
[3]Mean development times with an * are significantly different with a p-value of < 0.0001, those p-values with an * are significantly different

at 21˚C. These are suboptimal temperatures, which reinforces the assumption used in Taylor's (1981) study of physiological time that states the development rate declines symmetrically on both sides of the temperature optimum. However, it is important to note that only the *Ae. albopictus* population from Galveston reached the adult stage at 36˚C.

Another unusual pattern with development times at 36˚C occurred in the case of *Ae. aegypti*, which was not significantly different from that of *Ae. albopictus* at 27˚, 30˚ or 33˚C. These correspond with the fastest development rates for *Ae. albopictus*, but at the point in which the development rate declined for *Ae. aegypti* from its highest development rates.

The mean development times for each species fluctuated at each temperature similarly to the contrasting species within the same population (Table 15). For each population of a given species, the mean development times were significantly different at each temperature except at the 27˚ – 33˚C range with few exceptions. Among the populations from the Valley, there is no significant difference between the development times only at 30˚ and 33˚C for each species. The development time for the laboratory population of *Ae. aegypti* was the same in this regard, however, those for 21˚ and 30˚C are not significantly different from each other; however, they were different for populations reared at 27˚C. This is likely an effect of the lab population being reared under this temperature for a long period of time (years). In the case of *Ae. aegypti* in the Galveston and lab populations, there was no significant difference between the means over a broader range of temperature, 27˚ – 36˚C. Oddly enough, none of the

Table 15. Significantly-different mean development times for each population of the same species of *Ae. albopictus* and *Ae. aegypti* by temperature (Columns) and for each temperature by population and species (Rows) according to Dunnett's T3 test.

		14˚C	17˚C	21˚C	27˚C	30˚C	33˚C	36˚C
Ae. albopictus	Galv[1]	-	A[5] x[6]	B x	C x	C x	C xy	D
	Valley[2]	-	A y	B y	C x	D y	D x	-
	Lab[3]	A	B y	C y	D y	CE x	E y	-
Ae. aegypti	Galv	-	A x	B x	C x	C x	C x	C x
	Valley	-	A y	B x	C y	D y	D x	E y
	Lab	A	B x	C y	D x	D x	D x	ABCD xy
Total[4]		A	B	C	D	D	D	E

[1]Populations from Galveston County, TX.
[2]Populations from the Texas, Rio Grande Valley.
[3]Laboratory population.
[4] Species and populations of each temperature combined.
[5]Different temperature treatments within the population of the given species for which the developmental mean time is not significantly different (Dunnett's T3, $p < .05$) have the same letter (A, B, C, D or E).
[6]Different populations of the same species in columns with the same letter (x, y or z) are not significantly different (Dunnett's T3, $p < .05$)

developmental means for *Ae. aegypti* from the lab population were significantly different from the developmental mean at 36˚C. This can be attributed to the large variation in developmental times at 36˚C.

There was a lot of variation in development rates among the different populations within a species at the different temperatures. At 17˚ and 21˚C, the Valley and lab populations of *Ae. albopictus* were significantly different from the Galveston population. At 27˚C, the lab population of *Ae. albopictus* had a significantly faster development rate than the Galveston or Valley populations. *Ae. albopictus* from the Valley population had a significantly faster development rate at 30˚C than the other two, but only significantly faster than the lab population at 33˚C.

Interestingly, the *Ae. aegypti* Galveston population was only significantly different from that of the lab population at 21˚C in which the lab population was significantly faster than the other two. The Valley population of *Ae. aegypti* was significantly different from the other two at most temperatures, but not at 33˚C for which none of them were significantly different. At 36˚C, the Valley population of *Ae. aegypti* was significantly different from that of the Galveston population, but neither was significantly different from the lab population due to latter's large variance.

Development Rate Curves. The temperature-dependent development rate curve for each of the six populations was constructed. When plotted all together (Fig. 10), it is apparent that they are all different from each other, yet they still group together by species. All of the *Ae. aegypti* development rate curves exhibit an overall higher rate of

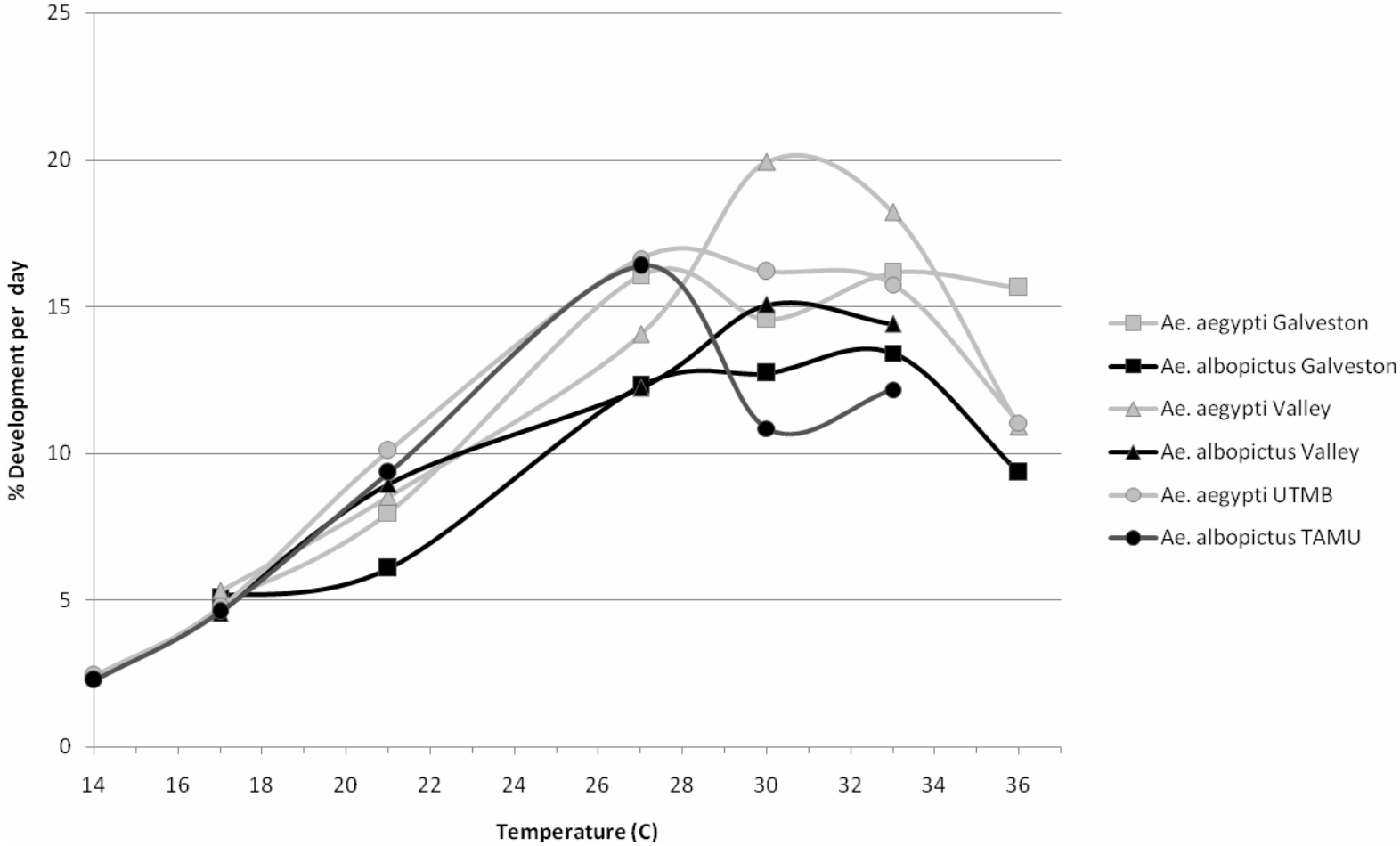

Figure 10. Temperature-dependent development rates (% development per day) for all study populations of *Ae. aegypti* and *Ae. albopictus.*

development than *Ae. albopictus*. However, to be able to clearly distinguish the individual characteristics of each population's development rate curve, they were plotted separately and the temperature optimum (Tm) and the maximum rate of development (Rm) were determined.

An interesting pattern was observed for the development rate curve at 30˚C for four of the six populations excluding those species from the Valley (Fig. 11 & 12). At 30˚C, the development rate decreased by a small amount in the lab populations (Fig. 13 & 14) and the Galveston *Ae. albopictus* population (Fig. 15) and even more in the case of *Ae. aegypti* from Galveston (Fig. 16), followed by another increase in the development rate. This deviation from the typical exponential curve has been observed before in *Ae. aegypti* in studies by Bar-Zeeve (1958) and Sames (1999), but in both instances, the decrease was at 32˚C followed by a small increase at 34˚C. They made no mention of this decrease, but it was prominent in this study.

The decrease in development rate at 30˚C in the Galveston and laboratory populations might be due to the lack of selection, i.e. there is little need for the mosquito larvae to mature faster at this temperature. Perhaps there is more competition at a slightly lower and slightly higher temperature such as 27˚ and 33˚C, at which point the costs of developing faster such as a resulting smaller size, outweigh the benefits of developing slower, and that the opposite is true at 30˚C. It is also possible, that the lower development rate at 30˚C is due to experimental error. However, the Valley population was in the same environmental chamber as the other populations at the same time and all chambers were checked twice daily with no marked deviation from 30˚C.

