DEVELOPMENTAL SYNCHRONIZATION OF THE PURPLE PITCHER PLANT MOSQUITO, *WYEOMYIA SMITHII,* AS A RESULT OF INCREASING ENVIRONMENTAL TEMPERATURES

by

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Developmental synchronization is the evolutionary phenomenon wherein individuals of a given population are able to achieve life cycle milestones in synchrony, independent of when development was initiated. Synchronization is important for population survival and reproduction. For ectothermic insect species, environmental temperature plays an important role in growth and development; increasing temperatures are positively correlated with developmental rates between the thermal threshold of development and the thermal optimum. The purpose of this study was to determine whether or not developmental synchronization occurs in the pitcher plant mosquito, *Wyeomyia smithii,* during the termination of larval diapause and subsequent development in response to increasing environmental temperatures. I sought to answer this question by gradually introducing four geographically distinct *W. smithii* populations into an increasing thermal environment (4-30 °C) and observing the time of pupation for individual larvae. I determined that *W. smithii* undergoes developmental synchronization from diapause termination to pupation between temperatures of 4 °C and 20 °C; however higher temperatures result in the decline of synchronization and delay of development. These results have important implications for mosquito control programs; programs may use this information to determine the optimal time and duration to deploy chemical or biological agents for control of mosquito vectors of serious human pathogens.

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Introduction

In nature, as the seasons change throughout the year, it is not unusual to notice the presence or absence of plant and animal activity. For example, many people enjoy the bioluminescent display of fireflies during the summer season – their appearance in the late-spring or early-summer is as sudden as their disappearance in the fall. This phenomenon is true for the firefly *Photinus carolinus,* found in the Great Smoky Mountains National Park on the border of Tennessee and North Carolina. For *P. carolinus,* environmental temperatures are very important to the species' life cycle. Temperature has been shown to impact their emergence as adults – coordinating when adults appear in the wilderness – and the synchronized flashing pattern of males' reproductive behavior (Faust & Weston 2009; Faust 2010).

The example of *P. carolinus* demonstrates environmental temperatures are able to impact the behavior and life events of a species Fireflies undergo synchronization of their development and reproductive behaviors

The synchronization of development – or **developmental synchronization*** – is an important and scientifically relevant form of coordinated activity that occurs passively. Developmental synchronization has been shown to occur in species beyond *P. carolinus*, including the development of the phantom midge *Chaborus americanus* (Bradshaw 1974) the emergence of hatchlings for the sea turtle *Chelonia mydas* (Santos 2016), and the pupation of tree hole mosquito *Aedes sierriensis* (McDowell 2009).

In this study, I addressed the question of whether the purple pitcher plant mosquito *Wyeomyia smithii* undergoes developmental synchronization from the

*Definitions for bolded terms can be found in the glossary at the end of the thesis.

termination of diapause through pupation under controlled conditions; and, if synchronization does occur, to what extent.

I determined that as larvae terminated diapause and developed to the pupal state, developmental synchronization occurred. However, I also determined synchronization declines and led to a developmental delay for larvae which began development at higher environmental temperatures.

Figure 1. Female *W. smithii* specimen.

Retrieved from Tom Murray, *Pitcher Plant Mosquito* – Wyeomyia smithii – *Female*. June 20, 2016, Digital image. Source: BugGuide.net, https://bugguide.net/node/view/1242204 (retrieved October 17, 2017).

These findings are relevant for the control of *W. smithii* mosquito populations. A better understanding of developmental synchronization for *W. smithii* will enable mosquito control programs to define an appropriate window for the release of chemical (i.e. pesticides) or biological agents (i.e. **sterile insect technique**) to limit mosquito populations. Ultimately, these findings have a potential to impact public health, aiding

in the control and management of disease-carrying vector populations. Additionally, these results may be used as reference data for predicting the effects of *W. smithii* developmental patterns as a result of climate change.

Background

Evolutionary Importance of Developmental Synchronization

Developmental synchronization may be applied to the concept of **synchronization of emergence**. The synchronization of emergence is a highly beneficial evolutionary adaptation for two primary reasons: predator evasion and reproduction.

When large numbers of a prey population emerge at a single time, prey often outnumber predators and the capacity for predators to consume them; this is termed **predator swamping** or predator satiation (Ims 1990). This swampping is true for the periodical cicada *Magicicada* (Karban 1982) and the previously-mentioned sea turtle, *Chelonia mydas*. For the sea turtle, mass hatching and migrating to the ocean increases the probability that some hatchlings will survive and avoid gull and crab predation (Santos 2016).

For reproductive purposes, synchronized emergence allows for members of a given generation to reach sexual maturity at the same time (**reproductive synchrony**) and produce a maximal number of offspring for the succeeding generation. This synchrony is particularly important for species which reproduce at specific times of the year. Individuals which do not reach sexual maturity in synchrony will not be able to reproduce (Ims 1990).

Effect of Temperature on Developmental Rates

Many insect species are **ectotherms**, deriving their body temperature from external sources, typically the surrounding environment. For ectothermic species

developmental rates are positively correlated with environmental temperatures. However, this correlation is only true between a species' **thermal threshold for development** and **thermal optimum**. As the environmental temperature increases from the thermal threshold for development, development rates will increase; the increase in rate will continue until environmental temperatures approach and surpass the thermal optimum for the species, typically occurring at 25 to 35 °C. Increasing the environmental temperatures past the thermal optimum will slow or stop development, or result in death from heat stress (Ratte 1985).

The thermal threshold for development can be found by manipulating the equation for the **Rule of Thermal Summing**:

$$C = \sum_{i}^{Pupation\ date} (T_{E,i} - T_0)$$

In the equation above, *C* represents the species-specific **thermal constant**, *i* represents the hatch date (day of larval emergence from an egg), $T_{E,i}$ represents the average daily temperature on day *i*, and T_0 represents the thermal threshold for development. It is important to note the thermal constant, *C*, and the thermal threshold for development, T_0, vary between species and may vary between populations of a given species (Ratte 1985).

Experimentally, the thermal threshold for development can be calculated by exposing discrete larval populations to varying constant thermal environments and determining their respective developmental rate. When temperature is plotted against developmental rates and a linear regression is performed, the slope of the regression line

represents the thermal constant and the x-intercept represents the thermal threshold for development (Ratte 1985, McDowell 2009)

For *W. smithii*, the thermal threshold for development is 12 °C; however, the thermal optimum is not known (Bradshaw & Holzapfel 2018).

