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The upper thermal tolerance of the secondary screwworm, *Cochliomyia macellaria* Fabricius (Diptera: Calliphoridae)

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ABSTRACT

Determining the thermal tolerance of an organism is important when assessing its activity time and survival rate in a given environment. However, thermal tolerance is not a static trait and may be influenced by a number of environmental and organismal factors. We report the results of three experiments investigating the effects of environmental temperature, exposure duration, age, sex, and nutrient availability on the upper thermal tolerance of the adult secondary screwworm, *Cochliomyia macellaria*. The probability of knockdown and survival was determined using a static method for different environmental temperatures (22, 40, 42, 44, or 45 °C), exposure durations (1, 2, 4, or 6 h), and nutrient availabilities (no food or water, water only, or both food and water) for both sexes and two age classes (young = 7–9 days post pupal emergence, old = 10–12 days post pupal emergence). In general, environmental temperature and exposure duration had the greatest effects on both the probability of knockdown and survival. As temperature or duration increased, the probability of knockdown increased while the probability of survival decreased. The availability of nutrients (water only or food and water) increased thermal tolerance at moderate temperatures (42 and 44 °C), but had no effect at 45 °C. Female flies were more thermally tolerant than males, regardless of nutrient availability. And age exhibited negligible effects on the probabilities of knockdown or survival, regardless of nutrient availability. These data show multiple environmental factors affected the thermal tolerance of *C. macellaria*. Thus, such aspects of basic thermal biology should feature more prominently in applied fields using blow flies, including but not limited to forensic entomology, disease ecology, and pollination ecology.

1. Introduction

Temperature affects the physiology, development, and behavior of all organisms (Angilletta, 2009; Cossins, 1987; Harrison et al., 2012). Therefore, determining thermal tolerance is an important first step in understanding how temperature limits the activity and survival of organisms (Huey and Stevenson, 1979; Pörtner, 2002; Sunday et al., 2011). Most ectotherms function over a broad range of temperatures described by either thermal reaction norms or thermal performance curves (Angilletta, 2009; DeWitt and Scheiner, 2004; Huey and Kingsolver, 1989; Stearns, 1989). Both measures peak at optimal temperatures (Angilletta et al., 2002; Bennett, 1990; Byrd and Butler, 1996) and are bound by critical temperatures (CTmin and CTmax) defining a thermal tolerance range (Angilletta, 2006; Huey and Kingsolver, 1989; Huey and Stevenson, 1979). Exposure to extreme temperatures outside a thermal tolerance range is potentially injurious and eventually lethal (Addo-Bediako et al., 2000; Huey et al., 1992; Lutterschmidt and Hutchison, 1997). And since environmental temperature varies both temporally and spatially, organisms typically experience some thermal extremes throughout their lifetime (Feder et al., 2000; Gibbs et al., 2003; Pörtner, 2002). Thus, the ability of an organism to remain active at or survive exposure to extreme temperatures has significant evolutionary fitness consequences (Huey and Bennett, 1990; Huey and Kingsolver, 1993; Loeschcke and Hoffmann, 2007).

However, thermal tolerance is not a static trait, but rather a function of both environmental and organismal factors. For instance, both the magnitude of temperature and the duration of exposure differentially affect an organism's thermal tolerance (Chown and Nicolson, 2004; Denlinger and Lee, 2010; Rezende et al., 2011; Terblanche et al., 2011). Chidawanyika and Terblanche (2011) found mortality increased in codling moths (*Cydia pomonella*) with either an increase in the severity of treatment temperature, or an increase in the duration of exposure at

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any given temperature. Similarly, factors such as age, sex, and metabolic state often affect thermal tolerance (Bowler and Terblanche, 2008; Gomez et al., 2009; Scharf et al., 2016). Pappas et al. (2007) found the upper thermal tolerance decreased with age in an insect (*Drosophila melanogaster*), while Xu and Ji (2006) found the upper thermal tolerance increased with age in a reptile (*Eremias brenchleyi*). Similarly, Folk et al. (2006) found male vinegar flies (*D. melanogaster*) tolerated warmer temperatures than female flies, while Winne and Keck (2005) found neonate female snakes (*Nerodia rhombifer*) tolerated higher temperatures than neonate males. Additionally, Nyamukondiwa and Terblanche (2009) found that fed fruit flies (*Ceratis capitata* and *Ceratitis rosa*) exhibited greater thermal tolerances (i.e., higher CTmax and lower CTmin) compared to fasted flies, and Claussen (1977) found hydrated salamanders (*Ambystoma tigrinum* and *Ambystoma jeffersonianum*) displayed greater heat resistance than dehydrated salamanders, indicating that food and water may help buffer the effects of extreme temperatures. All cases presented demonstrate variability across species in terms of thermal tolerance. Therefore, it is critical to consider and report potential confounding factors when measuring and comparing the thermal tolerances of organisms.

