The author(s) shown below used Federal funding provided by the U.S. Department of Justice to prepare the following resource:

Document Title:	**Investigating Upper Thermal Limits of Forensically Important Blow Flies to Improve Testimony in Forensic Entomology**
Author(s):	**Aaron M. Tarone, Travis W. Rusch, Jeffery K. Tomberlin**
Document Number:	**300811**
Date Received:	**May 2021**
Award Number:	**2016-DN-BX-0204**

Federal Agency & Organization Element to Which Report is Submitted:	NIJ FY 16 Research and Development in Forensic Science for Criminal Justice Purposes
Federal Grant or Other Identifying Number Assigned by Agency:	2016-DN-BX-0204
Project Title:	Investigating upper thermal limits of forensically important blow flies to improve testimony in forensic entomology.
PD/PI Name, Title and Contact Information:	Aaron M. Tarone 2475 TAMU Department of Entomology Texas A&M University College Station, TX 77843-2475
Co-Authors:	Travis W. Rusch, Jeffery K. Tomberlin
Name of Submitting Official, Title, Contact information if other than PD/PI:	NA
DUNS and EIN Numbers:	DUNS: EIN:
Recipient Organization (Name & Address):	Texas A&M Research Foundation 400 Harvey Mitchell Parkway, Suite 100 College Station, TX 77845
Recipient Identifying Number or Account Number:	504561
Project/Grant Period (Start Date, End Date):	01/01/2017 – 06/30/2020
Reporting Period End Date:	06/30/2020
Report Term or Frequency:	Final Report
Signature of Submitting Official:	

Project Design: Because blow flies regularly colonize and consume decomposing materials, including humans, forensic entomologists use them as evidence in death investigations (Greenberg 1991, Catts and Goff 1992, Byrd and Castner 2010). The most common application for blow flies in forensics uses larval development time as a biological clock to predict forensically important timelines, such as time of colonization, which can be interpreted as time of death given certain assumptions (Catts 1992, Tomberlin et al. 2011, Tarone and Sanford 2017). This method seems accurate and precise within moderate temperature ranges (Byrd and Butler 1996, 1997, Anderson 2000), but remains largely untested against more extreme temperatures observed on decomposing carcasses (Image 1). Without additional experimentation that exposes blow fly larvae to extreme temperatures, there is little that can be done to increase precision in estimates using larval development across environments.

To further complicate matters, larvae cannot develop on a dead body if adults cannot reach and colonize one. As with larval development, environmental temperature strongly affects adult locomotion, oviposition, and survival (Taylor 1963, Ody et al. 2017, Rusch et al. 2019), which ultimately determines colonization ability. Thus, if adults experience environmental temperatures above or below their thermal tolerance range, there may be certain conditions under which forensic entomologists should expect no or delayed colonization. For instance, Wells (2019) documented a death investigation in Las Vegas, NV USA where a dead body was found with no insect activity or colonization though it was outdoors and exposed to the elements. The forensic entomologists involved in the case (pers. comm.) determined that the lack of fly activity was because the remains had not been available long enough for blow flies to locate and colonize the body. Although this explanation is certainly plausible, it violates a core assumption for estimating the postmortem interval, that blow flies immediately colonize a body after death (Catts and Haskell 1990, Byrd and Castner 2010, Wells 2019). It is also important to consider that Las Vegas, NV USA is the #1 urban heat island in the United States (Kenward et al. 2014) and the body was found in July on concrete, which absorbs solar radiation and consequently gets much warmer than surrounding air temperatures (Myint et al. 2015). Thus, if the temperature of the concrete surrounding the body, or the temperature of the body itself, was above the thermal tolerance of blow flies, an alternative explanation for the absence of blow flies is simply that it was too warm for them to be active on or around the body.

Ideally, increasing the knowledge of blow fly thermal tolerance would be a collective effort by forensic entomologists as has been the case in building reference larval development datasets. It is generally recognized that

temperature limits more phenotypes than larval development, but few studies have quantified the effects of temperature on adults, such as oviposition (Ody et al. 2017), locomotion (Taylor 1963), and survival (Rusch et al. 2019). For this reason, we investigated the thermal biology of two blow fly species *Cochliomyia macellaria* Fabricius (Diptera: Calliphoridae) and *Chrysomya rufifacies* Macquart (Diptera: Calliphoridae) as both larvae and adults. Specifically, we defined the upper and lower survival, knockdown, and oviposition temperatures (aims #1 and 3), which provides information as to when and where blow flies can be active based on environmental temperature. Such information could provide evidence that a body has been transported after death, and could provide an alternative explanation as to why some dead bodies are found uncolonized (e.g., Wells, 2019). We also investigated the effects of exposing both immatures and adults to suboptimal temperatures to simulate different ecologically relevant scenarios (aims #2 and 5). These results provide information as to how suboptimal environmental conditions, such as heat waves or cold snaps, affect adult colonization and larval development. Lastly, we quantified the thermal preferences of both adult and immature (i.e., larval) blow flies (aim #4). Such information will help predict larval behavior and adult oviposition behavior.

Purpose and Goal: This project attempts to better understand basic aspects of blow fly thermal biology in order to improve our use of blow flies as evidence in death investigations. The data generated here will ideally be employed in actual casework to increase precision of development data sets and temperature thresholds used when estimating forensically important timelines, such as the time of colonization and the postmortem interval.

Methods:

***Colony Care*:** Both blow fly species (*Cochliomyia macellaria* and *Chrysomya rufifacies*) were captured in College Station, TX USA and reared using established methods (Byrd and Castner 2010) in the Forensic Laboratory for Investigative Entomological Sciences (FLIES) facility at Texas A&M University in College Station, TX USA.

Aim #1 Define thermal knockdown and mortality limits for immature and adult blow flies: We defined thermal knockdown as the inability to effectively locomote (Gilchrist and Huey 1999), and defined survival as active flies (e.g., walking, crawling, or flying) that responded to stimuli such as gentle pushing or prodding (Chidawanyika and Terblanche 2011) 24 h post treatments. For immature blow flies, an aluminum stage was constructed and partially submerged in a water bath. When measuring either the upper or lower thermal knockdown, the starting temperature was ~25°C and 10 larvae of the same life stage and species were placed on the surface of the aluminum plate together. For measuring the

upper thermal knockdown, the water bath was heated at a rate of ~0.7°C/min until all larvae knocked down. As larvae knocked down they were removed from the plate and placed in a Petri dish with ~5 g of beef liver where they recovered for 24 h, after which survival was recorded. Measuring the lower knockdown temperature and survival followed the same methods described above, but the water bath was cooled at a rate of ~0.7°/min by inserting ice at ~2 minute intervals. We measured the upper and lower thermal knockdowns for the four larval life stages (first, second, third, and post-feeding third instar) for both *C. macellaria* and *C. rufifacies*.

Measuring the upper and lower thermal knockdown and survival of adult blow flies consisted of a warm water bath to heat flies (described above) until they knocked down. Individual adults were placed in 15 mL plastic test tubes that were submerged in the water bath. A digital thermal couple was placed in the center of each test tube to record air temperature. As each fly knocked down, the temperature was recorded and the fly was transported to a 1 L glass jar where it was provided sugar and water and allowed to recover with four other flies for 24 h, after which survival was recorded.

Aim #2 Define thermal survival curves for immature and adult blow flies with different thermal exposure times: Immature blow flies of the same age (first, second, third, or post-feeding third instars) and species were placed in groups of 10 inside 50 mL plastic cups. The cup consisted of one of two nutrition treatments; 1) no food, or 2) food (20 g beef liver). Larvae inside the cups were placed in a Percival incubator set to s specific temperature (25, 35, 45, or 50°C) for a given duration (0.5, 1, or 2 h). After a given treatment, larvae were removed from the incubator and immediately scored for knockdown. Then, all 10 larvae from a given cup were placed in a Petri dish and provided ~20 g beef liver and allowed to recover for 24 h, after which they were scored for survival.

Aim #3 Define thermal limits of oviposition: Adult blow flies aged 9-11 days post pupal emergence were removed from colony cages and placed into experimental insect cages (20 cm^3). Groups consisting of 20 males and 20 females were placed in each experimental cage with sugar, water, and ~20g of beef liver (placed inside a 50 mL plastic cup for oviposition site). Experimental cages with adult blow flies were then placed inside a Percival incubator (n = 3 cages per incubator) set to a specific temperature ranging from 10-45.5°C (see Table 1 for full list of treatments by species) with a12:12 light:dark cycle and relative humidity of 70%. For each treatment the presence or absence of eggs was recorded, and any eggs laid were massed. The lowest and highest temperatures where eggs were found were deemed the upper and

lower thermal limits of oviposition, while the highest and lowest temperatures at which eggs hatched and completed development to adult emergence were considered the thermal limits for egg viability.

We conducted an additional experiment using adult *C. rufifacies* where we heat shocked virgin flies and assessed how this type of exposure affected oviposition. Immediately after emergence, adult flies were sexed and separated into sex specific insect rearing cages. After 24 hours, flies were exposed to one of three non-lethal heat shock treatments for 1 h; 1) 25°C (i.e., control), 2) 42°C, or 3) 44°C. Twenty four hours after the heat chock treatment, untreated virgin flies of the opposite sex were added to the experimental cages (n = 25 males and 25 females per cage) so flies could mate. Each cage also contained sugar, water, and ~20g of liver for an oviposition site. The liver in each cage was checked and replaced every 12 h for eggs for 14 days. When eggs were found, the day, time, and mass of eggs per cage were recorded.

Aim #4 Define thermal preferences of immature and adult blow flies: To determine the thermal preferences of immature and adult blow flies, an aluminum stage was constructed where each end was bent at 90° and dipped in either a warm or cold water bath (Image 2), thus providing flies a range of surface temperatures (~10-50°C). Four digital thermal couples were affixed to the surface of the thermal gradient to record temperatures and were used to estimate the slope of the temperature gradient by plugging the values into a second order polynomial equation. This allowed us to estimate the temperature of the gradient at any position. During experiments, 10 adult flies were placed in the center of the arena and were allowed to explore the gradient for 20 min before data collection began. Data were collected for 60 minutes by video recording the flies. Videos were analyzed using the computer tracking software Tracker©, where the position of each fly was recorded every 10 minutes. The x-coordinate of each position was then plugged into the polynomial equation derived from the digital thermal couples and the output estimated the temperature where each fly was located at each time point.

The preferred temperature of immature blow flies (i.e., larvae) was conducted using the same thermal gradient setup for adults (described above) with the addition of minced hamburger substrate (~1 cm thick) across the center of the gradient to provide a food source for the larvae during trials. Groups of 200 larvae were placed across the gradient and allowed to feed for 2.5 hours. After this feeding time, the positions of larval masses were recorded. In addition to the thermal couples affixed to the surface of the thermal gradient, a FLIR digital thermal camera was used to record the both the temperatures of the larvae and the temperature of the gradient.

Aim #5 Define developmental responses of blow flies to high temperatures: To examine the effects of suboptimal temperatures on blow fly development we gravimetrically weighed 500 *C. macellaria* eggs and placed them in 1 L glass Mason jars containing 200 mL of sand and 50 g of beef liver. These jars were then placed in Percival incubators set to one of three temperature regimens; 1) constant 25°C, 2) 25 ± 5°C, or 3) 25 ± 10°C. All incubators were set to 60% RH and maintained a 14:10 light-dark cycle. To simulate morning and evening oviposition events, we also varied the direction of initial temperature ramping. That is, all experiments began at 25°C, but one group experienced temperatures that initially ramped up (i.e., warmed) to simulate a morning oviposition event that starts cool and gradually warms as the day progresses, while the other set of trials initially ramped down to simulate an evening oviposition event that starts warm and gradually cools as temperatures drop overnight in absence of direct solar radiation. For each trial, six jars were placed in three incubators set to one temperature regimine and one ramping direction. Note, the constant 25°C groups did not ramp up or down as they remained at a constant 25°C. Three jars from each incubator were sampled every 12 hours after hatch by removing three larvae and storing them in 70% ethanol. This way larvae can be processed to compare differences in body size and when different instars stages are reached (in progress). The other three jars in a given incubator were not sampled. This allowed us to assess whether our sampling methods had any adverse handling effects on the larval development. Jars were left in the incubators until 7 days after the first adult emergence, after which experiments were stopped. Total time at each development stage (egg, larvae, and pupae) were quantified and compared across treatments.

Findings:

In defining the upper and lower thermal knockdowns of blow flies (Aim 1), we found adult *C. macellaria* exhibited a broader thermal knockdown range than *C. rufifacies*; *C. macellaria*'s lower thermal knockdown = 6.0 ± 0.9°C (avg ± 1 SD) and an upper thermal knockdown = 46.6 ± 0.6°C, while adult *C. rufifacies's* lower thermal knockdown = 7.8 ± 1.0°C and upper thermal knockdown = 46.0 ± 0.5°C (Figures 1-2). Thus it appears that *C. macellaria* is more of a thermal generalist compared to *C. rufifacies* and may benefit from thermal refugia both below and above the thermal tolerance of *C. rufifacies*. In defining the thermal knockdowns of immature blow flies (i.e., larvae), we found both the lower and upper thermal knockdown temperatures differed by age and species. The lower thermal knockdown of *C. macellaria* first instars = 6.5 ± 2.7°C, second instars = 5.6 ± 1.7°C, third instars = 7.0 ± 1.0°C, and post feeding third instars = 4.7 ± 0.8°C (Figure 3) while the lower thermal knockdown of *C. rufifacies* first instars = 14.8 ± 1.6 °C, second instars = 9.1 ± 1.9°C,

third instars = 8.4 ± 1.4°C, and post feeding third instars = 8.2 ± 0.9°C (Figure 4). The upper thermal knockdown of *C. macellaria* first instars = 39.2 ± 4.9°C, second instars = 48.4 ± 1.9°C, third instars = 53.1 ± 1.1°C, and post feeding third instars = 52.4 ± 1.4°C (Figure 5). The upper thermal knockdown of *C. rufifacies* first instars = 41.9 ± 3.4°C, second instars = 49.9 ± 2.0°C, third instars = 53.7 ± 1.1°C, and post feeding third instars = 53.5 ± 1.2°C (Figure 6).

In defining the thermal survival curves for immature and adult blow flies with different thermal exposure times (Aim 2), we found that temperature and duration had the largest effect sizes, increasing the probability of knockdown and decreasing the probability of survival as either temperature or duration increased both when flies were provided food or deprived food (Figures 7-8, 11-12, and 15-18). For adults, sex had a moderate effect size (Figures 9 and 13), with females having a lower probability of knockdown and a higher probability of survival. While age had minimal effects on the probability of knockdown and survival in adults (Figures 10 and 14), age had a strong effect on the probabilities of knockdown and survival in immature blow flies (Figures 15-18). These results follow general patterns of thermal tolerance, where increasing temperatures and exposure durations lead to the breakdown of systems (e.g., reaction rates, protein function, and cellular performance) required for maintaining homeostasis. Furthermore, providing food or water resulted in a buffering effect for both immature and adult blow flies (i.e., improved thermal tolerance compared to flies provided no nutrients), where flies likely were able to replenish depleting energy stores and thus were able to better tolerate the temperature treatments.