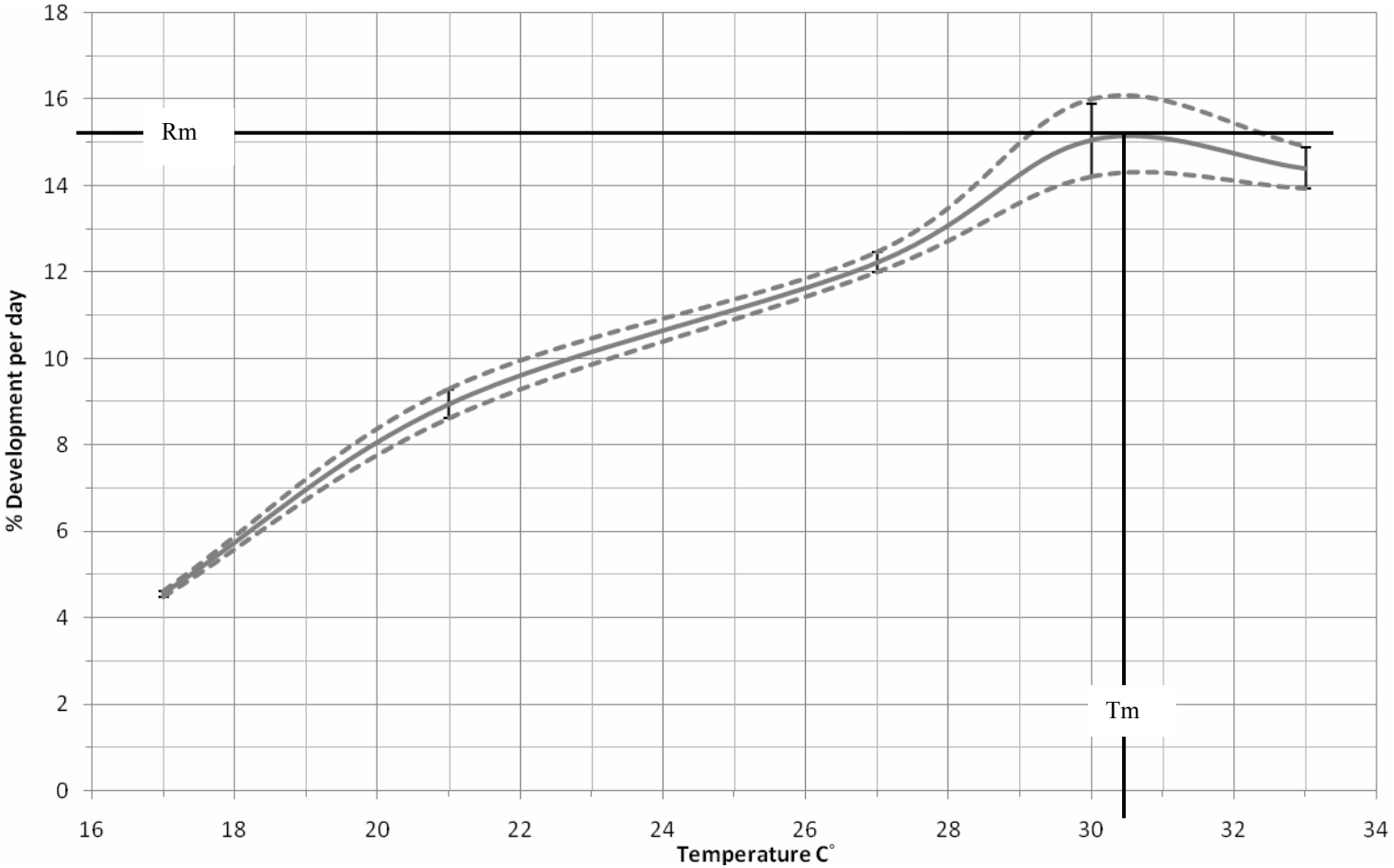

Figure 11. Percent development per day by temperature for the Valley population of *Ae. albopictus* [Temperature (Tm) at the maximum rate of development (Rm) derived from mean development time at each testing temperature of 14, 17, 21, 27, 30, 33 and 36˚C] (standard error shown).

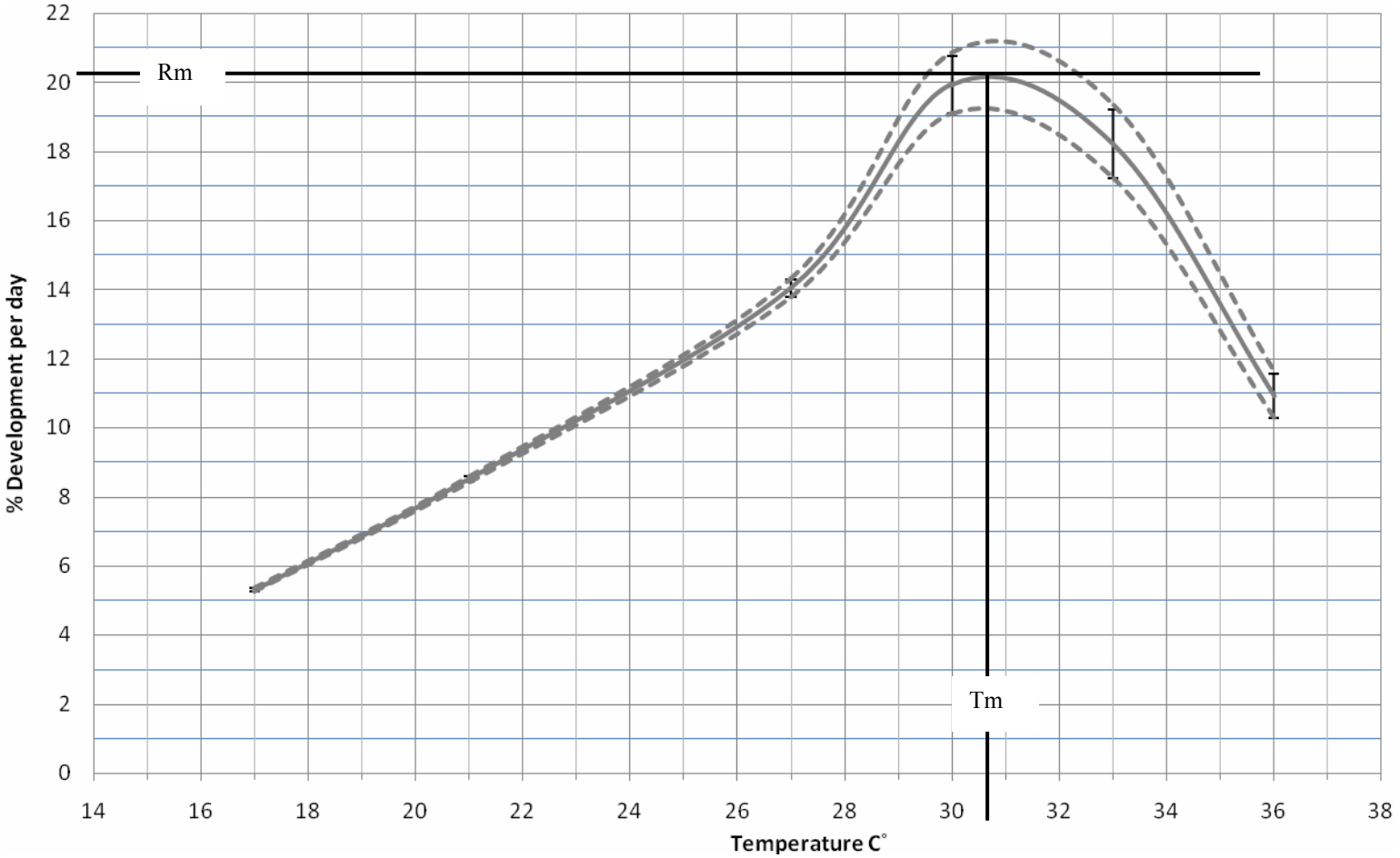

Figure 12. Percent development per day by temperature for the Valley population of Ae. *aegypti* [Temperature (Tm) at the maximum rate of development (Rm) derived from mean development time at each testing temperature of 14, 17, 21, 27, 30, 33 and 36˚C] (standard error shown).

