Critical Thermal Maxima of *Bombus impatiens*: from Castes to Colonies

Tiffany Bretzlaff

Thesis submitted to the University of Ottawa
in partial fulfillment of the requirements for the degree of
Doctorate of Philosophy in Biology

Department of Biology
Faculty of Science
University of Ottawa

Abstract

Bumblebees are experiencing declines and range contractions globally that are, in some cases, independent of anthropogenic pesticide- or land-use change, leaving rising global temperatures as the primary driver of such loses. With ambient temperature (T_a) and thermal limitations being a crucial component in these observed declines, I sought to determine the physiological limitations that high T_a imposes on both individuals and colonies of a temperate bumblebee species, *Bombus impatiens*. Through Chapter 2, I first established the upper thermal tolerance (CTmax) of the species, testing both adults and larvae to determine which of these colony castes are most thermally sensitive to heat. Collective thermoregulation at the colony-level is then important to ensure that the most heat sensitive individuals are protected from changes in optimal nest temperature (T_n). I thus identified the energetic costs associated with colonial thermoregulation and whether large colonies could successfully achieve thermal homeostasis under various T_a. Chronic bouts of heat stress are also of concern as colonies invest time and energy into thermoregulation, especially given that heatwave events are becoming more frequent. In Chapter 3, I examined whether there exists a trade-off between thermoregulation and foraging effort for colonies under chronic heat stress and how various measures of colony success are impacted. Finally, foraging requires individuals to employ flight for the procurement of resources. In Chapter 4, I investigated if the temperate adaptation of an insulative pile layer would hinder flight performance under high T_a by assessing the metabolic rates of adult castes during flight. I found that larvae were more thermally sensitive compared to bumblebee adults, which emphasizes the importance of colonial thermoregulation – a task successful at low T_a. Under heat stress, however, T_n could not be maintained despite elevated energetic investments (Chapter 2). These findings suggest that T_a which exceeds optimal T_n may pose significant

challenges to colonies; not only energetically but also to the health of thermally sensitive larvae within. A trade-off between thermoregulation and foraging effort did not emerge for colonies experiencing chronic exposure to high T_a. Instead, only high incidences of thermoregulation were observed which failed to prevent increases in T_n. Furthermore, a greater number of individuals were found to abandon the colony at high T_a, and fewer offspring were produced (Chapter 3). Here, findings suggest that chronic high T_a may pose the greatest risk to the production of thermally sensitive offspring by way of reduced worker population and failed thermoregulation. Finally, the metabolic output during flight at high T_a was not found to be affected by an insulative layer of pile (Chapter 4), indicating that either pile may play a role in limiting other measures of flight performance at high T_a, or that alternate physiological mechanisms may be responsible instead. Together the findings from this thesis broaden the understanding of how a temperate species of bumblebee responds physiologically to high T_a both at the individual and colonial level, providing further evidence on thermal limitations in a changing climate.

Acknowledgements

To begin, I would like to thank my supervisors Drs. Charles Darveau and Jeremy Kerr for their support throughout my degree. Charles' guidance has been invaluable to me, helping me develop into an independent researcher. His feedback along the way has greatly improved my skills both in the lab and as a writer. I'd also like to thank Jeremy for welcoming me into his lab and fostering a remarkable community of brilliant young scientists. Kirsten, Olga, Cat, Anouk, Peter, Susan, Sarah, Bé, Emily, Serena and Rosie are just a few of the amazing people I came to know as a result. I have also been lucky to be part of the Darveau lab, working alongside exceptional people such as Jane, Zoë, Ariane, Nadia, Tera, Zacc and Jamie. I would like to extend my thanks to the members of my thesis advisory committee, Heather Kharouba, Kathleen Gilmour and Gabriel Blouin-Demers, for their direction and feedback during my degree. I also wish to acknowledge Dr. Adam Brown for building my confidence as a communicator.

To Zoë Perkins, Jamie Thompson, Mireille Cardinal, Samantha Zabudsky and Laurence Lecavalier, I give a huge shout-out for helping collect data and sort through tedious bee videos. I also thank everyone at the Science Store for always being friendly and helpful, especially when it came to my endless orders of bee colonies and duct tape. And of course, I thank the bumblebees themselves; without whom I would not have data nor a severe allergy from their generous stinging donations.

Finally, I would like to thank my family and friends. To my parents, your endless love and unconditional support has taught me that anything is possible. To Fred, your words of wisdom are always on my mind and I know you'd be proud of how far I've come. To Kelli Knox, Jane Allison and Zoë Perkins, thank you for taking care of me just like family. And lastly, to a little squirrel named Kitty who taught me that a little determination can go along way.

Official statement of contributions

Each of the following chapters, including the research ideas, written content, figures and analyses, represent my own work. I led the design, analyses, interpretation, and writing, though my supervisors provided advice, guidance, support and feedback on each step of my work.

Chapter 2 has been accepted for publication in the *Journal of Thermal Biology*.

> Bretzlaff, T., Kerr, J. T., & Darveau, C. A. (2023). High temperature sensitivity of bumblebee castes and the colony-level costs of thermoregulation in *Bombus impatiens*. Journal of Thermal Biology, 103710. https://doi.org/10.1016/j.jtherbio.2023.103710

Author's contributions. Conceptualization: T.B., C.-A.D.; Data curation: T.B., C.-A.D.; Formal analysis: T.B., C.-A.D.; Funding acquisition: C.-A.D., J.T.K.; Investigation: T.B.; Methodology: T.B., C.-A.D.; Project administration: C.-A.D.; Resources: C.-A.D., J.T.K.; Supervision: C.-A.D., J.T.K.; Visualization: T.B.; Writing – original draft preparation: T.B. C.-A.D.; Writing – review and editing: T.B., C.-A.D., J.T.K.

Funding. This work was supported by a Natural Sciences and Engineering Research Council of Canada Discovery Grants awarded to C.-A.D. (RGPIN-2014-03584) and J.T.K. (RGPIN-2017-06008); T.B. was supported by a B1X Research Scholarship provided by the Fonds de Recherche du Québec – Nature et technologies (FRQNT).

Acknowledgements. I would like to thank Laurence Lecavalier and Mireille Cardinal, who helped collect CTmax data as well as the additional lab members who participated in colony maintenance including Zoë Perkins, Ariane Rondot, Nadia Stec, and Jamie Thompson.

Chapter 3 has been submitted for publication in *Conservation Physiology* and is currently under revision.

> Bretzlaff, T., Kerr, J. T., & Darveau, C. A. (2023). Handling heatwaves: trade-offs between thermoregulation, foraging and bumblebee colony success. *Conservation Physiology*, manuscript ID CONPHYS-2023-086. (*in revision)*.

Author contributions. Conceptualization: T.B., C.-A.D.; Data curation: T.B.; Formal analysis: T.B.; Funding acquisition: C.-A.D., J.T.K.; Investigation: T.B.; Methodology: T.B., C.-A.D.; Project administration: C.-A.D.; Resources: C.-A.D., J.T.K.; Supervision: C.-A.D., J.T.K.; Visualization: T.B.; Writing – original draft preparation: T.B.; Writing – review and editing: T.B., C.-A.D., J.T.K.

Funding. This work was supported by a Natural Sciences and Engineering Research Council of Canada Discovery Grants awarded to C.-A.D. (RGPIN-2014-03584) and J.T.K. (RGPIN-2017-06008); T.B. was supported by a B1X Research Scholarship provided by the Fonds de Recherche du Québec – Nature et technologies (FRQNT).

Acknowledgements. I would like to thank Samantha Zabudsky, Laurence Lecavalier, Mireille Cardinal, Jamie Thompson and Zoë Perkins who aided in processing fanning and IR videos.

Table of Contents

List of Figures and Tables

Figure S2.2. An example of a clear trace used for CTmax determination of a worker *B. impatiens*. A) Activity CTmax determined using the residual absolute difference sum (ADS) where the peak coincides with the final movement detected using infrared activity detector (red arrows). B) Respiratory CTmax where the residual ADS coincides with the cessation of spiracular activity identified by the transformed CO_2 release trace using the 'differentiate' then 'square' function in Expedata (red arrows) (see Vorhees and Bradley, 2012). The blue arrows correspond to the post-mortal peak (Turner and Lighton, 2004) where a final peak of the residual ADS is noted.

Figure S2.4. An example of trace used for CTmax determination of a *B. impatiens* larvae. A) Activity CTmax determined using the residual absolute difference sum (ADS) where the peak coincides with the final movement detected using infrared activity detector (red arrows). B) Respiratory CTmax could not be determined as the residual ADS yielded a peak after the post-mortal peak (see Figure S4). The cessation of spiracular activity could not be identified by the transformed CO2 release trace using the 'differentiate' then 'square' function in Expedata.

Figure S2.5. Compiled thermolimit respirometry raw traces from *B. impatiens* larvae. A final CO_2 release is noted by the circled area in red, which is interpreted as the post-mortal peak typical of such assays with corresponding timeline (Lighton and Turner, 2004; Vorhees and Bradley, 2012). No clear objective landmark could be identified for larvae (i.e., residual ADS, CSA) and therefore only relied on activity CTmax that could be determined using the ADS approach as in adults (see Fig. S3 for details).

Table S2.1. Mean CTmax (±SE) of queens and drones does not differ between colonies.

Table S2.2. Number of workers and their mean body mass of the colonies used in the whole-colony respiration experiment.

Figure 3.1 Colony setup of chronic foraging experiment. Bumblebee (*Bombus impatiens*) colonies within their nest box were placed within a 38L Styrofoam cooler of 1.5cm thickness and sealed with the corresponding Styrofoam lid. The colony entrance was fitted with tubing of an internal diameter approximately 1.30cm wide. Tubing was run to two RFID reader boxes and then connected to an entrance in a large flight cage (60x60x180cm). This permitted the movements of the 43 RFID tagged workers to be recorded as they exited and re-entered the colony. The total distance between the colony and the flight cage measured approximately 60cm. Within the flight cage, a 2kg container of BIOGLUC® solution was placed near the entrance allowing bees to forage for a nectar-like solution. Bumblebee colonies were exposed to 25, 30 and 35°C (n=5 colonies per temperature) for two-weeks. Two thermochron iButton® digital temperature loggers were placed among the brood cells containing developing larvae to monitor internal colony temperature. A plexiglass cover was fit overtop the colony with two holes cut into it for access to administer pollen balls every second day and to film the colony for the purposes of quantifying fanning incidence. When not filming, glass coverings were kept over the holes in the plexiglass and the Styrofoam lid remained in place.

