Effects of developmental state on low-temperature physiology of the alfalfa leafcutting bee, *Megachile rotundata*

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Abstract

The success of agriculture relies on healthy bees to pollinate crops. Commercially managed pollinators are often kept under temperature-controlled conditions to better control development and optimize field performance. One such pollinator, the alfalfa leafcutting bee, *Megachile rotundata*, is the most widely used solitary bee in agriculture. Problematically, very little is known about the thermal physiology of *M. rotundata* or the consequences of artificial thermal regimes used in commercial management practices. Therefore, we took a broad look at the thermal performance of *M. rotundata* across development and the effects of commonly used commercial thermal regimes on adult bee physiology. After the termination of diapause, we hypothesized thermal sensitivity would vary across pupal metamorphosis. Our data show that bees in the post-diapause quiescent stage were more tolerant of low temperatures compared to bees in active development. We found that commercial practices applied during development decrease the likelihood of a bee recovering from another bout of thermal stress in adulthood, thereby decreasing their resilience. Lastly, commercial regimes applied during development affected the number of days to adult emergence, but the time of day that adults emerged was unaffected. Our data demonstrate the complex interactions between bee development and thermal regimes used in management. This knowledge can help improve the commercial management of these bees by optimizing the thermal regimes used and the timing of their application to alleviate negative downstream effects on adult performance.

Introduction

Pollinators face thermal challenges in both natural and managed settings. The most intensively managed solitary pollinator, the alfalfa leafcutting bee, *Megachile rotundata* (Fabricius, 1793), is no exception to these challenges (Pitts-Singer and Cane, 2011). Much like most mass-reared insect species, artificial thermal regimes are used at multiple points in the life cycle of *M. rotundata*. These incubation periods are used to control the progression of development, and to better synchronize adult emergence with crop bloom. For example, bees are stored under low temperatures during winter while in the prepupal stage (Yocum *et al.*, 2006; Rinehart *et al.*, 2011). In the spring, low-temperature incubation is used to slow down metamorphosis to better synchronize emergence with peak crop bloom (Undurraga and Stephen, 1980; Richards, 1984; Yocum *et al.*, 2010). However, the parameters of these temperature treatments used during active development in spring can be stressful, decreasing survival and causing sub-lethal effects in adults (Yocum *et al.*, 2010; Rinehart *et al.*, 2011; Bennett *et al.*, 2015). Potentially transgenerational effects of low temperatures exist, although this has yet to be studied in this species. One critical issue with these storage periods is that very little is known about the thermal physiology of this species. Lack of this information is a knowledge gap that requires empirical data to optimize thermal conditions for mass rearing.

Some studies have examined the effects of low-temperature incubations during development on adult performance in *M. rotundata*. For example, low-temperature exposure during development affected survival of adult bees, where fluctuating thermal regimes (FTRs) were less detrimental than static thermal regimes (STRs) (Yocum *et al.*, 2006, 2010; Rinehart *et al.*, 2011). However, another study by our group found these same temperature regimes caused numerous sub-lethal effects in the resultant adult (Bennett *et al.*, 2015). Sub-lethal effects are hypothesized to be due to chill-injuries, which are thought to affect ion-channel function, disrupting proper muscle and nervous system function (MacMillan and Sinclair, 2011; Marshall and Sinclair, 2012). Incorporating multiple performance assessments and

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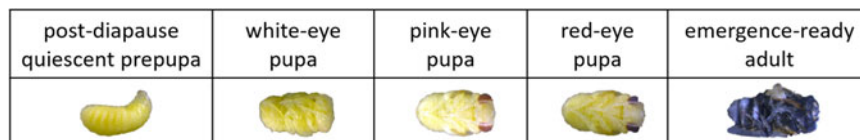| post-diapause quiescent prepupa	white-eye pupa	pink-eye pupa	red-eye pupa	emergence-ready adult

Figure 1. Photos of developmental stages throughout pupal metamorphosis of *M. rotundata*.

several stages of metamorphosis is important to better describe the response of *M. rotundata* to low-temperature regimes. Implications of select thermal regimes used in management practices are shown to affect adult flight performance and physiology (Bennett *et al.*, 2015). One problem is the lack of knowledge about the thermal thresholds for this species, which may inhibit the effective design of artificial treatments. Furthermore, whether artificial thermal regimes have downstream effects on adult thermal performance and pollination efficiency in the field is unknown. Thermal thresholds can restrict the range, or performance of a pollinator in the field. Managed *M. rotundata* are utilized for pollination services spanning North America, ranging from northern Canada down to New Mexico, as far east as Delaware, and as far west as California (https://www.gbif.org/species/1335648). Thus, *M. rotundata* pollinating at latitudes where risk of spring cold snaps occur may suffer from repeated exposure to low temperatures, the consequences of which are still elusive.