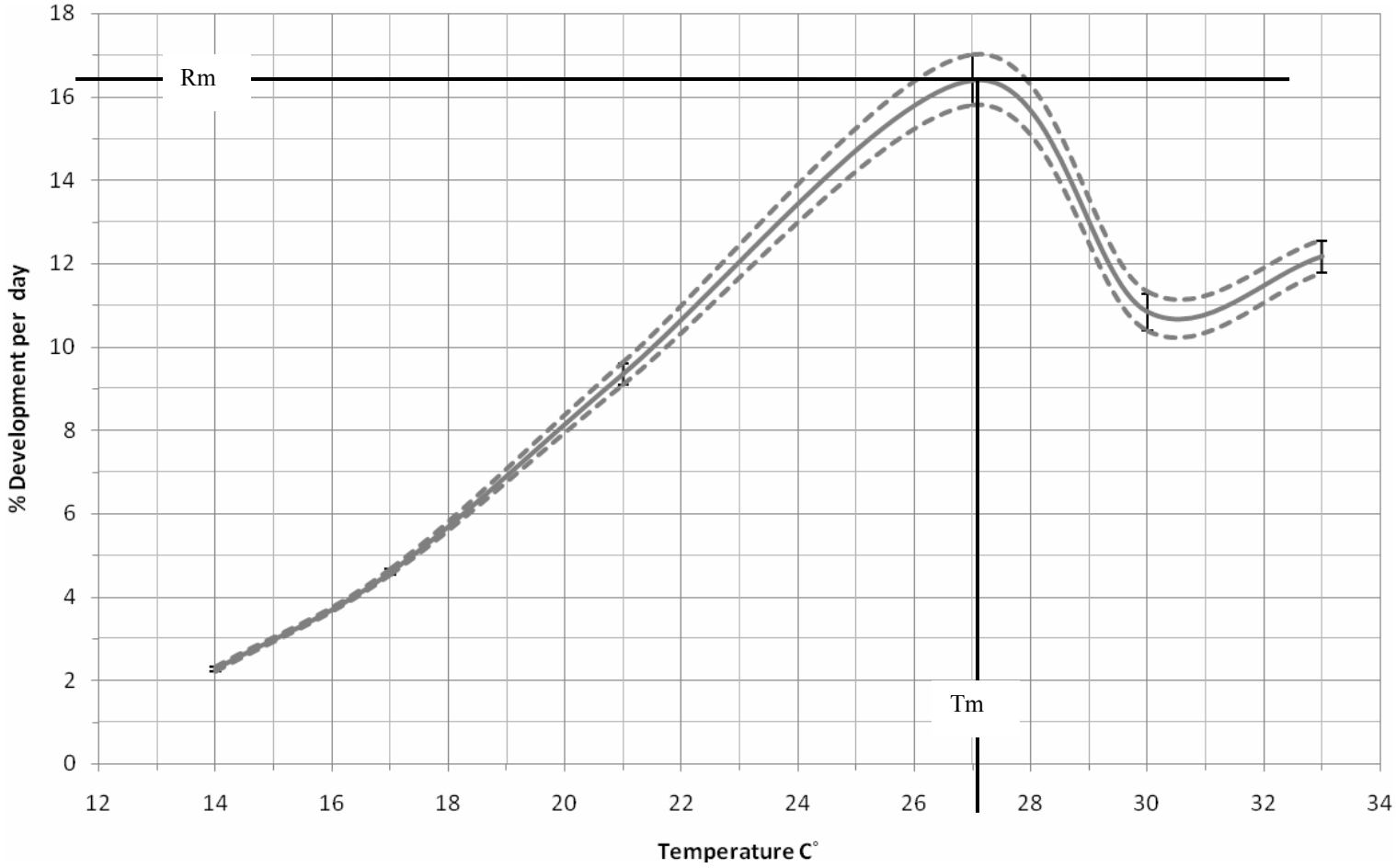

Figure 13. Percent development per day by temperature for the TAMU (lab) population of *Ae. albopictu* [Temperature (Tm) at the maximum rate of development (Rm) derived from mean development time at each testing temperature of 14, 17, 21, 27, 30, 33 and 36˚C] (standard error shown).

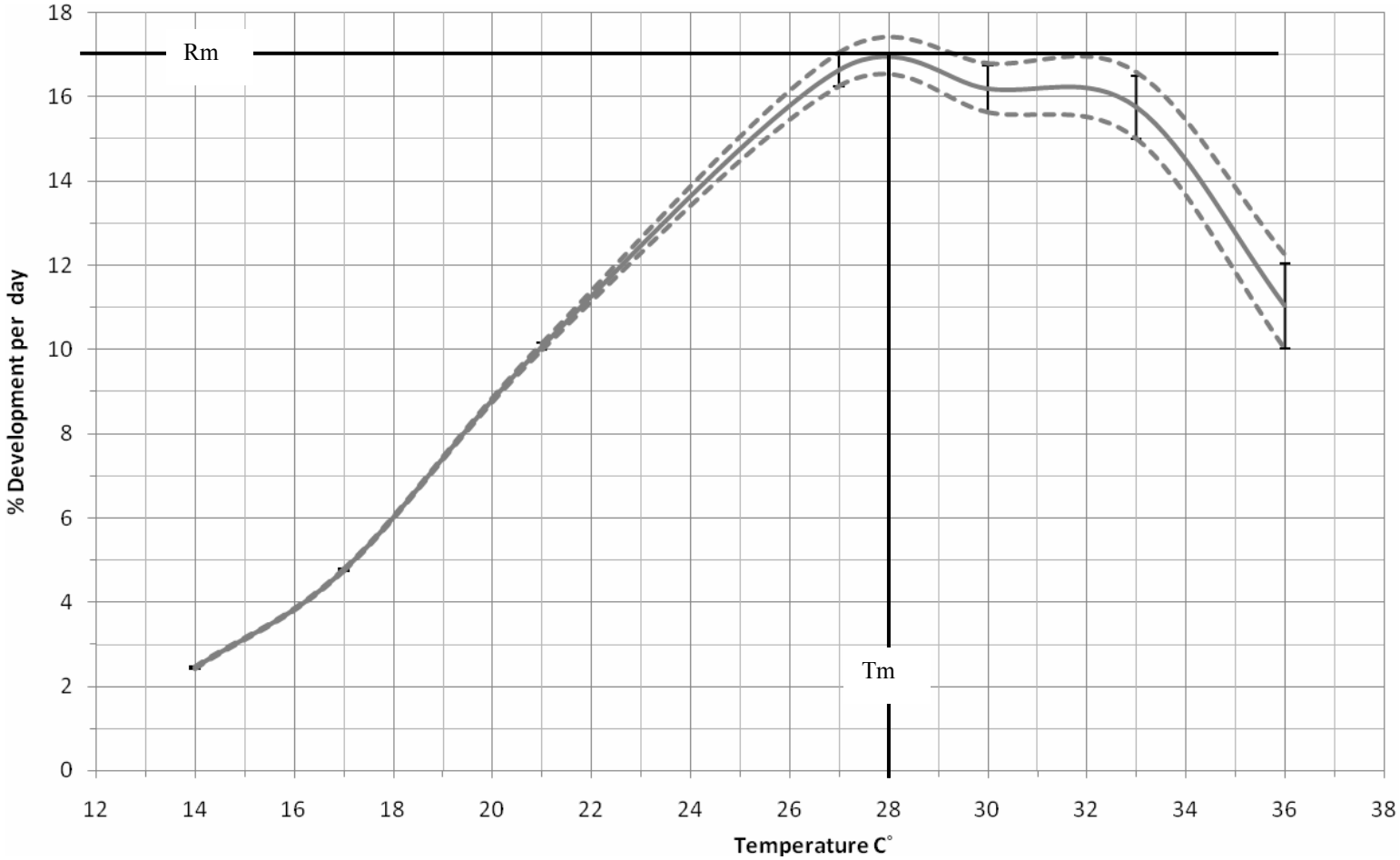

Figure 14. Percent development per day by temperature for the UTMB (lab) population of *Ae. aegypti* [Temperature (Tm) at the maximum rate of development (Rm) derived from mean development time at each testing temperature of 14, 17, 21, 27, 30, 33 and 36˚C] (standard error shown).

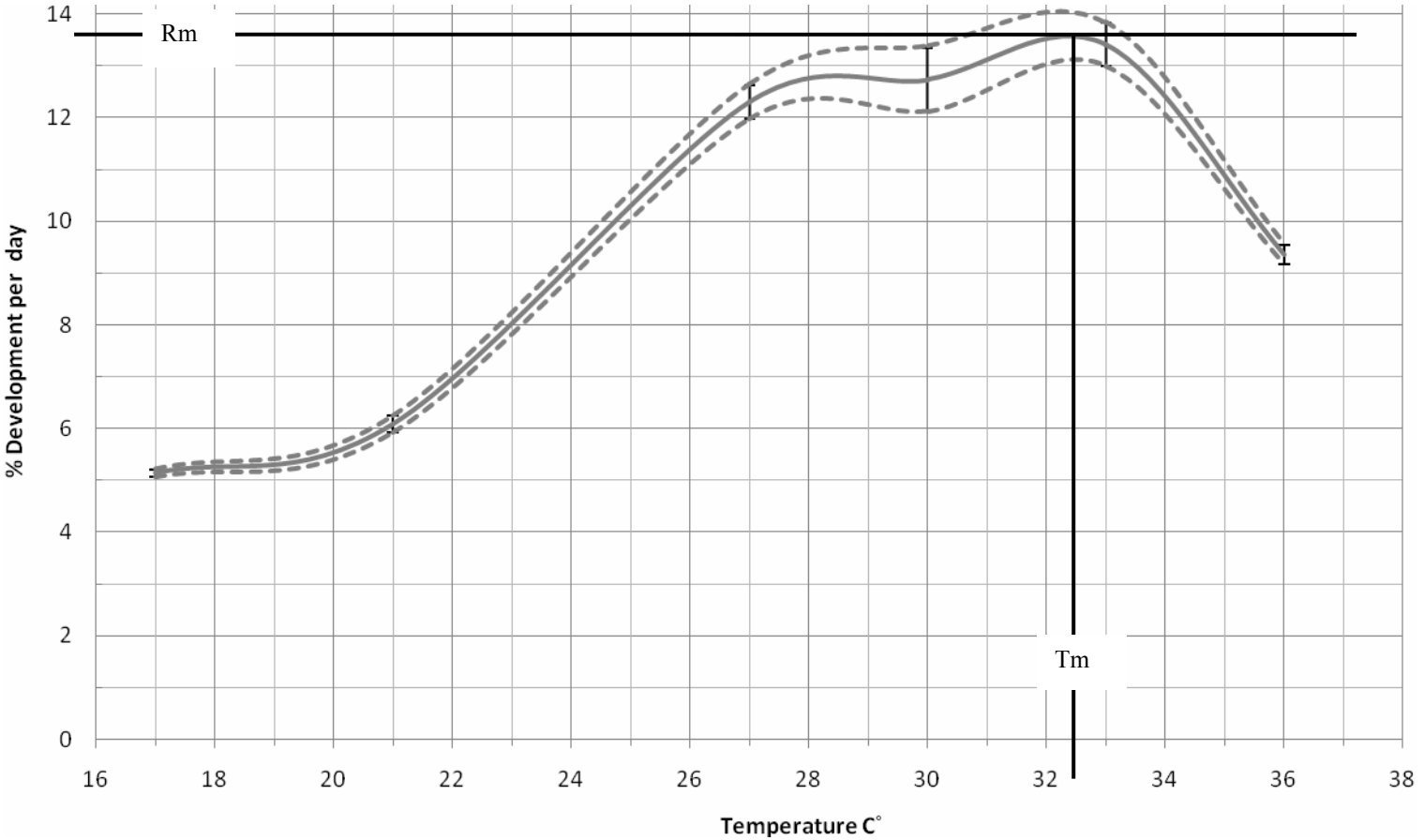

Figure 15. Percent development per day by temperature for the Galveston population of *Ae. albopictus* [Temperature (Tm) at the maximum rate of development (Rm) derived from mean development time at each testing temperature of 14, 17, 21, 27, 30, 33 and 36˚C] (standard error shown).