Figure 3.2. The percentage of *B. impatiens* workers that forage shows no clear trend. Bumblebee colonies of 43 workers were exposed to 25°C (yellow), 30°C (orange) or 35°C (red) for two-week periods (25°C, n=3 colonies; 30, 35°C, n=5 colonies). The percentage of tagged workers who engaged in foraging (10+ trips total) did not differ across T_a treatments (P=0.230). Box plots represent percentiles with the black bar across equalling the median

value. Also present are the individual data points to show the spread of the colonies tested. The average value is denoted by an (x) within each box.

Figure 3.3. Fanning incidence of *B. impatiens* workers increases under chronic heat stress. Bumblebee colonies of 43 workers were exposed to 25°C (yellow), 30°C (orange) or 35°C (red) for two-week periods (n=5 colonies per ambient temperature, T_a). The incidence of fanning was quantified daily by counting workers engaged wing fanning for 10+ seconds within a 165cm^2 area overtop the brood. The daily totals were averaged across each T_a trial. Fanning incidence at 35°C was significantly higher than at lower T_a (*$P \leq 0.049$). Box plots represent percentiles with the black bar across equalling the median value. Also present are the individual data points to show the spread of the colonies tested. The average value is denoted by an (x) within each box.

Figure 3.4. Internal colony temperature cannot be maintained under chronic heat stress. Bumblebee colonies (*B. impatiens*) of 43 workers were exposed to 25°C (yellow), 30°C (orange) or 35°C (red) for two-week periods (n=5 colonies per ambient temperature, T_a). Internal colony temperature was determined using iButtons placed within colony brood clumps. Optimal nest temperature (Barrow and Pickard, 1985; Heinrich, 2004; Schultze-Motel, 1991; Vogt, 1986a) is represented by the green shaded area on each panel. A) Average internal colony temperature, calculated across each T_a trial, was significantly higher at 35°C than at lower T_a (*$P<0.001$). Box plots represent percentiles with the black bar across equalling the median value. Also present are the individual data points to show the spread of the colonies tested. The average value is denoted by an (x) within each box. B) The daily average internal colony temperature, calculated as the mean on each experimental day, generally did not differ on a daily basis across two-week expose periods with the exception of an increase on days 8 and 14 at 30°C ($P \leq 0.016$). Individual data points are scattered about the mean line and standard error points for each colony tested.

Figure 3.5 The mean highest thorax temperature observed within *B. impatiens* colonies is not altered by chronic heat stress. Bumblebee colonies of 43 workers were exposed to 25°C (yellow), 30°C (orange) or 35°C (red) for two-week periods (n=5 colonies per ambient temperature, T_a). An infrared (IR) digital camera recorded the maximum thorax temperature (T_{th}) within a 165cm^2 field of view, daily. Daily totals were averaged across each experimental trial where mean values for a given T_a were not significantly different ($P=0.312$). Box plots represent percentiles with the black bar across equalling the median value. Also present are the individual data points to show the spread of the colonies tested. The average value is denoted by an (x) within each box.

Figure 3.6. Chronic heat stress does not alter the percent of adult emergence of *B. impatiens* colonies. Bumblebee colonies of 43 workers were exposed to 25°C (yellow), 30°C (orange) or 35°C (red) for two-week periods (n=5 colonies per ambient temperature, T_a). The percentage of workers that emerged during an experimental trial was calculated to determine adult emergence, which ultimately did not depend on T_a ($P=0.765$). Box plots represent percentiles with the black bar corresponding with the median value. Also present are the individual data points to show the spread of the colonies tested. The average value is denoted by an (x) within each box.

Figure 3.7. Chronic heat stress does not change the percentage of mortality in *B. impatiens* colonies. Bumblebee colonies of 43 workers were exposed to 25°C (yellow), 30°C (orange) or 35°C (red) for two-week periods (n=5 colonies per ambient temperature, T_a). The percentage of bees who were found deceased during or at the end of each experimental trial represents

mortality rate. No significant differences were found between tested values of ambient temperature, T_a (P=0.073). Box plots represent percentiles with the black bar across equalling the median value. Also present are the individual data points to show the spread of the colonies tested. The average value is denoted by an (x) within each box.

Figure 3.8. Chronic heat stress results in a higher percentage of *B. impatiens* workers abandoning their colony. Bumblebee colonies of 43 workers were exposed to 25°C (yellow), 30°C (orange) or 35°C (red) for two-week periods (n=5 colonies per ambient temperature, T_a). The percentage of workers who were found outside the colony and in the flight cage either deceased during or alive at the end of each experimental trial was used to quantify percent abandonment. This value was significantly higher at 35°C than at lower T_a (*P≤0.044). Box plots represent percentiles with the black bar across equalling the median value. Also present are the individual data points to show the spread of the colonies tested. The average value is denoted by an (x) within each box.

Figure 3.9. Offspring production is negatively impacted by chronic heat stress. Bumblebee colonies (*B. impatiens*) of 43 workers were exposed to 25°C (yellow), 30°C (orange) or 35°C (red) for two-week periods (n=5 colonies per ambient temperature, T_a). The total number of offspring present was dependent on T_a (P=0.0026). The number of larvae and pupae present in dissected broods following the end of each experimental trial quantifies offspring production. Significantly fewer offspring were found at 35°C when compared to lower T_a (*P≤0.007). Box plots represent percentiles with the black bar across equalling the median value. Also present are the individual data points to show the spread of the colonies tested. The average value is denoted by an (x) within each box.

Figure 4.1. The absence of pile does not alter the metabolic output across high ambient temperature for *B. impatiens* workers during agitated flight. Worker with their pile removed (light grey) and intact (dark grey) were flown under constant agitation at 25, 30 and 35°C to obtain their CO_2 output. Ambient temperature was not a significant predictor of flight metabolic rate (FMR; P=0.684). Box plots represent percentiles with the black bar across signalling the median value. Also present are the individual data points indicating the distribution of FMR values across individual workers. The average FMR value within each temperature group is indicated by an (x).

Figure 4.2. Pile differentially affected the metabolic output of large and small *B. impatiens* workers during agitated flight. Workers with pile removed (light grey) and intact (dark grey) were flown under constant agitation at 25, 30 and 35°C to obtain their CO_2 output. The regression lines with 95% confidence intervals of pile removed and intact bees are represented by grey shading. The interaction between mass and pile was significant (P=0.017), producing different slopes between pile treatments (removed: $FMR = 32.34(mass) + 3.05$; intact: $FMR = 49.76(mass) - 0.44$).

Figure 4.3. *B. impatiens* worker mass predicts their metabolic output during free flight. Workers with pile removed (●) and intact (■) were flown without constant agitation at 25°C (yellow), 30°C (orange) and 35°C (red) to obtain their CO_2 output (flight metabolic rate, FMR). The regression between FMR and mass was significant (P<0.0001; Adj R^2=0.84; slope: $FMR = 46.65(\text{mass}) + 0.15$).

Figure 4.4. Neither pile nor ambient temperature affects the metabolic output of *B. impatiens* workers during free flight. Workers with their pile removed (light grey) and intact (dark grey) were flown without constant agitation at 25, 30 and 35°C to obtain their CO_2 output.

Flight metabolic rate (FMR) did not vary across ambient temperatures (P=0.838) and neither was it significantly different between pile treatments (P=0.069). Box plots represent percentiles with the black bar across signalling the median value. Also present are the individual data points indicating the distribution of FMR values across individual workers. The average FMR value within each temperature group is indicated by an (x).

Figure 4.5. ***B. impatiens*** **drone mass predicts their metabolic output during free flight.** Drones with pile removed (●) and intact (■) were flown without constant agitation at 25°C (yellow), 30°C (orange) and 35°C (red) to obtain their CO_2 output (flight metabolic rate, FMR). The regression between FMR and mass was significant (P<0.0001; Adj R^2=0.84; slope: $FMR = 46.65(\text{mass}) + 0.15$).

Figure 4.6. **Neither pile nor ambient temperature affects the metabolic output of** ***B. impatiens*** **drones during free flight.** Drones with pile removed (light grey) and intact (dark grey) were flown without constant agitation at 25, 30 and 35°C to obtain their CO_2 output. Flight metabolic rate (FMR) did not vary across ambient temperatures (P=0.279) and neither was it significantly different between pile treatments (P=0.207). Box plots represent percentiles with the black bar across signalling the median value. Also present are the individual data points indicating the distribution of FMR values across individual drones. The average FMR value within each temperature group is indicated by an (x).

Figure 4.7. ***B. impatiens*** **queen mass predicts their metabolic output during free flight.** Queens with pile removed (●) and intact (■) were flown without constant agitation at 25°C (yellow), 30°C (orange) and 35°C (red) to obtain their CO_2 output (flight metabolic rate, FMR). The regression between FMR and mass was significant (P<0.0001; Adj R^2=0.25; slope: $FMR = 29.89(\text{mass}) + 3.37$).

Figure 4.8. **The absence of pile does not alter the metabolic output of queens under high T_a.** Queens with pile removed (light grey) and intact (dark grey) were flown without constant agitation at 25, 30 and 35°C to obtain their CO_2 output. There was an interactive effect of T_a and pile on flight metabolic rate (FMR; P=0.045) but no combination of T_a and pile produced significant differences in FMR ($P \geq 0.162$, pairwise analysis). Thus, FMR neither varied across T_a nor between bees with pile removed or intact. Box plots represent percentiles with the black bar across signalling the median value. Also present are the individual data points indicating the distribution of FMR values across individual queens. The average FMR value within each temperature group is indicated by an (x).