When we investigated the effects of temperature on blow fly oviposition (Aim 3), we first assessed the effects of a brief but non-lethal heat shock on oviposition behavior. We found that temperature treatments of 25 and 42°C had minimal effects, while females exposed to the temperature treatment of 44°C laid eggs sooner, laid eggs more frequently, and laid the most eggs over a 14 day period. Thus, a brief heat shock early in adulthood, such as a heat wave, could induce female *C. rufifacies* to colonize a carcass sooner and lay more eggs, potentially affecting estimates of the time of colonization and the postmortem interval (Figures 19-21). Our second experiment investigated the thermal preference of both *C. macellaria* and *C. rufifacies* when placed on a thermal gradient. Both species exhibited distinct thermal preference, though *C. macellaria* oviposited over a broader range of temperatures compared to *C. rufifacies* (mean ± SD; 35.9 ± 4.0°C vs 37.7 ± 1.9°C; Figure 22). The third sets of experiments investigated the effects of temperature on oviposition over a range of temperatures (10-45.5°C) and determined both the lower and upper thermal limits (of

oviposition) for both *C. macellaria* and *C. rufifacies*. Similar to thermal preference, *C. macellaria* oviposited over a broader range of temperatures compared to *C. rufifacies* (15 – 44.5°C vs 22.5 – 40.0°C; Table 1). However, it is important to note that although *C. macellaria* laid eggs at 43.5 or greater resulted in non-viable eggs (i.e., eggs did not hatch after 3 days held at 25°C), and eggs laid by *C. rufifacies* at 40°C or greater were also not viable. Thus the actual ranges of viable eggs for *C. macellaria* and *C. rufifacies* in our experiments were 15 – 42.5°C and 22.5 – 37.5°C. Nevertheless, the general trend that *C. macellaria* is more of a thermal generalist compared to *C. rufifacies* was observed again. Furthermore, concerning oviposition, *C. macellaria* has both an upper and lower advantage over *C. rufifacies* in colonizing remains.

Our fourth aim determined the thermal preferences of adult and immature blow flies. Adult *C. macellaria* selected a mean temperature of 33.8 ± 8.3°C while *C. rufifacies* selected a mean temperature of 31.6 ± 6.7°C (Figure 22). Larvae were divided again by instar, where *C. macellaria first instars* = 32.1 ± 2.9°C, second instars = 40.6 ± 1.0°C, early feeding third instars = 40.6 ± 1.4°C, and late feeding third instars = 42.5 ± 1.7. Similarly, larvae of *C. rufifacies* exhibited age specific preferred temperatures; first instars = 33.0 ± 1.2°C, second instars = 32.4 ± 2.9°C, early third instars = 39.4 ± 1.5°C, and late feeding third instars = 40.1 ± 1.4°C (Figures 28 and 29). These results provide further support that *C. rufifacies* is a thermal specialist compared to *C. macellaria* as it once again selected a tighter range of temperatures.

In defining developmental responses of blow flies to high temperatures (Aim 5), we found that the magnitude of fluctuating temperatures and the initial direction of the temperature ramp (i.e., warm or cold) affected development time, including hatch time, larval time, and total development time, but had no directional effect on pupal duration time (Figures 23-26). When the initial temperature fluctuation ramped up (i.e., warmed), development was accelerated, whereas, when the initial temperature fluctuation ramped down (i.e., cooled), development was slowed. Although the mean exposure time was the same across treatments, early thermal exposures (i.e., ramping up vs ramping down) revealed lasting downstream effects that either extended or reduced total development time. Furthermore, the magnitude (i.e., ± 5 vs ± 10°C) exacerbated the differences in total development time, with the larger magnitude having the strongest effects.

Implications for Criminal Justice Policy and Practice:

The results found through our experiments suggest temperature has major implications on various aspects of blow fly behavior and development. For instance, understanding the thermal limits of adult survival provides information as to when and where specific species of blow flies can be active. These data can aid forensic investigations in at least two

ways; 1) if we know the thermal limits of blow flies, we can potentially explain why some cases lack blow fly colonization (e.g., bodies found in cold or hot environments), such as the case reported by Wells (2019), and 2) in cases where a body is colonized by larvae in suboptimal temperature conditions, the presence of blow flies provides evidence that the body was moved after death. Furthermore, our work went beyond the critical temperatures of blow fly activity and survival, and investigated the thermal critical limits of oviposition, an often overlooked but vital part of colonization. Our results found that oviposition critical temperatures are even more limiting than adult thermal knockdown and survival temperatures. Thus, even though adult blow flies may be active in a region, they may opt to not colonize a body if the temperatures fall outside their oviposition thermal limits. Our results concerning larval development are useful to forensics as blow flies have been documented reducing their activity at temperature of only 35-40°C (Nicholson 1934, Mohr and Tomberlin 2015), and thus are more likely to colonize a carcass in the morning or evening when temperatures are cooler (in warm regions). Thus, our findings that initial ramping direction (i.e., warming or cooling) affects total development time, could provide evidence as to morning or evening oviposition events. That is, if blow flies colonize a body in the morning and the temperatures increase during the day, one may overestimate of time of colonization since they don't take into account that an initial warming could accelerate blow fly development. Conversely, one could underestimate the time of colonization if the colonization event occurred in the evening and the immature blow flies experience an initial cooling period overnight, which may delay their development time. Thus, any information on the potential disruption to adult blow fly colonization or larval development times is helpful in reducing the error in estimating forensically important timelines

Scholarly Products:

Theses:

1) Thesis (MSc) in Entomology. 2019. Texas A&M University. Lauren Beebe. "Thermal Tolerance of the Larval Stadia of Two Forensically Important Blow Fly Species, *Chrysomya rufifacies* (Macquart) and *Cochliomyia macellaria* (Fabricius) (Diptera: Calliphoridae)". – Graduated with MSc and currently working a research assistant in Tarone Laboratory at Texas A&M.

2) Jeffrey Yung (MSc) in Entomology. 2020. Texas A&M University. Jeffrey Yung. "Ecologically Relevant Thermal Performances of Immature *Cochliomyia macellaria* (Fabricius) (Diptera: Calliphoridae) and Associated Bacteria". Starting a PhD at Univ. of Windsor in Fall 2020.

Collaborating Researchers:

1) Abena Adutwumwaah. MSc research on the upper thermal tolerance of *Cochliomyia macellaria*. University of Lincoln, Lincoln-Lincolnshire, United Kingdom. MSC 2017.

2) Aline Spindola. PhD research on the thermal preferences and stress of the black soldier fly (*Hermetia illucens*). Texas A&M University. Currently working as a production manager at a black solider fly facility in Malaysia.
3) Samantha Sawyer. Collaborative project on the effects of heat shock on *Chrysomya rufifacies* oviposition behavior (Aim 4). Texas A&M University. Currently finishing PhD at Texas A&M University.

***Undergraduate Researchers*:**

1) Nicholas Richter. Independent research on the effects of heat shock on *Chrysomya rufifacies* oviposition behavior (Aim 4). Texas A&M University. Graduated with BS in 2018.
2) Abigail Orr – Independent research on the development of *Cochliomyia macellaria* to fluctuating temperatures (Aim 5). Currently working on PhD in Tarone Laboratory at Texas A&M University.
3) Lauren Gagner. Independent research on the effects of heat shock on *Chrysomya rufifacies* oviposition behavior (Aim 4). Texas A&M University. Graduated with BS in 2019.
4) David Sohn. Independent research on the effects of heat shock on *Chrysomya rufifacies* oviposition behavior (Aim 4). Texas A&M University. Graduated with BS in 2019.
5) Alexandria Smith Independent research on the effects of heat shock on *Chrysomya rufifacies* oviposition behavior (Aim 4). Texas A&M University. Currently finishing BS.

***Peer Reviewed Publications*:**

1) Rusch, T. W., A. Adutwumwaah, L. E. J. Beebe, J. K. Tomberlin, and A. M. Tarone. 2019. The upper thermal tolerance of the secondary screwworm, *Cochliomyia macellaria* Fabricius (Diptera: Calliphoridae). *Journal of Thermal Biology* 85:102405.
2) Rusch, T. W., A. M. Faris, *L. E. J. Beebe, J. Tomberlin, and A. Tarone. The upper thermal tolerance of the hairy maggot blow fly *Chyrsomya rufifacies* (Diptera: Calliphoridae). *Ecological Entomology*, (In Press).

Presentations:

1) The potential application of operative temperature in accumulated degree day models. Rusch, T. W., J. K. Tomberlin, and A. M. Tarone. North American Forensic Entomology Association annual meeting, Orlando, Florida 2018 (invited oral)
2) A discussion of approaches: Incorporating core concepts from thermal biology into forensic entomology. Rusch, T. W., J. K. Tomberlin, and A. M. Tarone. North American Forensic Entomology Association annual meeting, Orlando, Florida 2018 (oral)
3) Where to dump the kids? Oviposition site selection of a forensically important blow fly (*Cochliomyia macellaria*). Rusch, T. W., J. K., Tomberlin, and A. M. Tarone. Ecological Society of America national meeting, Vancouver, British Columbia 2018 (oral)
4) Spindola, A., E. Walsh, T.W. Rusch, A. M. Tarone, J. Rangel, and J. Tomberlin. Eating Insects, Athens, Georgia. 2018, oral.
5) Development Responses of a Forensically Important Blow Fly (*Cochliomyia macellaria*) to Fluctuating Temperatures. Rusch, T. W., J. K. Tomberlin, and A. M. Tarone. Entomological Society of America, St. Louis, Missouri 2019 (invited oral).
6) Consideration of Thermoregulation by Blow Fly Larvae to Improve Estimates of Development. Rusch, T. W., T. Chappell, J. Hayter, and A. M. Tarone. North American Forensic Entomology Association, Indianapolis, Indiana 2019 (oral).
7) Bebee, L., T. W. Rusch, and A. M. Tarone. North American Forensic Entomology Association, Indianapolis, Indiana 2019 (oral – won first prize in graduate student competition).
8) Hayter, J., T. W. Rusch, A. M. Tarone, and T. Chappell. North American Forensic Entomology Association, Indianapolis, Indiana 2019 (oral).
9) Malawey, A., J. S. Oliveira, E. Walsh, T. W. Rusch, A. M. Tarone, J. Rangel, and J. K. Tomberlin. INSECTA, Potsdam, Germany 2019.
10) Rusch, T. W., A. Orr, J. K. Tomberlin, and A. M. Tarone. American Academy of Forensic Sciences, Anaheim, California 2020 (oral invited – DOJ Research and Development Symposium).

11) Beebe, L., T. W. Rusch, and A. M. Tarone. Thermal Tolerance of the Larval Stadia of Two Forensically Important Blow Fly Species, *Chrysomya rufifacies* (Macquart) and *Cochliomyia macellaria* (Fabricius) (Diptera: Calliphoridae). Entomological Society of America Joint North Central Branch and Southwestern Branch Annual meeting. Oklahoma City, Oklahoma 2020 (abstract accepted).
12) Chappell, T., A. M. Tarone, T. W. Rusch, and J. Hayter. Toward improved arthropod phenology modeling methods North American Forensic Entomology Association, online 2020 (oral).
13) J. Hayter, A. M. Tarone, T. W. Rusch, and T. Chappell. Phenological Consequences of Blow Fly Larval Thermoregulation: A Simulation Approach. North American Forensic Entomology Association, online 2020 (oral).
14) L. E. J. Beebe, T. W. Rusch, and A. M. Tarone. Extreme Temperatures Impacting the Larval Stadia of the Hairy Maggot Blow Fly, *Chrysomya rufifacies* (Macquart), and the Secondary Screwworm, *Cochliomyia macellaria* (Fabricius) (Diptera: Calliphoridae). North American Forensic Entomology Association, online 2020 (oral).
15) T. W. Rusch, J. K. Tomberlin, and A. M. Tarone. Considerations for the critical and preferred temperatures for oviposition of two forensically relevant blow fly species *Chrysomya rufifacies* Macquart (Diptera: Calliphoridae) and *Cochliomyia macellaria* Fabricius (Diptera: Calliphoridae) North American Forensic Entomology Association, online 2020 (oral).

References:

Anderson, G. S. 2000. Minimum and maximum development rates of some forensically important Calliphoridae (Diptera). Journal of Forensic Sciences **45**:824-832.

Byrd, J. H., and J. F. Butler. 1996. Effects of temperature on *Cochliomyia macellaria* (Diptera: Calliphoridae) development. Journal of Medical Entomology **33**:901-905.

Byrd, J. H., and J. F. Butler. 1997. Effects of temperature on *Chrysomya rufifacies* (Diptera: Calliphoridae) development. Journal of Medical Entomology **34**:353-358.

Byrd, J. H., and J. L. Castner. 2010. Forensic entomology: the utility of arthropods in legal investigations. 2nd edition. CRC press.

Catts, E. 1992. Problems in estimating the postmortem interval in death investigations. Journal of Agricultural Entomology **9**:245-255.

Catts, E. P., and M. L. Goff. 1992. Forensic entomology in criminal investigations. Annual review of Entomology **37**:253-272.

Catts, E. P., and N. H. Haskell. 1990. Entomology & death: a procedural guide. Forensic Entomology Assocs.

Chidawanyika, F., and J. S. Terblanche. 2011. Rapid thermal responses and thermal tolerance in adult codling moth *Cydia pomonella* (Lepidoptera: Tortricidae). Journal of Insect Physiology **57**:108-117.

Gilchrist, G. W., and R. B. Huey. 1999. The direct response of *Drosophila melanogaster* to selection on knockdown temperature. Heredity **83**:15-29.

Greenberg, B. 1991. Flies as forensic indicators. Journal of Medical Entomology **28**:565-577.

Kenward, A., D. Yawitz, T. Sanford, and R. Wang. 2014. Summer in the city: Hot and getting hotter. Climate Central.

Mohr, R. M., and J. K. Tomberlin. 2015. Development and validation of a new technique for estimating a minimum postmortem interval using adult blow fly (Diptera: Calliphoridae) carcass attendance. International journal of legal medicine **129**:851-859.

Myint, S. W., B. Zheng, E. Talen, C. Fan, S. Kaplan, A. Middel, M. Smith, H.-P. Huang, and A. Brazel. 2015. Does the spatial arrangement of urban landscape matter? Examples of urban warming and cooling in Phoenix and Las Vegas. Ecosystem Health and Sustainability **1**:1-15.

Nicholson, A. 1934. The influence of temperature on the activity of sheep-blowflies. Bulletin of Entomological Research **25**:85-99.

Ody, H., M. T. Bulling, and K. M. Barnes. 2017. Effects of environmental temperature on oviposition behavior in three blow fly species of forensic importance. Forensic science international **275**:138-143.

Rusch, T. W., A. Adutwumwaah, L. E. Beebe, J. K. Tomberlin, and A. M. Tarone. 2019. The upper thermal tolerance of the secondary screwworm, *Cochliomyia macellaria* Fabricius (Diptera: Calliphoridae). Journal of Thermal Biology:102405.

Tarone, A. M., and M. R. Sanford. 2017. Is PMI the Hypothesis or the Null Hypothesis? Journal of Medical Entomology **54**:1109-1115.