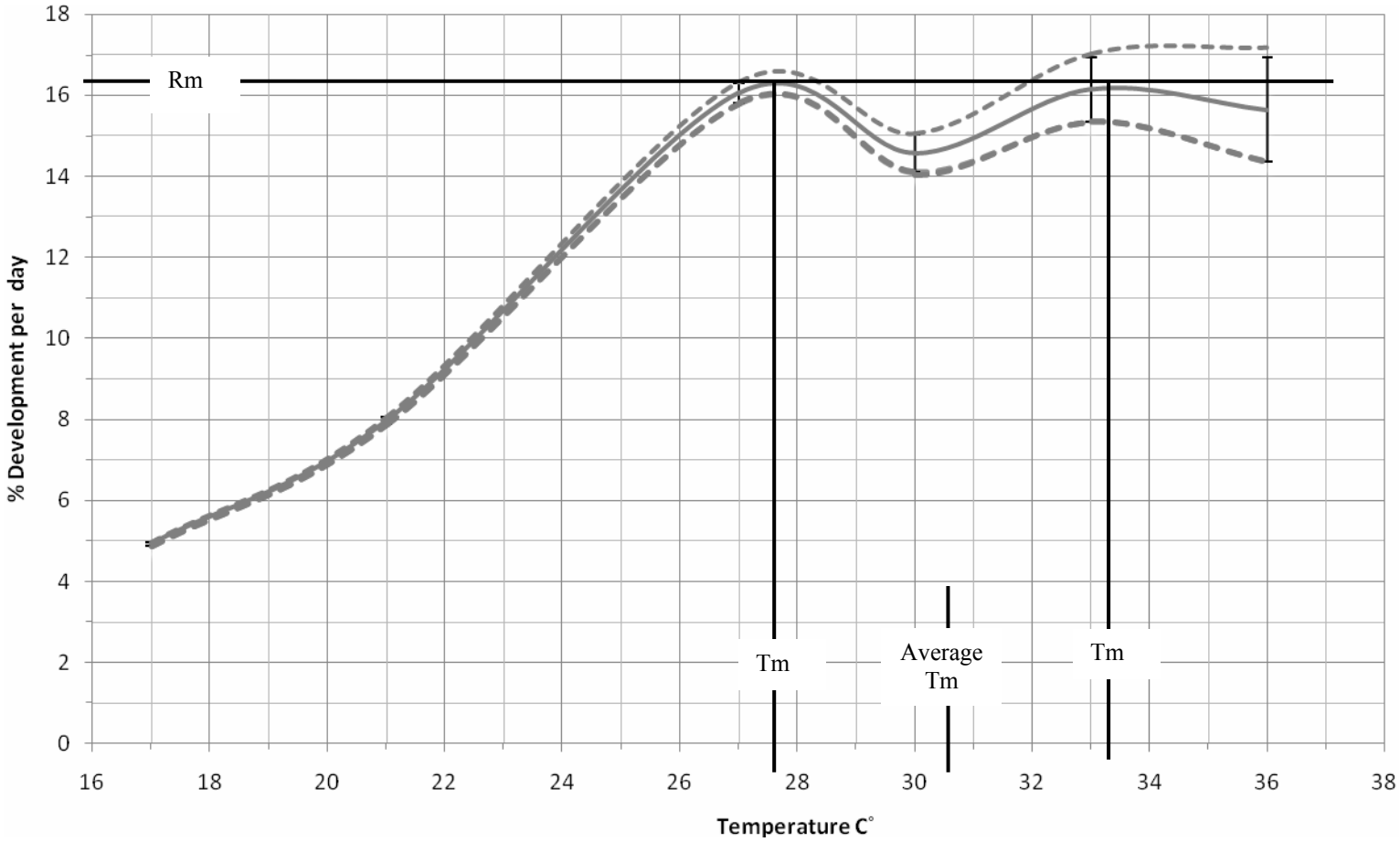

Figure 16. Percent development per day by temperature for the Galveston population of *Ae. aegypti* [Temperature (Tm) at the maximum rate of development (Rm) derived from mean development time at each testing temperature of 14, 17, 21, 27, 30, 33 and 36˚C] (standard error shown).

In the case of the population of *Ae. aegypti* from Galveston, the temperature at which the rate of development is fastest, the temperature optimum, appears to occur in two places along the development rate curve with a 0.1% development per hour difference (Fig. 16). Due to the possibility that this is a result of experimental error, the two temperatures were averaged together. The combined development means for all the populations of *Ae. albopictus* (Fig. 17) and *Ae. aegypti* (Fig. 18) were also plotted so that the optimal temperature (Tm) and maximum rate of development (Rm) could be determined. All of the values of these two parameters for each of the six populations was then tabulated (Table 16).

The maximum development rate (Rm) was faster in the *Ae. aegypti* populations than in the *Ae. albopictus*. The laboratory population of *Ae. albopictus* had the fastest rate for its species, but it was merely equal to the lowest rate of *Ae. aegypti*. The optimal temperatures for all populations were actually all very similar to one another. Overall, there were not many differences between *Ae. aegypti* and *Ae. albopictus* as a whole, but when compared by origin of population there are many differences.

Galveston, TX. *Ae. aegypti* is not abundant in Galveston (personal contact with Keith Haas, Galveston County Mosquito Control district), so Galveston is an area in which *Ae. albopictus* is the dominate species. In this study, *Ae. albopictus* from Galveston had a broader development rate curve than did sympatric *Ae. aegypti*. This was the only instance in this study for which that was true. Additionally, the temperature optimum for *Ae. albopictus* Galveston was higher than all the other species and populations. The

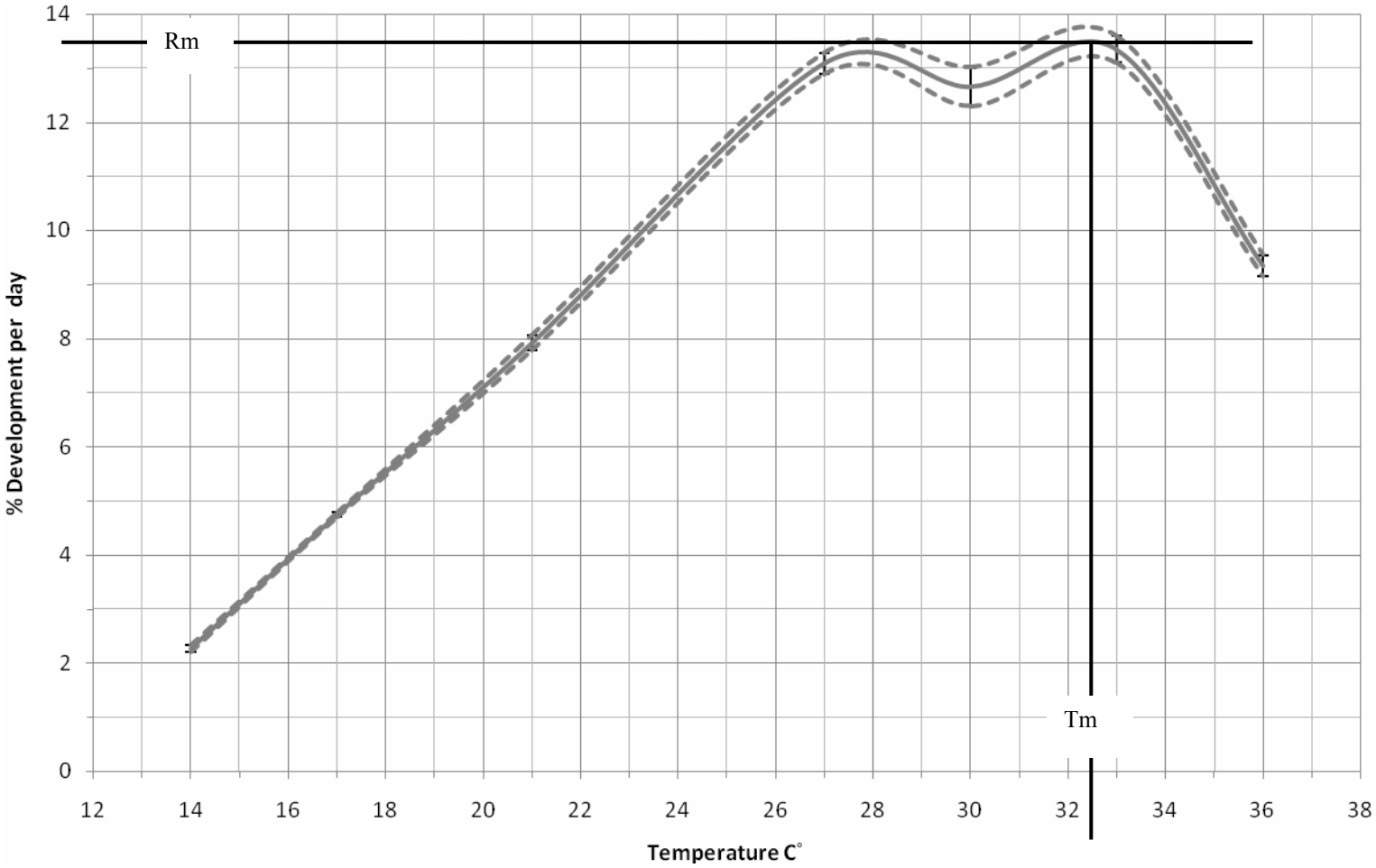

Figure 17. Percent development per day by temperature for all *Ae. albopictus* populations combined [Temperature (Tm) at the maximum rate of development (Rm) derived from mean development time at each testing temperature of 14, 17, 21, 27, 30, 33 and 36˚C] (standard error shown).