Physiological threshold temperatures are commonly used as assessments of responses to low temperatures because they are easily identifiable metrics that have functional significance. For example, supercooling point (SCP), critical thermal minimum (CT_{min}), and chill coma recovery (CCR) are three frequently measured thresholds. SCP is the temperature just before intra- or extra-cellular freezing occurs. CT_{min} is the temperature at which loss of muscle coordination occurs, termed chill coma, while CCR is the time it takes an insect to recover from chill coma or a standard low-temperature exposure (reviewed in Hazell and Bale, 2011; Overgaard and MacMillan, 2017). Some of these physiological thresholds, like CT_{min} and CCR have yet to be described for *M. rotundata*. Physiological thresholds, and how they may be shaped by low-temperature exposure, are base knowledge for designing and understanding the effects of artificial thermal regimes used in commercial insect management.

Ontogenetic changes in cold tolerance vary widely across species and are not necessarily linear in all cases (Bowler and Terblanche, 2008). Metamorphosis is a critical period where adult structures are growing and undergoing differentiation and, as such, can be susceptible to temperature stress. Although some species are more cold tolerant during pupation, like in the flesh fly *Sarcophaga crassipalpis* (Chen *et al.*, 1987), other species are less cold tolerant during pupation, like the Indianmeal moth, *Plodia interpunctella* (Carrillo *et al.*, 2005). One of our previous studies revealed that *M. rotundata* is more sensitive to low temperatures during pupation than during the prepupal stage. Bennett *et al.* (2015) found *M. rotundata* exposed to temperature stress during pupal development displayed numerous physiological differences and deformities as adults. Namely, *M. rotundata* exposed to temperature stress during pupal development took longer to complete metamorphosis than the controls, the adult life span of males was shortened, and over 50% of resultant adults had damaged wings resulting in decreased flight performance compared to controls with 10% of resultant adults displaying damaged wings (Bennett *et al.*, 2015). Thus, we hypothesize cold tolerance will decrease post-diapause when *M. rotundata* is actively undergoing pupation, and then increase when bees have

reached adulthood. We examined how exposure to low temperatures used in commercial management during development affects thermal physiology and behavior of adult bees. Understanding how low temperatures affect adult performance is important to improve bee health and pollination efficacy. Together these data are a first attempt at characterizing the thermal physiology of *M. rotundata*, and effects of low-temperature exposures during development on downstream adult performance and potential consequences on ecological service delivery efficiency.

Materials and methods

Animals

Loose brood cells containing pre-pupal *M. rotundata*, purchased from JWM Leafcutter, Inc. (Nampa, ID) in the spring of 2016, were kept at 6°C for approximately 2 months in darkness until use in experiments. Individual brood cells were placed into 24-well plates (Greiner Bio-one, Monroe, NC) and incubated at the standard commercial rearing temperature (control, 29°C) to stimulate metamorphosis (Kemp and Bosch, 2000). We used previously studied (Yocum *et al.*, 2010; Rinehart *et al.*, 2011; Bennett *et al.*, 2015) low-temperature treatments STR and FTR (Supplementary fig. 2), to assess their effects on CT_{min}, chilling recovery, and emergence behavior. The STR was 1 week at 6°C and the FTR was 1 week at 6°C with daily 1 h pulses of 20°C, with 1 h ramp periods. These treatments were applied during the red-eye pupal stage (approximately 14 days post-diapause at 29°C). The red-eye pupal stage was selected because commercial use of low-temperature interruptions usually occurs around this stage, slowing development to better match the timing of flower bloom. Temperature treatments for developmental stages for each experiment are outlined in Supplementary tables 1 and 2.

Supercooling point

We assayed SCP throughout metamorphosis to examine how development stage affects lower lethal temperatures. Bees from five developmental stages (fig. 1; post-diapause quiescent prepupa ($n = 15$), larval-pupal molt ($n = 12$), pink-eye pupa ($n = 15$), red-eye pupa ($n = 12$), and emergence-ready ($n = 12$)) were weighed and adhered to iButtons (Maxim Integrated, San Jose, CA) with petroleum jelly. Bees were weighed using a microbalance Mettler model AE-100 (Mettler Toledo, Columbus, OH). Data points were discarded if the bee fell off the iButton. We used calibrated DS1922L thermochron iButtons running with 11-bit resolution, sampling temperature every 5 s. To ensure maximum sensitivity to the latent heat of fusion, insects were attached to the bottom of the iButton so that they would be as close to the temperature sensor as possible. They were placed in a controlled cooling rate container (Nalgene 'Mr. Frosty'; Cole-Parmer, Vernon Hills, IL), starting at 23°C and placed inside a −80°C freezer to attain a cooling rate of approximately −1°C min^{-1}. After 45 min, temperature data were extracted from the iButtons

using a 1-Wire adapter (Maxim Integrated, San Jose, CA) and analyzed to identify the SCP.