Because thermal inertia decreases with mass, small ectotherms (e.g., insects) quickly equilibrate with their microenvironment (Kaspari et al., 2015; Porter and Gates, 1969; Stevenson, 1985). This phenomenon makes small ectotherms particularly vulnerable to rapid temperature changes (Seebacher et al., 1999; Stevenson, 1985), constraining activity in both time and space (Adolph and Porter, 1993; Gunderson and Leal, 2015; Willmer, 1983). Therefore, defining organismal thermal tolerances are not only important from a basic research perspective, but are also important from an applied research perspective. For example, adult blow flies (Diptera: Calliphoridae) are small (~1 cm in length) ectothermic insects that typically colonize decomposing flesh (Byrd and Castner, 2010). Therefore, they are regularly used in death investigations to estimate forensically important timelines such as time of colonization (Amendt et al., 2007; Catts and Goff, 1992; Greenberg, 1991), which can be inferred as a minimum postmortem interval (i.e., time since death) given certain assumptions (Anderson, 2001; George et al., 2013; Ody et al., 2017; Tarone and Sanford, 2017; Tomberlin et al., 2011a, 2011b). Furthermore, the colonization of decomposing flesh causes blow flies to actively transport potential pathogens leading to the spread of diseases (Basson et al., 2018; Greenberg, 1965; Olsen, 1998). And while blow flies are not typically recognized as active pollinators, there is an entire syndrome of plants evolved specifically to lure carrion-feeding insects through the mimicry of various carrion-like traits (e.g., smell of rotting flesh) for the purposes of pollination (Jürgens and Shuttleworth, 2016; Urru et al., 2011; Vereecken and McNeil, 2010). However, exposure to temperatures above or below their thermal tolerance range decreases blow fly locomotor function (Nicholson, 1934; Vogt, 1988; Williams, 2003). Thus, knowing blow fly thermal tolerance is important across at least three fields as these flies provide several ecosystem services. Specifically, the loss of locomotion prevents blow flies from; 1) colonizing bodies, which is important in forensic entomology, 2) spreading pathogens from contact with contaminated substrates (i.e., fecal matter or decomposing bodies), which is important to disease ecology, and 3) pollinating plants, which is important to pollination ecology.

Thermal tolerance is typically determined using one of two methods (Lutterschmidt and Hutchison, 1997). The *static* method exposes an organism to a constant temperature for a given duration and measures specific traits such as knockdown (i.e., the time at which an organism loses locomotor function and can no longer remain upright when exposed to a given temperature; Folk et al., 2006; Huey et al., 1992; Gilchrist and Huey, 1999) or survival (Brett, 1944; Fry et al., 1942; Klockmann et al., 2017). Alternatively, the *dynamic* method involves ramping the environmental temperature (up or down) until an endpoint (e.g., knockdown, CTmax, or death) is reached (Cowles and Bogert, 1944; Folk et al., 2006; García-Robledo et al., 2016). In this study, we used the *static* method to quantify the effects of age, sex, and nutrient availability on the upper thermal tolerance of a common blow fly (*Cochliomyia macellaria*) in the southern USA. We exposed adult flies to different temperatures for varying durations with or without nutrients (i.e., no food or water, water only, or both food and water) and quantified the probability of knockdown and survival. Because *C. macellaria* have relatively low thermal inertia and rapidly equilibrate to environmental temperatures (Kaspari et al., 2015; Stavenga et al., 1993; Stevenson, 1985), we predicted the probability of knockdown to increase and the probability of survival to decrease as treatment temperature or duration increased. Additionally, we predicted the availability of nutrients (i.e., food or water) would improve thermal tolerance by decreasing knockdown and increasing survival.

2. Methods

2.1. Species collection, identification, and colony maintenance

From May–July, 2017 adult *C. macellaria* (> 500 individuals) were collected from decomposing animal remains in College Station, TX, USA to initiate a laboratory colony. After capture, flies were placed in a 30 x 30 x 30 cm Bugdorm mesh cage (BioQuip, Rancho Dominguez, CA) and returned to the Texas A&M University Forensic Laboratory for the Investigation of Entomological Science (FLIES Facility). Specimens were identified prior to experiments using morphological features described by Whitworth (2010), and voucher specimens can be found in the Texas A&M University Insect Collection (voucher #736). Cages containing flies were held in a temperature-controlled room (~22 °C, 50% relative humidity, and a photoperiod of 14:10 [L:D] h). Adult flies were provided water from a reverse osmosis system in 200 ml glass mason jars with paper towel wicks, and fed a 50:50 diet of table sugar and milk powder *ad libitum*. Additionally, 4 days after pupal emergence adult flies were provided ~5 ml beef cattle blood every other day for 8 days (n = 4 blood meals) as an additional protein source. After the fourth blood meal, ~20 g beef liver in a 90 ml plastic cup was offered to the flies in each cage as an oviposition site. Liver was checked twice daily and eggs deposited were used in the subsequent experiments. Eggs and beef liver were transferred to a glass mason jar (79 × 178 mm; 946 ml, Ball Inc., Daleville, IN, USA; ~250 eggs per jar) filled half full with vermiculite (Sungro Agriculture, Agawam, MA, USA) and capped with a breathable cloth lid (WypAll, Kimberly-Clark Inc, Roswell, GA, USA). These jars were then held on a shelf in the room previously described. Larvae were monitored daily and fed additional beef liver as needed until they pupated. Four jars containing pupae were placed in each Bugdorm (n = 8) until ~200 adults emerged (colony consisted of ~1600 flies with ~200 flies per cage). This procedure was repeated for each generation. Additionally, wild flies were periodically captured and identified with methods previously described and added to the laboratory colony to minimize laboratory acclimation and maintain genetic diversity. Each time wildtype flies were added, the laboratory colony was set back to generation zero and the laboratory colony never exceeded 10 generations without additions of wildtype flies. All flies used in the experiments were between F_4-F_6 generations.