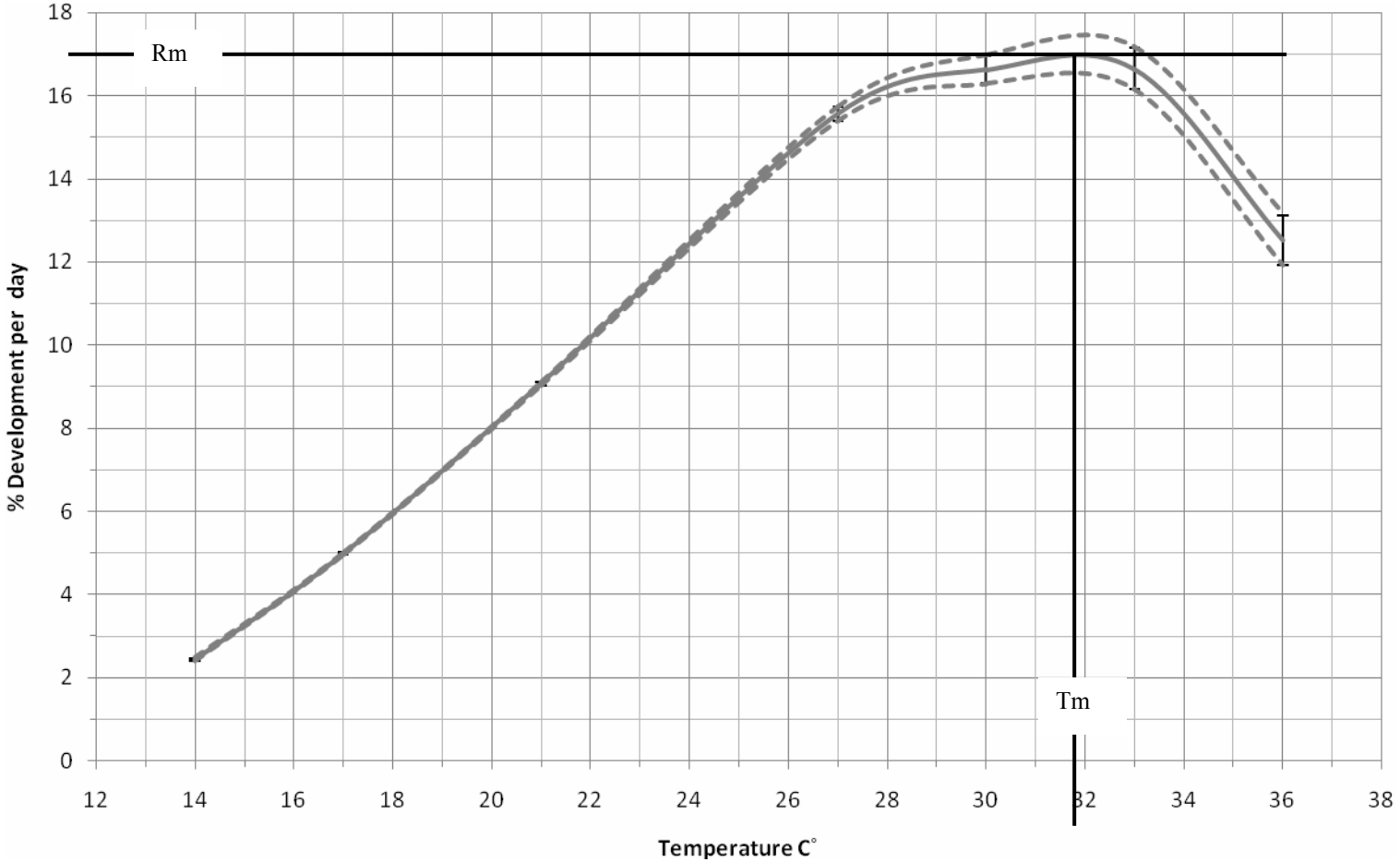

Figure 18. Percent development per day by temperature for all *Ae. aegypti* populations combined [Temperature (Tm) at the maximum rate of development (Rm) derived from mean development time at each testing temperature of 14, 17, 21, 27, 30, 33 and 36˚C] (standard error shown).

Table 16. Developmental parameters for *Ae. aegypti* and *Ae. albopictus* by population and species, derived development curves of mean percent development per hour, at each temperature.

Species	Population	Tm[1] (C°)	Rm[2]
Ae. albopictus	Galveston	32.4	13.6
	Valley	30.5	15.2
	Lab	27.1	16.4
	All	32.4	13.5
Ae. aeygypti	Galveston	30.45[3]	16.3
	Valley	30.65	20.2
	Lab	28	17
	All	31.9	17

[1]Temperature at the maximum rate of development, optimal temperature.
[2]Maximum rate of development.
[3]Average temperature optimum for *Ae. aegypti* Galveston.

maximum rate of development was faster in Galveston's *Ae. aegypti* (Rm = 16.3%) as compared to *Ae. albopictus* (Rm = 13.6%), but this faster development rate might lead to the production of smaller, less fit mosquitoes, which might help explain why *Ae. albopictus* is a better competitor and the dominate species in Galveston County.

Texas Rio Grande Valley. *Ae. albopictus* and *Ae. aegypti* occur at equal levels in Brownsville, Texas throughout the mosquito season which spans April to September (Personal communication with Jose Hinjosa, Brownsville Health department). The average temperature in the Rio Grande valley cities of McAllen and Brownsville, TX (50 mile separation) at the beginning of the mosquito season are between 23 and 33˚C according to The Weather Channel archives. At these temperatures, *Ae. albopictus* and *Ae. aegypti* would be developing at their optimal temperature (30.5˚ and 30.65˚C). The corresponding rate of development for *Ae. aegypti* is 20.5% per day, which is much faster than *Ae. albopictus* (Rm = 15.2%). It is likely that this faster development rate in the *Ae. aegypti* population is what allows them to maintain a population at all, given that *Ae. albopictus* is a superior competitor. The average temperature increases to 25˚ - 35˚C in July and August (The Weather Channel), and because of the very narrow range that *Ae. albopictus* can develop optimally, it is likely their population decreases. Additionally it was demonstrated in this study that they cannot reach the adult stage at 36˚C (Table 10). So under these circumstances, it is highly probable that *Ae. aegypti* would become the prevalent species in these regions. However this does not consider

microhabitats in which shading is likely to provide microclimates that favor *Ae. albopictus*, which is probably why the two species exist in equal numbers in this region.

Laboratory Populations. As for the laboratory populations, they both had the same optimal temperature that corresponds to the temperature at which they have been maintained for so many years. This is the lowest of the optimal temperatures for *Ae. aegypti*, but it corresponds with the largest range of temperatures (14˚C) at which development is at its fastest and it has the fastest, maximum rate of development (Rm = 17%). It is possible that in the wild at warmer temperatures *Ae. aegypti* populations evolve to withstand the warmer temperature, and the ability to develop at a high rate over a broad range of temperatures is lost or reduced.

The optimal temperatures plotted against the corresponding maximum rate of development (Fig. 19) depict the main difference between the two species, which is *Ae. aegypti* develops at a faster rate as compared to *Ae. albopictus*. Optimal temperatures are similar for each species of the same origin; however, the difference in development rates is greatest between the two species with the same origin in the case of the wild populations. This may be indicative of the separate microclimates each species inhabits in its city of origin.