Post-cold exposure survival

To determine how low temperatures affect adult survival, we exposed developing bees to lower temperatures and shorter time periods than the STR treatment. Three developmental stages were selected: (1) post-diapause quiescent, (2) red-eye pupae (14 days after initiation of metamorphosis at 29°C), and (3) emergence-ready (adults that have eclosed but not emerged from the brood cell). Bees were exposed to −5 or −10°C for 1–4 days (24, 48, 72, and 96 h) ($n = 72$ for each developmental stage and time point). These temperatures were chosen because they were above the SCP and below the commercially used STR treatment (Yocum et al., 2006; Rinehart et al., 2011; Bennett et al., 2015; Torson et al., 2015) in order to determine other lethal temperatures outside these thresholds. Brood cells were placed in 15 ml Conical tubes (Corning, Tewksbury, MA) held upright by a plastic rack and submerged in a water bath, Neslab model RTE-21 (Neslab, Inc., Portsmouth, NH) set to maintain constant temperatures. Antifreeze was added to the water bath to achieve below-freezing temperatures. Bees were removed from the water bath after 24, 48, 72, and 96 h, placed into 24-well plates, and incubated at 29°C to resume development to adult emergence. The plates were checked daily for adult emergence until bees ceased to emerge over numerous days in a row.

CT$_{min}$ and chilling recovery

We assessed how adult CT$_{min}$ and chilling recovery were affected by low-temperature exposure during pupal development. We exposed red-eye pupae to STR ($n = 30$), FTR ($n = 30$), and control ($n = 27$; constant 29°C with no low-temperature interruption) conditions and measured if these treatments had an effect on CT$_{min}$ in emerged adults. The CT$_{min}$ of adult bees was measured by recording the temperature at which the loss of muscle function occurred and the insects were unable to self-right. Individual bees were placed in a 15 ml conical tube containing a toothpick for the bees to perch on. Tubes were then placed into an incubator programmed to decrease the temperature from 25 to 0°C at a rate of −1°C min^{-1}. A thermocouple was taped to the side of each tube and temperature data were recorded using a custom-built multi-channel apparatus consisting of MAX31855 thermocouple amplifier breakout boards (Adafruit, New York, NY) connected to an Arduino Nano microcontroller (Adafruit, New York, NY). The board was programmed to display the temperature of each of six thermocouples on a 20×4 LCD screen (Adafruit, New York, NY), with values refreshed once per second. Six bees were run simultaneously, and closely monitored visually by the experimenter throughout the entirety of the temperature decrease. The CT$_{min}$ was designated as the temperature recorded on a thermocouple the moment at which the respective bee could no longer hold onto its toothpick. We measured CT$_{min}$ for male and female leaf-cutting bees separately to account for sex-specific differences.

During the red-eye pupal stage, bees were exposed to the above-mentioned STR or FTR thermal regimes. Chill recovery of the adults from these pupae was then assessed by first placing the newly eclosed insects individually inside conical tubes submerged in a water bath at 0°C for 48 h. Once removed from the water bath, chilled immobile adult bees were placed into 24-well plates lying on their back. Upon return to room temperature,

bees were observed at 1, 3, and 24 h for recovery. Recovery was determined as the ability to exhibit a righting response. Bees were scored on whether they were recovered at 1, 3, or 24 h after exposure to 0°C. We chose these time points so that we could get an idea of recovery in the short term (1 or 3 h) vs. a day later (24 h). Even if a bee recovered at 3 h and survived to 24 h, they were also scored at 24 h as recovered.

Emergence behavior

To determine how thermal history could affect complex emergence behavior, we assessed the timing of adult emergence after exposure to low-temperature stress during the red-eye pupal stage. Because STR and FTR had previously been shown to be stressful (Bennett et al., 2015), we chose those thermal regimes. The control (29°C with no low-temperature interruption), FTR, and STR treatments were conducted as described above, with the exception that instead of being placed in 29°C to stimulate metamorphosis, bees were exposed to a Δ4°C thermoperiod consisting of a 27°C cryophase and a 31°C thermophase in constant darkness and with 12 h spent at each phase. A Δ4°C thermoperiod was used because this species is known to synchronize emergence to the thermophase of this treatment (Yocum et al., 2015). The time of emergence was determined to the nearest second as previously described (Bennett et al., 2018, 2019).