Chapter 1: General Introduction

Temperature is an influential abiotic factor which, in part, acts to determine where animals may thrive successfully. This influence becomes apparent when examining species thermal tolerance and distribution across broad scale analyses. In general, lower thermal tolerance limits (LTLs) decrease with increasing latitude and altitude for both terrestrial endotherms and ectotherms, as well as for aquatic ectotherms (Addo-Bediako et al., 2000; Calosi et al., 2010; Clusella-Trullas et al., 2011; Huey et al., 2009; Snyder and Weathers, 1975; Sunday et al., 2019, 2011; Van Berkum, 1988), meaning that animals who are tolerant to cooler temperatures will inhabit regions with ambient temperatures (T_a) that reflect these thermal limits. While the climate variability hypothesis infers that high latitude species should survive over larger geographical areas due to their broad thermal tolerances (see Calosi et al. 2010), predicting species distribution based on thermal limits alone becomes complicated when using upper thermal tolerance limits (UTLs). In this case, only aquatic ectotherms show moderate to strong declines in UTLs with increasing latitude and altitude (Sunday et al., 2019), while there is an apparent lack of variation across both terrestrial endotherms and ectotherms (Addo-Bediako et al., 2000; Clusella-Trullas et al., 2011; Cruz et al., 2005; Huey et al., 2009; Pimsler et al., 2020; Snyder and Weathers, 1975; Sunday et al., 2019, 2011; Van Berkum, 1988). Additionally, previous studies demonstrate that species distribution is better predicted by different variables, such as how the operative foraging thermal limits of ants best correspond to their realized niches (Guo et al., 2020), or how the UTLs of drosophila from hot-dry regions reflect the climate, while those from wetter regions do not (Kellermann et al., 2012). Contradictions to broadscale analyses also exist wherein UTLs vary across latitudes among different populations of similar lizard species (Herrando-Pérez et al., 2020). Therefore, determining the thermal environments where an

animal may thrive successfully is not uniform for all species, and because temperature affects all levels of biological organization, there is a need to understand how it acts to shape species thermal limits within an ecological context (Pörtner et al., 2017).

Determining the effect of T_a on the responses of ectothermic species is especially important given their inability to regulate body temperature (T_b) through internal physiological processes. Thus, T_a has a more direct influence on ectotherm T_b (see Clarke 2017) and they must rely more heavily on behaviour to warm up or avoid overheating (see Morash et al., 2018; Sunday et al., 2014). Thermal performance curves (TPC) can be used to evaluate the resulting consequences of T_a (Sinclair et al., 2016) by plotting the relationship between temperature and some metric of an animal's performance, such as respiration, growth, locomotion or reproduction (Huey and Stevenson, 1979; Schulte et al., 2011). These curves take on the shape of an inverted parabola, bound on either end by the minimum (LTL) and maximum (UTL) temperatures the individual can tolerate. Between these extremes, performance peaks at a thermal optimum (T_{opt}; Huey and Stevenson 1979, Pörtner 2001, Schulte et al. 2011) and when T_{opt} is surpassed, performance declines until the UTL is reached. High T_a can thus have many detrimental effects on ectotherm performance by increasing oxygen demand through elevated respiration, altering neuron firing frequency within the central nervous system (CNS), and destabilizing molecular structures like proteins, DNA and cell membranes (see Neven 2000). While behavioural avoidance and the expression of heat shock proteins in response to high T_a may help buffer against these detrimental effects (see Harrison et al., 2012; Scharf et al., 2016), temperatures which reach an individual's UTL cause death via failure of oxygen delivery systems to keep pace with oxygen demand (Pörtner, 2001; Schulte et al., 2011). For insects, oxygen is supplied efficiently due to the presence of a tracheal system, thus making oxygen delivery an unlikely

cause for insect death at high T_a (Klok et al., 2004; McCue and De Los Santos, 2013). Mortality at the UTL of an insect may instead be attributed to a failure of a heat sensitive mechanism within the CNS (Lighton and Turner, 2004). Given that global temperatures are on the rise as a result of anthropogenic climate change (The Core Writing Team IPCC, 2015), TPCs thus provide a physiological scaffold from which to build our understanding of how climate change will impact species across the globe (Deutsch et al., 2008).

Thermal responses, when viewed on a TPC, are asymmetric, being that an individual's T_{opt} will lie only a few degrees below their UTL while it will exists several degrees above the LTL (Bozinovic et al., 2011). Therefore, small increases in temperature may result in more significant declines in performance when compared to a reduction in temperature (Morash et al., 2018). Thermal variation is also of concern, especially if species already live near their T_{opt} (Bozinovic et al., 2011). Thermal safety margins (TSM) quantify this difference between the UTL of an individual and the regional maximum T_a experienced within its habitat (Sunday et al., 2014). Generally, TSMs broaden with latitude (Deutsch et al., 2008), with tropical species more at risk of being pushed towards their UTL (Deutsch et al., 2008; Sunday et al., 2012; Tewksbury et al., 2008) or already existing in areas where T_b exceeds UTL, providing no TSM (Pinsky et al., 2019; Sunday et al., 2014). Temperate species, despite supposedly broad TSMs, are also put at risk from climate change. Temperate climates fluctuate annually and not all species will remain active year-round. Thus, when considering mean active season T_a, as opposed to mean annual T_a, the TSM and warming tolerance of temperate species are no longer as robust as they were previously thought to be (Johansson et al., 2020). Climate change alters both daily T_a ranges and means (see Huey et al. 2012) with increased frequencies of heat stress (see Kingsolver et al. 2013). It is thus predicted that mid-latitude species will be especially vulnerable to variation in

T_a caused by climate change (Kingsolver et al., 2013), highlighting the importance of understanding how various aspects of physiological fitness will be limited for terrestrial ectotherms experiencing thermal stress.

One group of particular concern are the large-bodied pollinators, such as bees, who provide invaluable services to both the environment and human agriculture (Gill et al., 2016; Klein et al., 2006). Research to date demonstrates declines in bee pollinator abundance (Iserbyt and Rasmont, 2012; Jacobson et al., 2018), diversity (Biesmeijer et al., 2006; Powney et al., 2019) and species richness (Fourcade et al., 2019; Soroye et al., 2020; Vray et al., 2019) implicating climate change as a major driver of such declines in most cases. Moreover, bumblebee range constrictions at local (Jacobson et al., 2018) and continental (Kerr et al., 2015) scales imply that dispersion rates may be too slow to track climate warming (Sirois-Delisle and Kerr, 2018). Additional evidence reveals that bumblebees live closest to their UTLs within urban areas, reducing their TMSs (Burdine and McCluney, 2019) and that bee species with the lowest heat tolerances have declined the most within those areas (Hamblin et al., 2017). Together, these findings emphasize the value of determining which aspects of bee physiology act to limit them within their thermal environment and how they may cope with future increases in global temperature.

Bumblebees, like other large-bodied insect pollinators such as honeybees and moths, are endothermic poikilotherms, meaning that they are capable of allowing T_b to conform to the environment as well as regulating it for flight (Heinrich, 1995, 1974a, 1971). This unique thermal strategy denotes that their response to T_a is less straightforward than other ectothermic insects. Flight is an energetically expensive behaviour and the high-energy food sources utilized by moths and bees allow for high metabolic rates, elevating T_b and permitting endothermy

(Heinrich, 1995, 1974a). Over evolutionary time, flight thus evolved to require high muscle temperature where the minimum thorax temperature (T_{th}) of bumblebees must be 30°C to facilitate the behaviour (Heinrich, 1974a). Endogenous heat produced through internal physiological processes (see Heinrich, 2004; Masson et al., 2017; Staples et al., 2004) generate heat while physiological and morphological adaptations, such as a counter-current heat exchange system (Heinrich, 1976) and an insulative layer of fur (pile), respectively, maintain T_{th} (Heinrich, 2004, 1974a). These adaptations have allowed bumblebees to successfully colonize temperate climates, promoting activity at low T_a (Heinrich, 2004, 1974a) and even extending some species ranges into arctic regions (Colla et al., 2014; Richards, 1973). Not only is thermoregulation important on an individual level, but for colonial species, it enables thermal control over internal colony temperature (T_n) as individuals work cooperatively as a superorganism. This then leads to the central question of my thesis, where I ask whether a eusocial, temperate-adapted species of bumblebee is able to physiologically cope with high T_a at an acute level, for individuals and colonies, as well as how colonies respond to chronic thermal stress using the Common Eastern Bumblebee, *Bombus impatiens*, as a model species. Easily reared in a lab and commercially available to purchase for greenhouses and gardens (Velthuis and Van Doorn, 2006), *B. impatiens* makes an ideal candidate for study. It has a vast distribution in North America across a wide range of habitats including woodlands, grasslands, farmlands, wetlands and urbanized areas (Colla et al., 2014). Though *B. impatiens* is primarily a cavity nester (underground; Colla et al. 2014), it, like many other bumblebee species, are also commonly found at aboveground nesting sites (Liczner and Colla, 2019). These locations may experience wide fluctuations in T_a of up to 24°C throughout the day (Mullan, 2022), making studies which focus on replicating aboveground

nesting conditions key to understanding how a colony is able to cope with such thermal challenges.

Some species of bumblebees have remained fairly robust in the face of recent climate change. For example, *B. impatiens* is capable of expanding into the ranges of species undergoing extirpation (Jacobson et al., 2018), and when investigating site occupancy, recent work finds no evidence of genus-wide declines (Jackson et al., 2022). Despite these findings, bumblebee site occupancy is still strongly related to temperature and more species of bumblebees will likely "lose" in the face of climate change rather than thrive (Jackson et al., 2022). Thus, recent works call for species-specific analyses of occupancy (Guzman et al., 2021; Jackson et al., 2022) as well as investigation into physiological responses to temperature change to best understand the impact of climate change on these important pollinators (Leroy et al., 2023).