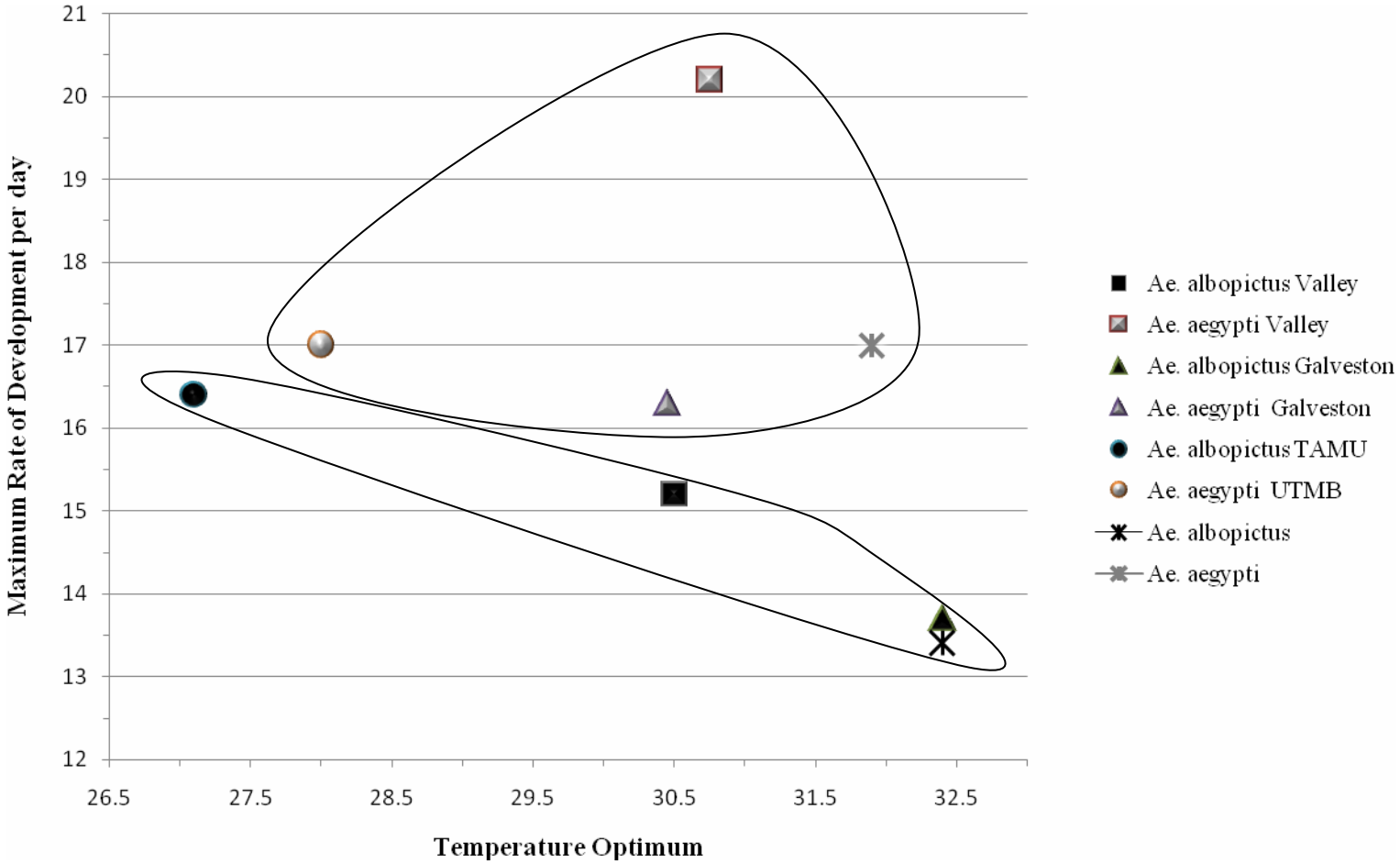

Figure 19. Maximum rate of development per day and the corresponding optimal temperature for various populations of *Ae. albopictus* and *Ae. aegypti*.

CHAPTER VI

SUMMARY AND CONCLUSIONS

The purpose of this study was to determine what environmental factors impact the success of *Ae. aegypti* and *Ae. albopictus* populations in different geographic areas, particularly in South Texas where there is an increasing threat of Dengue Fever carried by these mosquitoes.

The percent hatch for eggs of *Ae. albopictus* and *Ae. aegypti* collected from McAllen and Brownsville, Texas, as well as from laboratory populations were determined after they had been subjected to 25 different temperature and relative humidity combinations: 15˚, 21˚, 27˚, 32˚, 35˚C in combination with 15%, 35%, 55%, 75%, 95% RH, for up to three months. The 5-way interactions of all the variables (i.e., species, population, temperature, relative humidity and time) was significant. This indicated that the percent egg hatch for *Ae. albopictus* and *Ae. aegypti* eggs is dependent on the effects of several climatic variables in combination. The effect of temperature on the percent egg hatch was such that, in most treatments, *Ae. aegypti* eggs had a significantly greater percent hatch than did those of *Ae. albopictus*, but to a much lesser extent at the lower temperatures of 15˚ and 21˚C. With an increase in relative humidity, the hatching percentages for both species increased, but at the higher temperatures of 32˚ and 35˚C, the amount of time the eggs were exposed to those temperatures had a greater negative effect on the percent hatch than did the positive effect of increase in relative humidity. Overall, percent hatch decreased during the eighth and twelfth weeks, except in the case of *Ae. aegypti* at 15˚C for which the opposite was true. The greater impact of

temperature with the increase in time the eggs are exposed to that temperature indicates it may be wise to reduce watering of yards and cemeteries in regions that experience high temperatures such at 32˚ and 35˚C as a means of mosquito population control.

Overall, this study on percent hatch, leads to the conclusion that *Ae. aegypti* eggs, particularly from Texas, hatch at a much higher percentage than *Ae. albopictus,* especially in warmer climates. In warmer climates this increase in percent hatch coupled with the paralleled decrease in percent hatch of *Ae. albopictus* eggs, could translate into more individuals of *Ae. aegypti,* which may be the advantage necessary to overcome the competitive superiority *Ae. albopictus* has demonstrates in the larval (Sucharit et al. 1978, Sames 1999, Juliano 1998, Blackmore et al. 1995) and adult stages (Costanzo et al. 2005, Galliard 1962, Hein 1976, Nasci et al. 1989, Soekiman et al. 1984).

Desiccation of the mosquito eggs via the flow of water from the eggs to the air could have also been analyzed by measuring the drying power of the air. The interaction of relative humidity and temperature could also be explained by calculating the saturation deficit as a measure of the drying power of in the test environments. Water loss from arthropods is increased by high saturation deficiencies in the environment due to low relative humidity and high temperatures (Edney 1957).

In search of a structural mechanism on the mosquito egg that aids in desiccation resistance, 50 eggs of each population of *Ae. albopictus* and *Ae. aegypti* collected in McAllen and Brownsville, as well as from the laboratory populations, were dyed to view and measure the chorionic egg pad (a gelatinous pad formed by the swelling of the epichorion). Sota and Mogi (1992) have found a high correlation between large egg

volume and an increase in desiccation resistance, and that *Ae. aegypti* eggs are significantly larger than *Ae. albopictus* eggs. Thus, the volume of each egg was also calculated. Moisture is lost primarily through the cuticle (Cooper 1983), so larger amounts of cuticle or surface area on the eggs may increase desiccation. The significance of surface area is increased with a decrease in volume. For these reasons, the surface area and volume of the eggs with and without the chorionic egg pad, the size of the chorionic egg pad and the surface-area-to-volume ratio with and without the egg pad was calculated. Following a significant multivariate affect of the variables by species, population and species-population, the means for each variable were compared.

The egg volume with and without the chorionic egg pad was significantly larger in *Ae. aegypti* from the wild populations, but the opposite was true with the laboratory populations. Both species from the McAllen population had a significantly larger volume as compared to the other populations. The surface area with and without the chorionic egg pad was significantly larger in the *Ae. aegypti* eggs than the *Ae. albopictus* eggs as was the surface area to volume ratio, which could indicate greater susceptibility to desiccation. However, the chorionic egg pad which can be likened to a wet sponge was significantly larger on *Ae. aegypti* eggs than *Ae. albopictus* eggs and may contribute to the increased desiccation resistance in *Ae. aegypti* by providing a source of water to the eggs in contact with the egg cuticle. This water source could be utilized for active water vapor absorption or as a means of reducing the contact of drying air with the egg cuticle. A larger egg pad could then lead to a longer association with this water source.

The egg volume of *Ae. albopictus* Brownsville population was the smallest among the populations. However, in the study on percent egg hatch this population of *Ae. albopictus* had a higher percent hatch then the other populations, and at the lowest level of humidity, did not have a percent hatch significantly different from that of *Ae. aegypti*. Additionally, the *Ae. albopictus* population from Brownsville was the only population of either species that did not have a significant decrease in percent hatch between the exposure time of eight and twelve weeks. This is important, because *Ae. albopictus* from Brownsville had the smallest surface-area-to-volume ratio only when including the egg pad. The size of the egg pad was not significantly different from the other populations, however, in calculating the surface-area-to-volume ratio with the egg pad, the size of the egg pad is related to the overall size of the egg in which case the egg pad on the eggs of *Ae. albopictus* from Brownsville could be said to have a larger surface of contact with the eggs than in the case of the other populations. This suggests that the size of the egg pad in relation to the overall size of the egg can indicate a greater ability to resist desiccation, and that this chorionic egg pad may play a role in the high desiccation resistance seen in the eggs of *Ae. albopictus* Brownsville and those of *Ae. aegypti*.

Development rate curves for *Ae. albopictus* and *Ae. aegypti* of mosquitoes from populations collected in Galveston and Brownsville, Texas, as well as from the laboratory populations, were created by measuring the development time from a hatched egg to the adult at seven temperatures; 14˚, 17˚, 21˚, 27˚, 30˚, 33˚ and 36˚C. The temperature at which development is the fastest, i.e. the optimum temperature, was very

similar in all populations tested. However, the rate of development for the *Ae. aegypti* populations was significantly faster at this optimum. In the Galveston population, *Ae. albopictus* had a wider range of temperatures for which the development rate was high, as compared to the sympatric *Ae. aegypti* species, which may attribute to why *Ae. albopictus* is the dominate species in Galveston, Texas. In the Brownsville population, *Ae. aegypti* developed at a high rate over a broad range of temperatures as compared to the narrow range of *Ae. albopictus*, which may attribute to why *Ae. albopictus* is not the dominate species in Brownsville, Texas. Due to the favorable climate, it is possible that *Ae. aegypti* can sustain numbers equal to those of *Ae. albopictus* even though *Ae. albopictus* is a superior competitor. The laboratory populations responded differently from wild populations, indicating the need to perform these types of analyses on wild populations specific to the region of interest.

These studies determined several factors that indicate why *Ae. aegypti* are more common in geographic areas that have higher temperatures and less frequent rains (Fontenille and Rodhain 1989 and Mogi et al. 1988), such as the fact that their eggs have the ability to resist desiccation at these warm, dry conditions and that they can do so for extended periods of time while waiting for rain. Results indicate that *Ae. albopictus* are more common in areas that have lower temperatures (15˚ -21˚C), because their eggs hatched at a high percent, similar to *Ae. aegypti* eggs at these temperatures, allowing *Ae. albopictus* to fully employ their competitive abilities at the larval and adult stages. However, *Ae. albopictus* eggs hatched at a lower percentage with an increase in

time, which may explain their reduced abundance in areas of little or infrequent rainfall (Khan 1980, Fontenille and Rodhain 1989, Mogi et al. 1988, and Juliano et al. 2002).