Taylor, L. 1963. Analysis of the effect of temperature on insects in flight. The Journal of Animal Ecology:99-117.

Tomberlin, J., R. Mohr, M. Benbow, A. Tarone, and S. Vanlaerhoven. 2011. A roadmap for bridging basic and applied research in forensic entomology. Annual Review of Entomology **56**:401-421.

Wells, J. D. 2019. A Forensic Entomological Analysis Can Yield an Estimate of Postmortem Interval, and Not Just a Minimum Postmortem Interval: An Explanation and Illustration Using a Case. Journal of Forensic Sciences.

Appendix:

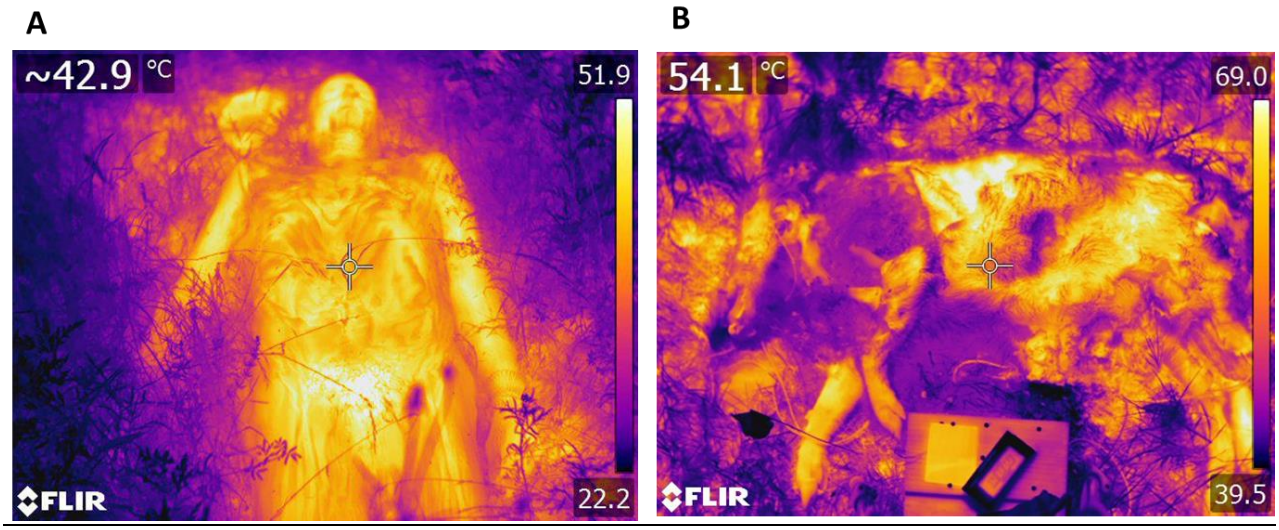

Image 1. Thermal image of human (A) and Pig (B) carcasses depicting temperatures available to blow flies during summer months in Texas, USA.

Image 2. Thermal gradient used to quantify preferred temperatures of blow flies (left – photo image, right – thermal image).

Figure 1. Lower knockdown temperatures of adult *C. macellaria* and *C. rufifacies*.

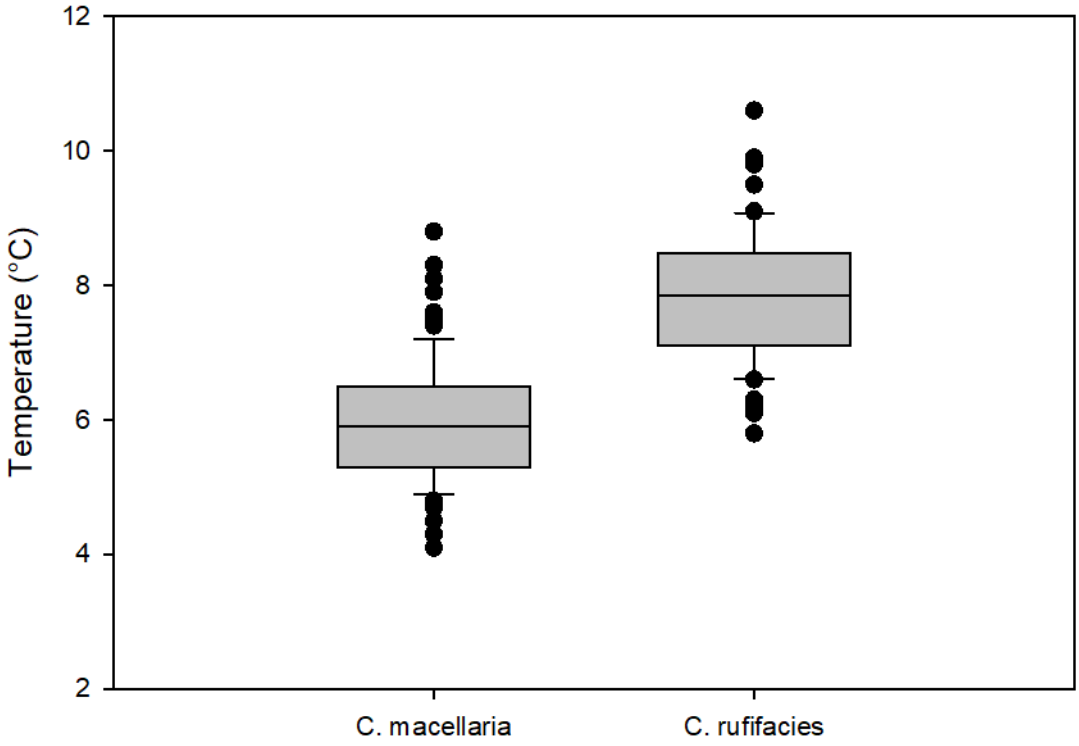

Figure 2. Upper knockdown temperatures of adult *C. macellaria* and *C. rufifacies*.

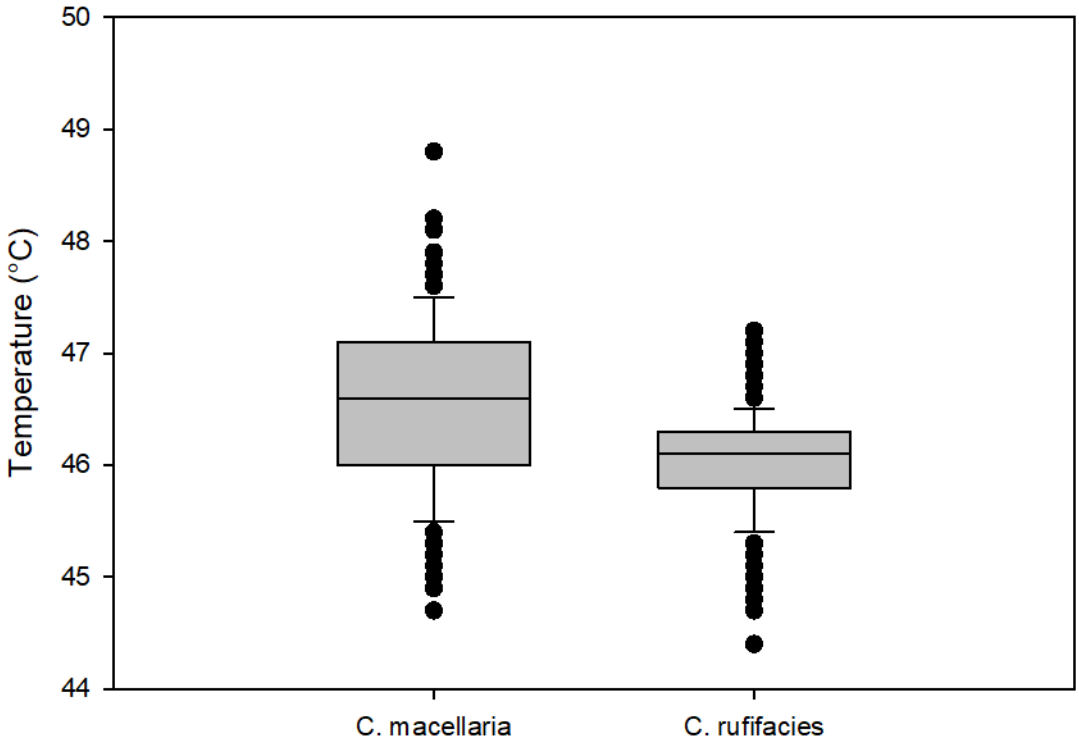

Figure 3. Lower knockdown temperatures of *C. macellaria* at four different ages.

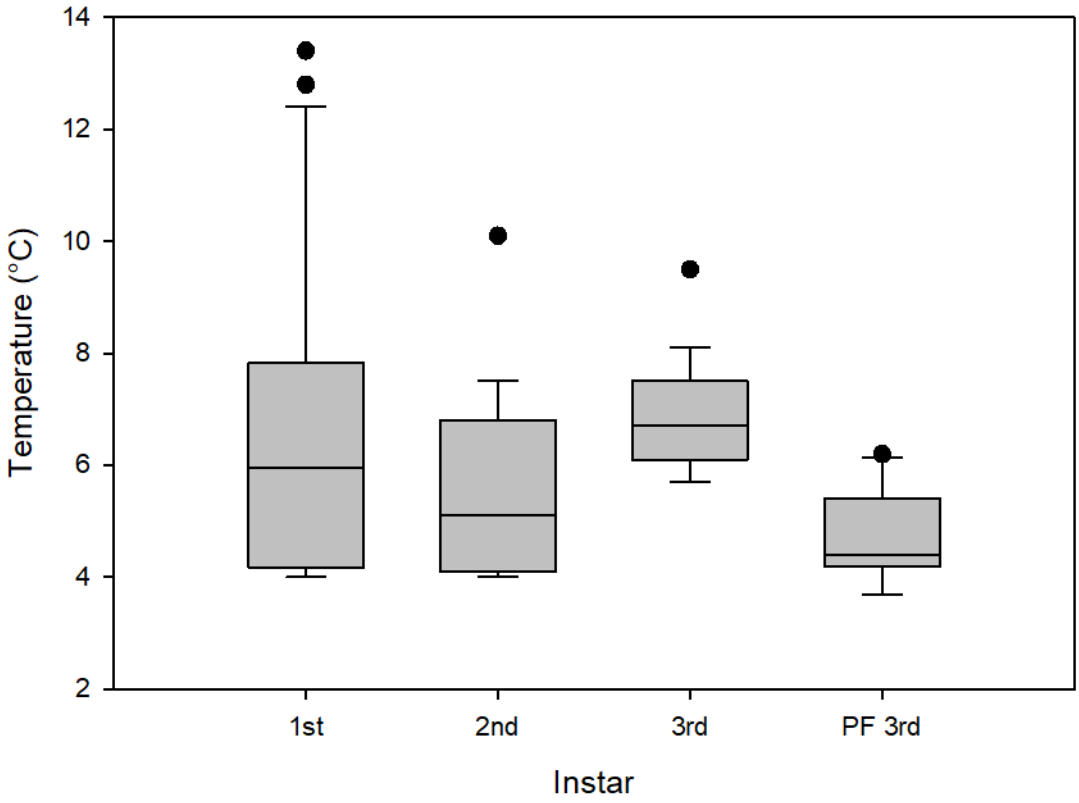

Figure 4. Lower knockdown temperatures of *C. rufifacies* at four different ages

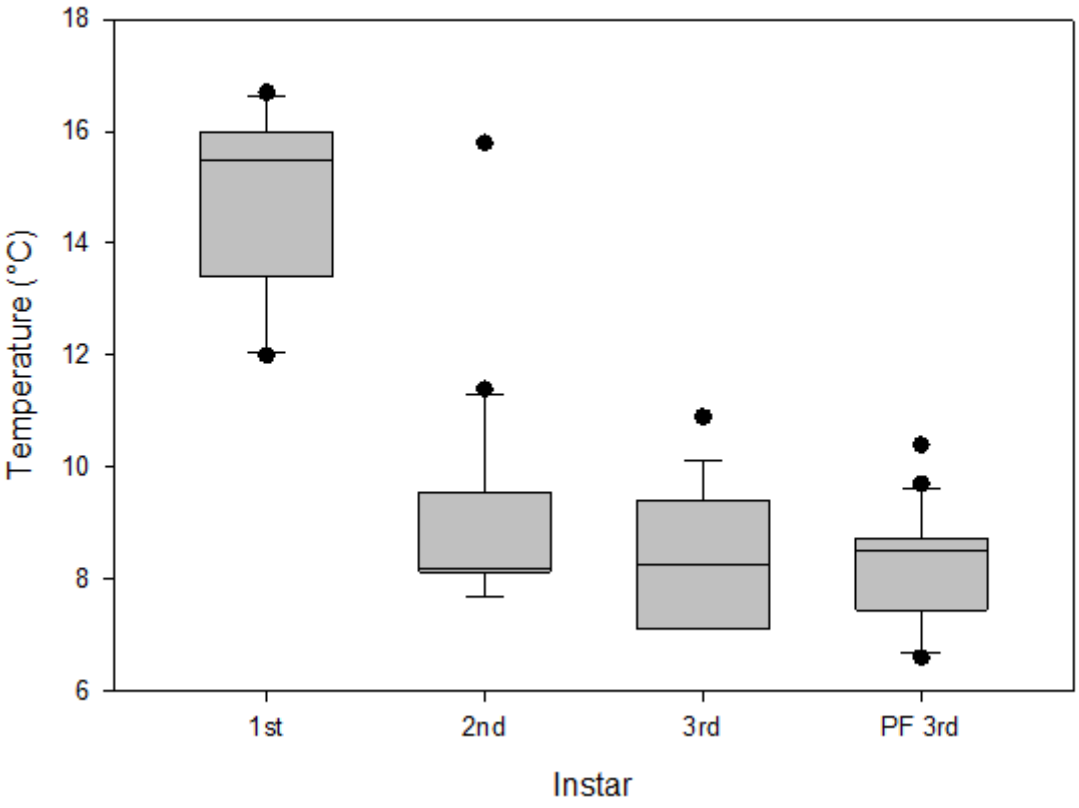

Figure 5. Upper knockdown temperatures of *C. macellaria* at four different ages.