Chapter 2 of this thesis considers acute individual thermal tolerance and whole-colony thermoregulation. Bee colonies are comprised of workers, drones, queens and juveniles and previous works define a "weak link" concept, theorizing that colonial members with the lowest heat tolerance will ultimately dictate the colony's overall susceptibility to heat stress (Baudier and O'Donnell, 2017). Furthermore, insect heat tolerance has been linked with ontogeny, where the developmental stages with least mobility, such as juveniles, would require higher thermal tolerances to compensate for a lack of behavioural avoidance (Bowler and Terblanche, 2008). For social bees, whose offspring are protected within an enclosed colony, nest temperatures (T_n) are maintained within narrow thermal windows (30-33°C for bumblebees; Barrow and Pickard, 1985; Heinrich, 2004; Schultze-Motel, 1991; Vogt, 1986a) to prevent developmental defects stemming from altered rearing temperatures (Groh et al., 2004; Heinrich, 2004; Jones et al., 2005; Medrzycki et al., 2010; Tautz et al., 2003; Wang et al., 2016). Maintaining T_n within this

optimal range is achieved through thermogenesis to warm the nest (Heinrich, 1976, 1974b, 1972a) and through fanning to convectively cool it down (Heinrich, 2004; Vogt, 1986a); both of which incur energetic costs (Vogt, 1986a). Therefore, I first sought to determine which caste member was the most thermally sensitive to heat, using critical thermal maxima (CTmax) to quantify the UTLs of adult workers, drones, queens as well as juvenile larvae. I predicted that the thermal tolerance of larvae would be less than their adult counterparts, emphasizing the importance of colonial thermoregulation to buffer against changes in optimal T_n. This next led to determining how effective colony thermoregulatory efforts are at maintaining optimal T_n and how energetically costly it is to the colony superorganism. To accomplish this, flow-through respirometry was conducted to quantify the metabolic outputs of colonies experiencing acute thermal stressors over a range of temperatures, while simultaneously gaging internal T_n using digital temperature loggers. I predicted that the energetic requirements of colonies under low and high thermal stress would be greater than at T_a which fall within the range of optimal T_n. Due to *B. impatiens* being a temperate-adapted species, I also expected that colonies would be most successful at thermoregulating their nests at T_a which were at or below optimal T_n.

The concept that high energetic costs are associated with thermoregulation then led to the central focus of Chapter 3, which considered the potential trade-offs between resource procurement and thermoregulation that arise for colonies experiencing bouts of chronic thermal stress. The duration of heat exposure can reduce UTLs (Maia-Silva et al., 2021) and increase mortality (Cane and Neff, 2011) for some bee species, indicating that the effects of prolonged heat events, such as a heatwave, are important to consider. Moreover, the frequency and severity of heatwave events are predicted to increase in the near future (Meehl and Tebaldi, 2004). Elevations in T_a are known to increase fanning incidence within bee colonies (Vogt, 1986a;

Wynants et al., 2021), while reducing foraging (Kwon and Saeed, 2003; Rami Reddy et al., 2015), thus leading to questions regarding the sustainability of thermoregulation over extended periods of time. Patterns in worker bee behaviours correspond to the colony's requirements (Free, 1955) and, when under both heat and nutritional stress, colonies suffer developmentally (Vanderplanck et al., 2019). Given that nutritional intake correlates to colony growth (Vaudo et al., 2018), it is imperative to determine whether chronic high T_a exposure poses a significant thermal threat such that colonies forgo resource collection in favor of thermoregulation and what the resulting impacts to colony success will be. To study this potential trade-off, I exposed *B. impatiens* colonies to chronic high T_a while quantifying foraging and fanning efforts, internal T_n as well as metrics of colony success. I predicted that T_a which exceed optimal T_n would induce a trade-off, reducing foraging while increasing fanning incidence. It was also predicted that chronic heat stress would cause thermoregulation to be unsuccessful, resulting in reduced adult emergence and offspring production with increased mortality.

A bee colony's reproductive success is primarily dependent on the amount of energy available to rear offspring (Heinrich, 2004) and thus, flight is a key behaviour in ensuring that foraging is accomplished. Previous works propose that thermal constraints on flight will limit the effectiveness of pollination (Corbet et al., 1993) and that heatwaves could limit the load carrying capacities for endothermic insects due to impaired performance (Glass and Harrison, 2022). Additionally, high T_a is associated with a reduction in foraging trips (Couvillon et al., 2010), time spent in flight (Woods et al., 2005) and likelihood to fly (Kenna et al., 2021) for various bees. Since these insects possess an insulative layer of pile to help maintain minimum T_{th} for flight, observations of reduced flight endurance at T_a greater than 25°C are thought to be linked with poor heat dissipation (Kenna et al., 2021). Bumblebees specifically, appear to overheat

when flown at 35°C as T_{th} cannot be prevented from rising towards the UTL of the individual (Heinrich, 1975) with T_{th} becoming elevated by both full syrup (Heinrich, 1975) and pollen loads, which in turn could push T_{th} towards individual UTLs (Naumchik and Youngsteadt, 2023). As such, Chapter 4 aimed to determine whether the temperate adaptation of an insulative pile layer impedes the essential behaviour of flight by hindering a bee's ability to dissipate excess heat. To do so, bee flight performance was quantified using flight metabolic output (FMR) and compared across different T_a for bees with and without their pile intact. I predicted that the absence of pile would alter the relationship between T_a and FMR for bees with and without pile, where bees flown at high T_a would have lowered FMR if pile is absent, ultimately indicating that pile removal aids in heat dissipation by reducing the overall energetic cost of flight. Additionally, FMR differs according to the size of the bumblebee (Billardon and Darveau, 2019; Darveau et al., 2014; Skandalis and Darveau, 2012) and each adult caste engages in flight at some point in their lifecycle, making it essential for foraging and reproduction. Therefore, the experiment was repeated for workers, drones and queens.

In this thesis I established the caste that is most thermally sensitive to heat, the energetic costs and success of thermoregulation at the colonial level, the chronic effects of high T_a stress on colonial success, as well as whether a metric of flight performance would be hindered by the temperate adaptation of pile. Together, these chapters help illustrate the potential physiological limitations of high temperature on both individuals and colonies of a temperate bumblebee species, *B. impatiens*.

Chapter 2: High temperature sensitivity of bumblebee castes and the colony-level costs of thermoregulation in Bombus impatiens

2.1 Abstract

Physiological thermal limits often reflect species distribution, but the role that ambient temperature (T_a) plays in limiting species within their thermal environment remains unclear. Climate change-linked declines in bumblebees, an important pollinator group, leave questions regarding which aspect of their physiology is hindered under high T_a. As a eusocial species, bumblebees utilize their ability to thermoregulate as a superorganism to maintain nest temperature (T_n) within a narrow thermal window to buffer developing larvae from developmental defects. Thermoregulatory behaviours, such as thermogenesis to warm up and fanning to cool down the nest, are energetically expensive and it is uncertain how successful large colonies are at maintaining T_n within its optimal range. Using a common bumblebee species, *Bombus impatiens*, my study first established the critical thermal limits (CTmax) of workers, queens, drones and larvae to determine which caste is most thermally sensitive to heat. I found that larvae had significantly lower heat tolerance than adults, highlighting the importance of colonial thermoregulation. I then measured the energy expenditure of large colonies under acute thermal stress (5-40˚C) using flow-through respirometry while simultaneously quantifying T_n. Colonies that experienced T_a at or below optimal T_n (≤30˚C) were successful at thermoregulation. At 35˚C and above, however, T_n increased despite high energetic costs to the colony. Together my results demonstrate that high T_a poses a risk to colonies that fail to buffer thermally sensitive larvae from changes in T_n.

2.2 Introduction

The capacity to function in variable environmental temperatures is central to animal species success and distribution. Macrophysiological patterns emerging to date indicate that measures of thermal tolerance are linked with species distribution, though this association is not straightforward. Terrestrial cold tolerance has greater latitudinal and altitudinal variation than upper thermal tolerance both on a broad scale across taxon groups (Sunday et al., 2019, 2014, 2011) and within taxa including lizards (e.g., Clusella-Trullas et al., 2011; Huey et al., 2009; Van Berkum, 1988), amphibians (e.g., Snyder and Weathers, 1975) and insects (e.g., Addo-Bediako et al., 2000; Calosi et al., 2010; Oyen et al., 2016). Other factors than thermal tolerance may be better predictors of species distribution including foraging activity thermal limits (e.g., Guo et al. 2020), growing degree day and precipitation (e.g., Tremblay et al. 2021) as well as moisture and predator-prey interactions (e.g., Amundrud and Srivastava 2020). Nevertheless, at the population-level, upper thermal tolerance may respond to local climate and habitat (Herrando-Pérez et al., 2020; Vorhees et al., 2013). Recent findings suggesting that the ability to cope with temperatures that exceed species' historical upper thermal limits explained the decline of endothermic poikilothermic bumblebees (Soroye et al., 2020) and shrinking distributions at the southern ranges of this group of bees (Kerr et al., 2015). Whether physiological properties limit their capacity to cope with environmental temperature variation remains unclear.

Other factors than thermal tolerance may be better predictors of species distribution including foraging activity thermal limits (e.g., Guo et al. 2020), growing degree day and precipitation (e.g., Tremblay et al. 2021) as well as moisture and predator-prey interactions (e.g., Amundrud and Srivastava 2020). Nevertheless, at the population-level, upper thermal tolerance may respond to local climate and (Herrando-Pérez et al., 2020; Vorhees et al., 2013). Recent findings suggesting that the ability to cope with temperatures that exceed species' historical

upper thermal limits explained the decline of endothermic poikilothermic (Soroye et al., 2020) and shrinking distributions at the southern ranges of this group of (Kerr et al., 2015). Whether physiological properties limit their capacity to cope with environmental temperature variation remains unclear.