Factors facilitating coexistence of these species have also been determined, i.e. *Ae. aegypti* eggs hatch at a higher percentage in warm/dry climates, which gives them an advantage over *Ae. albopictus,* possibly equal to the advantage *Ae*. *albopictus* has shown at the larval and adult stages. Populations of *Ae. aegypti* would likely increase with an increase in temperature and/or a decrease in moisture in the form of rain or humidity. The opposite is likely the case with *Ae. albopictus*. However, in a region or city that favors one species, there are likely microhabitats that favor the other one, such as an exposed tire yard (favoring *Ae. aegypti*) or a well-watered and shaded cemetery (favoring *Ae*. *albopictus*). In an effort to reduce the incidence of Dengue fever, microhabitats that favor *Ae. aegypti* could be targeted while maintaining *Ae. albopictus* populations.

There was a significant difference in response of the two species to the various factors tested in relation to a given population's location of origin, indicating that it is beneficial to study the local populations. This is also particularly helpful in explaining the discrepancy in the results of the many studies on *Ae. aegypti* and *Ae. albopictus* and should encourage researchers to interpret experimental findings on a subject of interest with the consideration of the subject's origin and be more apt to include this information in their own results.

This study revealed environmental factors affecting hatching percentages of populations of *Ae. aegypti* and *Ae. albopictus*. Due to the superior vector potential of

Ae. aegypti in the transmission of Dengue, it would be less harmful to have *Ae. albopictus* than *Ae. aegypti* in an area. It is likely that the prevalence of *Ae. albopictus* in the Texas Rio Grande Valley largely contributes to the minimal amount of Dengue transmission in the region despite the large number of cases across the border in Mexico. It is thus suggested that perhaps a control measure for Dengue fever may be to select for the properties in the lab that have been determined to attribute to the ability for *Ae. albopictus* to withstand warmer and dryer climates. Coupled with their ability to compete with *Ae. aegypti*, and the fact that the males of this species have been found to interfere with the mating of *Ae. aegypti*, perhaps a release of *Ae. albopictus* into regions with a high prevalence of *Ae. aegypti* could greatly reduce their numbers. Also, the adult males emerge a day or two before the blood feeding females, so it may be possible that an effective ratio of males to females could be obtained and used to fulfill the necessary high numbers of individuals that would likely need to be released in order to suppress the *Ae. aegypti* population without increasing the biting rates and/or the disease prevalence. Obviously, a great deal more research would be needed to truly assess this possible control method, but this study found that the ability to survive and perhaps thrive in the warmer, drier climates is certainly within the genetic variability of *Ae. albopictus*.

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APPENDIX

Table A1. Mean percent hatch at 15˚C for all populations of *Ae. aegypti* and *Ae. albopictus* at each week in combination with the different experimental relative humidities.

			Weeks						
RH	Pop[1]	Species[2]	1	2	*3*	*4*	8	*12*	*Total*
15%	BWN	Albo	46.24	40.12	51.92	69.62	51.65	48.27	51.30
		Aeg	26.96	58.17	47.65	61.38	68.06	59.92	53.82
	McA	Albo	55.88	59.07	54.45	69.76	60.75	51.96	47.94
		Aeg	n/a	n/a	n/a	n/a	n/a	n/a	n/a
	Lab	Albo	41.55	26.85	46.08	40.83	29.22	38.93	37.24
		Aeg	37.72	67.34	32.33	47.41	35.06	55.88	55.48
35%	BWN	Albo	62.69	41.23	31.93	73.11	59.45	45.96	58.00
		Aeg	46.63	62.73	41.11	50.04	47.45	58.61	51.09
	McA	Albo	43.61	44.44	76.39	51.58	48.35	42.51	51.15
		Aeg	45.60	40.35	56.93	66.73	44.63	77.27	55.25
	Lab	Albo	49.45	20.92	27.92	45.85	41.98	23.39	36.79
		Aeg	46.14	47.37	65.59	73.70	67.69	54.94	61.04
55%	BWN	Albo	60.38	73.67	63.23	47.84	67.44	70.73	63.88
		Aeg	46.51	68.12	49.13	82.56	68.73	74.43	64.91
	McA	Albo	51.90	48.31	36.50	47.06	46.58	52.60	47.16
		Aeg	45.25	55.90	51.35	70.85	76.86	44.47	57.45
	Lab	Albo	56.01	49.63	52.45	54.98	50.30	56.28	53.27
		Aeg	35.03	56.15	48.81	60.90	72.78	66.29	56.66
75%	BWN	Albo	63.10	78.30	36.10	70.03	69.36	72.78	64.94
		Aeg	46.33	56.59	55.35	76.07	59.76	68.86	60.49
	McA	Albo	45.99	38.57	53.22	47.99	61.36	69.35	52.75
		Aeg	43.92	61.65	61.50	76.35	76.26	83.99	67.28
	Lab	Albo	78.04	90.90	60.88	60.37	59.56	58.71	68.08
		Aeg	67.49	38.58	41.33	55.37	58.85	82.72	57.39
95%	BWN	Albo	66.46	64.20	62.16	76.17	57.06	79.53	67.60
		Aeg	67.65	77.68	69.67	83.44	66.22	71.89	72.76
	McA	Albo	45.12	42.98	44.17	57.24	58.24	70.91	53.11
		Aeg	47.25	72.96	56.16	69.58	78.12	85.46	68.26
	Lab	Albo	86.49	74.12	72.01	69.81	63.59	71.42	72.91
		Aeg	52.01	63.91	61.04	66.64	63.72	81.40	64.78

[1]BWN is population collected in Brownsville, McA is the population collected in McAllen, and Lab refers to the laboratory colony.
[2] Albo refers to the *Ae. albopictus* species and Aeg refers to the *Ae. aegypti* species.

Table A2. Mean percent hatch at 21˚C for all populations of *Ae. aegypti* and *Ae. albopictus* at each week in combination with the different experimental relative humidities.

			Weeks						
RH%	Pop[1]	Species[2]	1	2	3	4	8	12	Total
15%	BWN	Albo	63.43	74.88	68.76	61.89	54.69	58.43	63.68
		Aeg	29.71	72.48	86.27	87.33	68.58	57.23	66.93
	McA	Albo	54.38	70.24	70.44	80.70	56.76	43.86	56.28
		Aeg	59.93	71.73	66.40	70.31	27.52	41.80	62.73
	Lab	Albo	58.04	56.10	61.66	48.97	36.28	35.83	49.48
		Aeg	89.21	69.93	74.39	53.31	77.20	69.84	72.31
35%	BWN	Albo	86.24	82.76	70.46	78.09	74.89	53.00	75.25
		Aeg	84.30	81.48	76.98	69.79	74.68	74.36	76.93
	McA	Albo	77.54	81.55	78.84	83.30	69.39	54.16	74.13
		Aeg	66.45	85.13	73.54	83.25	74.11	58.31	73.46
	Lab	Albo	74.67	71.29	85.82	87.71	73.95	44.34	72.96
		Aeg	95.47	90.48	76.54	84.59	85.19	80.97	85.54
55%	BWN	Albo	97.70	85.49	97.13	79.01	62.42	67.69	81.57
		Aeg	76.15	64.75	59.28	80.29	78.24	72.75	71.91
	McA	Albo	77.14	79.42	87.38	60.69	72.51	69.58	74.45
		Aeg	71.48	74.78	78.70	92.32	85.74	75.84	79.81
	Lab	Albo	84.95	69.54	79.08	93.28	67.33	74.36	78.09
		Aeg	97.70	79.76	92.37	82.96	87.98	79.18	86.66
75%	BWN	Albo	62.76	93.39	71.68	55.71	73.81	75.84	72.20
		Aeg	82.18	71.94	78.05	74.43	71.68	68.50	74.46
	McA	Albo	79.07	85.92	85.56	83.76	80.12	81.85	82.71
		Aeg	97.70	94.28	93.40	93.36	78.04	80.20	89.50
	Lab	Albo	84.89	71.60	85.96	93.39	69.25	69.94	79.17
		Aeg	92.17	90.35	92.12	67.67	80.19	78.96	83.58
95%	BWN	Albo	97.84	100.00	96.83	86.97	86.97	83.56	92.03
		Aeg	91.62	91.71	81.24	89.04	72.77	82.81	84.87
	McA	Albo	96.10	94.10	96.27	86.39	88.16	81.99	90.50
		Aeg	89.37	87.71	100.00	87.31	90.11	79.76	89.05
	Lab	Albo	85.97	71.58	86.42	81.84	75.20	77.49	79.75
		Aeg	93.16	95.87	100.00	91.68	91.95	84.80	92.91

[1]BWN is population collected in Brownsville, McA is the population collected in McAllen, and Lab refers to the laboratory colony.
[2] Albo refers to the *Ae. albopictus* species and Aeg refers to the *Ae. aegypti* species.

Table A3. Mean percent hatch at 27˚C for all populations of *Ae. aegypti* and *Ae. albopictus* at each week in combination with the different experimental relative humidities.