2.2. Experiment design and treatments

The upper thermal tolerance of adult blow flies (*C. macellaria*, n = 1920) was assessed over the course of three experiments using two metrics, knockdown and survival. Knockdown was defined as the inability to effectively locomote and cling to an inclined surface (Folk et al., 2006; Gilchrist and Huey, 1999), and was recorded immediately following treatments. Survival was defined as active flies (e.g., walking, mating, feeding, or flying) that responded to stimuli such as gentle tapping or pushing (Chidawanyika and Terblanche, 2011) and was recorded 24 h after treatments. Each experiment followed the same static heating methods, but differed in nutrient availability (no food or water,

water only, or both food and water) and temperature exposure (22, 40, 42, 44, or 45 ± 2 °C). The water only treatment consisted of ~1 g of presoaked water storage crystals (Root Naturally, Denver, Colorado, USA), while the food and water combination treatment consisted ~1 g of water storage crystals saturated with a blended beef liver and water mix (1:1). Treatment temperatures and durations were determined from an initial pilot study exposing flies to upper temperatures regularly observed on carcasses during summer months in College Station, TX (36, 38, 40, 42, 44, and 46 °C; Rusch et al., unpublished data). When exposed to either 36 or 38 °C for 6 h, less than 5% of flies were knocked down and thus were excluded from the study as the flies tolerated these temperatures as easily as they did room temperature (22 °C). Similarly, no flies resisted knockdown or survived 1 h of exposure to 46 °C. Thus 46 °C was also excluded from the study as it fell outside the thermal tolerance range. For all experiments, individual flies of known ages (7–12 days post pupal emergence) and sex were placed into plastic 1.5 mL microcentrifuge tubes (Thermo Fisher Scientific, Waltham, MA, USA) with a breathable cap. Microcentrifuge tubes containing flies were then randomly placed into an already warmed analog block heater (VWR, Irving, TX, USA) for a given duration (1, 2, 4, or 6 h) and temperature (22, 40, 42, 44, or 45 ± 2 °C). This resulted in 30 flies per each temperature-duration treatment in experiments 1 and 2, and 20 flies per each temperature-duration-nutrient treatment in experiment 3. Each experiment (1–3) was replicated three times.

2.3. Experiments conducted

Experiment 1 exposed each fly to one temperature-time treatment (22, 40, 42, 44, or 45 ± 2 °C for 1, 2, 4, or 6 h) and provided no nutrients within the tubes. Experiment 2 exposed flies to the same temperature-time treatment as in experiment 1, but provided a food and water combo treatment within all tubes. After observing an apparent buffering effect (i.e., reduced knockdown and increased survival) at more extreme temperatures (42 and 44 °C) when visually comparing experiments 1 and 2 (Figs. 1–2), experiment 3 was conducted on a subsample of temperature-time treatments (42, 44, or 45 ± 2 °C for 1, 2, 4, or 6 h) where all three nutrient treatments (no food or water, water only, or both food and water) were supplied to assess; 1) interactive effects of temperature treatment and nutrient availability, and 2) if water only could explain the buffering effect. Temperatures of each heat block (n = 4) were recorded using a four-channel thermometer with type K thermocouples (Ametek Arizona Instrument LLC, Chandler, AZ, USA). These thermal couples were validated using two NIST certified thermometers before each treatment (Thermo Fisher Scientific, Waltham, MA, USA). Immediately following treatments, the microcentrifuge tubes containing the flies were removed from the heat block and the number of knocked down flies was counted. Flies from the same treatment were then transferred to glass mason jars (79 × 178 mm; 946 ml, Ball Inc., Daleville, IN, USA) where they were kept at room temperature (~22 °C) and provided sugar and water (n = 5 flies per jar). After 24 h of recovery the number of living flies was counted to determine survival.

To ensure that environmental temperatures inside the microcentrifuge tubes were consistent across treatments, we conducted a follow-up experiment where we placed two thermal couples inside each tube, one inside the nutrient media and one in the air above the nutrient media, and placed the tubes inside two heating blocks set to 42 °C for 6 h, with temperature readings recorded every hour. The tubes containing no food or water still contained two thermocouples, one at the bottom and one near the top. All thermocouples were inserted through the air hole in the lid of the tubes. One set of tubes contained adult *C. macellaria* and the second set of tubes contained no flies. A seventh treatment consisted of a probe in water in the center of the heat block (i.e., how temperature was recorded during experiments described above). The greatest within tube difference was 1.2 °C with the treatment being an empty tube (i.e., no food or water) containing a fly. Probes inside food/water had mixed results of either being warmer or colder than the air above them in the tube regardless if the tube contained a fly or not. We conducted an generalized linear model to test if the mean temperature across treatment, probe, or time was significantly different and found no significant differences ($P > 0.05$) for any treatment, probe, or time point (seetable S13 for statistical output). The maximum difference between any two probes inside a single tube was 1.1 °C and probes above or in the food varied as to whether they were warmer or colder. Thus we are confident that the food did not create a heat sink and create a cooler (or warmer) environment for the flies.