The ability to cope with temperature variation is not easily characterized in animals such as eusocial bumblebees given their multifaceted thermal physiology. An individual's capacity to tolerate acute changes in environmental temperature can be characterized by measuring their critical thermal maxima (CTmax), a point of failure locomotion is no longer possible (Berrigan and Hoffmann 1998). Bumblebee species show variation in CTmax according to altitude (Oyen et al., 2016) as well as sociality and nesting strategy, for example cavity-nesting bumblebees are less thermally tolerant than other ground-nesting bee species (Hamblin et al., 2017). CTmax values obtained for a species are fairly robust with no changes found according to acclimation temperature, feeding status, adult age (Oyen and Dillon, 2018) or caste (workers vs. queens; Maebe et al. 2021). Bumblebee colonies also include male caste as well as developing larvae and pupae, which may represent thermally vulnerable colony members. The "weak link" hypothesis discusses how individuals with the lowest CTmax are more easily thermally stressed and thus impact colony performance (Baudier and O'Donnell, 2017). Therefore, bumblebee colonies with diverse phenotypes, and possibly variable abilities to cope with heat stress, should be considered.

The endothermic poikilothermic capacity of bumblebees provides multiple benefits for individuals and the colony. Individuals can warm up and maintain flight muscle temperature above that of the environment, making them particularly successful at foraging in colder climates (e.g., Corbet et al., 1993; Heinrich, 1974a). To achieve flight, bumblebee thorax muscles must reach approximately 30°C (Heinrich, 1974a) accomplished through wing shivering as well as

proposed non-shivering mechanisms including the use of mitochondrial substrate glycerol 3-phosphate (Masson et al., 2017) and futile cycling (Staples et al., 2004). With this capacity for thermogenesis, the secondary benefit of nest temperature (T_n) regulation arises. Social bee T_n is therefore maintained within narrow thermal windows of 34-36°C for honeybees (Medrzycki et al., 2010; Stabentheiner et al., 2021, 2010) and 30-33°C for bumblebees (Barrow and Pickard, 1985; Heinrich, 2004; Schultze-Motel, 1991; Vogt, 1986a). Deviations from these optimal nest temperatures lead to detrimental effects on individuals within a colony. For instance, reductions in pupal-stage rearing temperature are well documented to result in impairments to the dance communication, olfactory senses, and short-term memory of adult honeybees (Groh et al., 2004; Jones et al., 2005; Tautz et al., 2003; Wang et al., 2016) and elevated temperatures of 3°C can prevent pupae from emerging as adults (Groh et al., 2004). Larvae also represent a crucial stage of bee development, requiring incubation and feeding by workers to maintain their growth and development to reach the pupal stage (Heinrich, 2004). Previous works document that larvae reared under suboptimal thermal conditions experience higher adult mortality in honeybees (Medrzycki et al., 2010) as well as stunted growth and wrinkled wings in bumblebees (Heinrich, 2004). In a eusocial termite, larvae are also more sensitive to high temperature than adults with a 2 to 3°C lower CTmax, in part attributed to the more constant thermal environmental found in their subterranean nest (Mitchell et al., 1993). The effects of temperature that exceed optimal nest conditions, however, are less explored. Cook et al. (2016) provide evidence that larvae play an influential role in the thermoregulatory behaviours of adult honeybees under elevated temperatures, thus indicating that the larval stage of bee development is thermally sensitive and requires careful nest thermoregulation by the colony. The numerous negative effects on growth

and development that result from fluctuations in nest temperature, illustrate that collective thermoregulation is vital for bee colonies to maintain thermal stability within their nests.

Maintaining narrow T_n ranges involves collective thermoregulation. When T_a falls, bees facilitate conductive heat transfer towards their abdomen pressed against brood clumps (see Heinrich, 2004, 1976) resulting in incubation. An individual bumblebee queen incubating her brood at temperatures 10°C or lower have a metabolic rate similar to free flight (see Heinrich 2004). For honeybees, colony metabolic rates triple when temperatures drop from 30°C to 20°C, with bees forming tight clusters to prevent dissipation of metabolic heat as T_a cools (Kronenberg and Heller, 1982). When T_a rises, behavioural wing fanning acts to reduce colony temperatures through evaporative and convective heat loss (Heinrich, 2004; Vogt, 1986a). When T_a rises above optimal values, the incidence of honeybees fanning increases and individuals disperse to facilitate heat loss; metabolic rates simultaneously tend to decrease as temperatures approach 40°C (Kronenberg and Heller, 1982). Small bumblebee colonies (10-42 workers) exposed to temperature lower than 31°C increased the incidence of incubation behaviour and colony metabolic rate tripled below 10°C (Vogt, 1986a). Temperatures above optimal range resulted in higher percentages of workers fanning and a 20% increase in colony metabolic rate (Vogt, 1986a). The energetic burden of maintaining nest temperature in cold conditions is clear in both groups, but the energetic implication of fanning remains unclear as large honeybee colonies (1500-2500 workers) show no clear effect of warm temperatures on colony metabolic rate, and small bumblebee colonies (10-42 individuals in 2 colonies of *B. impatiens* and 1 *Bombus affinus*) show a slight increase (Vogt, 1986a). The size of the colonies and the conditions experienced (presence or absence of insulation) may impact the energetic cost of coping with warm environmental conditions.

This study's main objective was to characterize the upper temperature tolerance limit of a eusocial endothermic poikilothermic insect, *B. impatiens*, at the individual and colonial level. I first determined the relative thermal tolerance of adult colony castes, also incorporating the larval stage of development into my study to determine if a juvenile stage may present as a thermally sensitive "weak link" when compared to adults. Larvae are predicted to be more sensitive to high temperature as observed in eusocial termites (Mitchell et al., 1993), and studies used this developmental stage to successfully compare CTmax between juvenile and adult insects (e.g. Davison, 1969; Klok and Chown, 2001; Li et al., 2019). Second, the energetic costs of thermoregulation for typical-sized bumblebee colonies of 200 or more workers were measured. The energetic cost of thermoregulation was predicted to rise as T_a deviates away from optimal T_n. The presence of insulation on colony thermoregulation was also investigated as small colonies better maintained T_n in such conditions (Vogt, 1986b). The success of colony thermoregulatory efforts in maintaining T_n was monitored by measuring the temperature of the brood.

2.3 Methods

2.3.1 Bumblebee colonies and holding conditions

The Common Eastern bumblebee, *Bombus impatiens*, is a native underground nesting species with a wide North American distribution (Colla et al., 2014). Commercial colonies of this species were purchased from Biobest Canada Ltd. (Leamington, ON, Canada) to use in both CTmax and whole-colony thermoregulation experiments. All colonies were contained in the supplier's housing boxes in a room maintained at approximately 25°C on a 12h:12h light:dark photoperiod. BIOGLUC® sugar solution from the supplier was available *ad libitum* to colonies and pollen was provided every second day. Individuals used in CTmax experiments were

sampled randomly from multiple colonies housed within the lab. An additional ten colonies were used specifically for whole-colony respiration testing (see Supplementary material 2.8).

2.3.2 Individual CTmax

CTmax was determined using the thermolimit respirometry method (Lighton and Turner, 2004). Mature individual workers, progeny drones and queens, as well as similar-sized late instar larvae that were removed from individual pollen cell (see Supplementary material 2.8), were collected from multiple colonies maintained in the laboratory and weighed on an analytical balance prior to being transferred into a 20 ml glass respirometry chamber. The potential effect of colony was tested for a subset of individuals where no effect was found and not further considered in the analyses (Supplementary material 2.8). The chamber was placed in an activity detector to monitor bee movements via infrared detection [AD-1; Sable Systems International (SSI), Las Vegas, NV, USA], which in turn, was contained within a temperature-controlled cabinet (PTC-1, SSI). A copper coil was used to allow incurrent air from the flow-through respirometry system to equilibrate with the temperature inside the cabinet prior to entering the respirometry chamber. A FOXBOX Respirometry System (SSI) was used to push air, scrubbed free of water using a drierite column, at a rate of approximately 50 ml min^{-1}. The CO_2 production rate of the animal was measured.

A temperature ramping protocol was used to identify CTmax and each adult bee was subjected to the following temperature sequences using a PELT-5 temperature control unit (SSI): a 20 min soak period at 25°C to allow the bee to equilibrate within the respirometer; a ramping period of 0.25°C min^{-1} until a maximum of 56°C was reached; a subsequent soak period at 56°C for 25 min; a final ramping phase to reduce temperature by 2.0°C min^{-1}. The total ramping protocol elapsed for 190 min. Thermolimit respirometry was also used to determine CTmax of

larvae where movement could also be detected using the AD-1 infrared detector. Larvae were not as active as adults and a 10-minute initial equilibrium phase was used. Respirometry, temperature and activity outputs were obtained using an analog to digital converter (UI2, SSI) and acquired using Expedata (SSI). Activity and respiratory CTmax were estimated using the absolute difference sums (ADS) method described by Lighton and Turner (2004). This approach led to difficulties identifying respiratory CTmax of adult castes (see also Vorhees and Bradley, 2012 and Appendiz A for details and examples), so an alternative approach was used, the cessation of spiracular activity (CSA), known to yield indistinguishable CTmax values in other insect species (Vorhees and Bradley, 2012). For larvae, only activity CTmax could be determined as respirometry traces did not provide the usual cues (see Supplementary material 2.8); activity CTmax is commonly used to determine larval thermal tolerance (Cooley et al., 2016; Li et al., 2019; Mitchell et al., 1993). Furthermore, given that respiratory- and activity-CTmax (Kovac et al., 2014; Lighton and Turner, 2004; Vorhees et al., 2013) or CTmin (MacMillan et al., 2012) are highly correlated and not significantly different, activity-CTmax alone remains a relevant metric for quantifying larval thermal tolerance.

2.3.3 Whole colony thermoregulation

Five colonies were used for experiments where the insulating cotton batten supplied with the colony was removed for visual observation of the colony after each experiment. Removing the insulation disturbed the nest and colonies were allowed two weeks to recover. An additional set of five colonies were used with the insulating cotton batten remaining in place. These colonies were used for the experiment five days after arrival to obtain colonies approximately the same size of those used for the uninsulated group. Colonies sizes were measured after experimentation and averaged 316±17 (SE) workers each.

To record internal nest temperature, a thermochron iButton® (iButtonLink Technology, Whitewater, WI, USA) was placed among the brood cells with developing larvae on the day of experimentation for uninsulated colonies. For insulated colonies, this was done on the day of delivery to minimize the disturbance to the hive. Internal colony temperature was sampled at 2-minute intervals for the duration of the experiment.