Life Cycle and Development of W. smithii

The purple pitcher plant mosquito, *W. smithii*, is an **arthropod** native to North America. *Wyeomyia smithii* is termed the purple pitcher plant mosquito because of its life cycle and geographic distribution being related to that of the purple pitcher plant, *Sarracenia purpurea* (Figure 2). The mosquito reproduces by laying its eggs in the water-filled leaves of the plant. (Bradshaw & Lounibos, 1977; Bradshaw & Holzapfel 1986; Istock & Weisburg 1987); *Wyeomyia smithii* and *S. purpurea* range from the Gulf Coast of the United States to the Canadian province of Labrador; they are also found in the more western portions of the continent, including the American Midwest and Canadian province of Mantioba (Bradshaw & Lounibos 1977). *Wyeomyia smithii* can be geographically and genetically categorized into northern and southern clades, differentiation occurring around the mid-Atlantic region (mainland Maryland) of the American east coast (Miller, Bradshaw & Holzapfel 2018; Merz, *et. al.* 2013).

Figure 2. Example of the purple pitcher plant, *Sarracenia purpurea*.

Retrieved from Thomas G. Barnes, Sarracenia purpurea L. *purple pitcher plant*. Digital image: Source: USDA-NRCS PLANTS Database: https://plants.usda.gov/core/profile?symbol=sapu4 (retrieved October 26, 2017).

Wyeomyia smithii hatch from their eggs within the purple pitcher plant as first **instar** larvae; the larvae develop through morphologically distinct larval stages (first through fourth larval instars). Finally, larvae undergo **pupation** to become **pupae** and later emerge as adult mosquitoes (Bradshaw & Holzapfel 1990; Mahmood & Crans 1998).

The development of *W. smithii* larvae may be interrupted by the onset of larval **diapause**. The initiation and termination of diapause is amongst one of the most important life cycle events of any arthropod. Evolutionarily, diapause is intended to provide an organism or species with the ability to withstand harsh environmental conditions, temporarily halt development, and increase the storage of lipids (Tauber, Tauber & Masaki 1986; Bradshaw 1974).

For *W. smithii*, day-length (**photoperiod**) is the most important environmental cue for initiation and termination of diapause; photoperiod serves as a reliable predictor for the changing seasons and the environmental conditions which characterize those seasons (Bradshaw 1974; Bradshaw & Holzapfel 2007). Annually, photoperiod is at a maximum during the summer and a minimum during the winter (summer and winter solstices, respectively). In the fall, when photoperiod decreases to a critical day-length (**critical photoperiod**), diapause is initiated; the opposite is true in the spring – once photoperiod increases to a critical day-length, diapause is terminated. For *W. smithii*, the critical photoperiod is a light:dark (L:D) between 12:12 and 15:9 for southern and northern populations, respectively (Bradshaw & Lounibos 1977; Tauber & Tauber 1976; Neuman 2018). An L:D cycle where the day length is shorter than the critical photoperiod is referred to as a short-day; an L:D cycle where the day length is longer than the critical photoperiod is referred to as a long-day.

Northern and southern clades of *W. smithii* will diapause at different larval instars and at different depths where depth of diapause refers to the number of long-days required to induce diapause termination. Northern populations diapause in the third larval instar and have a deep diapause whereas southern populations diapause in the fourth larval instar and a have a shallow diapause (Bradshaw & Lounibos 1977).

Methods & Materials

Four populations of *W. smithii* were used for this experiment, each native to distinct geographic regions within the United States. These populations were from Florida (WI), Alabama (LI), Maine (KC), and Wisconsin (ML) (Figure 3). The populations used for this experiment were laboratory stock populations. Stock populations were collected as larvae from their native habitats in the summer of 2016. The original collected samples have since gone through two successive generations before the experiment began; the progression of the populations through two generations minimized environmental and maternal effects on the experimental populations.

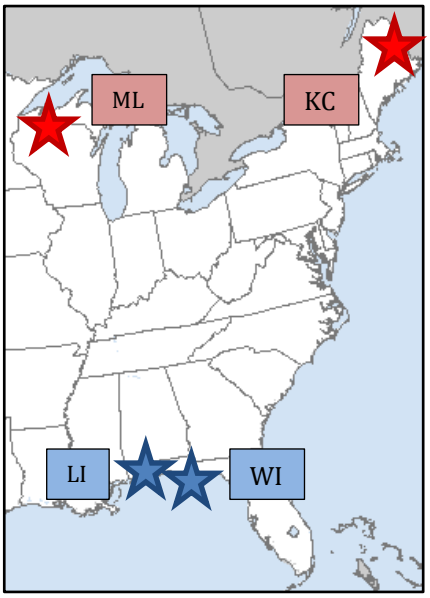

Figure 3. Geographic location of experimental populations.

WI: 30 °N 85 °W; elevation = 10 m

LI: 30 °N, 91 °W; elevation = 15 m

KC: 46 °N 68 °W; elevation = 365 m

ML: 46 °N 90 °W; elevation = 500 m

Storage and Rearing of Laboratory Populations

The collected wild populations were stored as distinctly separately in a light and temperature-controlled room. Long-day conditions were set to an L:D of 18:6 and temperatures of 15 °C to 30 °C in a sine-wave temperature cycle lagging the light cycle by four hours. These conditions were previously found to be optimal for inducing pupation of the larvae (Zani 2005). Once pupated, the pupae from each population were transferred to a cup filled with clean deionized water (DI) water and placed in separate enclosures. Each enclosure contained a single purple pitcher plant leaf and moistened layers of paper towel with a top layer of filter paper, covering the entirety of the enclosure's floor. The enclosure was stored in the light-and-temperature-controlled room to allow the pupae to develop into mature adults. Dried pesticide-free raisins were placed on top of the enclosures, accessible by the adult mosquitos as a carbohydrate nutrient source.

The enclosures were checked periodically three times per week to observe deposition of eggs onto the moistened filter paper. Eggs from each population were collected into separate petri dishes and allowed to hatch as new larvae. New larvae were counted and placed into individual dishes (35 larvae per dish), separated by population and labeled by their collection date. The larval dishes were filled with tetracycline antibiotic water (0.108-grams per liter of DI water) as to prevent bacterial growth in the dishes. Larval dishes were then stored in another light and temperature controlled room, programmed for short-days (L:D of 8:16 to induce diapause). Weekly, the stock populations were fed with approximately 2-mL of food solution (see below).