2.4. Statistical analyses

Because the three experiments were conducted at different times, each data set was analyzed separately. For experiments 1 and 2, the effects of age, sex, temperature, and duration were modeled on the probabilities of knockdown and survival. For experiment 3, the effects of age, sex, temperature, duration, and nutrition were modeled on the probabilities of knockdown and survival. All analyses modeled these

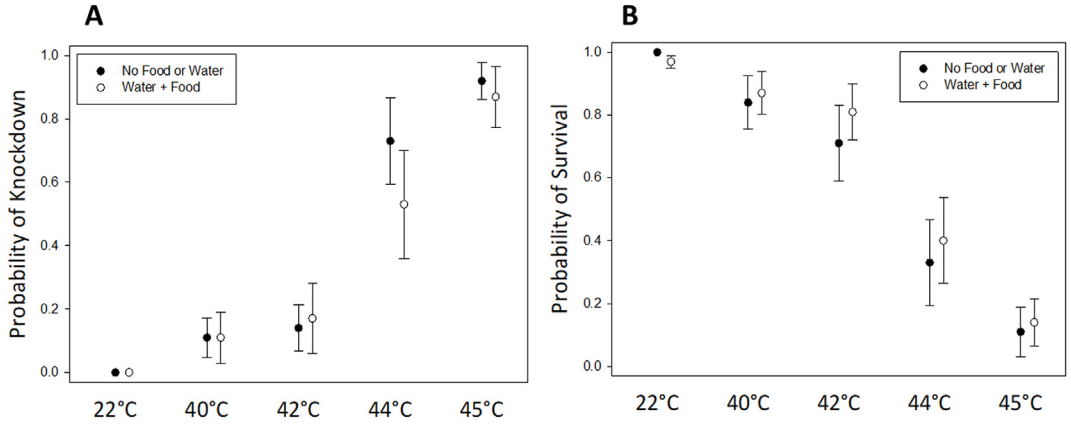

Fig. 1. Combined results of experiments 1 and 2. The probability of knockdown increased with increasing temperatures (A) while the probability of survival decreased with increasing temperatures (B), regardless of nutrient availability. Filled and open circles depict estimated mean probabilities of knockdown and survival while error bars depict the standard deviation of the given temperature and nutrient treatment. Means and standard deviations were computed by multimodel averaging for experiments 1 (filled circles = no food or water) and 2 (open circles = water and food). Experiments 1 and 2 were not statistically compared as they were conducted as independent experiments with different treatments. However, the plots were combined to visually show the buffering effects of adding food and water during experiment #2, which inspired experiment #3 that contained three different nutrient treatments (see Figs. 4–6).

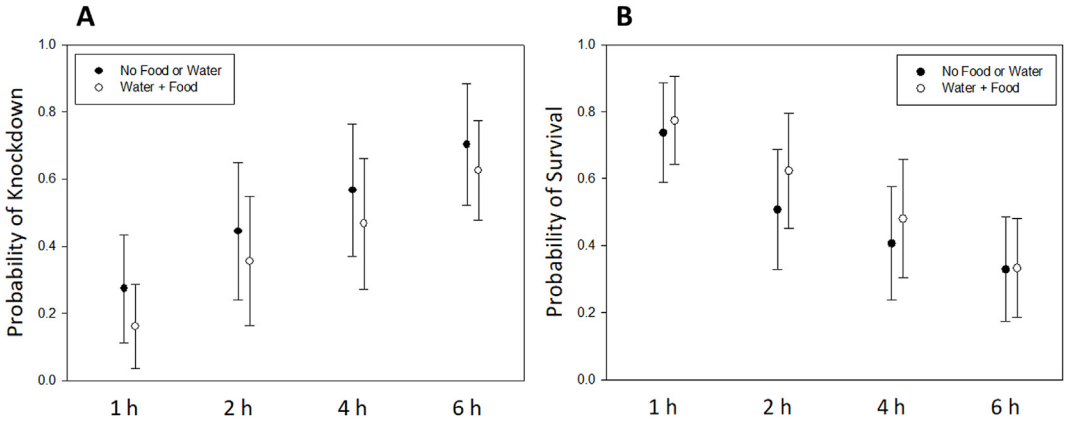

Fig. 2. Combined results of experiments 1 and 2.The probability of knockdown increased with increasing exposure duration (A) while the probability of survival decreased with increasing exposure duration (B), regardless of nutrient availability. Filled and open circles depict estimated mean probabilities of knockdown and survival while error bars depict the standard deviation of the given duration and nutrient treatment. Means and standard deviations were computed by multimodel averaging for experiments 1 (filled circles = no food or water) and 2 (open circles = water and food). Experiments 1 and 2 were not statistically compared as they were conducted as independent experiments with different treatments. However, the plots were combined to visually show the buffering effects of adding food and water during experiment #2, which inspired experiment #3 that contained three different nutrient treatments (see Figs. 4–6).

factors as fixed factors and modeled trial as a random intercept. Age was binned into two classes (young = 7–9 days post pupal emergence, and old = 10–12 days post pupal emergence) to increase statistical power.