The metabolic rate of the colony containing both adults and brood was measured using flow-through respirometry. A single colony within its housing container and accompanying sugar solution was placed in a 30 L plexiglass chamber. Air was pulled from the chamber using a total of three pumps connected in parallel: two SS-3 Gas Analyzer Subsamplers (SSI) with flow rates of approximately 2 L min^{-1} and one FlowBar 8 Multichannel Mass Flow Meter (SSI) with a flow rate of approximately 2.5 L min^{-1}. Outflow was then combined, totalling between 6-6.6L min^{-1}, which was confirmed using a rotameter, and subsampled at 0.2 L min^{-1} to analyze CO_2 concentration (ppm) using Li-7000 CO_2/H_2O analyzer (LI-COR, Inc. Lincoln, NE, USA).

Temperature treatments were obtained by placing the respirometry chamber containing the bee colony within a temperature-controlled incubator (VWR International). Each single colony underwent 6 temperature treatments, one temperature per day, in the following sequence: 25, 5, 35, 15, 30 and 40°C. This sequence was chosen to vary the degree of thermal challenge from day to day, ending with the most challenging treatment at 40°C that induced damaged to the colony. The temperature was held constant for a total of 4 hours during respirometry measurements. The first 60 minutes of each measurement were considered an equalization period for colonies to adjust to experimental conditions and was not included in the analysis. Following the 40°C treatment, the entire colony was sacrificed by placing it in a -80°C freezer, the number of workers were counted and their total mass measured.

Respirometry measurements were collected using Expedata Analysis Software (SSI) and colony metabolic rate was expressed as the rate of CO_2 production per gram (VCO_2 ml $hr^{-1}g^{-1}$). Colony metabolic rate was expressed as the maximum rate corresponding to the 10 min period with the highest CO_2 production, in addition to the average colony metabolic rate per hour over the last three-hours of measurement. Average T_n was calculated over the last three-hours of measurement as well.

2.3.4 Data analysis

Statistical analyses were performed using R (R Core Team, 2014) and values are reported as mean ± standard error of the mean. Differences in CTmax measurements among castes were tested using ANCOVAs with body mass as a covariate. The final model presented in the results was simplified by first removing the non-significant interaction term. The simplified model including castes and body mass had non-normally distributed residuals that could not be resolved using data transformations. I confirmed that body mass had no effect on CTmax within each caste through linear regressions and further removed this term from the model. The effect of castes on CTmax was finally tested using a nonparametric Kruskal-Wallis test given that the normality assumption could not be met. The difference in between activity and respiratory CTmax was determined using the Wilcoxon signed-rank exact test.

Whole-colony data analyses were conducted using the lme4 and lmerTest packages (Bates et al., 2015; Kuznetsova et al., 2017) to perform linear mixed models. To accommodate the assumptions of normality and homogeneity of variance, maximum and mean hourly colony metabolic rate were log-transformed, while nest temperature was raised to the third power. Linear mixed models were conducted to test for differences in colony maximum metabolic rate and nest temperature using the REML estimation method where colonies were considered as a

random variable and ambient temperature and insulation factor were fixed effects. Mean hourly metabolic rate was also tested for changes over time where hour (1st, 2nd and 3rd) was further added as fixed effect in the model. Tukey pairwise comparisons were performed for each analysis.

2.4 Results

2.4.1 Bumblebee caste CTmax

Activity-CTmax differed between adults and larvae (Kruskal-Wallis χ^2=31.767, df=3, P<0.001; Fig. 2.1A) with higher activity-CTmax for adult castes (drone: 45.65 ± 0.37˚C (n=21), queen: 46.39 ± 0.32˚C (n=19), worker: 46.19 ± 0.14˚C (n=21) and approximately 2.5˚C lower for larvae (43.44 ± 0.32˚C (n=20)). Adult casts did not differ in activity-CTmax values (P≥0.648), and they were all significantly higher than larvae (P≤0.001).

Respiratory-CTmax that could be determined for workers, drones and queens was found to be significantly influenced by caste (Kruskal-Wallis χ^2=9.4371, df=2, P<0.001; Fig. 2.1B) where drones (47.18 ± 0.27˚C) had significantly higher respiratory-CTmax compared to workers (46.53 ± 0.13˚C; P=0.008) but not to queens (46.57 ± 0.27˚C; P=0.128). Respiratory-CTmax was greater than activity-CTmax in drones (Z=3.98, P<0.001) and workers (Z=3.98, P<0.001), but not for queens (Z=0.282, P=0.78).

2.4.2 Whole-colony thermoregulation

2.4.2.1 Colony size

The mean number of individuals in colonies measured at the end of the experiment differed between the uninsulated colonies averaging 284±15 workers and the insulated colonies with an average of 348±22 workers (t(7.17)=2.44, P=0.044). This difference in size was due to one colony that was substantially larger than the other colonies with 404 individuals; analysis

conducted without this large colony show no difference between groups ($t(5.71)=1.93$, $P=0.105$). All further analyses were done both with and without this large colony to assess its influence on the results.

2.4.2.2 Maximum whole-colony energy expenditure

Maximum colony metabolic rate was affected by T_a and the presence of insulation in the colony (Fig. 2.2; T_a: $F_{5,45}=16.88$, $P<0.001$; insulation: $F_{1,8}=6.24$, $P=0.037$); the interactive effect between T_a and insulation was not significant and was removed from the model. The maximum metabolic rate of insulated colonies was approximately 15% lower than uninsulated colonies ($P=0.037$). When comparing the maximum metabolic rate of colonies at the various T_a (Fig. 2.2), the lowest values were observed at 25°C and 30°C and did not differ from one another ($P=0.927$). As T_a decreases from 25°C down to 5°C and 15°C, the maximum metabolic rate increased by approximately 26% and 31%, respectively ($P\leq0.001$). Similarly, when T_a rose from 30°C up to 35°C and 40°C, the maximum metabolic rate also increased by around 22% and 36%, respectively ($P\leq0.001$). The absence of the large, insulated colony did not alter the overall statistical outcome of maximum colony metabolic rate (T_a: $F_{5,40}=13.815$, $P<0.001$; insulation factor: $F_{1,7}=6.39$, $P=0.039$).

2.4.2.3 Change in whole-colony energy expenditure over time

The average hourly metabolic rate of the colonies showed a significant interaction between the temperature treatment and the hour measured, and the presence of insulation was not significant and removed from the model (Fig. 2.3; T_a: $F_{5,9}=31.47$, $P<0.001$; hour: $F_{2,108}=0.51$, $P=0.603$; $T_a\times$hour: $F_{10,108}=40.37$, $P<0.001$). At T_a of 5-30°C, there were small differences in the metabolic rate over the three consecutive hours of measurement (15°C, 1st to 3rd hour: $P=0.044$; 30°C, 1st to 3rd hour: $P=0.002$). Larger changes were observed for colonies exposed to 35°C,

where it increased by 17% from the 1st to the 2nd hour (P<0.001), 19% from the 2nd to 3rd hour (P<0.001). Colonies exposed to 40°C showed a continuous decline in metabolic rate falling by 17% from the 1st to 2nd hours (P<0.001) and 12% from the 2nd to 3rd hours (P<0.001). Removing the large, insulated colony from analysis did not change the overall results for hourly metabolic rate (T_a: $F_{5,136}$=52.125, P<0.001; hour: $F_{2,136}$=0.1393, P=0.870; T_a-hour: $F_{10,136}$=6.593, P<0.001).

2.4.2.4 Internal colony nest temperature

T_a: $F_{5,53}$=106.852, P<0.001; insulation: $F_{1,53}$=4.409, P=0.041); but the interactive effect between T_a and insulation was not significant and removed from the model. Despite a significant overall effect, pairwise analysis revealed that mean T_n of insulated colonies was not different from uninsulated colonies (P=0.069). Excluding the large, insulated colony from the analysis slightly alter the statistical outcomes of colony T_n where the overall effect of insulation on T_n became insignificant (T_a: $F_{5,47}$=91.882, P<0.001; insulation: $F_{1,47}$=2.431, P=0.126). Examining T_n between various T_a conditions, it did not differ between 15, 25 or 30°C with T_n of 32.3, 32.0 and 33.0°C, respectively (P>0.05); these temperature values falling within the range of optimal T_n (Fig. 2.4). When T_a was reduced to 5°C, T_n declined by approximately 3.0°C when compared to T_n at 30°C (P≤0.001). At T_a of 35°C and 40°C, T_n increased by 2.0°C and 6.9°C, respectively (P≤0.003). At 35°C, both insulated and uninsulated colonies experienced T_n that was dependent on the hour of measurement (uninsulated, hour: $F_{2,8}$=53.51, P<0.001; insulated, hour: $F_{2,8}$=151.40, P<0.001) where T_n increased over each hour of exposure (P<0.001) for a total increase from the first to the third hour of 1.5 and 1.9°C, respectively. Similarly, at 40°C, T_n was also dependent on hour (uninsulated, hour: $F_{2,8}$=317.79, P<0.001; insulated, hour: $F_{2,8}$=73.53, P<0.001) where T_n increased by 1.5 and 1.8°C, respectively.

2.5 Discussion

Current efforts attempt to relate physiological thermal limits of species with their thermal environment and ultimately geographical distribution. However, for social endothermic poikilotherms, such as bumblebees, understanding their response to thermal variation within the environment involves investigation into both individual- and colony-level thermal tolerance. The present study first assessed the acute upper thermal tolerance limits of *B. impatiens* castes and developmental stages in order to identify which members of a colony were the most thermally sensitive. I show that adult castes have similar CTmax and the larvae are the most sensitive, highlighting the importance of T_n regulation. The energetic costs of nest thermoregulation for peak-season sized colonies increase as T_a decreased and increased away from optimal T_n range, the presence of insulation reducing those costs. At high T_a, colony metabolic rate changed substantially over time indicating challenges in thermoregulating. Colonies experiencing high T_a failed to regulate T_n that increased to T_a. Together, I here show the central challenges of regulating nest temperature at high environmental temperature and protecting the thermally sensitive larvae.