This overall cycle was repeated two more times and stored as stock populations to be used for experimental purposes.

Preparation of Larval Food Solution

Stock larval populations were fed with 2-mL of larval food solution. The solution was created by thoroughly blending together a 1:4 mixture of ground shrimp brine and gerbil food pellets. The mixture was sifted with a 0.50-mm open sieve to remove large granules. A volume of 30-mL of the blended mixture was thoroughly mixed with the previously described tetracycline antibiotic water. The solution was allowed to settle before dispensing to the larval dishes as to avoid dispensing larger granules into the dishes.

Increasing Temperature Experimental Setup

Populations of WI, LI, KC, and ML were counted and placed into 120-mL petri dishes filled with approximately 2-mL of larval food solution and filled half-way with the tetracycline antibiotic water. For each population, two cohorts were established, A and B. A single cohort contained 14 petri dishes, each holding 30 larvae.

Two incubators were programmed to temperatures of 4 °C. One incubator was programmed to an L:D of 10:14 (short-day, SDI), the second incubator was programmed to an L:D of 18:6 (long-day, LDI). Temperature for both incubators were monitored and recorded with WatchDog B-Series Button Loggers, both set to record temperature every 10 minutes. Once the 4 °C temperatures of both incubators were established and consistent for seven consecutive days, populations of WI, LI, KC, and ML were placed in the SDI.

Increasing Temperature Experiment

On experimental day zero (0), one dish from each of the four populations and their respective cohorts (A and B) were placed in the LDI set to 4 °C; the rest of the dishes remained in the SDI. The dishes placed in the LDI were marked with the current temperature.

On experimental day two (2), larvae in the LDI were checked for pupation. If pupation occurred, the number marked on the dish and the number of pupae in the respective dish were recorded. Additionally, the temperatures of both the LDI and SDI were checked, recorded, and adjusted if necessary.

On experimental day four (4), the temperature of the LDI was increased 2 °C; the SDI was kept at a constant temperature of 4 °C (Figure 4). All larval dishes were replaced – larvae were transferred into fresh 120-mL petri dishes, prepared as described above. This procedure was done to ensure all larvae were properly fed and to maintain the cleanliness of the dishes. Following the temperature increase and placement of the larvae in new petri dishes, one A and one B dish from each population were moved from the SDI to the LDI. Finally, the temperatures of the LDI and SDI were checked and recorded.

The schedule described above was repeated until experimental day 52. Every-other-day (day 2, 4, 6, etc.), pupation and temperatures were checked and recorded; every fourth experimental day (4, 8, 12, etc.), pupation and temperatures were checked and recorded, the LDI temperature was increased 2 °C, larvae were transferred into fresh larval dishes, and one A and one B dish from each population were moved from the SDI to the LDI (Figure 5.) On experimental day 52, the LDI temperature was set to

the final temperature of 30 °C and no longer increased; at this time, all experimental larval dishes were in the LDI; temperature of the SDI was no longer recorded.

All tasks continued according to the described schedule until all viable larvae had pupated.

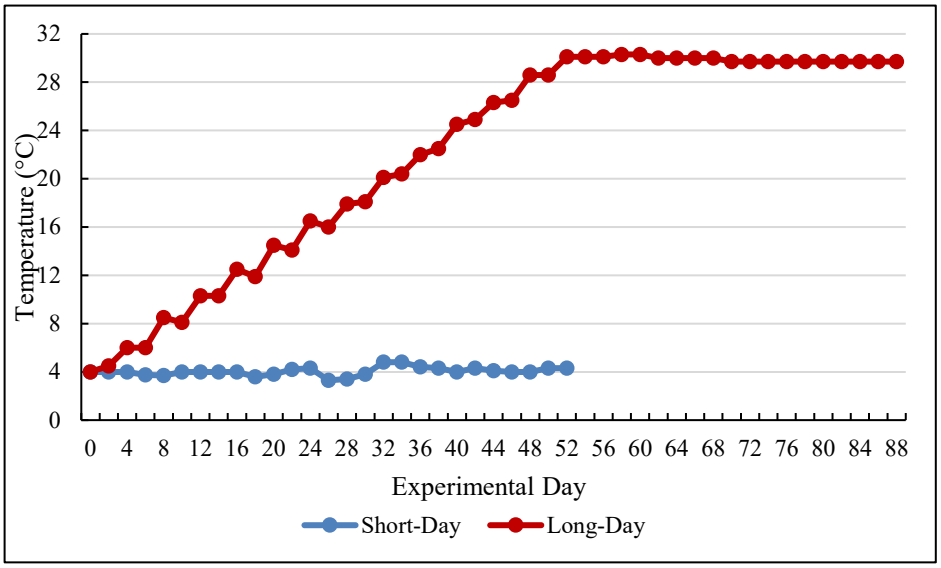

Figure 4. Short-day and long-day incubator temperatures.

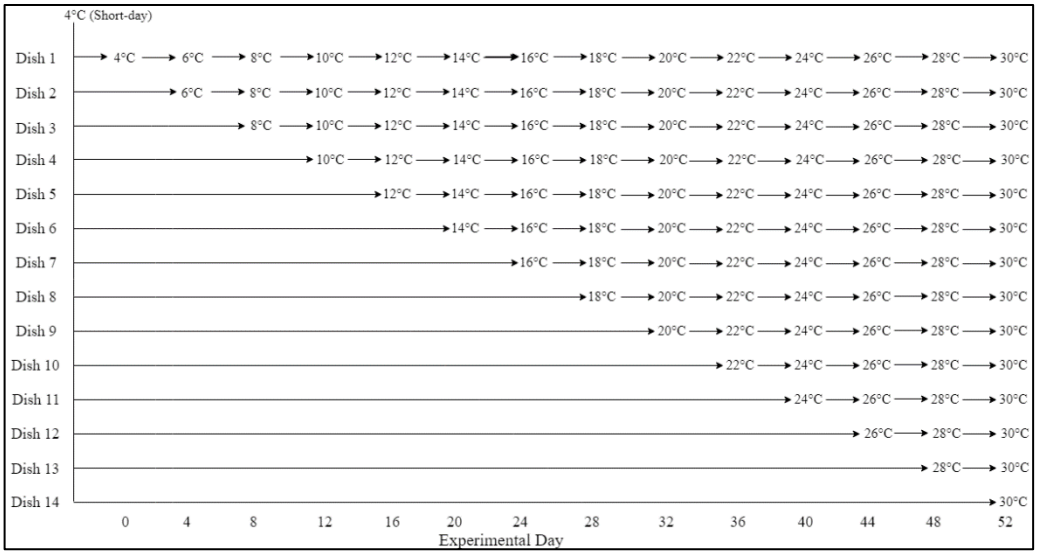

Figure 5. Temperature treatments for experimental dishes.