For both dependent variables (i.e., knockdown and survival), multimodel inference was used to determine the most likely probabilities of knockdown and survival. First, Akaike Information Criterion were used to determine the most likely random component of the model (Zuur et al., 2009). Then, all possible models of fixed effects were fit and the Akaike weight of each model was calculated using the *MuMIn* library (Bartoń, 2013) of the R Statistical Software (R-Core-Team, 2015). The *lme4* library was used (Bates et al., 2015) when modeling all dependent variables because of its ability to capture binomial error. Once the Akaike weight of each model (see Supplementary Materials; Tables S1–S6) was determined, the weighted average value of each parameter was calculated (see Supplementary Materials; Tables S7–S12). The resulting values of parameters were used to compute the most likely probability of each dependent variable (i.e., knockdown or survival) for each treatment. This approach eliminates the need to solely rely on *P* values and standard errors, because all models (including the null model) contributed to the most likely value of each probability.

3. Results

In experiments 1 and 2, treatment temperature, exposure duration, and sex strongly influenced the probabilities of knockdown and survival, regardless of nutrient availability (Tables S7–10). Conversely, fly age had minimal effects and only affected the probability of survival in experiment 1 when no food or water was available (Fig. 1B, Table S8). Specifically, as treatment temperatures increased, the probability of knockdown increased (from 0% at 20 °C to 92% and 87% at 45 °C for flies without and with nutrients) (Fig. 1A). Conversely, as treatment temperature increased, the probability of survival decreased (from 100% to 97% at 20 °C to 11% and 14% at 45 °C for flies without and with nutrients) (Fig. 1B). Similarly, as exposure duration increased, the probability of knockdown increased (from 27% to 16% after 1 h to 70% and 63% after 6 h for flies without and with nutrients) (Fig. 2A), while the probability of survival decreased (from 74% to 77% after 1 h to 33% after 6 h for flies without and with nutrients) (Fig. 2B). Furthermore, in experiments 1 and 2, male flies had a greater probability of knockdown (59% and 48% vs 41% and 36%) and a lower probability of survival (40% and 50% vs 59% and 61%) compared to female flies, regardless of nutrient availability (Fig. 3). Although fly age had minimal effects on the probability of knockdown in either experiment 1 or 2 (see Supplementary Materials; Fig. S1A), older flies had a lower probability of survival (42%) compared to younger flies (57%) in experiment 1 when nutrients were not available (see Supplementary Materials; Fig. S1B).

Experiment 3 yielded similar trends to those of experiments 1 and 2 with treatment temperature, exposure duration, and sex strongly influencing the probabilities of knockdown and survival. Nutrient availability also strongly influenced the probabilities of knockdown and survival. Age had minimal effect on the probabilities of knockdown and survival. And though the top models included an interactive effect between temperature exposure and nutrient availability, the effect sizes were small compared to main effects (see Supplementary Materials; Tables S11 and S12). Specifically, as treatment temperatures increased, the probability of knockdown increased (from 22%, 10%, and 11% at 42 °C to 96%, 97%, and 98% at 45 °C for flies provided no food or water, water only, or both food and water) (Fig. 4A). Conversely, as treatment temperatures increased, the probability of survival decreased (from 78%, 90%, and 88% at 42 °C to 5%, 11%, and 8% at 45 °C, for flies provided no food or water, water only, or both food and water) (Fig. 4B). Thus, the addition of either water only or food and water improved the thermal tolerance of flies at 42 °C and 44 °C, but had minimal effects at 45 °C (Fig. 4). Similarly, as exposure duration increased, the probability of knockdown increased (from 38% after 1 h to 80% after 6 h) (Fig. 5A), while the probability of survival decreased (from 70% after 1 h to 23% after 6 h) (Fig. 5B). Male flies had a greater probability of knockdown (65% vs 55%) and a lower probability of survival (37% vs 49%) compared to female flies, regardless of nutrient availability (Fig. 6). And age had minimal effects on either knockdown or survival, regardless of nutrient availability (see Supplementary Materials; Fig. S2).