2.5.1 Individual CTmax

The activity-critical thermal maximum of *B. impatiens,* more specifically the temperature at which muscular control was lost, was found to be on average 46.03 ± 0.18°C for adults. The respiratory-CTmax showed very similar values, although slightly higher for workers and a more pronounced difference observed for drones. For the larval developmental stage, only activity-CTmax could be determined and larvae are more susceptible to high temperature than adults with CTmax values approximately 2.5°C lower. These findings for adults are consistent with a previous study that determined *B. impatiens* workers to have a CTmax of 46.07°C using the

righting response (Hamblin et al., 2017). Conversely, other works found differing values for this species, ranging from 50 to 53°C for workers and queens when using the onset of spasm methodology (Maebe et al., 2021; Oyen and Dillon, 2018). The methodology used to determine thermal limits, including the method of assessing end-point temperature (e.g., thermolimit respirometry, onset of spasms or righting response) and ramping rate, is well known to affect the values obtained (see Gonzalez et al., 2022; Lighton and Turner, 2004; Terblanche et al., 2007), yet the merit of CTmax testing lies within the relative differences observed between individuals or groups tested. For example, the lack of difference in CTmax observed between workers and queens has also been reported on other species of bumblebees (Maebe et al., 2021), while neither age of the adult, mass, feeding or acclimation temperature significantly alter the temperatures which bees are capable of tolerating (Gonzalez et al., 2020; Oyen and Dillon, 2018). Having multiple metrics of CTmax also appears useful to detect the central emerging patterns as different measures sometimes yield slightly different outcomes such as drones that differ from workers for respiratory-CTmax but not when activity-CTmax is used. Furthermore, interspecific comparisons show that bees which nest in cavities have lower thermal tolerances versus species that nest in stems or in the ground (Hamblin et al., 2017), indicating that the thermal conditions experienced help govern insect thermal tolerance, just as low altitude species and populations of bees have been found to possess higher upper thermal limits than those found at higher elevations (Gonzalez et al., 2020; Oyen et al., 2016). Not only that, but CTmax also demonstrates predictive power for determining insect responses to warming both experimentally and within communities (Diamond et al., 2012; Hamblin et al., 2017) providing support for its relevance in understanding species' responses to environmental thermal challenges.

Baudier and O'Donnell (2017) indicate that most studies do not account for the negative effects temperature poses on the most thermally sensitive members of insect colonies and call for an inclusion of minimal thermal tolerance when considering a species' vulnerability to thermal stress. This "weak link" hypothesis is discussed in the context of worker castes only, but it is also important to consider additional castes or developmental stages as potential weak links within a species. Previous works review how juvenile, young adults and senescent insects differ in their thermal tolerance ranges (Bowler and Terblanche, 2008), emphasizing the importance of also including juveniles within studies on social insect thermal tolerance. Larvae have previously been used to compare the thermal tolerance of a juvenile stage to that of adults. For example, kelp fly larvae are more heat tolerant likely due to limited behavioural response to temperature resulting from restricted mobility (Klok and Chown, 2001). Similarly, wood wasp larvae are more tolerant than adults due, in part, to their emergence time during the year (Li et al., 2019). In contrast, the beetle larvae studied in Vorhees and Bradley (2012), demonstrate similar heat tolerances as pupae, yet both life stages are less tolerant than adults, potentially in response to the microclimate conditions experienced during each life stage. The reduced thermal tolerance of larvae observed in subterranean species such as termites may also be reflecting the nest microclimate conditions (Mitchell et al., 1993). Thus, in social hymenopterans, where colonies care for offspring and create an ideal thermal microclimate within the nest, larvae represent a vulnerable stage in the life cycle of species like the bumblebees tested within my study, and additional developmental stages should be included for a comprehensive assessment of the "weak links" of the species. As such, colony thermoregulation is vital in the protection of thermally sensitive larvae, but its associated costs and success are dependent on the T_a encountered.

2.5.2 Whole-colony thermoregulation

2.5.2.1 Maximum whole-colony energy expenditure

The energy expenditure of colonies of *B. impatiens* of sizes corresponding to peak season increases as T_a deviates away from the range of optimal T_n, at both the lower and upper thermal extremes. Colonies that experience 25°C and 30°C are within or near the range of optimal T_n and have the lowest colony metabolic rate as they likely expend less thermoregulatory effort to maintain nest conditions. This is consistent with the findings of Vogt (1986a) using small colonies of two bumblebee species.

The change in energy expenditure of bee colonies is likely attributed to increased incidence of behaviours associated with communal thermoregulation, especially when exposed to low temperatures. Honeybees cluster to incubate as T_a drops, elevating colony energy expenditure (Kronenberg and Heller, 1982). In bumblebees, a 50-80% incidence of incubation was reported when T_a dropped below 20°C, accompanied by an approximately 3-fold increase in energy consumption as T_a reached 3°C (Vogt, 1986a). In comparison, my larger colonies experienced an increase in metabolic rate of 1.3-1.5 times when T_a fell to 5-15°C. Thus, colony size probably has a strong influence on the cost of thermoregulation and remains to be studied systematically. My study using colonies of over 200 workers shows a reduced cost compared with Vogt (1986a), indicating the likely importance of thermal conductance with more individuals thermoregulating and possibly clustering at low temperatures (Rivière, 2012). Furthermore, insulated colonies had overall reduced metabolic rates in comparison to colonies lacking insulation. In colder conditions, insulation likely reduces heat loss and colony energy expenditure associated with thermogenesis.

High T_a also poses an energetic challenge and colonies expend more energy in such conditions, which may in part be due to mechanisms mobilized to dissipate excess heat. My large

colonies demonstrated a 22-36% increase in energy expenditure when T_a was greater than 30°C. Vogt (1986a) also described a 20% elevation in metabolic rate when T_a rose from 31 to 39°C. At T_a of 35 and 40°C, T_n increases and reaches T_a, which can impact the resting metabolic rate of individuals that cannot regulate their body temperature, such as developing brood. The elevation in resting metabolic rate with temperature documented in the honeybee shows that it is not straightforward and linear (Kovac et al., 2007) , but using a simple linear Q10 effect of 2 to 3 to approximate the impact of increased temperature predicts that larvae going from 33 to 35°C would lead to a 20 to 30% increase in metabolic rate. This proportional increase coincides with the elevation in colony metabolic rate observed, but the presence of a large number (>200) of endothermic workers in my colonies must, to some extent, contribute to the increased colony metabolic rate. Small bumblebee colonies will allocate between 20 and 100% of their adult workforce towards the fanning behaviour (Vogt, 1986a) in an attempt to thermoregulate the nest. The fanning behaviour involves flight muscle contraction that can generate heat as a by-product and contribute to temperatures experienced within the colony. Stabentheiner et al. (2021) showed the complex and dynamic changes observed in colonial thermoregulation in large honeybee colonies, where at high T_a (40°C) many workers leave the nest to reduce overall heat production. The contribution of fanning to the colony energy expenditure should be assessed further to evaluate the temperature range at which it is most effective where heat dissipation is greater than the heat gain associated with muscle contraction.

Colonies exposed to high T_a exhibit changes to their energy expenditure over time, pointing to the importance of fanning behaviour engaged by adults. When exposed to 35°C, a temperature slightly above optimal T_n, colonies increase their metabolic rate over a three-hour period (Fig. 2.3). In contrast, the highest tested temperature of 40°C, imposes a cost that does not

appear sustainable because colony metabolic rate decreases over time. My results coincide with observations in the literature where fanning incidence in honeybee and bumblebee colonies increases at temperatures that exceed 30°C (Kronenberg and Heller, 1982; Vogt, 1986a)**.** The recruitment of fanners occurs when individual worker thermal thresholds are surpassed for the behaviour to initiate. When heated to 30°C, *B. terrestris* exhibit thresholds between 27 and 28.7°C (Weidenmüller, 2004), yet individuals may each have differing thermal triggers, resulting in either an immediate or delayed response (Jandt and Dornhaus, 2014). The increase in metabolic rate over time observed at 35°C for my colonies may be indicative of more individual thresholds being triggered as the thermal threat persists. Accordingly, workers were observed vigorously fanning at the end of 35°C trials. Nonetheless, this is confounded by the concurrent increase in nest temperature also observed over the three-hour measurement period that may affect the brood metabolic rate. The contrasting pattern observed at 40°C, where T_n also rose gradually over the three-hour period, helps evaluate the contribution of fanning to the whole-colony energy expenditure. Despite the gradual increase in T_n, the whole-colony metabolic rate decreases gradually. A large number of individuals likely reached their thermal thresholds early, coinciding with initial high metabolic rates within the first hour. As T_a of 40°C persisted, the hive structures sustained heat damage with workers abandoning their fanning behaviour and attempting to leave the nest box, thus decreasing the colony metabolic rate over time. This reduction in colony metabolic rate over time, despite an increase in T_n over the same period, supports the contribution of the fanning behaviour to the colony energy expenditure. The T_n encountered which matched the T_a of 40°C, appears beyond the capacity of collective thermoregulation and is approaching the thermal limits of larvae found via activity-CTmax.

Bumblebee workers in a colony collectively act as a superorganism to thermoregulate T_n for the larvae, incurring energetic costs. When subjected to various T_a conditions, colony metabolic rates create a pattern that is reminiscent of vertebrate endothermic homeotherms. The optimal T_n range of colonies resembles the thermoneutral zone where the metabolic costs to regulate body temperature is minimal. For the present study, this range corresponds to 25-30°C where the lowest metabolic rates were observed. Below the thermoneutral zone, endothermic homeothermic animals must increase metabolic rates to maintain body temperature. The increase in energy expenditure above the 25-30°C thermoneutral zone can be due to the combined effects of increased nest temperature on ectothermic individuals within the colony, mostly the brood, but also mechanisms mobilized to thermoregulate, such as fanning. The changes in colony metabolic rate observed over the three-hour measurement period suggest that fanning contributes to the observed increase in energy expenditure at 35 and 40°C. The increase in colony metabolic rate over consecutive hours at 35°C is more substantial than the increase in nest temperature observed over the same period, suggesting greater investment in fanning efforts. Furthermore, at 40°C there is also an increase in nest temperature over the three consecutive hours of measurements, but colony metabolic rate shows a decrease over the same time period, indicating that colony metabolic rate is decoupled from nest temperature. I therefore suggest that a large part of the increased energy expenditure at 35 and 40°C is associated with the fanning behaviour. These comparisons highlight the importance of social cooperation in bee colonies, especially in consideration of the apparently vulnerable larvae within the nest.