All dishes were transferred from a 4 °C short-day incubator to a long-day incubator set to the first temperature listed in the dish's respective row; unless otherwise stated, all temperature treatments took place in a long-day incubator.

Results

The average pupation day (relative to experimental day zero) was organized by experimental population and its respective A and B replicates (Figure 6-9). For understanding the Figures 6-9 below, error bars represent +/- two standard errors; and data points for cohort B are slightly offset from cohort A for visual differentiation and clarity (the x-values for cohort A and B are identical) A comprehensive summary of the results can be found in Appendix A.

Average Pupation Day

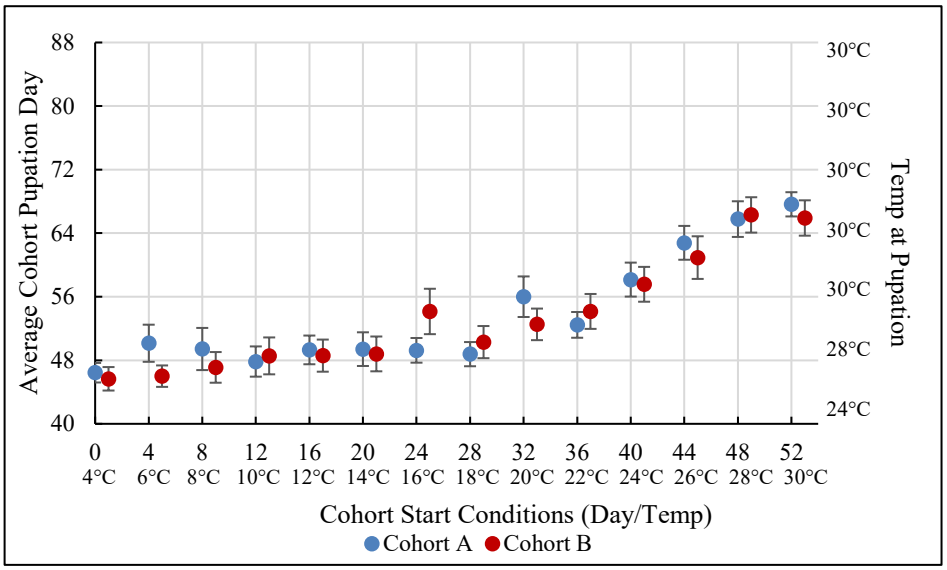

Figure 6. Average pupation day of the southern *W. smithii* population WI as it relates to start day and temperature of development.

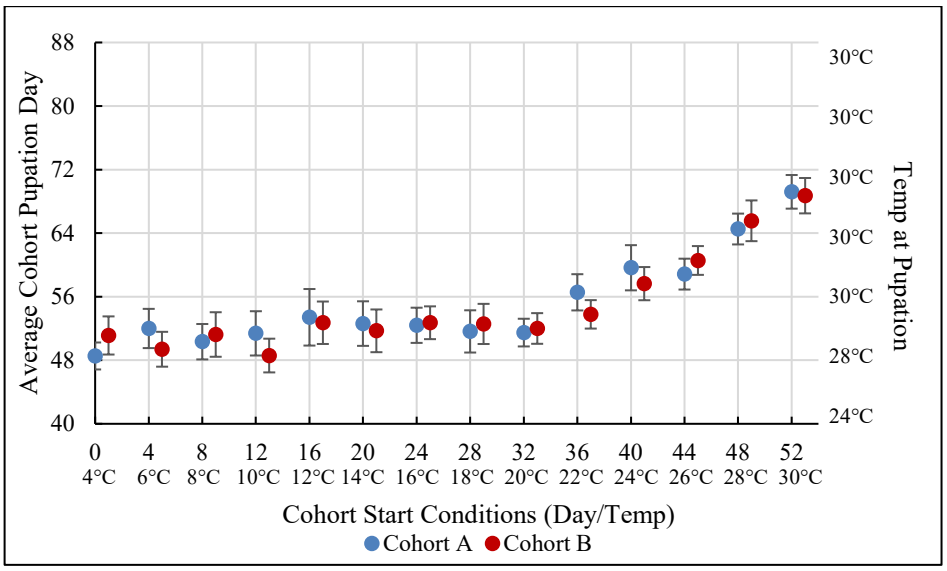

Figure 7. Average pupation day of the southern *W. smithii* population LI as it relates to start day and temperature of development.

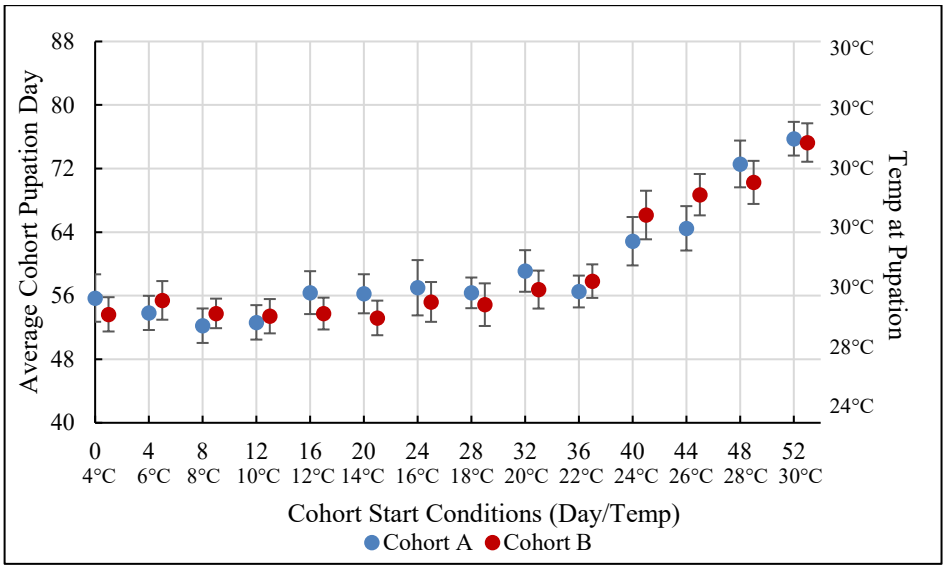

Figure 8. Average pupation day of the northern *W. smithii* population KC as it relates to start day and temperature of development.