Statistical analyses

Statistical analyses were conducted using JMP version 14.0.0 software (SAS Institute Inc., Cary, NC) and RStudio (version 1.1.453). Figures were generated using base graphics and 'ggplot2' R package (Wickham, 2016). We used non-parametric Kruskal–Wallis test to compare differences among SCPs across developmental stages because a Levene's test revealed unequal variances ($F = 2.4325$, $P = 0.0411$). Multiple comparisons were performed using a Steel–Dwass test to account for unequal sample sizes. We used the Wilcoxon non-parametric tests to compare temperature treatment effect on CT$_{min}$ because the data were non-normal in distribution. A generalized linear mixed model (GLMM) was constructed in RStudio using the 'lme4' R package to analyze survival data. The GLMM contained a binomial error distribution and logit transformation to assess recovery at four time points (24, 48, 72, 96 h). The response variable was a binomial response (recovered or did not recover) and the fixed effects were time points post-exposure to −5 or −10°C, and developmental stage (post-diapause quiescent, red-eye pupa, emergence-ready stage). We included bee ID and sex as random effects to account for individual variation. Pairwise comparisons were performed on fixed effects using the 'emmeans' R package. The same model was used to determine how temperature treatments (STR, FTR) affect recovery at 1, 3, and 24 h post-exposure to 0°C. Time points and temperature treatment were treated as fixed effects in the model. Effects of temperature treatment on days to emergence were calculated by totaling the number of days at 29°C post-diapause. The low-temperature treatment duration was subtracted from the total days. Because of the circular nature of the emergence data, circular analysis of variance was performed to determine if the average emergence time-of-day was affected by low-temperature exposure during development. Circular statistical analysis was conducted using circSASv1 SAS macros to calculate all circular statistics (http://statweb.calpoly.edu/ulund). Means ± SEM or circular means are presented throughout.

Results

Supercooling point

Developmental stage significantly affected SCP (fig. 2: χ^2(df 4) = 60.8245, $P < 0.001$). The post-diapause quiescent stage had the lowest SCP at -23.96 ± 0.54°C, and was significantly different from all developmental stages: pink-eye, white-eye, red-eye, emergence-ready (fig. 2). The pupal stages had a mean SCP of approximately -14.99 ± 1.46°C. The SCP did not significantly differ between the pupal stages (fig. 2, $Z = 1.58584$, $P = 0.5065$). However, the emergence-ready stage was significantly different from the pink-eye stage ($Z = -3.44005$, $P = 0.0053$), with an average SCP of -12.49 ± 1.07°C. Thus, cold tolerance directionally decreased as metamorphosis progressed; with the emergence-ready stage being the least cold tolerant in this assay.

Post-cold exposure survival

Developmental stage at the time of cold exposure had a significant effect on survival of adults (fig. 3; χ^2(df 2) = 41.179, $P < 0.001$). Furthermore, survival was affected by time spent at -5°C (χ^2(df 3) = 34.460, $P < 0.001$). Post-diapause quiescent bees had the highest survival across all time points, and emergence-ready bees had the lowest survival (fig. 3). There was no significant difference in survival across developmental stages at 24 h, but significant differences between the developmental stages emerge at time points 48, 72, and 96 h (fig. 3). Survival differed between the -10°C post-diapause quiescent stage and red-eye pupal stage, with none of the red-eye pupae surviving -10°C (χ^2(df 1) = 451.33, $P < 0.01$; Supplementary fig. 3).

CT_{min} and chilling recovery

We found there was no effect of STR and FTR on the CT_{min} of the adults compared to the control (fig. 4a; Wilcoxon, $P > 0.05$ for all pairs). Furthermore, CT_{min} was not significantly different between
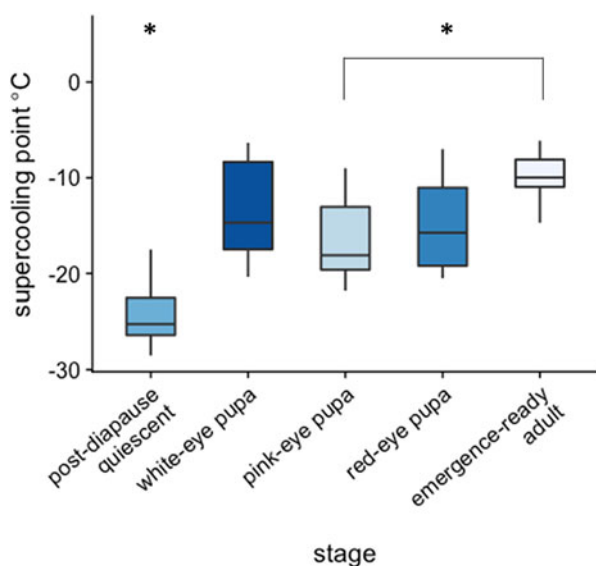

Figure 3. Percent survival vs. the amount of time spent at -5°C for *M. rotundata* at the post-diapause quiescent prepupa (long-dashed line), red-eye pupa (solid line), emergence-ready adult (short-dashed line). Asterisk indicates a significant difference in between the groups in percent survival at a specific time point.

males and females (fig. 4b; Wilcoxon, $Z = -1.560$, $P = 0.1186$). The average CT_{min} of adult bees when treatment and sex are pooled was 6.46 ± 0.25°C ($n = 81$).