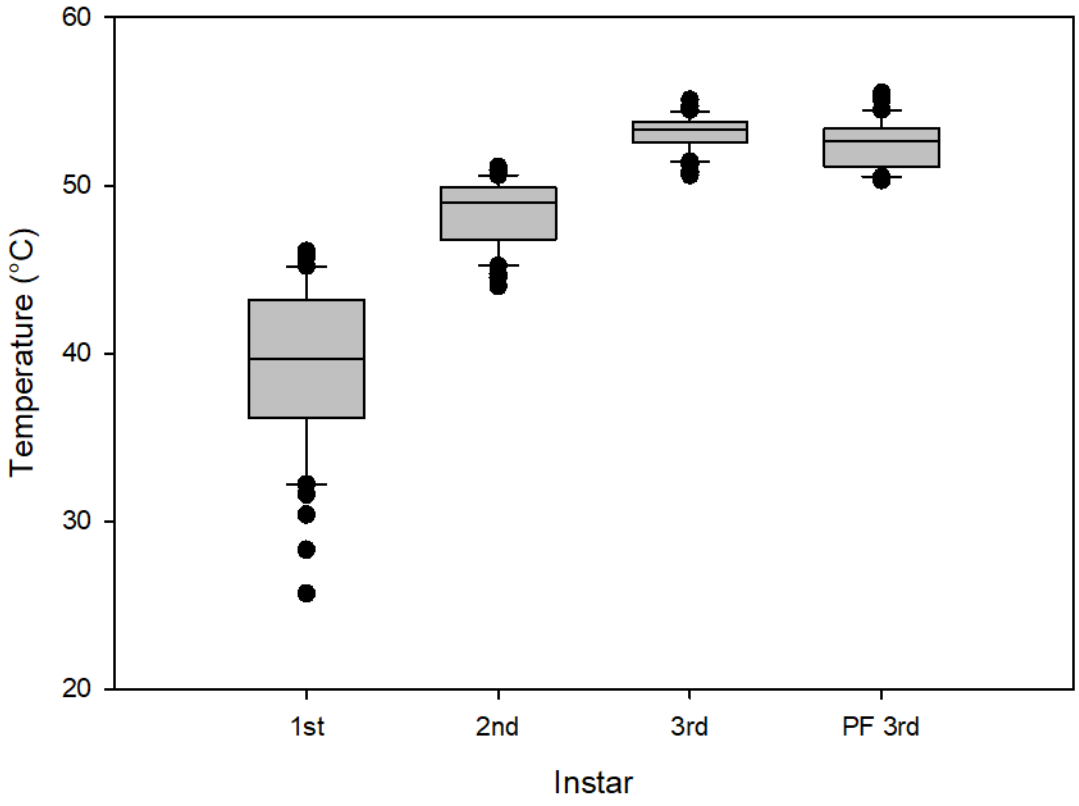

Figure 6. Upper knockdown temperatures of *C. rufifacies* at four different ages.

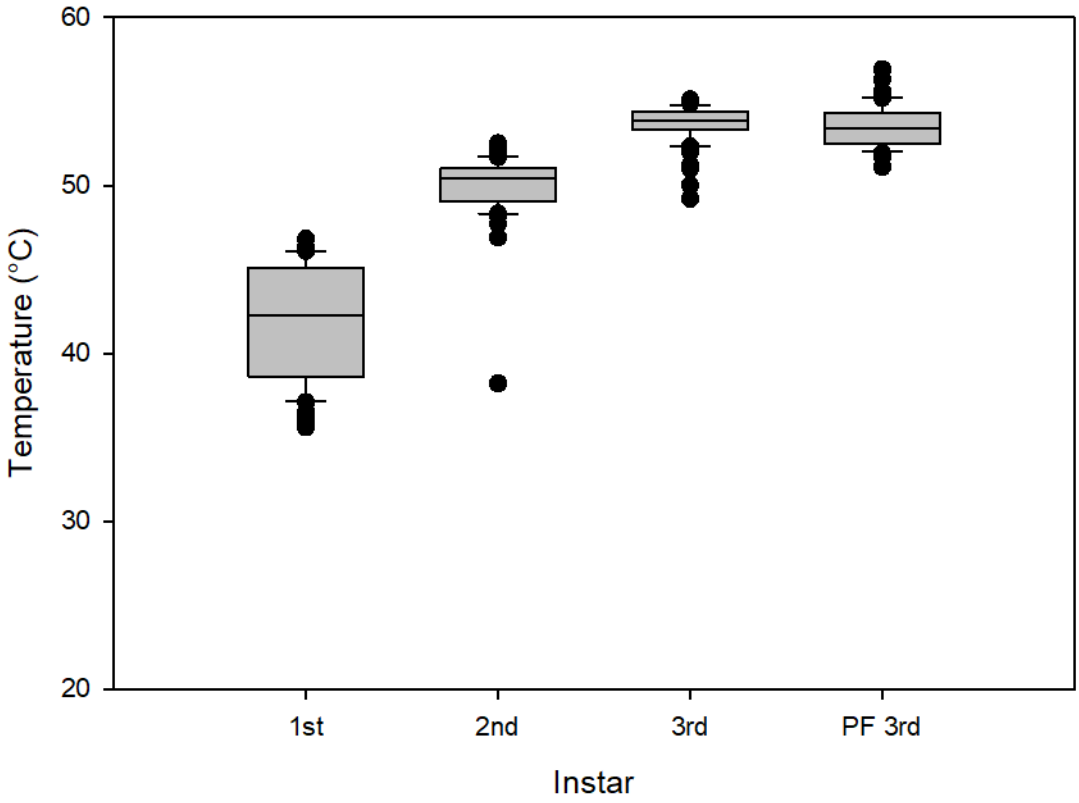

Figure 7. Probability (± 1 SD) of knockdown (A) and survival (B) of *C. macellaria* exposed to different temperatures provided 1 of 3 nutrients.

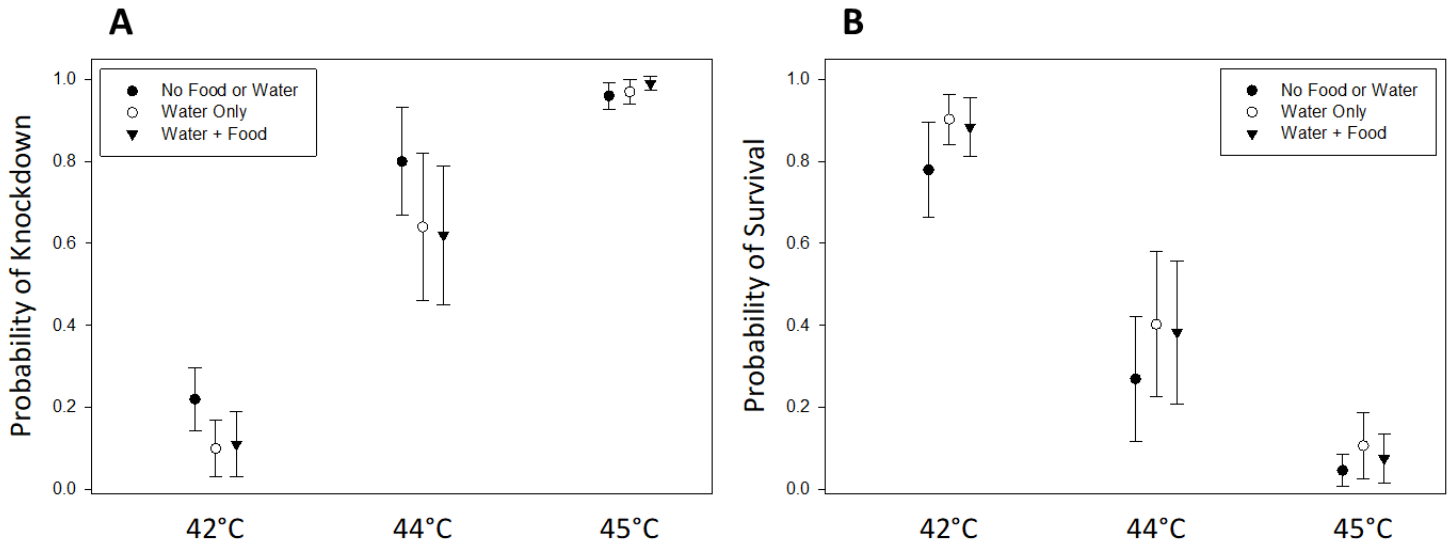

Figure 8. Probability (± 1 SD) of knockdown (A) and survival (B) of *C. macellaria* exposed to different temperatures for different durations of time.

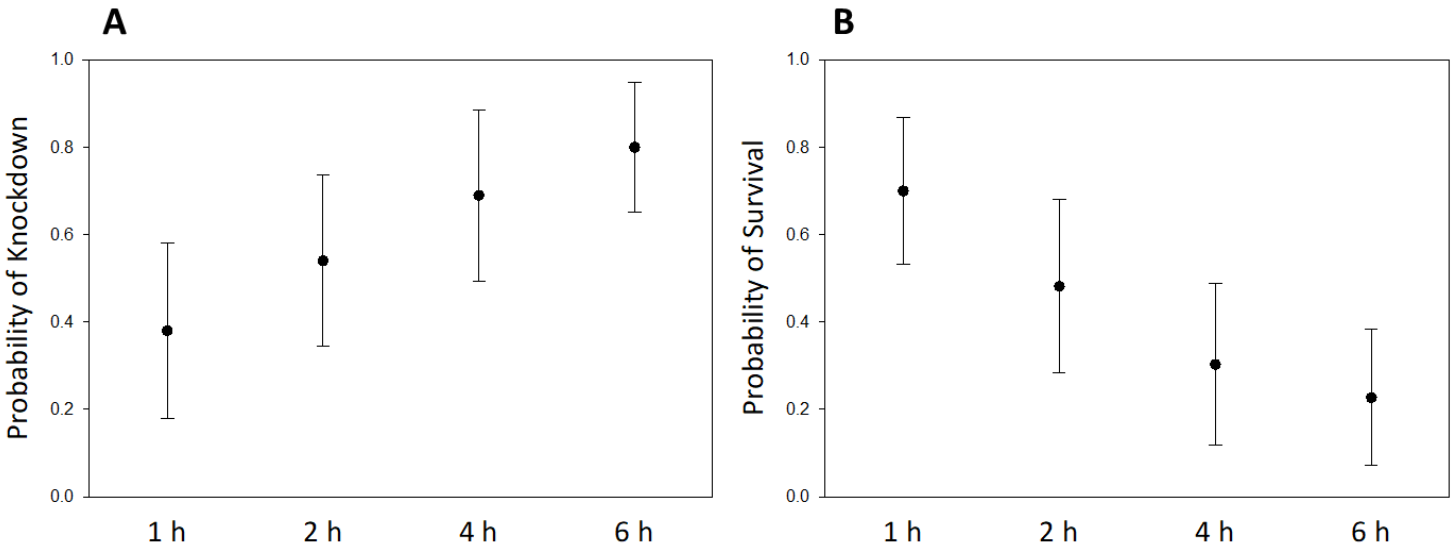

Figure 9. Probability (± 1 SD) of knockdown (A) and survival (B) of *C. macellaria* exposed to different temperatures for different durations of time by sex.

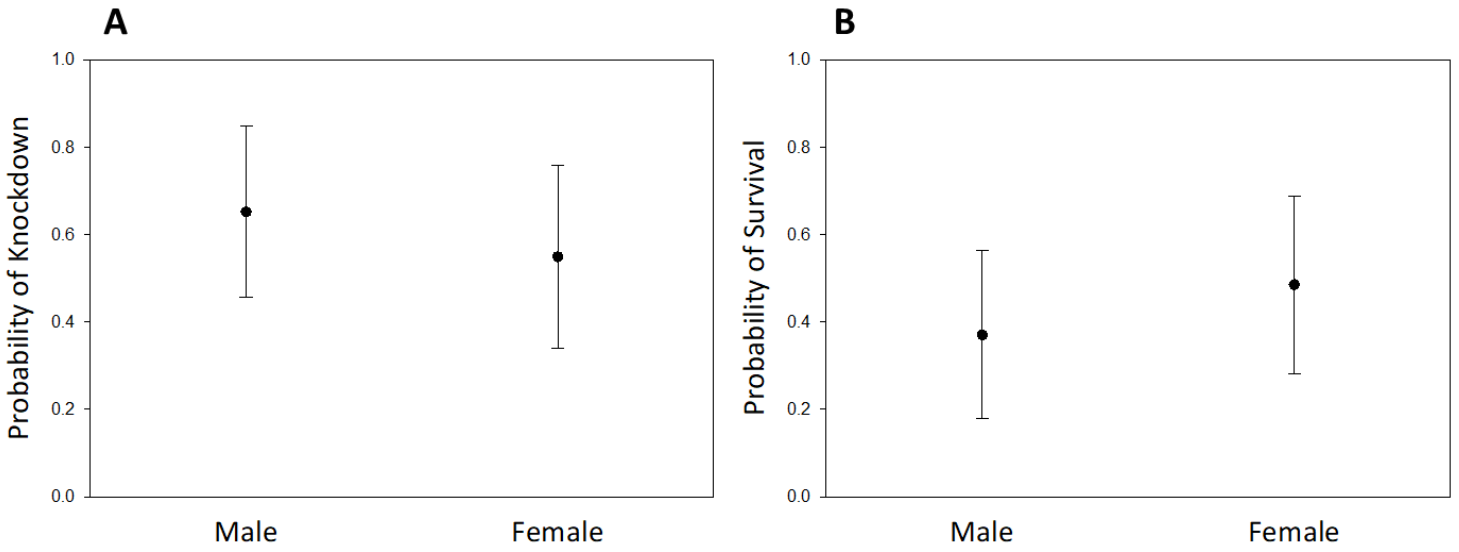

Figure 10. Probability (± 1 SD) of knockdown (A) and survival (B) of *C. macellaria* exposed to different temperatures for different durations of time by age.

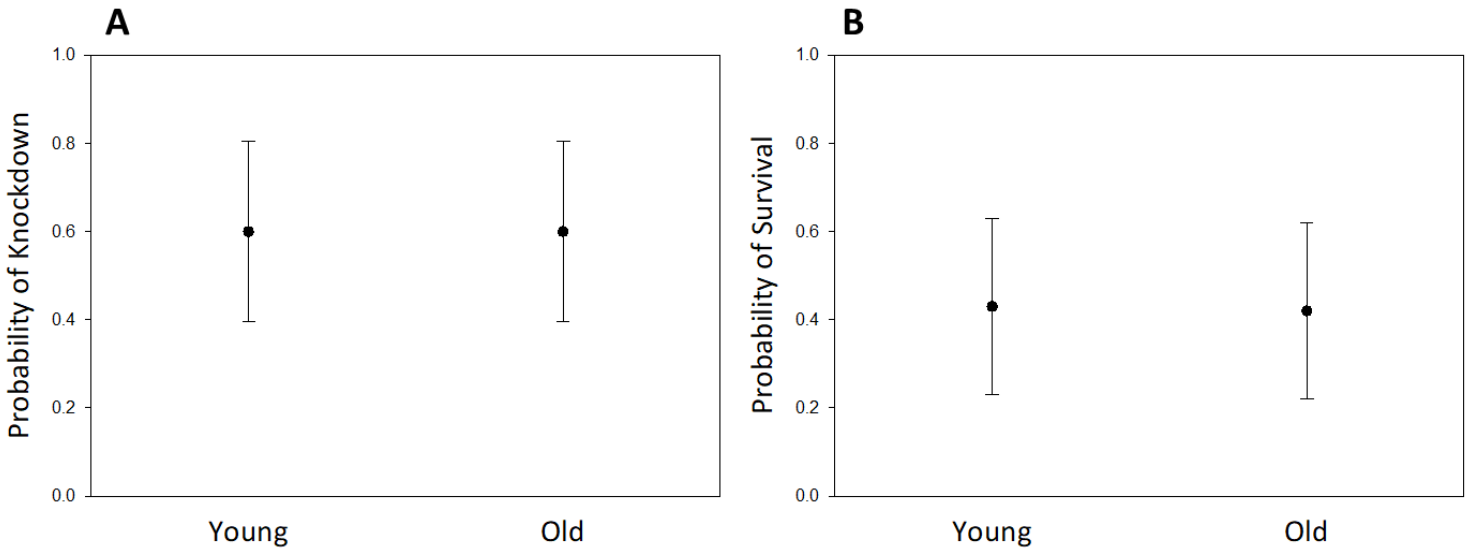

Figure 11. Probability (± 1 SD) of knockdown (A) and survival (B) of *C. rufifacies* exposed to different temperatures provided 1 of 3 nutrients.