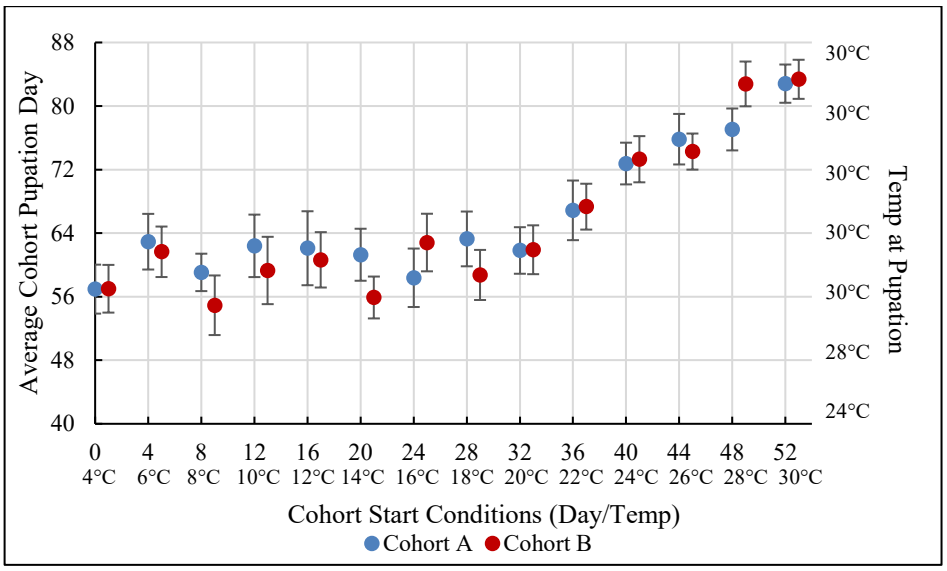

Figure 9. Average pupation day of the northern *W. smithii* population ML as it relates to start day and temperature of development.

Regression Analysis

For developmental synchronization to be demonstrated, it is expected that within a given cohort, the average day of pupation for all temperature treatments would occur on approximately the same day. A linear regression of these data would produce a slope of zero.

Visual evaluation of the graphs suggests all populations are undergoing developmental synchronization, evident by the linear data for lower developmental start temperatures. However, what was unexpected is the curve upward at the higher developmental start temperatures.

To identify objectively where developmental synchronization starts to decline, regression analysis was used to find two transitions: one, the temperature interval during which developmental synchronization was occurring; two, the developmental delay following the departure from synchronization.

To find the temperature intervals in which developmental synchronization was occurring, multiple linear regression analyses were performed between data points for a given cohort. For example, a regression was performed between developmental start days 0 and 8. The linear coefficient (x) was checked for significance ($\alpha = 0.05$); an non-significant result indicated the coefficient was not statistically different from zero and developmental synchronization occurred within that temperature interval. Similar analyses were performed (0-12, 0-16, 0-20, etc.) until the linear coefficient was significant, indicating developmental synchronization was no longer occurring. Once significant, this interval was termed the interval of significance for the linear coefficient (x) (Table 1).

To find the development delay following the failure of synchronization, multiple polynomial regression analyses were performed. The procedure for this analysis was identical to the previous linear regressions, however analyses compared the end of the dataset and earlier data points (44-52, 40-52, 36-52, etc.) and the quadratic coefficient (x^2) was being tested for significance. Significance of the quadratic coefficient indicated the regression line is no longer linear and developmental delay is not following a linear trend. Once significant, this interval was termed the interval of significance for the quadratic coefficient (x^2) (Table 1).

		ML					KC					
		Interval of significance		x or x^2 P-value (α=0.05)	Linear Coeff. (±SE)	x^2 Linear Coeff. P-value (α=0.05)		Interval of significance		x or x^2 P-value (α=0.05)	Linear Coeff. (±SE)	x^2 Linear Coeff. P-value (α=0.05)
		Exp. day	Temp. (°C)					Exp. day	Temp. (°C)			
A	x	0-40	4-24	0.0153	0.2299 ± 0.0770		A	0-32	4-20	0.0323	0.1431 ± 0.0537	
	x^2	40-52	24-30	0.0305	0.7849 ± 0.1553	0.3002		x^2	40-52	24-30	0.0289	1.1703 ± 0.2108	0.5040
B	x	0-40	4-24	0.0132	0.2869 ± 0.0932		B	x	0-36	4-22	0.0300	0.0871 ± 0.0331	
	x^2	36-52	22-30	0.0096	1.0385 ± 0.1636	0.8292		x^2	36-52	22-30	0.0130	1.2048 ± 0.1233	0.1953
		LI					WI					
		Interval of significance		x or x^2 P-value (α=0.05)	Linear Coeff. (±SE)	x^2 Linear Coeff. P-value (α=0.05)		Interval of significance		x or x^2 P-value (α=0.05)	Linear Coeff. (±SE)	x^2 Linear Coeff. P-value (α=0.05)
		Exp. day	Temp. (°C)					Exp. day	Temp. (°C)			
A	x	0-36	4-22	0.0296	0.117 ± 0.0443		A	x	0-36	4-22	0.0341	0.1459 ± 0.0572	
	x^2	36-52	22-30	0.0125	0.7543 ± 0.1584	0.2187		x^2	40-52	24-30	0.0272	0.7583 ± 0.1098	0.1898
B	x	0-36	4-22	0.0252	0.0910 ± 0.0333		B	x	0-12	4-10	0.0320	0.2440 ± 0.0447	
	x^2	40-52	24-30	0.0055	0.9553 ± 0.0699	0.5874		x^2	36-52	22-30	0.0097	0.8064 ± 0.1197	0.2041

Table 1. Intervals of Significance for Developmental Synchronization and Delay

x P-value represents the significance for the region in which synchronization decline has begun (slope $\neq 0$). x^2 P-value represents the significance for the region in which delay is non-linear. x^2 linear coeff. P-value represents the divergence of the x^2 linear coefficient from 1.

For the southern populations WI and LI, the regression analyses determined developmental synchronization occurred between developmental start temperatures of 4 and 20 °C and declines at higher temperatures (WI A, LI A, and LI B).