We determined if STR and FTR treatments experienced during the red-eye pupal stage affect recovery after a second bout of cold in adulthood (0°C for 48 h. Compared to the control, 29°C constant ($n = 60$), the STR- ($n = 47$) and FTR-treated bees ($n = 45$) were less likely to recover at 3 h post-exposure to 0°C (fig. 5; χ^2(df 2) = 6.076, $P = 0.048$), and 24 h after exposure (χ^2(df 2) = 7.945, $P = 0.19$). There was no significant effect of treatment at 1 h post-exposure (fig. 5; χ^2(df 2) = 0.995, $P = 0.608$). Results of GLM pairwise comparisons across time points are presented in Supplementary table 3.

Emergence behavior

We tested the hypothesis that low temperatures during development may affect the timing of emergence behavior. Neither the FTR ($n = 164$) or STR ($n = 77$) treatment during metamorphosis affected the time of day when bees emerge compared to the

Figure 2. SCP variation across major developmental stages of *M. rotundata*. Developmental stages are described on the *x*-axis, and SCP begins at -30°C and ascends to 0°C on the *y*-axis. Boxes indicate interquartile range, whiskers indicate minimum and maximum values, black bar is the median. Asterisk indicates a significant difference from all other treatment groups.
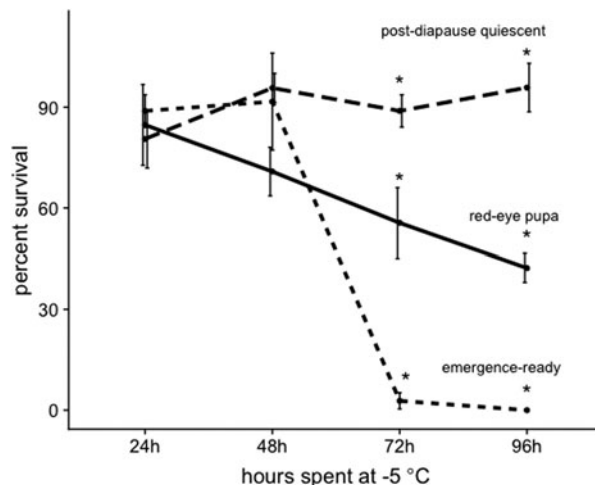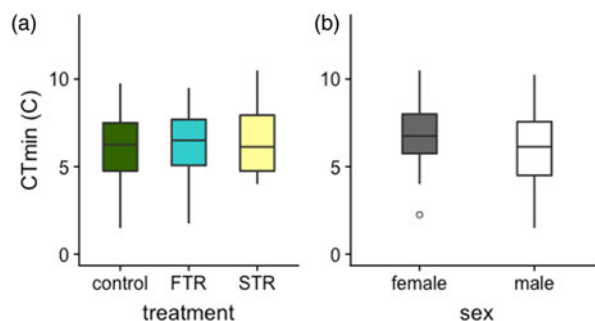

Figure 4. CT_{min} of newly emerged adult bees after exposure to a low-temperature treatment during development. Boxes indicate the interquartile range, whiskers indicate the minimum and maximum values, the black bar is the median, and points show outliers. The CT_{min} of control bees (green), FTR-treated (blue), and STR-treated (yellow) are shown in panel A. Pooled data for the CT_{min} of male (white) and female (gray) bees are shown in panel B.
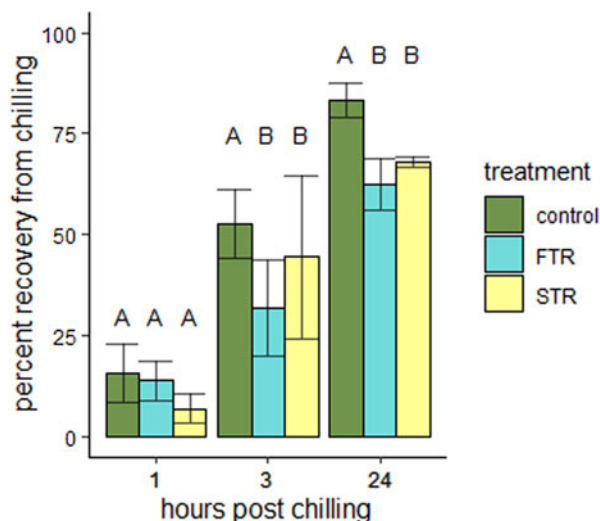
Figure 5. Percent of bees that recovered from chill coma vs. the number of hours after the exposure to 0°C with standard error bars. Developing red-eye pupae control (green), FTR (blue), and STR (yellow) were counted as recovered if they flipped over at 1, 3, or 24 h after exposure to 0°C. Significant differences among groups, within a time point, are denoted by different letters.