4. Discussion

Consistent with our theoretical perspective (Andrew et al., 2013; Chidawanyika and Terblanche, 2011; Tewksbury et al., 2008), environmental temperature and exposure duration had the strongest effects on knockdown and survival of *C. macellaria* adults (Tables S7–S12), with warmer temperatures and longer durations resulting in greater probabilities of knockdown and mortality (Figs. 1, 2, 4 and 5). Such temperature impairment relationships are well documented and explained by several potential factors; desiccation stress, impairment of evaporative cooling, denaturation of proteins, disruption of membrane structure and function, depletion of energy stores, or insufficient

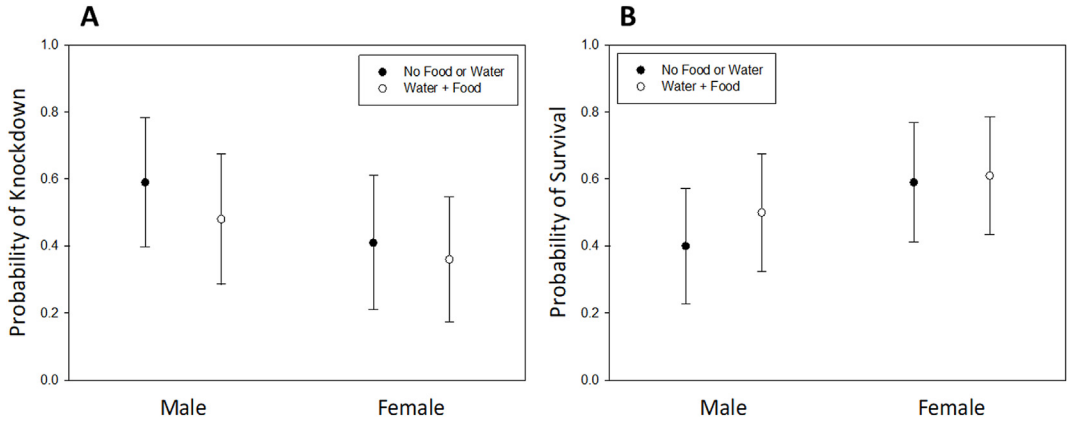

Fig. 3. Combined results of experiments 1 and 2. Male flies had a greater probability of knockdown (A) and a lower probability of survival (B) compared to female flies, regardless of nutrient treatment. Filled and open circles depict estimated mean probabilities of knockdown and survival while error bars depict the standard deviation of the given sex. Means and standard deviations were computed by multimodel averaging for experiments 1 (filled circles = no food or water) and 2 (open circles = water and food). Experiments 1 and 2 were not statistically compared as they were conducted as independent experiments with different treatments. However, the plots were combined to visually show the buffering effects of adding food and water during experiment #2, which inspired experiment #3 that contained three different nutrient treatments (see Figs. 4–6).

oxygen delivery (Chown and Terblanche, 2006; Klepsatel et al., 2016; Klose and Robertson, 2004; Pörtner, 2001; Verberk et al., 2016). All of these factors disrupt homeostasis, which reduces locomotor functions (i.e., potential knockdown) and leads to death if the temperature exposure is severe enough. Although the specific mechanisms limiting heat tolerance remains unknown for *C. macellaria*, the upper thermal tolerance was reached between 44 and 45 °C when exposed for at least 1 h, as more than half of the flies exposed to these treatments were knocked down and died (Figs. 1 and 4).

Thermal tolerance often depends on the biological state of the organism (Hoffmann et al., 2013; Nyamukondiwa and Terblanche, 2009; Wahid et al., 2007). Because blow flies are small bodied organisms, they are susceptible to desiccation stress and energy depletion when exposed to elevated temperatures for extended periods of time, especially in the absence of food or water. Providing *C. macellaria* either water only or food and water enhanced thermal tolerance at moderately extreme temperatures of 42 and 44 °C, revealing a buffering effect of nutrient availability on temperature exposures (Figs. 1 and 4), which possibly alleviated desiccation stress and energy depletion over shorter durations (Fig. 2). However, providing nutrients had no buffering effects at 45 °C, suggesting exposure to 45 °C may have overridden any desiccation stress or energy depletion resistance due to nutrient availability. Alternatively, exposure to 45 °C may have caused irreversible cellular damage that no amount of nutrient availability could compensate, as all organisms have thermal limits (Angilletta, 2009; Lutterschmidt and Hutchison, 1997). Nevertheless, our results coincide with the general literature indicating most organisms exhibit a greater heat tolerance when provided food or water resources up to a certain point (Andersen et al., 2010; Chidawanyika et al., 2017; Hoffmann et al., 2013; Mitchell et al., 2017; Nyamukondiwa and Terblanche, 2009). Future work is needed to identify the specific mechanisms of *C. macellaria*'s upper (and lower) thermal tolerance.

The upper thermal tolerance also differed by sex, with female *C. macellaria* having a greater heat tolerance than males, regardless of nutrient availability (Figs. 3 and 6). One possible explanation is female *C. macellaria* tend to be larger than males, and consequently have a greater volume to surface area ratio which reduces water loss (Chown, 2002; Harrison et al., 2012). Furthermore, a larger body may contain more energy reserves (e.g., lipids, glycogen, and water) that can be allocated to knockdown resistance (Arrese and Soulages, 2010; Canavoso et al., 2001; Lease and Wolf, 2011). As for males, a brief exposure to elevated temperatures can reduce reproductive output