After departure from synchronization, developmental delay followed a linear trend between 24 and 30 °C (WI B, LI A) or 26 and 30 °C (WI A, LI B). The linear coefficients for the developmental delay regions were not significantly different from one ($\alpha = 0.05$), indicating that development was delayed one day for every day development was not initiated; this demonstrates there was no developmental catch-up which characterizes developmental synchronization.

For the northern populations KC and ML, the regression analyses determined developmental synchronization occurred between developmental start temperatures of 4 and 18 °C (KC A), 4 and 20 °C (KC B), and 4 and 22 °C (ML A, ML B). After the departure from synchronization, developmental delay followed a linear trend between 24 and 30 °C (KC B, ML B) and 26 and 30 °C (KC A, ML A). Like the southern populations, the linear coefficients for the developmental delay regions were not significantly different from one ($\alpha = 0.05$), indicating that development was delayed one day for every day development was not initiated; this demonstrates there was no developmental catch-up which characterizes developmental synchronization.

A visual summary for the intervals of developmental synchronization and developmental delay can be found in Figure 10.

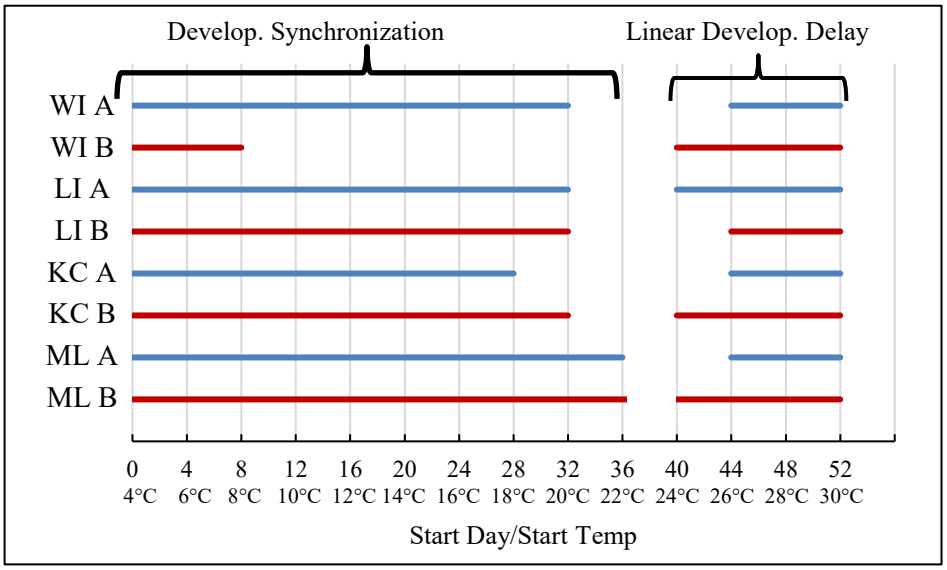

Figure 10. Developmental synchronization and delay summary

All – aside from fromWI B – experimental populations had a synchronic effect between 4 °C and 20 °C, +/- 2 °C; following the departure from synchronization, all populations underwent developmental delay, reaching a linear constant at 24-26 °C.

Discussion & Conclusions

Synchronization in Northern and Southern Clades

My results demonstrate developmental synchronization is not exclusive to either the southern or northern clades of *W. smithii*. Despite geographic and genetic differences of the two clades, populations underwent similar patterns of developmental synchronization and delay. Developmental synchronization occurred between temperatures of 4 and 20 °C, evident by the decreasing average days to terminate diapause and develop to the pupal state (days-to-pupate, DTP); the decreasing number of days represents developmental catch-up. Decline of synchronization and the onset of developmental delay occurred at temperatures exceeding 20 °C, evident by the constant average DTP (Figure 11).

Developmental synchronization and delay was similar for all populations and replicates, however the period of synchronization for WI B was the only exception. WI B underwent developmental synchronization between temperatures of 4 and 8 °C. Due to the dissimilarities with the WI A replicate and other experimental populations, the shorter period of developmental synchronization is likely an anomaly.

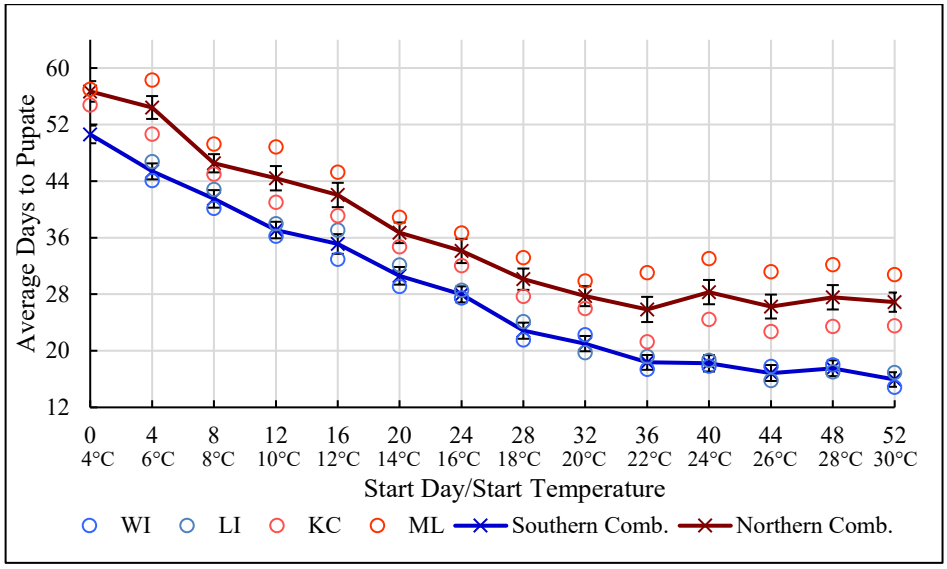

Figure 11. Average days to pupate for northern and southern clades of *W. smithii* as it relates to start day and temperature of development.

Data regarding individual populations (WI, LI, KC, ML) are composites of their respective A and B cohorts; data regarding the northern and southern clades are composites of their regional populations; error bars represent +/- two standard errors.

Additionally, my results confirm the phenomenon described by Bradshaw and Lounibos (1977) concerning the differences in southern and northern diapause. Despite the southern and northern populations undergoing identical experimental conditions, southern populations required fewer days to reach pupation than their northern counterparts. These findings are because of the differences in what larval instars the southern and northern populations diapause at and the depth of their diapause. Southern populations enter a shallow 4th instar diapause whereas the northern populations enter a deep 3rd instar diapause. The southern populations do not require the additional time and energy to grow, develop and, molt to the 4th instar. Southern populations also have a shallow depth of diapause in comparison to the deeper diapause of the northern populations, requiring fewer days beyond their critical photoperiod to terminate

diapause and initiate development. These two factors explain the consistent differences in days to pupation (Figure 11) between southern and northern populations.