control ($n = 196$) (fig. 6, $F_{3,557} = 0.66$, $P = 0.53$). However, the number of days to emergence significantly differed between the control and low-temperature treatments (Supplementary table 4, $F_{3,556} = 124.88$, $P < 0.01$). The control differed from the FTR and STR in the mean number of days to emergence ($P < 0.01$), but the STR did not significantly differ from the FTR treatment ($P = 0.96$). Specifically, the control bees on average emerged the earliest (22.12 ± 0.12 days), and the FTR treatment (25.84 ± 0.21 days) and STR treatment (24.39 ± 0.21 days) emerged 2–3 days later.

Discussion

Our data showed that sensitivity to low-temperature stress varies by developmental stage in *M. rotundata*. Previous studies
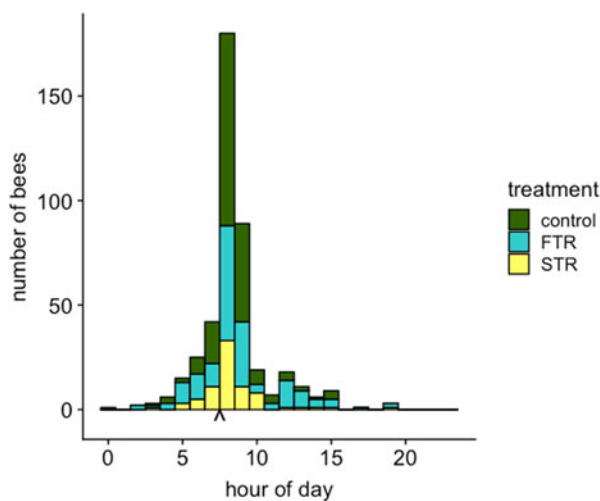

Figure 6. Frequency histograms of the number of bees emerging at a given time of day for control (green), FTR-treated (blue), and STR-treated (yellow). The thermophase ramp occurred between hour 7 and 8, denoted by the arrow below the bar.

suggested that cold tolerance of *M. rotundata* declines with age post-wintering (Rinehart *et al.*, 2011; Yocum *et al.*, 2012; Bennett *et al.*, 2015). Support for the hypothesis that cold tolerance decreases with development and increases with adulthood was mixed. Our hypothesis that prepupal *M. rotundata* that have recently finished overwintering are more tolerant to cold compared to those in active development was supported by both SCP and the survival data. Both of these assays revealed a linear pattern of cold tolerance, decreasing tolerance to cold as development progresses to the emergence-ready stage. However, when we assessed deleterious sub-lethal effects, later stages seemed more cold tolerant. One hypothesis is tissue reorganization during metamorphosis contributes to the variation in cold tolerance in this species. We also showed that low temperatures during the red-eye pupal stage affected adult physiology in some ways but not others. For example, after exposure to FTR or STR during metamorphosis, the likelihood of recovering from a second acute low-temperature exposure in adulthood decreased compared to controls reared at constant warm temperatures, but CT_{min} was unaffected. Had we not examined the effects of repeated cold exposure on thermal performance and only considered CT_{min}, the effects of our treatments may not have been fully recognized. Collectively, our data demonstrate the complex interactions between developmental stage and tolerance to low temperatures in *M. rotundata*.