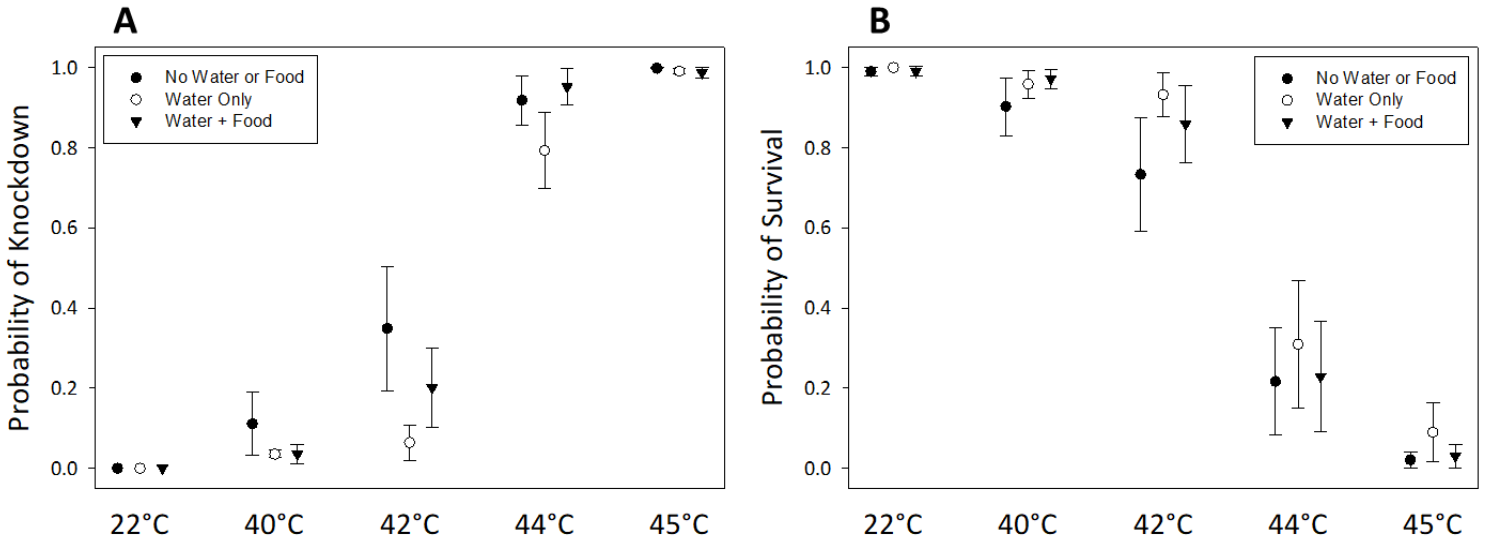

Figure 12. Probability (± 1 SD) of knockdown (A) and survival (B) of *C. rufifacies* exposed to different temperatures for different durations of time.

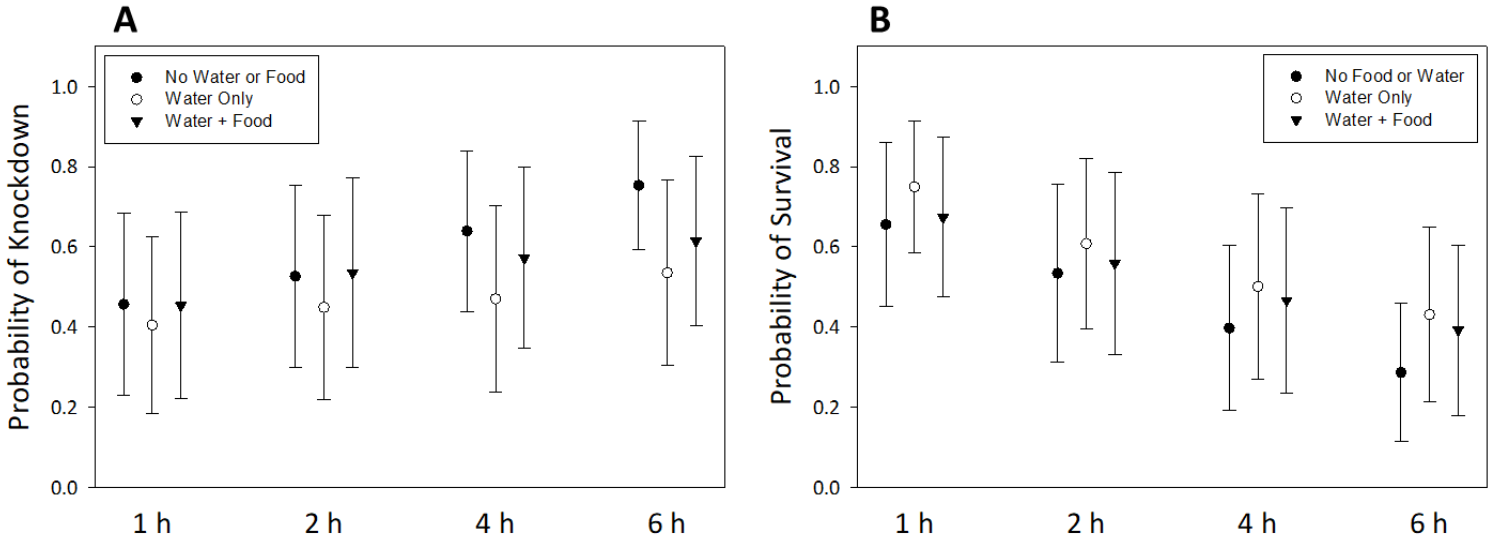

Figure 13. Probability (± 1 SD) of knockdown (A) and survival (B) of *C. rufifacies* exposed to different temperatures for different durations of time by sex.

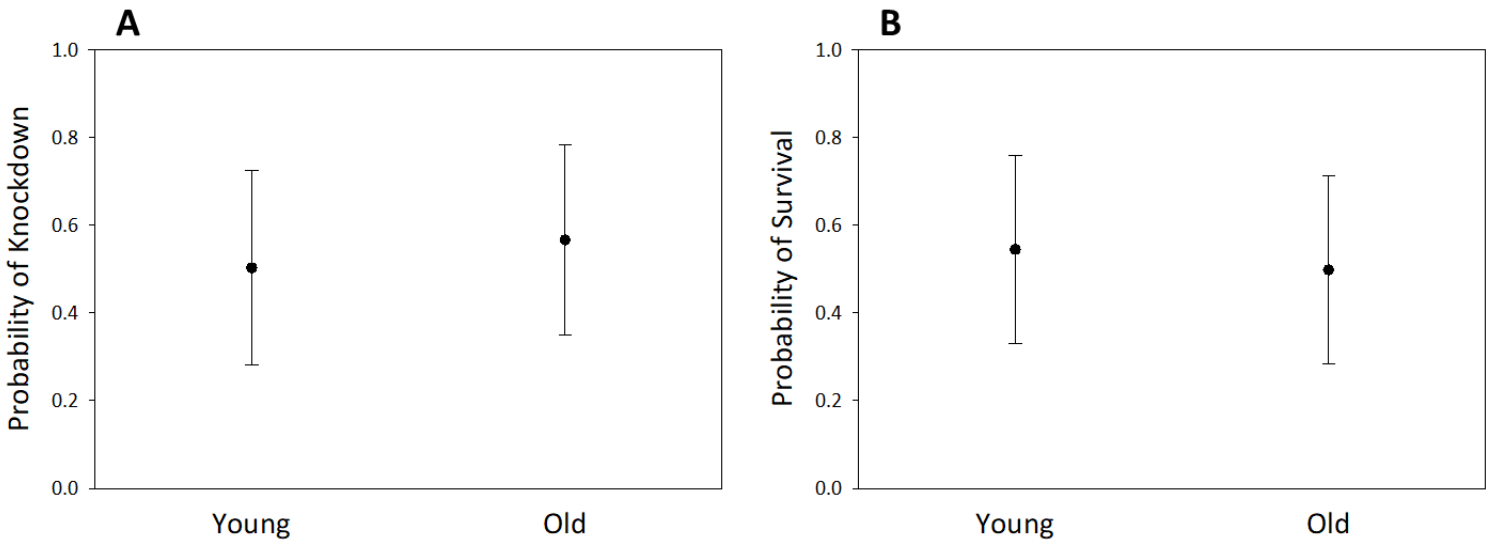

Figure 14. Probability (± 1 SD) of knockdown (A) and survival (B) of *C. rufifacies* exposed to different temperatures for different durations of time by age.

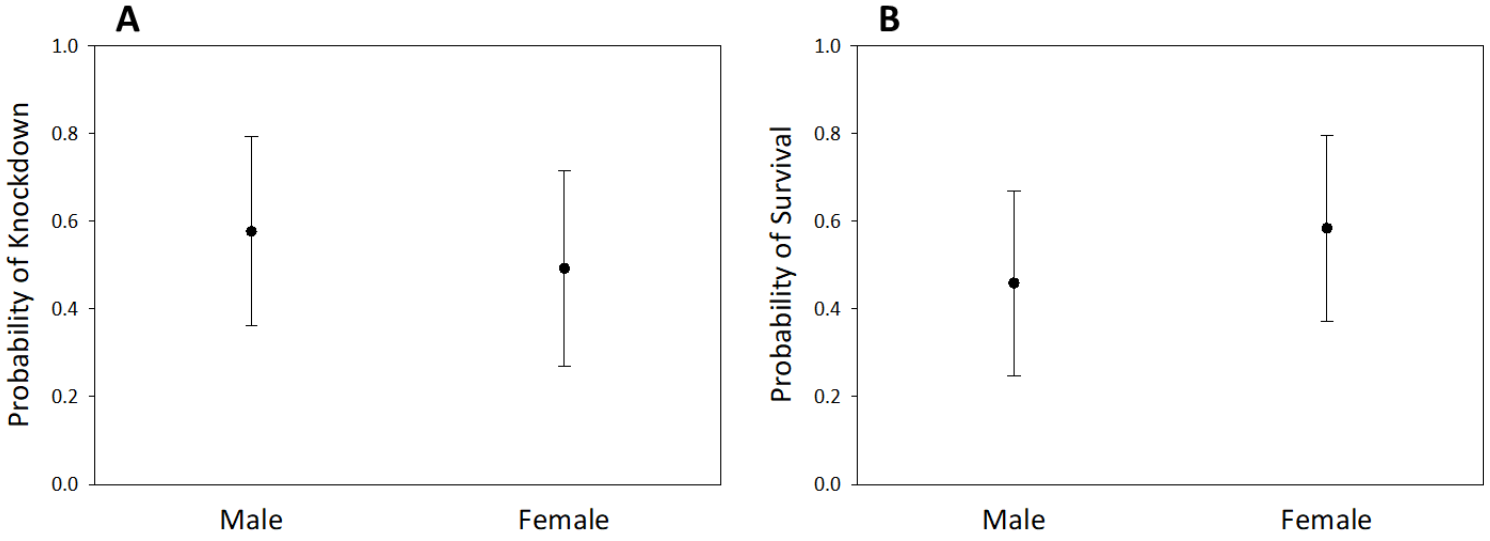

Figure 15. The probability of knockdown for immature *C. macellaria* exposed to different temperatures, durations, and food treatments. Red lines represent post feeding third instars, maroon lines represent feeding third instars, orange lines represent second instars, and yellow lines represent first instars.

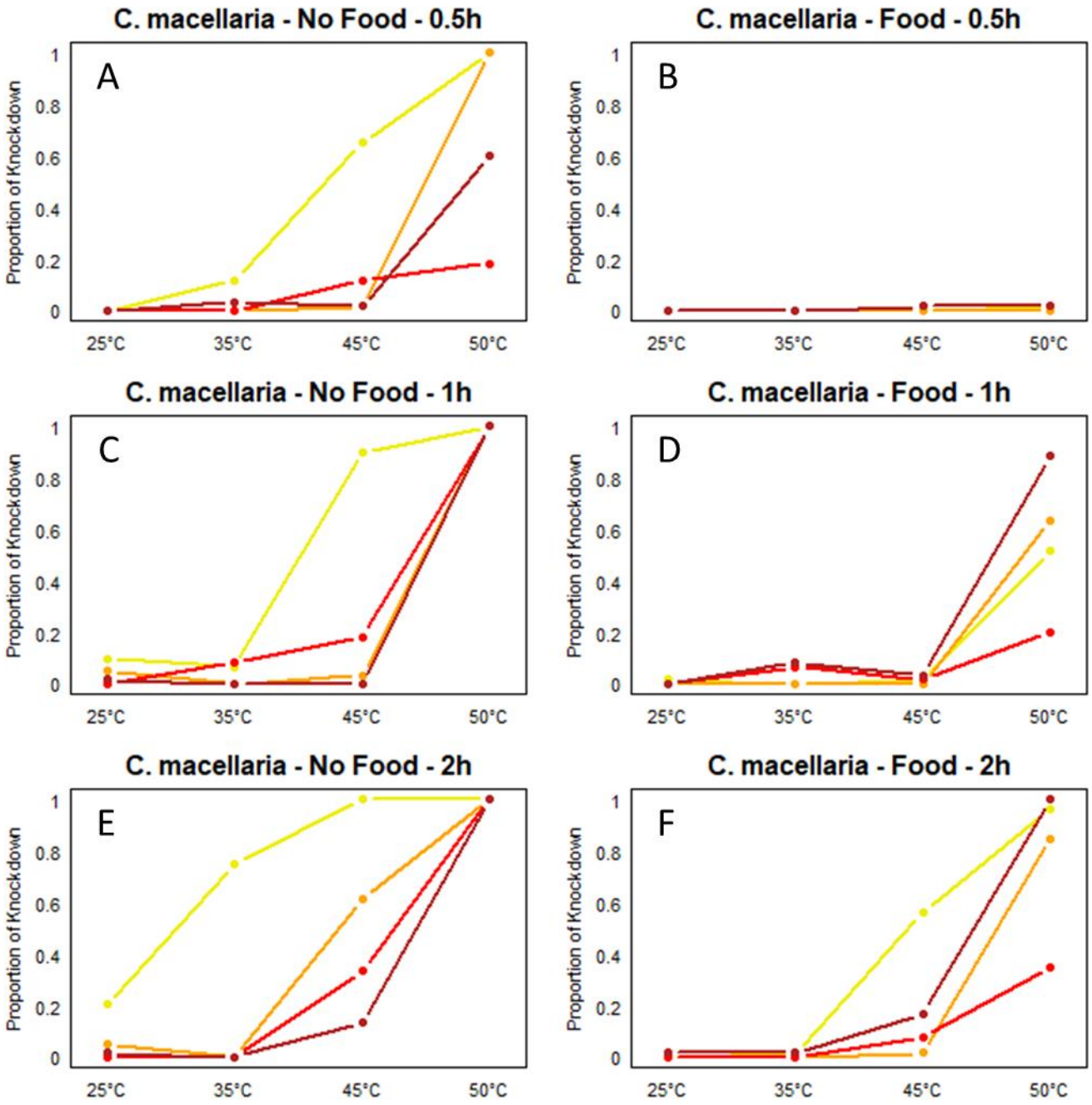

Figure 16. The probability of survival for immature *C. macellaria* exposed to different temperatures, durations, and food treatments. Red lines represent post feeding third instars, maroon lines represent feeding third instars, orange lines represent second instars, and yellow lines represent first instars.