Mosquito Population Control

Mosquito control programs seek to manage mosquito populations, utilizing methods which are effective, practical, and protect public health and the environment. Methods may include chemical or biological agents; chemical agents include releasing pesticides into the environment, biological agents include utilizing the sterile male technique, releasing invertebrate predators of the mosquito, parasites, or mosquito-targeted diseases.

The control of mosquito populations is particularly important because mosquitos are serious threats to public health. Many mosquitos are **vectors** for disease or disease-causing parasites, including malaria and yellow fever, and Zika viruses. A bite from a disease or parasite-carrying mosquito may lead to illness or death.

Having a better understanding of mosquito growth and development is a useful tool for mosquito control programs to determine the optimal time and duration for deploying their pest management strategies. Because of the cost, health-impacts, and environmental harm chemical pesticides may have, it is best to minimize the time at which they are being released into the environment. Based on my data, the most efficient time for pesticide deployment may be the 20-30 days following the date at which environmental temperatures reach 25 °C; this spraying schedule would target 80% mosquito populations (Figure 12).

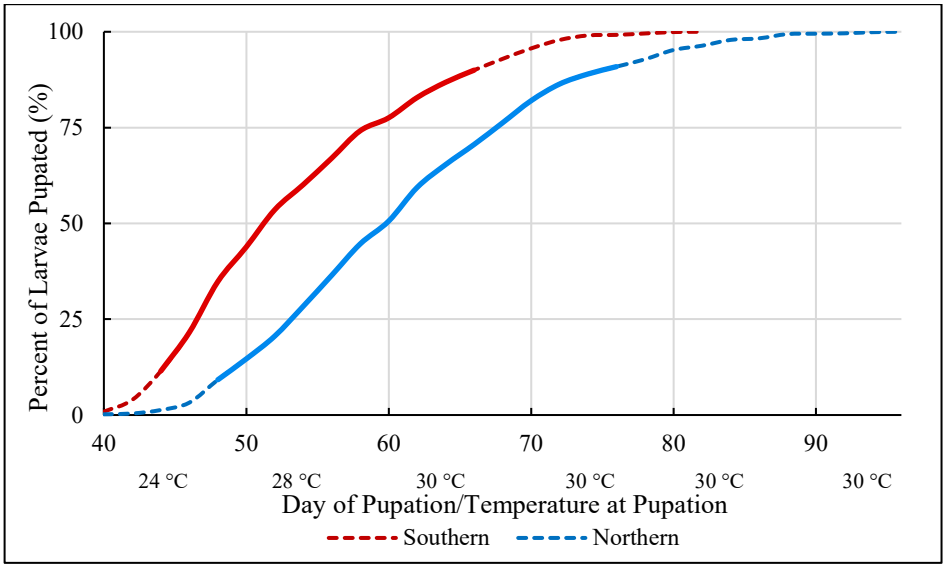

Figure 12. Cumulative percent pupation as it relates to temperature.

Solid lines represents the timeframe in which spraying chemical pesticides would target 80% of the population; for the southern populations, this is a 22 day timeframe (day 44-66); for the northern populations, this is a 28 day timeframe (day 48-76).

Although optimal deployment times may vary from species to species and population to population, the timeframe for deployment may be determined by the proportion of the population which have pupated or emerged as adults. This strategy may be used by other pest control programs to regulate mosquito and other insect populations.

Climate Change

Rapid climate change does not affect the photic year of Earth as it does not alter the rotational axis of the earth or the rotation of earth about the sun. However, climate change will ultimately increase the duration of the growing season – advancing the onset of spring and delaying the onset of fall and winter. Because the termination of diapause in *W. smithii* is controlled by photoperiod, there would be no effect on when *W. smithii* terminates and initiates diapause. However, as climate change continues, the

environmental temperatures of temperate regions (i.e. *W. smithii*'s geographic range) will increase and ultimately enhance the ability for *W. smithii* to reproduce (Bradshaw & Holzapfel 2006, 2008, 2010).

Based on my findings, as climate change continues we would expect to see a temporal shift in when *W. smithii* is undergoing synchronized development. When diapause is terminated at a population's critical photoperiod, environmental temperatures will be higher and larvae will develop at a faster rate. Because synchronization fails at approximately 20 °C, synchronized development will occur for a shorter period of time and thus lengthen the timeframe in which *W. smithii* are emerging as adults. This consequence is detrimental to mosquito control programs as it will prolong the period during which chemical or biological control agents are released.

Next Steps for Research

While the results reported in this thesis are significant regarding *W. smthii* under strictly-controlled conditions, the experiment performed did not reflect non-linear increase in temperature in natural environments. A similar experiment should be performed which utilizes realistic temperature patterns. Additionally, it would be beneficial to add more geographically distinct *W. smithii* populations to the study by evaluating developmental synchronization across altitude as well as latitude. Study should also be expanded to other mosquito species aside from *W. smithii* as many species of mosquito are detrimental to public health and the environment.

Glossary

Arthropod: an invertebrate organism which possesses an exoskeleton, a segmented body, and pair-jointed appendages; typically inhabits temperate climates; examples include mosquitoes, lobsters, and spiders

Critical photoperiod: the day-length that promotes 50% development and 50% diapause.

Developmental synchronization: development amongst individuals in a single population resulting in reaching certain developmental milestones at approximately the same time; development rates for individuals may vary during this process

Diapause: physiological life cycle event of many arthropods where development is temporarily halted; initiation is primarily triggered by photoperiod and termination by temperature.

Ectotherm: organism which uses external sources as a means of controlling body temperature, as opposed to internally-regulated body temperature; "cold-blooded" organism.

Instar: a phase between two periods of molting in the development of arthropod

Photoperiod: the duration of light in a light:day cycle; often expressed as light hours to dark hours (L:D) which normally sum to 24 hours of a day.

Predator swamping: when prey vastly outnumber predators, effectively allowing for the survival of a portion of the prey population.

Pupa: morphogenic state for mosquitos which corresponds to the final pre-adult stage; in this stage, the larval structures are broken and form adult mosquito structures were developed.

Pupation: the process in which a fourth instar larva becomes a pupa (see pupa).