Physiological thresholds are important descriptors of cold tolerance, because they might indicate temperatures at which lethal and sub-lethal effects occur. We found that SCP was approximately 10°C lower in post-diapause quiescent prepupae than the pupal and emergence-ready stages, consistent with findings from another *M. rotundata* study (Sheffield, 2008). When we exposed developing bees to low temperatures above the freezing point, we found that adult CT_{min} was unchanged by STR and FTR treatments (fig. 4). A study in bumblebees (*Bombus impatiens*) found that age and acclimation did not affect CT_{min} (Oyen and Dillon, 2018). The average CT_{min} of *M. rotundata* was approximately 6°C which is comparable to those of other insect species (Andersen *et al.*, 2015; Boardman *et al.*, 2016). SCP and CT_{min} can be used as a reference to better design commercial thermal regimes to minimize the amount of time near or below these points. Once an insect drops below its CT_{min}, it will enter chill coma where it is immobilized. We wanted to know if bees exposed to STR and FTR during development have a compromised ability to recover from chilling in adulthood. These chronic exposures to cool temperatures during development negatively affected recovery from an acute stress as adults in our study. In comparison with acute low-temperature exposures, chronic exposures often correlate with the increased severity of the injury (reviewed in Overgaard and MacMillan, 2017). One hypothesis is that both low-temperature rearing regimes caused an accumulation of chill injuries, leading to death. Thus, low-temperature storage could have serious implications for pollinator performance in an agricultural scenario. If commercial managers are using FTR or STR, bees foraging in fields at latitudes where the risk of spring cold snaps is high may be less resilient to even brief periods of low temperature. Our data suggest to reduce the time spent at low temperatures, and eliminate any exposure lower than the SCP.

The timing of adult emergence can be affected by low-temperature stress during development. For example, cold exposure during development shifts the timing of emergence to later in the thermophase in flesh flies, *S. crassipalpis* (Yocum *et al.*, 1994). In contrast, when we exposed metamorphosing pupae to low

temperatures, our treatments affected the days to emergence but not the time-of-day they emerged. It is interesting that even after the STR treatment, which we know is a severe chronic stress, that emergence remained synchronized. This suggests the circadian system is resistant to low-temperature stress in this species and bees exposed to low temperatures may still be able to emerge together, maintaining synchrony in emergence for mating and foraging.

Conclusions

Our study identified critical thermal thresholds and responses to low temperatures across development in an important pollinator, *M. rotundata*. Our data support the hypothesis that developmental stages closer to overwintering may be more tolerant of exposure to low temperatures, but the pupal stage might be vulnerable to sub-lethal effects. There are many deleterious sub-lethal effects that can negatively affect physiology, morphology, and behavior (Hutchinson and Bale, 1994; Yocum *et al.*, 1994; Kelty *et al.*, 1996; Rinehart *et al.*, 2011; Marshall and Sinclair, 2012; Bennett *et al.*, 2015). We know low-temperature interruptions can slow development, impacting day of adult emergence, which is an important factor in order to match peak bloom time. In previous studies we found that low-temperature treatments affect wing morphology and flight metabolic rate (Bennett *et al.*, 2015). Metrics like these could affect flight performance and dispersal ability of adult bees. Furthermore, identifying if these thermal regimes have transgenerational effects would be of interest. This is especially relevant because multiple generations of bees are sometimes needed to pollinate crops. Although we did not measure transgenerational effects, we found that recovery from low temperatures in adulthood was affected by low-temperature regimes during development. This result could be informative for stakeholders to help better inform decisions on whether to expose bees to repeated low-temperature exposure, in order to minimize unintended consequences on adult bees. These data are important to better understand the thermal physiology of *M. rotundata*, and ultimately, to contribute to the improvement of artificial thermal regimes that result in fewer off-target or sub-lethal effects.

References

Andersen JL, Manenti T, Sørensen JG, Macmillan HA, Loeschcke V and Overgaard J (2015) How to assess *Drosophila* cold tolerance: chill coma temperature and lower lethal temperature are the best predictors of cold distribution limits. *Functional Ecology* **29**, 55–65.

Bennett MM, Cook KM, Rinehart JP, Yocum GD, Kemp WP and Greenlee KJ (2015) Exposure to suboptimal temperatures during metamorphosis reveals a critical developmental window in the solitary bee, *Megachile rotundata*. *Physiological and Biochemical Zoology* **88**, 508–520.

Bennett MM, Rinehart JP, Yocum GD, Doetkott C and Greenlee KJ (2018) Cues for cavity nesters: investigating relevant zeitgebers for emerging leaf-cutting bees, *Megachile rotundata*. *Journal of Experimental Biology* **221**. doi: https://doi.org/10.1242/jeb.175406

Bennett MM, Rinehart JP, Yocum GD and Yocum I (2019) A precise and autonomous system for the detection of insect emergence patterns. *Journal of Visualized Experiments* **143**, e58362.

Boardman L, Sørensen JG, Koštál V, Šimek P and Terblanche JS (2016) Cold tolerance is unaffected by oxygen availability despite changes in anaerobic metabolism. *Scientific Reports* **6**, 32856.

Bowler K and Terblanche JS (2008) Insect thermal tolerance: what is the role of ontogeny, ageing and senescence? *Biological reviews of the Cambridge Philosophical Society* **83**, 339–355.

Carrillo MA, Cannon CA, Wilcke WF, Morey RV, Kaliyan N and Hutchinson WD (2005) Relationship between supercooling point and mortality at low temperatures in Indianmeal moth (Lepidoptera: Pyralidae). *Journal of Economic Entomology* **98**, 618–625.