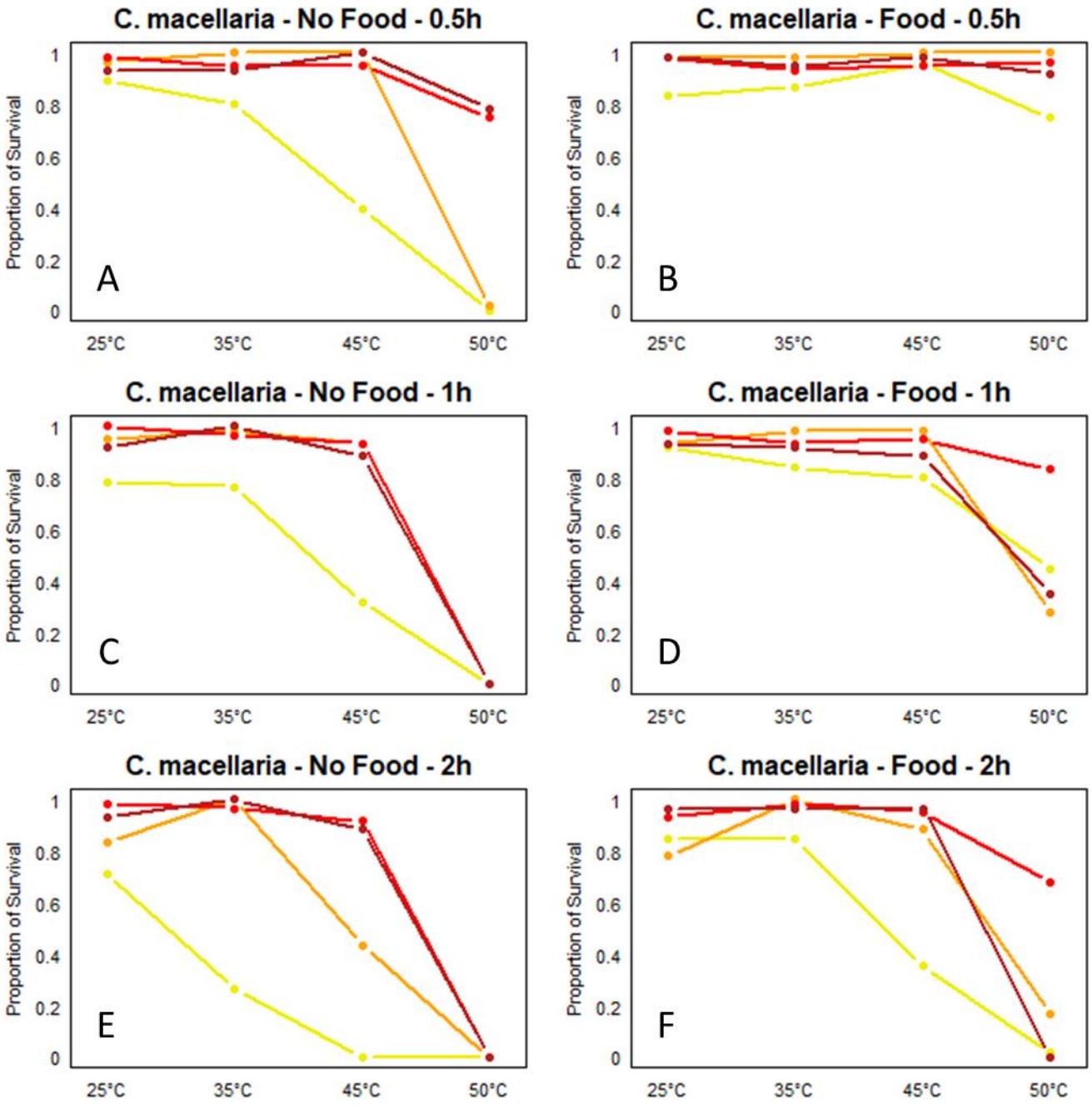

Figure 17. The probability of knockdown for immature *C. rufifacies* exposed to different temperatures, durations, and food treatments. Red lines represent post feeding third instars, maroon lines represent feeding third instars, orange lines represent second instars, and yellow lines represent first instars.

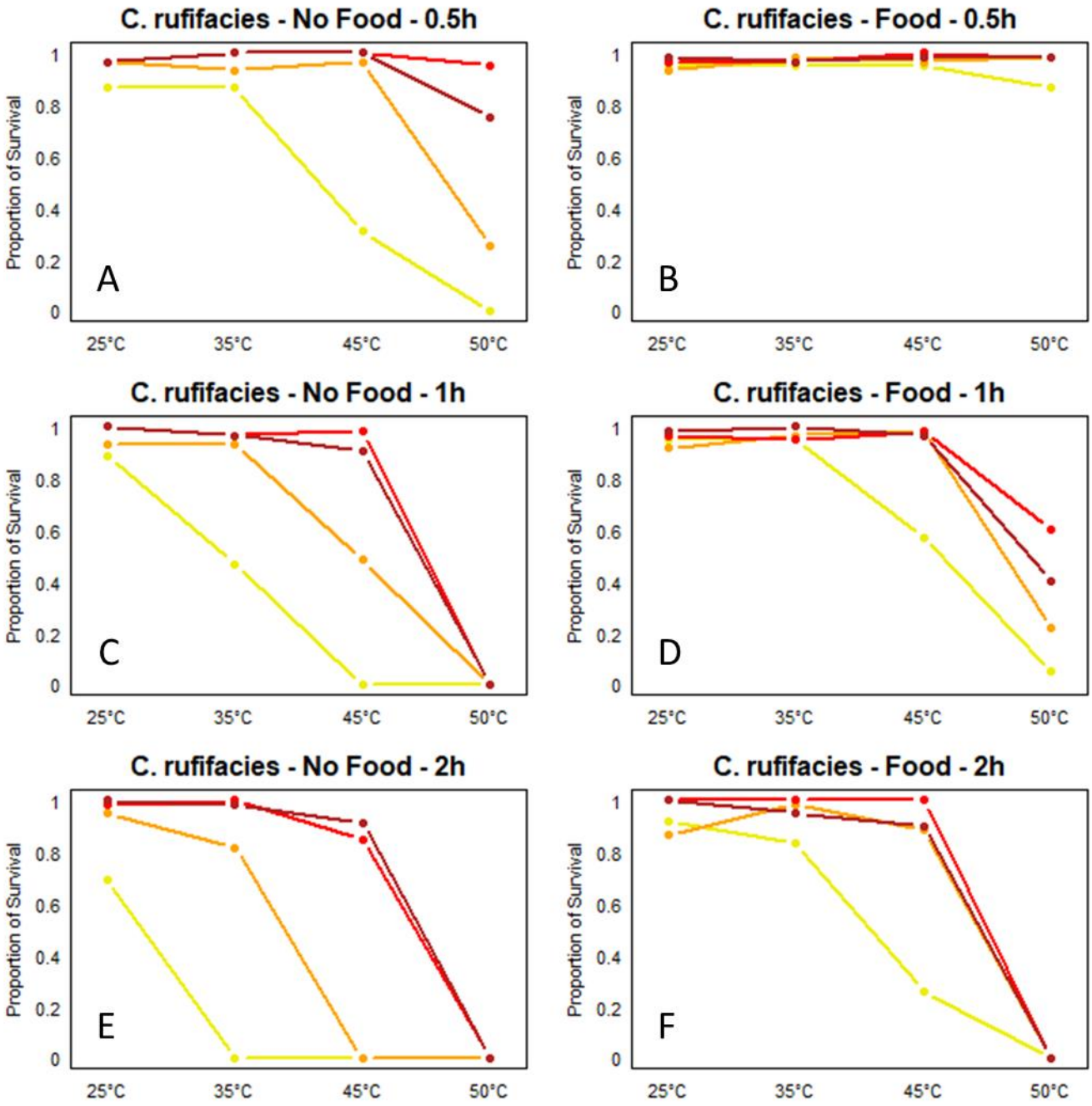

Figure 18. The probability of survival for immature *C. rufifacies* exposed to different temperatures, durations, and food treatments. Red lines represent post feeding third instars, maroon lines represent feeding third instars, orange lines represent second instars, and yellow lines represent first instars.

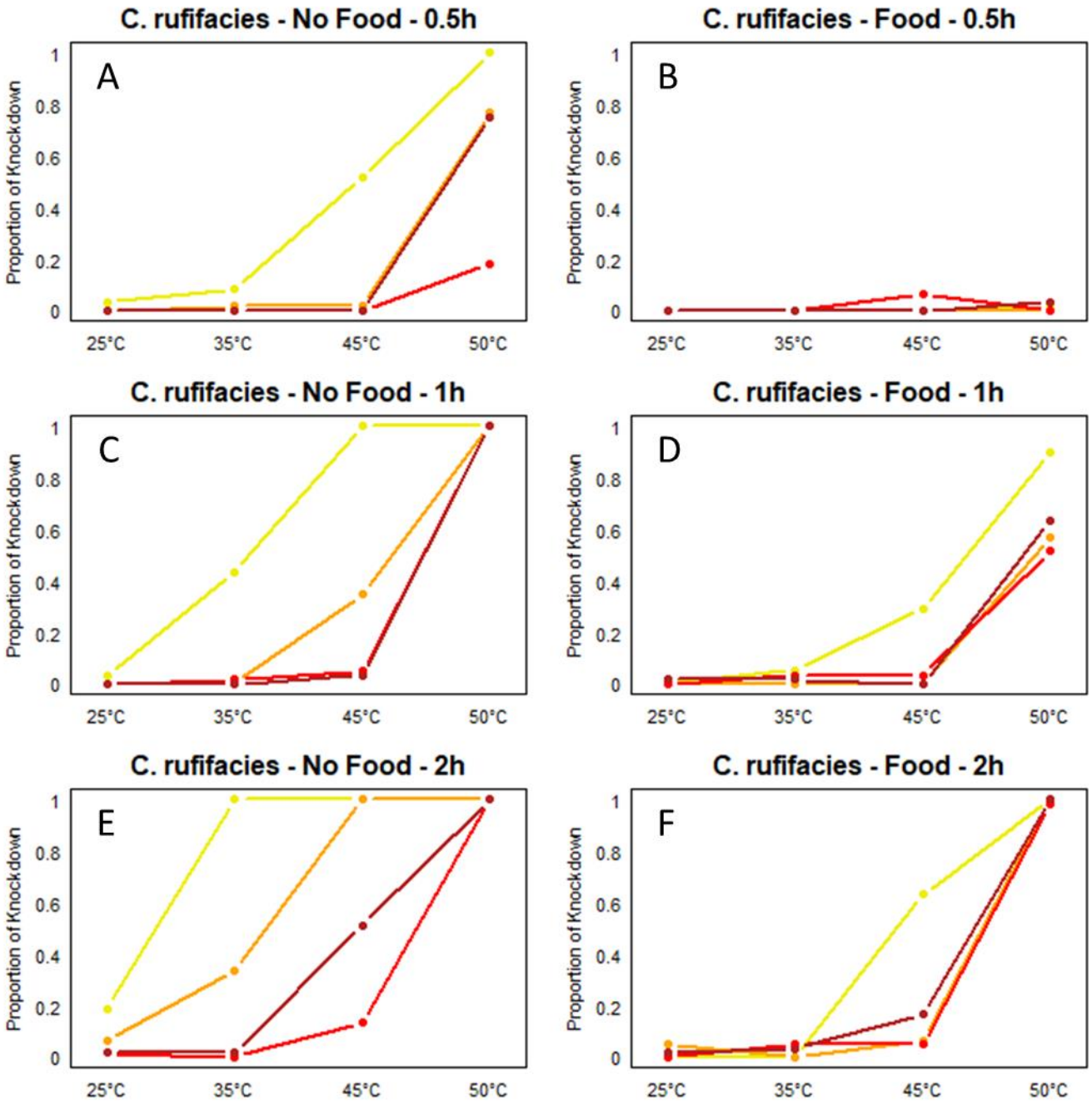

Figure 19. Days until first *C. rufifacies* oviposition event (y-axis) following a brief non-lethal heat shock (x-axis).

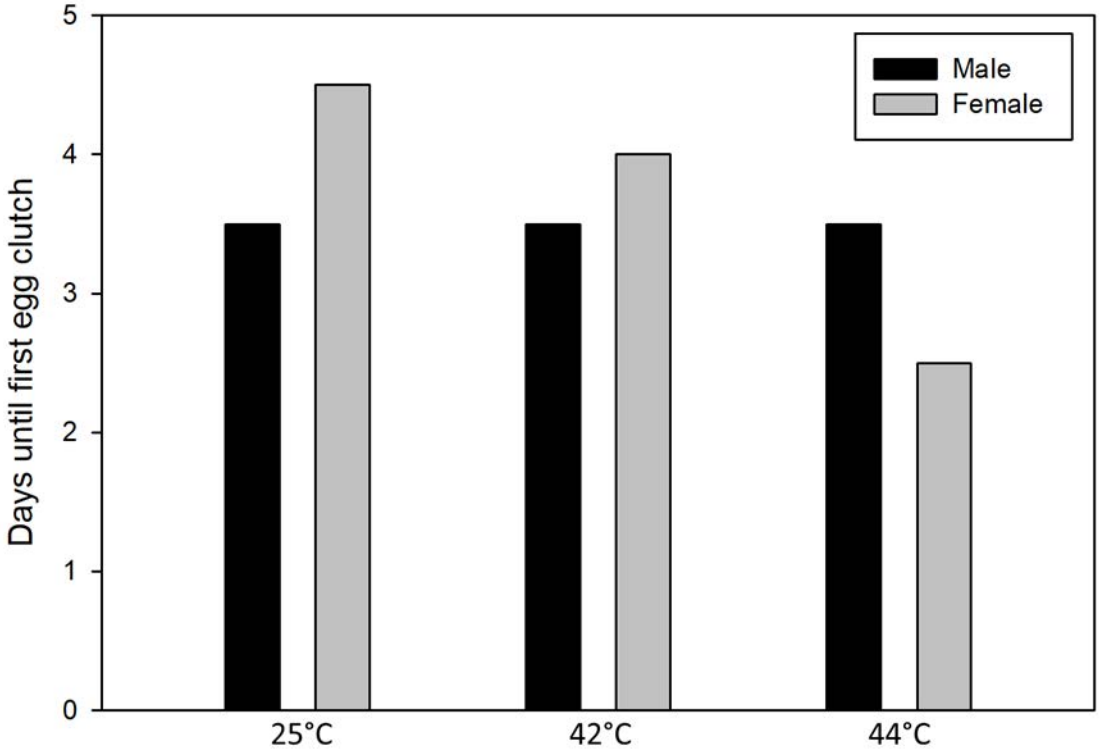

Figure 20. Egg laying frequency (y-axis) of *C. rufifacies* following a brief non-lethal heat shock (x-axis).