			Weeks						
RH%	Pop[1]	Species[2]	1	2	*3*	*4*	8	*12*	*Total*
15%	BWN	Albo	87.85	89.69	79.07	83.44	55.66	50.49	74.37
		Aeg	93.96	90.81	74.93	95.68	93.32	79.82	88.08
	McA	Albo	70.00	75.63	63.49	65.19	39.68	31.55	57.59
		Aeg	82.73	79.50	93.29	82.72	53.78	54.49	74.82
	Lab	Albo	66.23	57.09	61.48	57.14	47.73	37.68	54.56
		Aeg	83.86	81.76	78.57	79.17	75.81	44.98	74.03
35%	BWN	Albo	100.00	89.93	80.59	85.21	73.71	71.30	83.46
		Aeg	85.46	94.98	95.47	82.83	84.79	74.69	86.37
	McA	Albo	68.48	58.90	76.10	68.65	28.45	28.99	54.93
		Aeg	100.00	97.84	96.60	100.00	88.61	69.89	92.16
	Lab	Albo	91.50	92.60	77.01	66.80	40.54	47.49	69.32
		Aeg	89.17	84.83	86.58	94.35	90.71	75.33	86.83
55%	BWN	Albo	95.76	97.81	100.00	87.37	71.47	70.60	87.17
		Aeg	86.72	92.36	88.68	91.45	93.95	80.58	88.96
	McA	Albo	91.72	79.19	78.25	76.74	86.58	52.68	77.53
		Aeg	97.44	100.00	100.00	100.00	89.86	84.52	95.46
	Lab	Albo	77.77	82.09	84.18	78.44	78.19	44.32	74.16
		Aeg	87.40	80.32	89.25	96.97	87.85	90.77	88.76
75%	BWN	Albo	86.90	92.81	93.11	85.66	87.73	68.09	85.72
		Aeg	86.06	92.47	87.35	85.58	81.20	82.39	85.84
	McA	Albo	88.00	77.50	71.89	78.25	77.96	51.66	74.21
		Aeg	95.94	97.84	98.03	94.48	100.00	97.62	97.32
	Lab	Albo	84.82	88.08	100.00	81.39	68.22	61.76	80.71
		Aeg	95.40	95.95	97.93	100.00	91.77	83.22	94.04
95%	BWN	Albo	82.95	80.78	95.06	68.60	74.44	70.41	78.71
		Aeg	100.00	88.42	88.30	93.98	80.16	73.84	87.45
	McA	Albo	65.47	89.56	95.37	84.09	88.53	76.58	83.27
		Aeg	97.74	100.00	94.60	91.70	89.62	84.63	93.05
	Lab	Albo	84.56	79.11	59.95	74.97	69.15	63.12	71.81
		Aeg	74.86	70.48	70.20	71.56	77.78	84.09	74.83

[1]BWN is population collected in Brownsville, McA is the population collected in McAllen, and Lab refers to the laboratory colony.
[2] Albo refers to the *Ae. albopictus* species and Aeg refers to the *Ae. aegypti* species.

Table A4. Mean percent hatch at 32˚C for all populations of *Ae. aegypti* and *Ae. albopictus* at each week in combination with the different experimental relative humidities.

			Weeks						
RH%	Pop[1]	Species[2]	1	2	*3*	*4*	8	*12*	*Total*
15%	BWN	Albo	60.14	77.45	52.50	60.14	36.09	60.92	57.87
		Aeg	77.69	69.05	66.28	44.40	48.73	49.33	59.25
	McA	Albo	25.75	55.68	30.52	60.99	59.78	48.26	46.83
		Aeg	69.08	67.66	53.87	55.26	38.72	47.96	55.43
	Lab	Albo	45.52	45.87	26.51	69.31	41.59	40.43	44.87
		Aeg	57.35	59.52	64.49	48.06	50.64	46.83	54.48
35%	BWN	Albo	74.30	71.88	52.82	63.33	39.62	62.20	60.69
		Aeg	100.00	86.10	71.50	71.68	57.15	53.19	73.27
	McA	Albo	30.17	48.23	30.73	61.69	49.89	61.03	46.96
		Aeg	67.63	63.52	55.87	60.83	27.06	58.24	55.53
	Lab	Albo	47.50	65.41	33.25	23.38	30.21	52.05	41.97
		Aeg	59.39	56.58	64.66	48.06	46.71	54.50	54.98
55%	BWN	Albo	65.15	64.82	60.26	37.99	40.82	46.83	52.64
		Aeg	91.61	81.21	84.60	74.36	54.35	62.82	74.82
	McA	Albo	49.04	50.18	38.34	71.79	53.73	42.90	51.00
		Aeg	91.59	91.21	70.19	65.67	47.78	52.78	69.87
	Lab	Albo	90.31	64.03	12.55	31.17	42.41	35.75	46.04
		Aeg	96.27	93.66	78.31	63.86	56.82	54.57	73.92
75%	BWN	Albo	63.76	53.70	45.04	42.16	41.51	35.07	46.87
		Aeg	75.11	70.41	49.00	66.54	45.22	51.88	59.70
	McA	Albo	53.23	53.87	35.34	55.23	38.09	54.22	48.33
		Aeg	86.04	72.85	65.82	65.22	45.84	43.98	63.29
	Lab	Albo	96.67	67.07	48.21	57.81	25.57	40.72	56.01
		Aeg	81.97	94.97	87.48	75.94	42.89	42.29	70.92
95%	BWN	Albo	100.00	85.10	82.26	80.36	52.82	45.18	74.29
		Aeg	97.13	82.77	79.96	79.54	77.20	41.11	76.28
	McA	Albo	89.89	73.06	69.30	68.69	30.84	28.57	60.06
		Aeg	95.59	96.67	88.45	96.97	55.90	40.16	78.96
	Lab	Albo	61.69	78.44	68.70	62.23	27.86	28.56	54.58
		Aeg	90.63	95.55	84.87	82.38	75.74	52.83	80.33

[1]BWN is population collected in Brownsville, McA is the population collected in McAllen, and Lab refers to the laboratory colony.
[2] Albo refers to the *Ae. albopictus* species and Aeg refers to the *Ae. aegypti* species.

Table A4. Mean percent hatch at 35˚C for all populations of *Ae. aegypti* and *Ae. albopictus* at each week in combination with the different experimental relative humidities.

			Weeks						
RH%	Pop[1]	Species[2]	1	2	*3*	*4*	8	*12*	*Total*
15%	BWN	Albo	47.50	65.63	63.36	58.65	50.08	69.73	59.16
		Aeg	66.75	72.38	49.07	70.68	29.09	54.68	57.11
	McA	Albo	81.11	77.05	69.97	59.86	45.07	46.56	63.27
		Aeg	56.52	72.09	50.51	71.74	52.39	47.79	58.51
	Lab	Albo	38.22	43.65	41.65	29.03	49.03	35.97	39.59
		Aeg	51.57	75.33	64.58	60.33	61.64	65.91	63.23
35%	BWN	Albo	48.60	65.92	53.96	63.31	57.27	56.05	57.52
		Aeg	63.07	62.20	49.50	74.24	39.20	39.27	54.58
	McA	Albo	73.36	59.80	50.11	37.79	55.36	56.59	55.50
		Aeg	75.00	71.31	53.82	52.68	42.70	31.76	54.54
	Lab	Albo	47.81	45.96	34.07	39.00	43.10	20.41	38.39
		Aeg	40.18	87.16	56.87	66.80	73.34	57.03	63.56
55%	BWN	Albo	51.06	65.01	51.63	77.68	49.73	55.38	58.42
		Aeg	66.60	69.68	73.80	68.29	74.81	40.28	65.58
	McA	Albo	85.13	84.49	45.15	52.70	46.05	40.83	59.06
		Aeg	91.59	52.42	37.00	35.70	53.34	37.93	51.33
	Lab	Albo	50.47	27.43	32.82	28.69	46.42	41.22	37.84
		Aeg	50.56	95.67	72.63	63.11	58.61	58.29	66.48
75%	BWN	Albo	63.37	64.17	73.38	79.46	52.83	43.66	62.81
		Aeg	81.41	78.78	70.50	63.73	37.71	59.06	65.20
	McA	Albo	94.65	46.58	30.72	31.01	39.10	42.02	47.34
		Aeg	94.26	66.23	56.37	43.09	56.06	42.86	59.81
	Lab	Albo	62.47	34.27	44.13	26.97	34.08	25.28	37.87
		Aeg	63.53	67.44	70.09	54.66	56.07	51.63	60.57
95%	BWN	Albo	92.10	73.60	64.78	43.96	56.75	38.07	61.54
		Aeg	82.31	86.41	63.51	55.16	38.17	26.67	58.71
	McA	Albo	67.62	51.81	69.24	50.78	39.33	20.79	49.93
		Aeg	87.11	89.16	70.33	61.91	45.74	51.25	67.58
	Lab	Albo	79.72	68.95	67.67	55.43	32.47	11.64	52.65
		Aeg	77.31	84.67	85.28	82.30	67.73	30.48	71.30

[1]BWN is population collected in Brownsville, McA is the population collected in McAllen, and Lab refers to the laboratory colony.
[2] Albo refers to the *Ae. albopictus* species and Aeg refers to the *Ae. aegypti* species.

VITA

Catherine Zindler Dickerson, born Catherine Marlene Zindler grew up in the Houston suburb of Webster. She attended San Antonio Community College in 1995 and San Jacinto South Community College from 1996 to 1997 before attending Texas A&M University in 1998. She graduated from Texas A&M University in 2000 with a Bachelors of Science in Entomology. She worked as a biological science aid on cotton pests at the USDA ARS in College Station, the summer of 2000 before beginning graduate school at Texas A&M University under the expert tutelage of Dr. Jimmy K. Olson.

While at Texas A&M University, Catherine (Cat) Dickerson obtained the graduate certificate in Geographic Information Systems and became a fellow of the Center for Teaching Excellence. She taught over forty lab sections of six different Entomological topics as a Teaching assistant.

Catherine met her husband, Patrick Callen Dickerson while attending graduate school; they have one daughter. Catherine Dickerson can be reached at the USDA, ARS, CMAVE, 1600 S.W. 23rd Drive, Gainesville, Florida 32608.