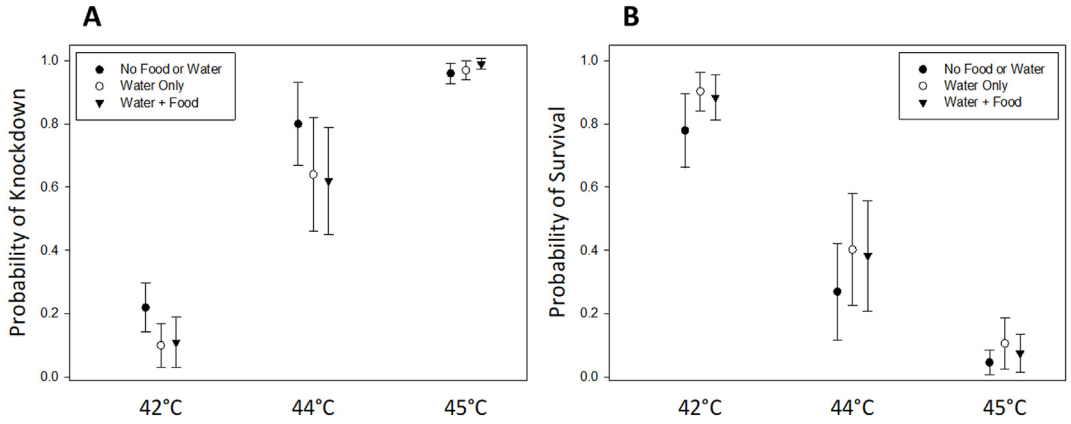

Fig. 4. Results of experiment 3. The probability of knockdown increased with increasing temperatures (A) while the probability of survival decreased with increasing temperatures (B), regardless of nutrient availability. However, both water only and water + food treatments reduced the probability of knockdown and increased the probability of survival at 42 and 44 °C compared to the no food or water treatments, while the availability of nutrients at more extreme temperatures of 45 °C had no effects on knockdown or survival. Filled circles, open circles, and filled triangles depict estimated mean probabilities of knockdown and survival while error bars depict the standard deviation of the given temperature and nutrient treatment. Means and standard deviations were computed by multimodel averaging. Filled circles = no food or water, open circles = water only, and filled triangles = water and food treatment.

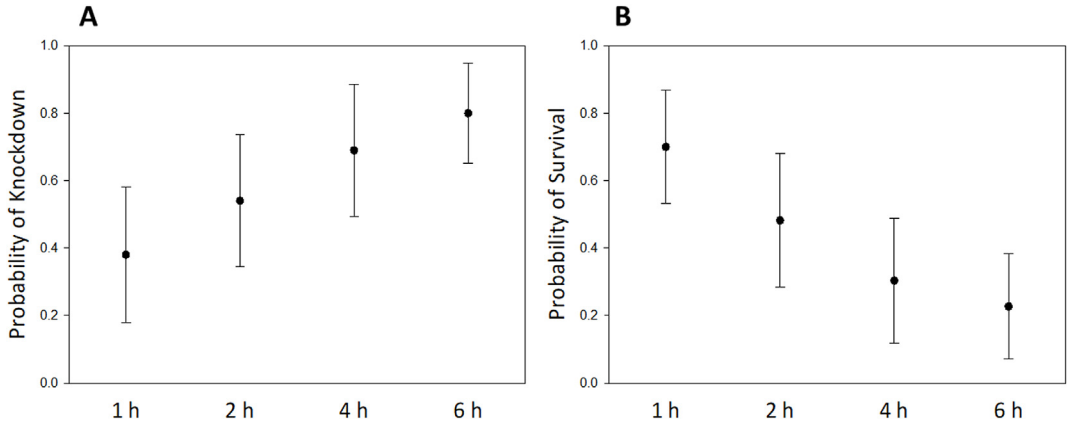

Fig. 5. Results of experiment 3. The probability of knockdown increased with increasing exposure duration (A) while the probability of survival decreased with increasing exposure duration (B). Filled circles depict estimated mean probabilities of knockdown and survival while error bars depict the standard deviation of the given duration treatment computed by multimodel averaging.

through sperm damage (Rinehart et al., 2000; Rukke et al., 2015; Sugai and Ashoush, 1968). Therefore, at the population level male *C. macellaria* may be the limiting sex as reproduction begins to be interrupted when temperatures approach the male thermal tolerance. However, sperm storage by females prior to exposure to elevated temperatures and reversible sterility by males following exposure to elevated temperatures is possible (David et al., 2005; Smith et al., 1988). Conversely, age had minimal effects on the upper thermal tolerance of *C. macellaria*. Theory predicts older and younger flies to be most sensitive to temperature extremes as they tend to be less resilient to and contain fewer energy reserves for combating exposure to environmental extremes (Bowler and Terblanche, 2008; Nyamukondiwa and Terblanche, 2009). However, blow flies can live 25 + days (Holmes, 2017), and we only tested blow flies aged 7–12 days (post pupal emergence). Thus, it is possible that our age range was too narrow to see a measurable response. It is worth noting that the age ranges in this study reflect the age break that defines reproductively immature and mature individuals in that colony.