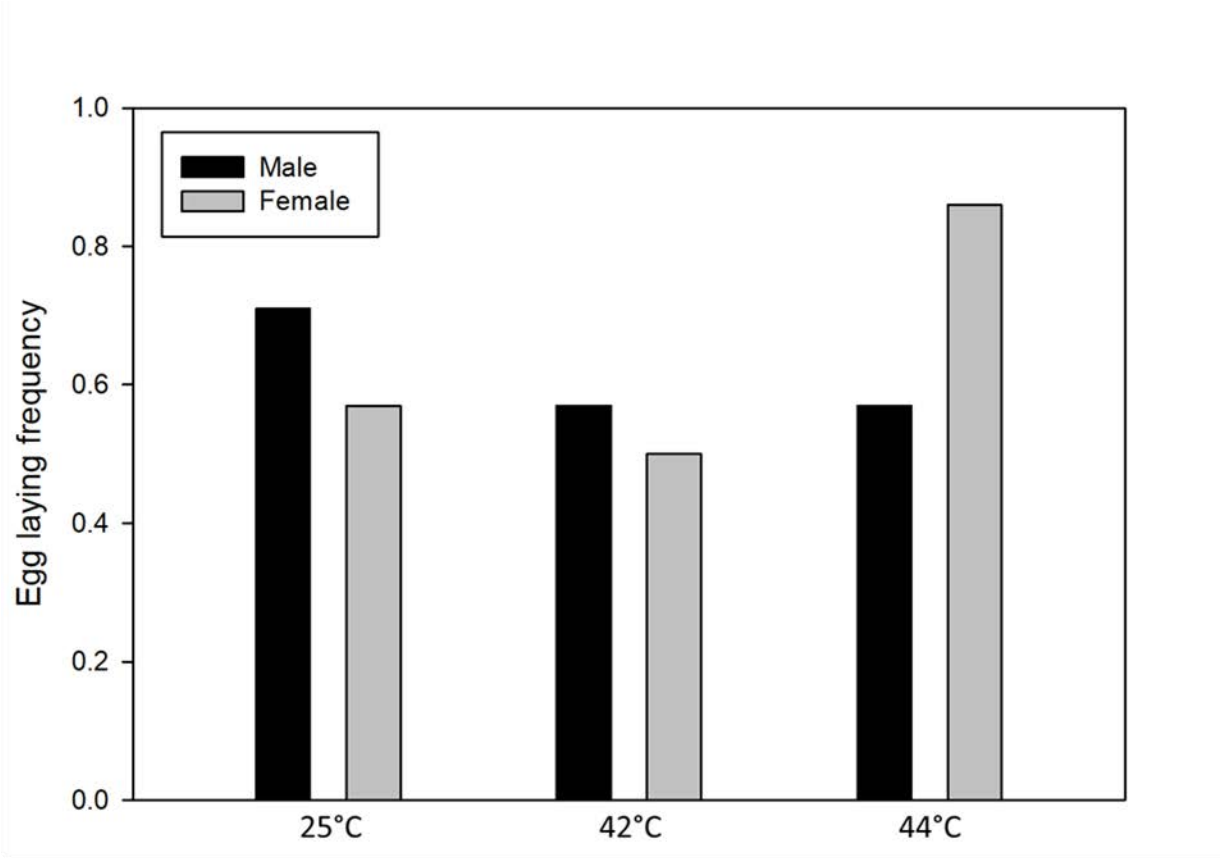

Figure 21. Total egg masses (y-axis) of *C. rufifacies* following a brief non-lethal heat shock (x-axis).

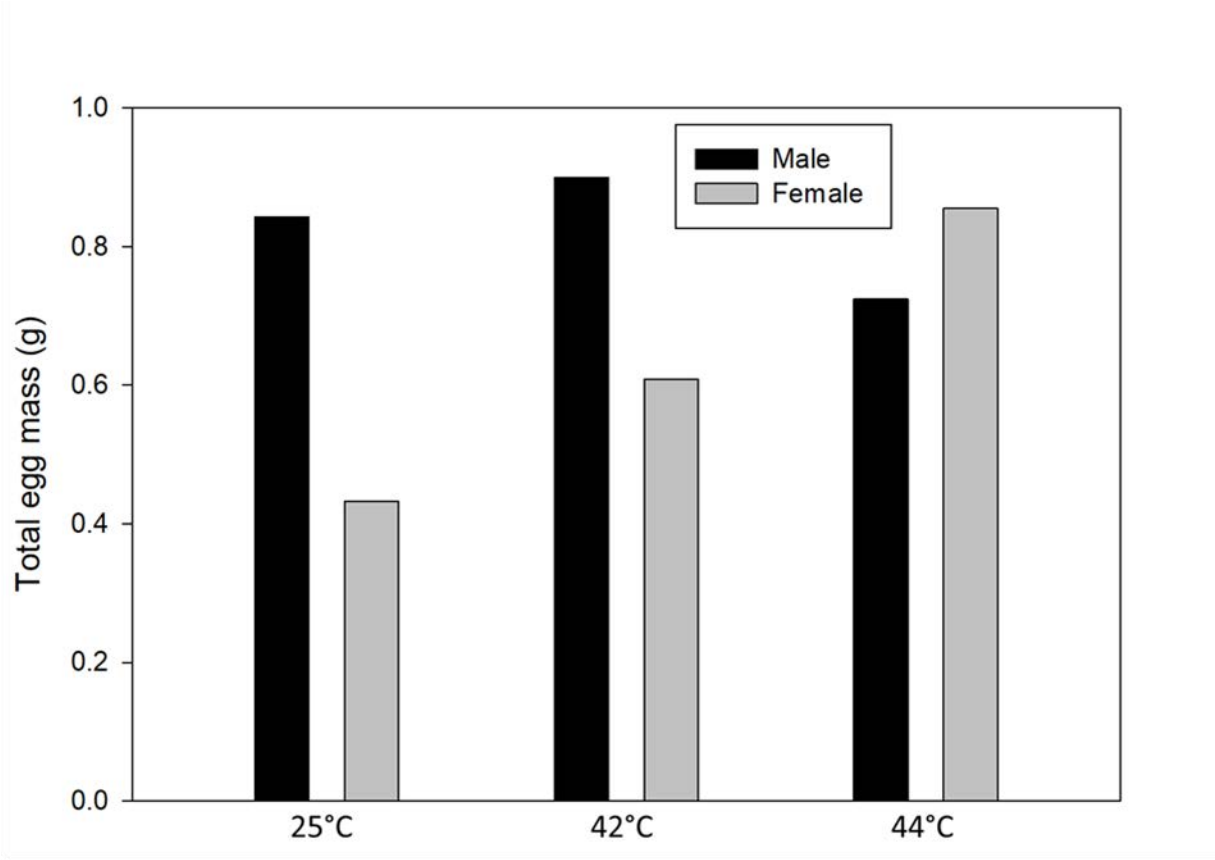

Figure 22. Mean (± 1 SD) oviposition thermal preference of *C. macellaria* and *C. rufifacies*.

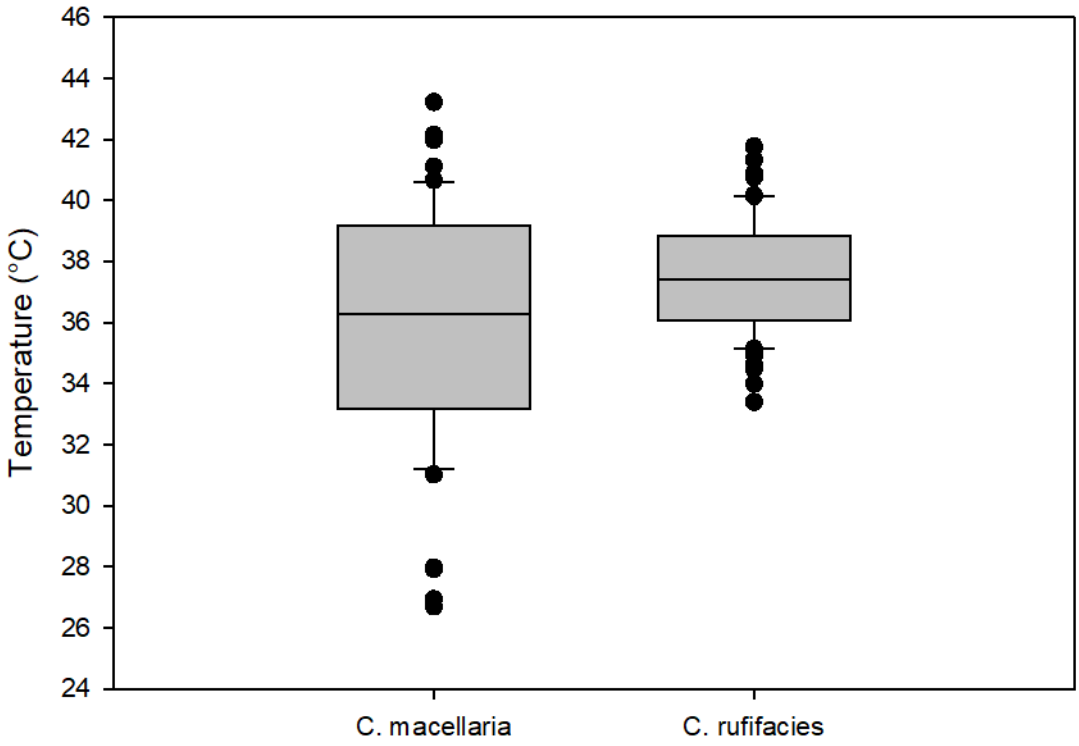

Figure 23. Mean (± 1 SD) preferred temperature of adult *C. macellaria* and *C. rufifacies*,

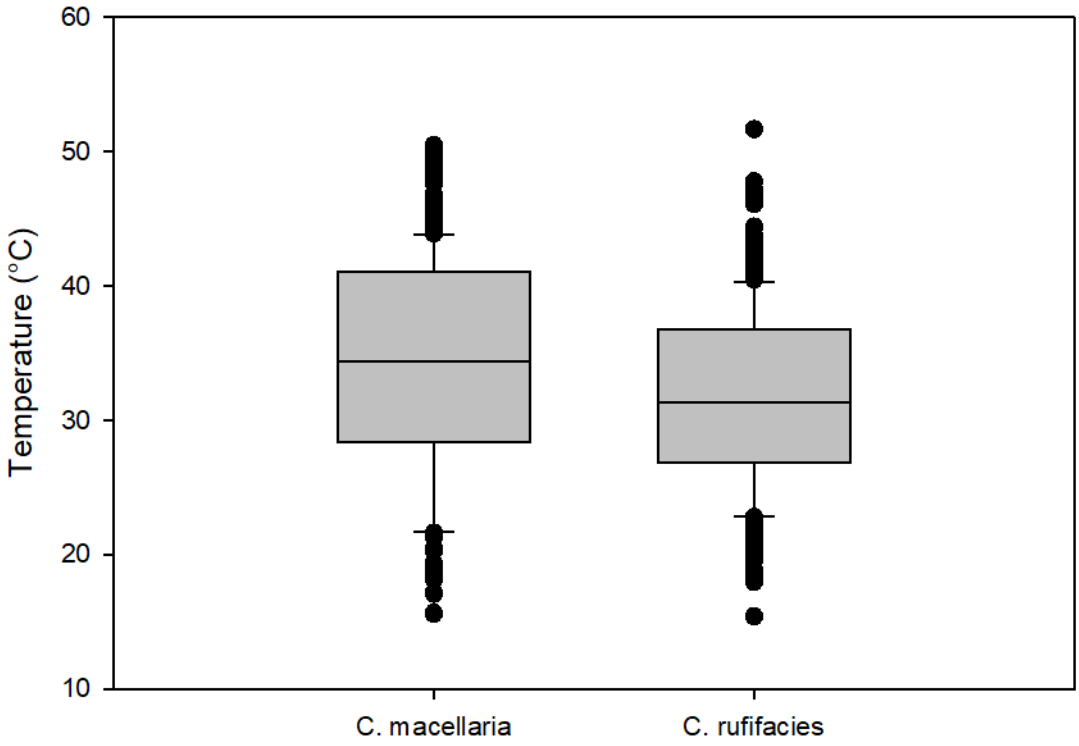

Figure 24. Mean (± 1 SD) egg duration for *C. macellaria* when exposed to one of three temperature treatments (x-axis) with either initial ramping up (i.e., warming) or ramping down (i.e., cooling).

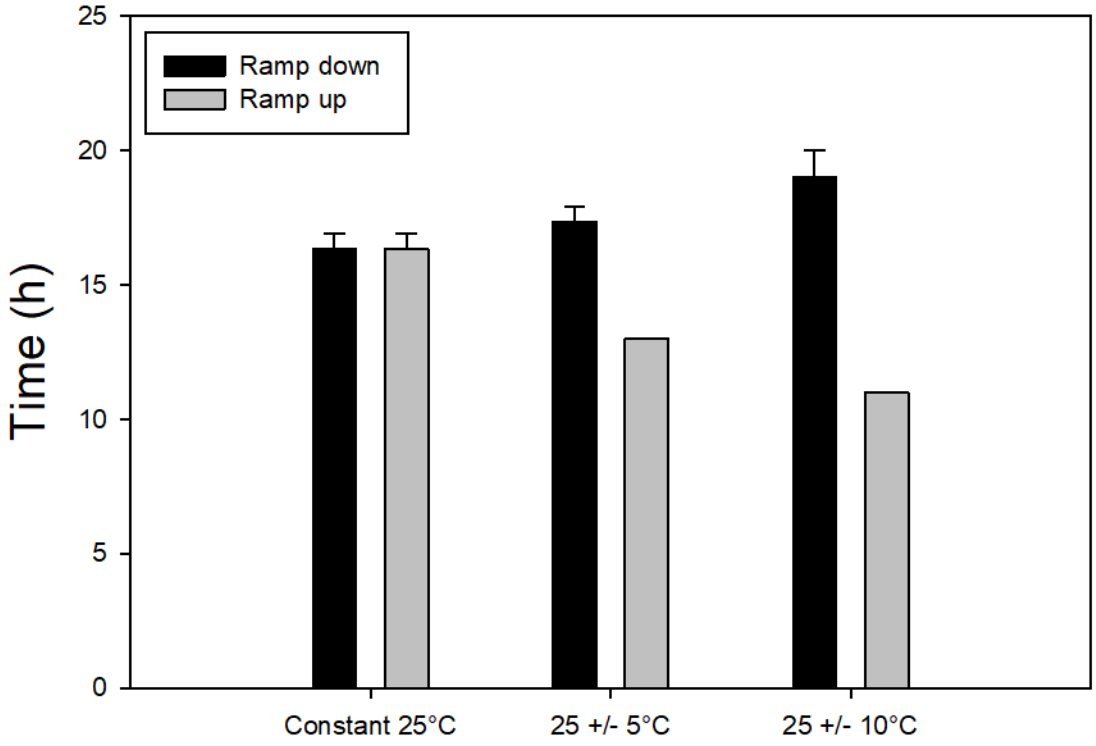

Figure 25. Mean (± 1 SD) larval duration of *C. macellaria* when exposed to one of three temperature treatments (x-axis) with either initial ramping up (i.e., warming) or ramping down (i.e., cooling).