Reproductive synchrony: when fertile members of a given population mate and conceive offspring at the same time or in temporal clusters.

Rule of Thermal Summing: principle which describes the accumulation of thermal energy (past the thermal threshold for development) required for development (thermal constant, thermal constant).

Sterile insect technique: biological insect population control method where a large number of sterile male specimens are released into a natural environment; sterile males will compete with wild males to reproduce, however they will not produce offspring.

Synchronization of emergence: emergence of adults from the pupal stage of development at the same time.

Thermal constant: describes the amount of thermal energy (day-degrees) required for an ectotherm to develop.

Thermal optimum: the environmental temperature at which developmental rates are the highest; exceeding the thermal optimum will result in the slowing or halting of development, or death.

Thermal threshold for development: the temperature at which development occurs; temperatures below the threshold may permit survival, but no development will occur.

Vector: an organism that transmits a disease or parasite from one plant or animal to another.

Appendix A

	Summary for Average Day of Pupation							
	Southern Populations				Northern Populations			
Start Day & Temp.	WI		LI		KC		ML	
	A	B	A	B	A	B	A	B
Day 0 4 °C	μ=46.43 σ=2.95 n=23	μ=45.67 σ=3.62 n=24	μ=48.54 σ=4.33 n=26	μ=51.12 σ=6.00 n=25	μ=55.70 σ=7.76 n=27	μ=53.65 σ=5.18 n=23	μ=56.95 σ=7.06 n=21	μ=57.00 σ=6.37 n=18
Day 4 6 °C	μ=50.14 σ=6.18 n=28	μ=46.00 σ=3.57 n=28	μ=52.00 σ=6.53 n=28	μ=49.38 σ=5.59 n=26	μ=3.83 σ=5.27 n=24	μ=55.41 σ=6.32 n=27	μ=62.92 σ=8.93 n=26	μ=61.65 σ=7.62 n=23
Day 8 8°C	μ=49.42 σ=6.50 n=24	μ=47.10 σ=5.20 n=29	μ=50.33 σ=5.46 n=24	μ=51.24 σ=7.55 n=29	μ=52.22 σ=5.64 n=27	μ=53.77 σ=4.74 n=26	μ=59.06 σ=4.85 n=17	μ=54.92 σ=6.76 n=13
Day 12 10 °C	μ=47.84 σ=4.76 n=25	μ=48.55 σ=6.25 n=29	μ=51.38 σ=7.10 n=26	μ=48.59 σ=5.51 n=27	μ=52.64 σ=5.74 n=28	μ=53.42 σ=5.29 n=24	μ=62.40 σ=8.79 n=20	μ=59.30 σ=9.48 n=20
Day 16 12 °C	μ=49.30 σ=4.33 n=23	μ=48.58 σ=4.95 n=24	μ=53.41 σ=9.25 n=27	μ=52.72 σ=6.68 n=25	μ=56.38 σ=6.88 n=26	μ=53.75 σ=4.91 n=24	μ=62.10 σ=10.41 n=20	μ=60.64 σ=8.71 n=25
Day 20 14 °C	μ=49.40 σ=5.80 n=30	μ=48.80 σ=5.48 n=25	μ=52.62 σ=7.15 n=26	μ=51.70 σ=6.99 n=27	μ=56.24 σ=6.15 n=25	μ=53.20 σ=5.45 n=25	μ=61.28 σ=8.18 n=25	μ=55.90 σ=5.89 n=20
Day 24 16 °C	μ=49.25 σ=4.39 n=32	μ=54.14 σ=7.56 n=28	μ=52.40 σ=5.54 n=25	μ=52.72 σ=5.16 n=25	μ=57.00 σ=8.53 n=24	μ=55.21 σ=6.63 n=28	μ=58.38 σ=8.43 n=21	μ=62.82 σ=8.50 n=22
Day 28 18 °C	μ=48.77 σ=3.88 n=26	μ=50.29 σ=5.35 n=28	μ=51.63 σ=6.91 n=27	μ=52.57 σ=6.69 n=28	μ=56.36 σ=5.11 n=28	μ=54.87 σ=6.43 n=23	μ=63.27 σ=8.08 n=22	μ=58.74 σ=6.87 n=19
Day 32 20 °C	μ=56.00 σ=6.75 n=28	μ=52.52 σ=5.16 n=27	μ=51.48 σ=.53 n=27	μ=52.00 σ=5.11 n=28	μ=59.11 σ=6.80 n=27	μ=56.77 σ=6.09 n=26	μ=61.82 σ=6.87 n=22	μ=61.91 σ=7.21 n=22
Day 36 22 °C	μ=52.45 σ=3.80 n=22	μ=54.15 σ=5.71 n=27	μ=56.55 σ=6.14 n=29	μ=53.78 σ=4.65 n=27	μ=56.53 σ=.36 n=19	μ=57.83 σ=5.17 n=24	μ=66.87 σ=9.00 n=23	μ=67.33 σ=5.59 n=15
Day 40 24 °C	μ=58.15 σ=5.45 n=26	μ=57.56 σ=6.01 n=30	μ=59.64 σ=7.52 n=28	μ=57.64 σ=5.53 n=28	μ=62.86 σ=8.06 n=28	μ=66.15 σ=7.80 n=26	μ=72.76 σ=6.02 n=21	μ=73.30 σ=6.97 n=23
Day 44 26 °C	μ=62.78 σ=5.11 n=23	μ=60.92 σ=6.84 n=26	μ=58.84 σ=4.23 n=19	μ=60.56 σ=4.53 n=25	μ=64.48 σ=6.98 n=25	μ=68.71 σ=6.89 n=28	μ=75.83 σ=7.63 n=23	μ=74.27 σ=5.50 n=15
Day 48 28 °C	μ=65.77 σ=5.72 n=26	μ=66.30 σ=5.78 n=27	μ=64.52 σ=5.04 n=27	μ=65.56 σ=6.66 n=27	μ=72.58 σ=7.21 n=24	μ=70.26 σ=6.50 n=23	μ=77.05 σ=5.75 n=19	μ=82.78 σ=6.76 n=23
Day 52 30 °C	μ=67.63 σ=3.96 n=27	μ=65.91 σ=5.22 n=22	μ=69.20 σ=5.29 n=25	μ=68.71 σ=5.89 n=28	μ=75.76 σ=5.30 n=25	μ=5.28 σ=6.05 n=25	μ=82.82 σ=5.65 n=22	μ=83.36 σ=5.77 n=22

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