Chen CP, Denlinger DL and Lee RE (1987) Cold-shock injury and rapid cold hardening response in the flesh fly, *Sarcophaga crassipalpis*. *Physiological Zoology* **60**, 297–304.

Hazell SP and Bale JS (2011) Low temperature thresholds: are chill coma and CT(min) synonymous? *Journal of Insect Physiology* **57**, 1085–1089.

Hutchinson LA and Bale JS (1994) Effects of sublethal cold stress on the aphid *Rhopalosiphum padi*. *Applied Ecology* **31**, 102–108.

Kelty JD, Killian AK and Lee REJ (1996) Cold shock and rapid cold-hardening of pharate adult flesh flies (*Sarcophaga crassipalpis*) effects on behaviour and neuromuscular function following eclosion. *Physiological Entomology* **21**, 283–288.

Kemp WP and Bosch J (2000) Development and emergence of the alfalfa pollinator *Megachile rotundata* (Hymenoptera: Megachilidae). *Annals of the Entomological Society of America* **93**, 904–911.

MacMillan HA and Sinclair BJ (2011) The role of the gut in insect chilling injury: cold-induced disruption of osmoregulation in the fall field cricket, *Gryllus pennsylvanicus*. *Journal of Experimental Biology* **214**, 726–734.

Marshall KE and Sinclair BJ (2012) The impacts of repeated cold exposure on insects. *Journal of Experimental Biology* **215**, 1607–1613.

Overgaard J and MacMillan HA (2017) The integrative physiology of insect chill tolerance. *Annual Review of Entomology* **79**, 187–208.

Oyen KJ and Dillon ME (2018) Critical thermal limits of bumblebees are marked by stereotypical behaviors and are unchanged by acclimation, age or feeding status. *The Journal of Experimental Biology* **221**, jeb165589.

Pitts-Singer TL and Cane JH (2011) The alfalfa leafcutting bee, *Megachile rotundata*: the world's most intensively managed solitary bee. *Annual Review of Entomology* **56**, 221–237.

Richards KW (1984) *Alfalfa Leafcutter Bee Management in Western Canada*. Agriculture Canada Publication. 1495/E. Ottawa, ON, Canada: Agriculture Canada.

Rinehart JP, Yocum GD, West M and Kemp WP (2011) A fluctuating thermal regime improves survival of cold-mediated delayed emergence in developing *Megachile rotundata* (Hymenoptera: Megachilidae). *Journal of Economic Entomology* **104**, 1162–1166.

Sheffield CS (2008) Summer bees for spring crops? Potential problems with *Megachile rotundata* (Fab.) (Hymenoptera: Megachilidae) as a pollinator of lowbush blueberry (Ericaceae). *Journal of the Kansas Entomological Society* **81**, 276–287.

Torson AS, Yocum GD, Rinehart JP, Kemp WP and Bowsher JH (2015) Transcriptional responses to fluctuating thermal regimes underpinning differences in survival in the solitary bee *Megachile rotundata*. *Journal of Experimental Biology* **218**, 1060–1068.

Undurraga JM and Stephen WP (1980) Effect of temperature on development and survival in post-diapausing leafcutting beepupae (*Megachile rotundata* (F.)). II. Low temperature. *Journal of the Kansas Entomological Society* **53**, 677–682.

Wickham H (2016) *ggplot2: Elegant Graphics for Data Analysis*. New York: Springer-Verlag. ISBN 978-3-319-24277-4. Available at https://ggplot2.tidyverse.org.

Yocum D, Zoarek JAN, Joplin KH, Lee REJ, Smith DC and Manter KD (1994) Alteration of the eclosion rhythm and eclosion behavior in the flesh fly, *Sarcophaga crassipalpis*, by low and high temperature stress. *Journal of Insect Physiology* **40**, 13–21.

Yocum GD, Kemp WP, Bosch J and Knoblett JN (2006) Thermal history influences diapause development in the solitary bee. *Journal of Insect Physiology* **52**, 1113–1120.

Yocum GD, Rinehart JP, West M and Kemp WP (2010) Interrupted incubation and short-term storage of the alfalfa pollinator *Megachile rotundata* (Hymenoptera: Megachilidae): a potential tool for synchronizing bees with bloom. *Journal of Economic Entomology* **103**, 234–241.

Yocum GD, Rinehart JP, Yocum IS, Kemp WP and Greenlee KJ (2015) Thermoperiodism synchronizes emergence in the alfalfa leafcutting bee (Hymenoptera: Megachilidae). *Environmental Entomology* **45**, 254–251.