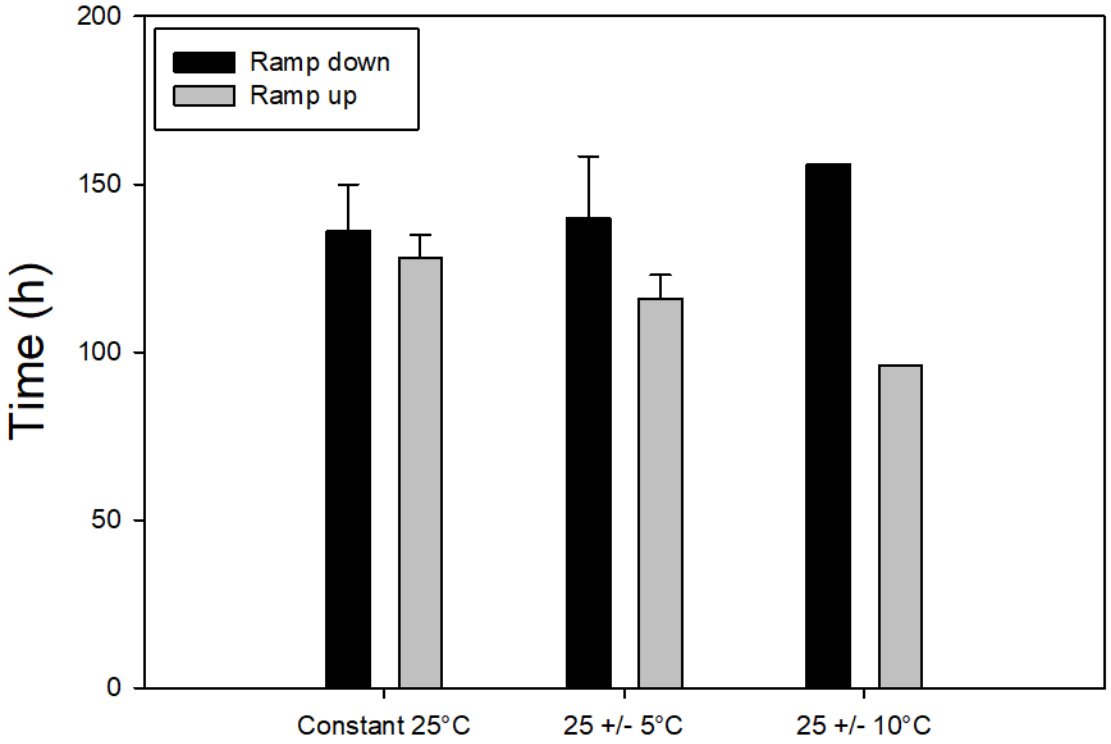

Figure 26. Mean (± 1 SD) pupal duration of *C. macellaria* when exposed to one of three temperature treatments (x-axis) with either initial ramping up (i.e., warming) or ramping down (i.e., cooling).

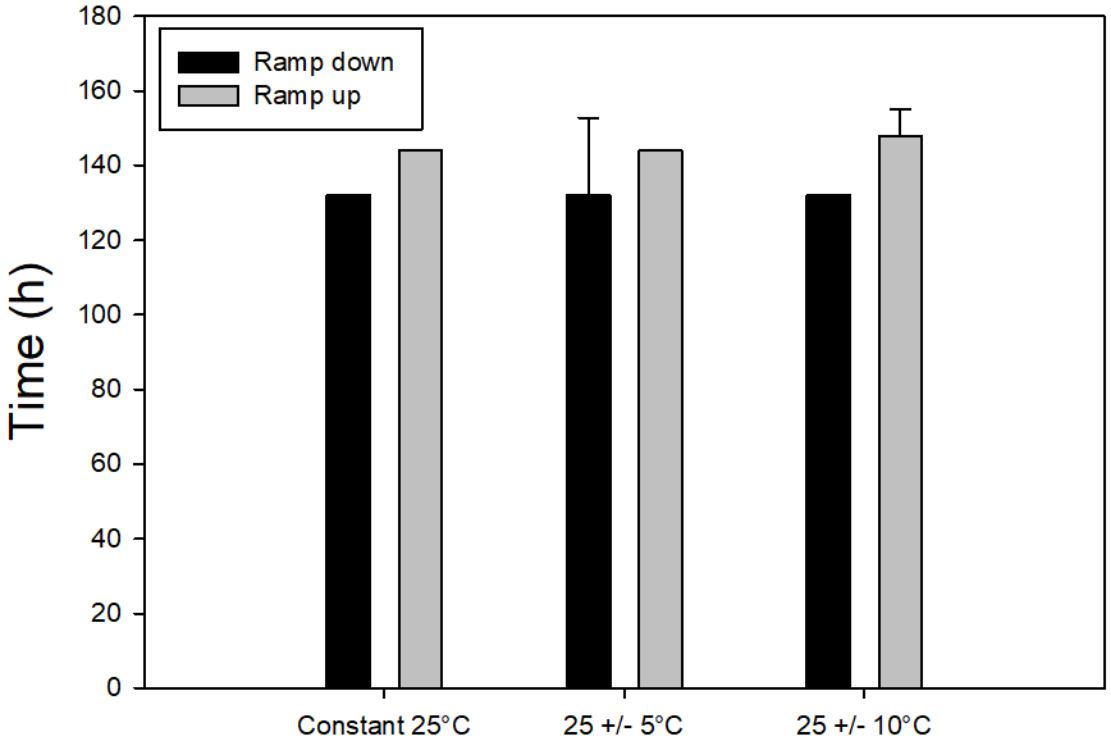

Figure 27. Mean (± 1 SD) total development time (i.e., time to emergence) of *C. macellaria* when exposed to one of three temperature treatments (x-axis) with either initial ramping up (i.e., warming) or ramping down (i.e., cooling).

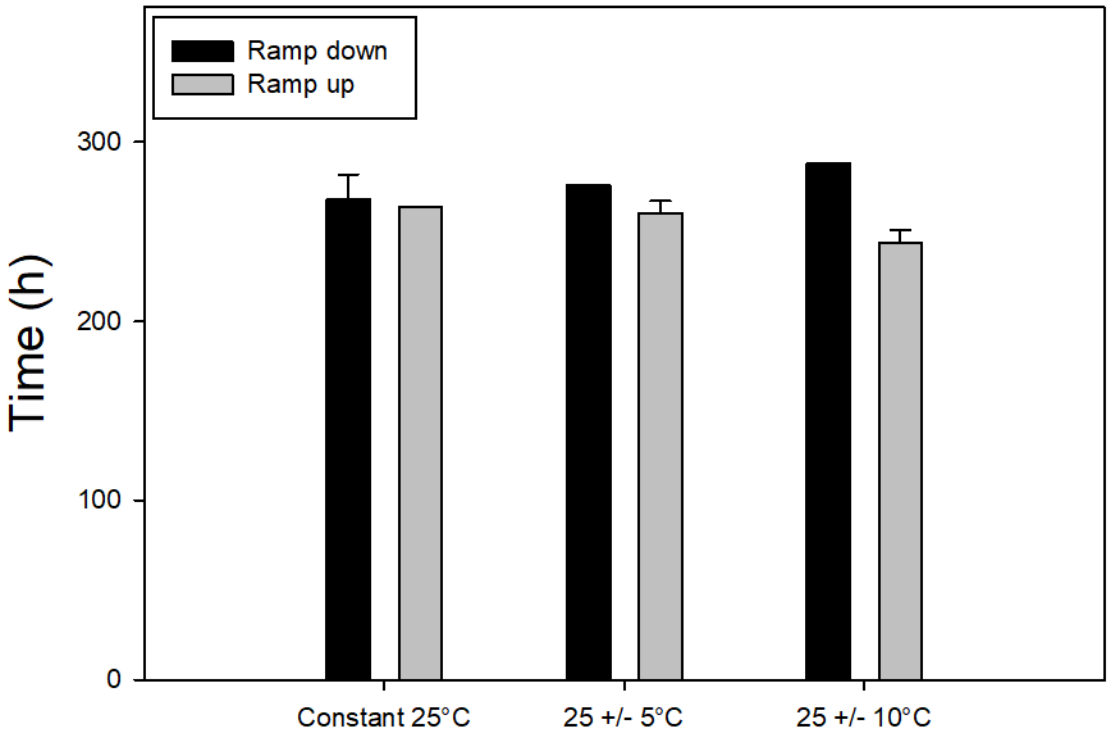

Figure 28. Mean (± 1 SD) preferred surface temperature for *C. macellaria* by age.

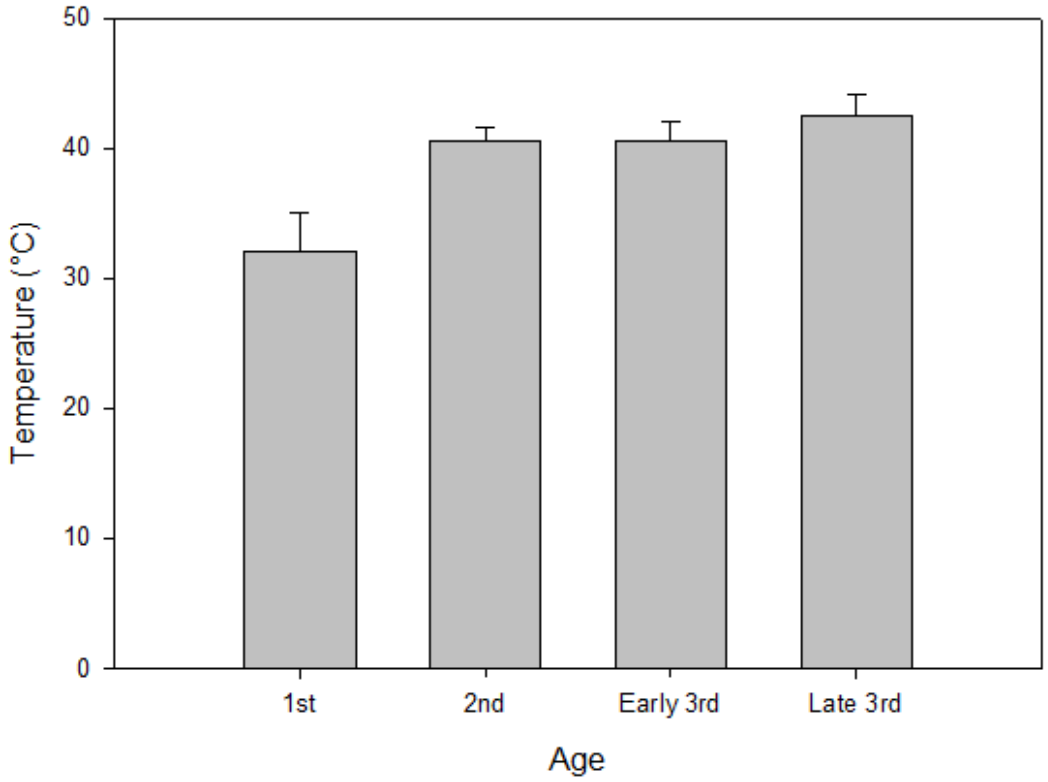

Figure 29. Mean (± 1 SD) preferred surface temperature for *C. rufifacies* by age.

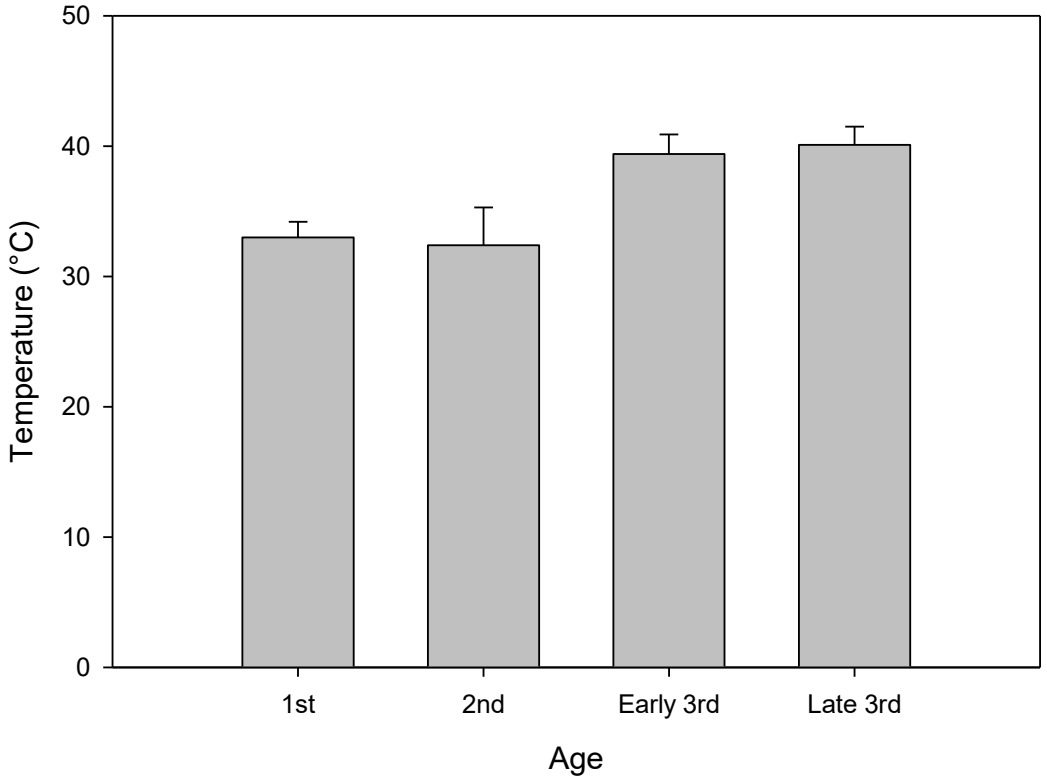

Table 1. Oviposition treatments and results by species.

	C. macellaria		*C. rufifacies*	
Temperature	**Oviposit**	**Viable Eggs**	**Oviposit**	**Viable Eggs**
10.0	No	NA	No	NA
12.5	No	NA	No	NA
15.0	Yes	Yes	No	NA
17.5	Yes	Yes	No	NA
20.0	Yes	Yes	No	NA
22.5	Yes	Yes	Yes	Yes
25.0	Yes	Yes	Yes	Yes
30.0	Yes	Yes	Yes	Yes
35.0	Yes	Yes	Yes	Yes
37.5	Yes	Yes	Yes	Yes
40.0	Yes	Yes	Yes	Yes
42.0	Yes	Yes	Yes	No
43.5	Yes	No	No	NA
44.5	Yes	No	No	NA
45.5	No	NA	No	NA