Knowing the thermal tolerances of blow flies is not only important from a basic science perspective, but also has value in the applied sciences. For instance, knowledge of blow fly upper thermal tolerances could aid forensic entomologists in predicting blow fly colonization performance, which could potentially explain rare cases where no insect colonization occurs. For example, Wells (2019) documented a homicide investigation in Las Vegas, NV, USA where a dead body was found with no insect activity or colonization. It was determined by several forensic entomologists involved in the case (pers. comm.) 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 is 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 and surrounded by brick walls, which gets much warmer than air temperature during this time of year (Myint et al., 2015). Thus, if the temperature of the materials surrounding the body, or the temperature of the body itself, was above the thermal tolerance of carrion-feeding insects, an alternative explanation for the lack of insects present is simply that it was too warm for them to be active on or around the body. For instance, Ody et al. (2017) determined oviposition performance by *Calliphora vicina* was inhibited at only 35 °C and greatly reduced in both *Calliphora vomitoria* and *Lucilia sericata* at 40 °C. Therefore, the upper thermal tolerance of blow flies ought to be considered in forensic entomology casework in warm and arid regions, and forensic entomologists should record body and surface temperatures during death investigations (Byrd and Castner, 2010).

Similarly, the interaction between blow flies and decomposing material (discussed above) also has ramifications for disease ecology. More specifically, blow flies are known to serve as mechanical vectors of a number of pathogens of human and other vertebrate species (Tomberlin et al., 2017). In fact, blow flies have deposited these pathogens on surfaces in which they have contact (Zurek and Ghosh, 2014). Given the activity of these flies is largely limited by temperature, pathogens have the potential to spread into new locations as climates change and become more appropriate for blow flies. Similarly, an

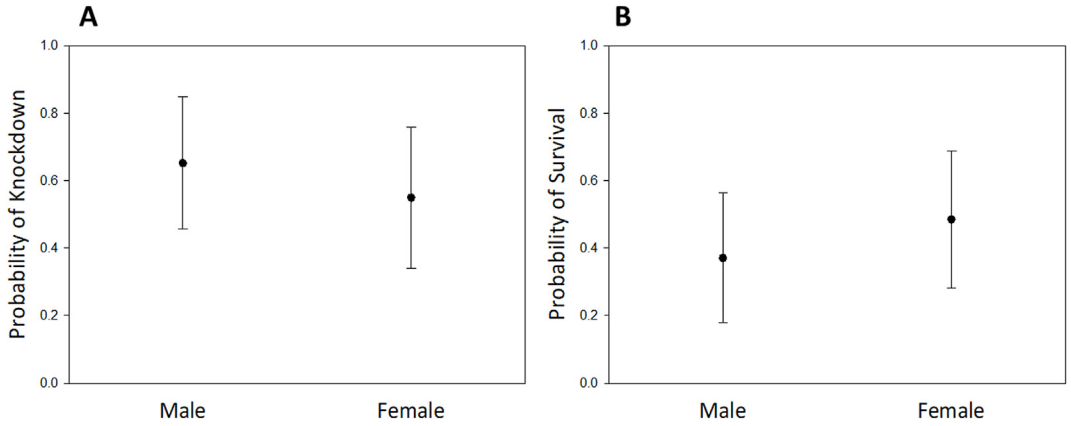

Fig. 6. Results of experiment 3. Male flies had a greater probability of knockdown (A) and a lower probability of survival (B) compared to female flies. Filled circles depict estimated mean probabilities of knockdown and survival while error bars depict the standard deviation of the given sex computed by multimodel averaging.

important but often unrecognized ecological service of adult blow flies is pollination. *Cochliomyia macellaria* specifically has been demonstrated to pollinate lychee in Florida (McGregor, 1976), tropical chestnuts (e.g., *Sterculia chichi*) in Brazil (Inouye et al., 2015), as well as mangroves in the Caribbean (Sánchez-Núñez and Mancera-Pineda, 2012). As with disease ecologists, researchers and agriculturalists interested in blow fly pollination would benefit greatly by knowing species and region specific thermal tolerances of blow flies, as they could better predict when and where blow flies will pollinate plants. However, the exact outcomes of these interactions are not known at this time. Thus, subsequent studies are needed to accurately model pathogen movement and pollination efficiency of blow flies.

In conclusion, we have shown that *C. macellaria* from College Station, TX, USA has an upper thermal tolerance of ~44–45 °C when exposed for at least 1 h. The availability of nutrients, either food or water, has a buffering effect of temperature exposure up to 44 °C and females have a greater thermal tolerance than males, while age has little to no effect. Admittedly, additional research is still needed before current data can be employed. For example, variation across species and populations should be known in order to extend this concept of thermal tolerance beyond the current lab study. Efforts to validate laboratory observations with field data will also be important. Nevertheless, these findings show the importance of determining the thermal tolerance of blow flies as they are associated with a variety of important applications.

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Disclaimer

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Author declaration

TWR, AMT, JKT, and AA designed the experiments. TWR, AA, and LEJB conducted experiments. TWR analyzed data. TWR, AMT, and JKT wrote first draft of manuscript and all authors contributed to revisions of manuscript.

Declarations of interest

None.

Ethics

All applicable institution and national guidelines were followed.

Data availability

The data generated and analyzed during the current study are available at Mendeley Data: https://doi.org/10.1016/j.jtherbio.2019.102405

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jtherbio.2019.102405.

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