2.5.2.2 Internal colony nest temperature

Overall, bumblebee colonies were successful at maintaining T_n when acute thermal challenges did not exceed that of optimal T_n, 30-33°C (*Bombus lapidarius* 31.7±1.0°C, Schultze-

Motel 1991; *B. impatiens* 28-32°C, Vogt, 1986a; *B. terrestris* 32.3±0.4°C, Weidenmüller et al., 2002). At 25 and 30°C, optimal T_n was achieved with the least energetic effort, while at 5°C and 15°C, this energetic cost rose due to incubation efforts as workers attempted to buffer against a drop in T_n. The capacity of *B. impatiens* colonies to maintain optimal T_n at low T_a further exemplifies how robust bumblebees are against low T_a. For example, the arctic species (*Bombus polaris*) possess exceptional thermoregulatory capacities that enable colonies to maintain T_n at 35°C when T_a falls to 7.5-11°C in the summers (Richards, 1973).

The presence of insulation also had an overall effect on T_n where insulated colonies were about half a degree warmer than uninsulated colonies, although this effect appears to be largely influenced by one larger colony in my study. Nevertheless, other work shows a reduced T_n value in the absence of insulation and also a reduced number of workers and drones by the end of their lifecycles (Vogt, 1986b). Moreover, insulation clearly lowers the overall energetic costs of thermoregulation, reducing the incidence of brood incubation necessary for maintaining T_n (Vogt, 1986b). My study simulates surface or aboveground nests with and without insulation. Underground nesting sites appear to be the most common across subgenera of bumblebees, including being the preferred nesting strategy of *B. impatiens* (Colla et al., 2014), though surface-level and aboveground nests are also frequented in both wild and artificial or human-made nest sites (Liczner and Colla, 2019). Simulating aboveground nesting sites also holds relevance given that bumblebees, like the *B. impatiens* colonies used in my study, are commercially available for use in greenhouse and garden pollination (Velthuis and Van Doorn, 2006). Nests located aboveground experience wider fluctuations in T_a as demonstrated in a study on *B. impatiens* using empty, artificial nests (Mullan, 2022), and choosing a thermally optimal nesting site implies success in the rearing of bee offspring (Potts and Willmer, 1997; Vickruck

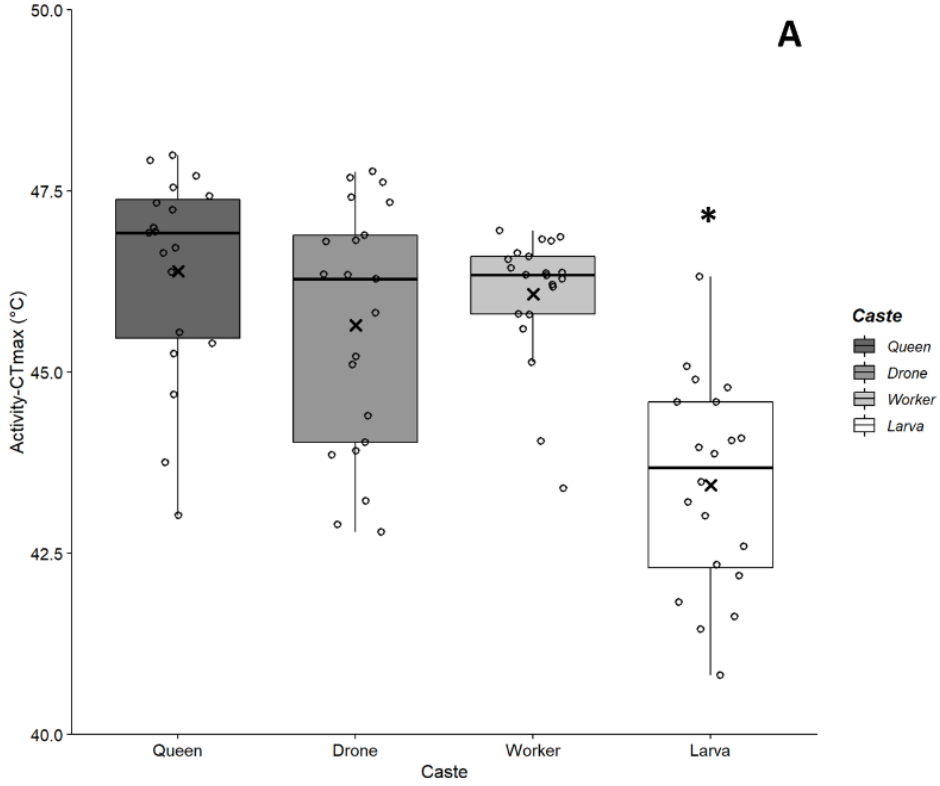
and Richards, 2012; Wuellner, 1999). Therefore, understanding how colonies may buffer temperature fluctuations, which can vary widely according to colony size and species (Gradišek et al., 2023), provides insight into whole-colony responses to thermal stress in common nesting locations. On the other hand, high T_a impedes a bumblebee colony's ability to maintain optimal T_n. At 35 and 40°C, T_n was equivalent to T_a, demonstrating that the high energetic costs associated with these temperatures do not result in successful thermoregulation. Similarly, Vogt determined that despite over 50% of the available workforce fanning within small colonies, optimal T_n could not be maintained and was consistently 1 to 2°C higher than T_a between 33 and 39°C (Vogt, 1986a). As such, fanning efforts cannot successfully dissipate sufficient heat through evaporative or convective means to lower T_n, ultimately posing potential consequences to the individuals within a colony.

2.6 Conclusions

The present study, as well as previous works investigating the physiological and behavioural consequences of changes in T_a and T_n, underscore the importance of understanding how both individuals and colonies respond to varying thermal conditions. Responses to temperature differ between individuals and the colony superorganism. The lower thermal tolerance found for larvae, emphasize the need for colonies to thermoregulate against changes within the thermal environment, however, thermal challenges where T_n rises above optimal, may prove to be energetically costly and unsustainable for colonies. Sustainability comes into question when considering that thermal stress decreases brood maintenance (Vogt, 1986a) and negatively impacts foraging activity (e.g., Hemberger et al., 2023; Kwon and Saeed, 2003), leaving fewer individuals available for these essential tasks. Given that both nutritional and thermal stress leads to reduced colony growth (Vanderplanck et al., 2019), elevated energetic

costs under high T_a in addition to the inability to successfully thermoregulate at high temperatures, may result in additional strain on colonies reducing growth or causing colony failure if thermally challenging conditions persist over longer periods of time.

2.7 Figures


A
50.0
47.5
45.0
42.5
40.0
Activity-CTmax (°C)
*
Queen
Drone
Worker
Larva
Caste
Caste
Queen
Drone
Worker
Larva

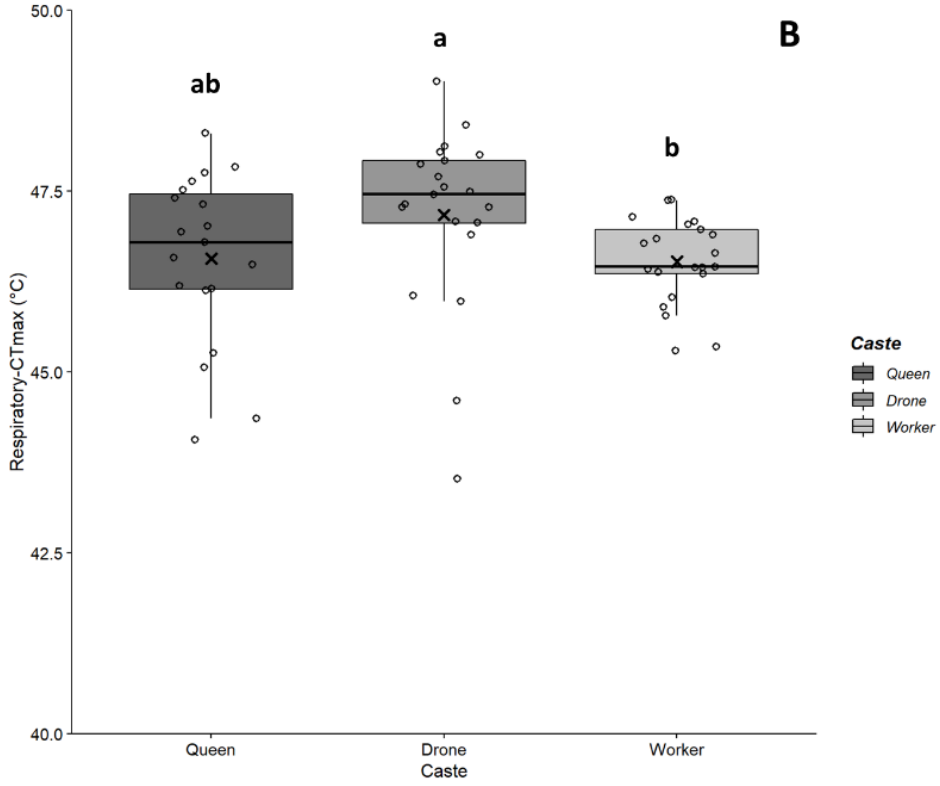

B
50.0
47.5
45.0
42.5
40.0
Respiratory-CTmax (°C)
ab
a
b
Queen
Drone
Worker
Caste
Caste
Queen
Drone
Worker

Fig. 2.1. CTmax comparisons across bumblebee (*B. impatiens*) castes. Individual queens, drones, workers and larvae were ramped at 0.25°C min^{-1} to determine their critical thermal maxima (CTmax). A) The temperature at which muscular control is lost (activity-CTmax) is significantly lower for larvae when compared to all adult castes (*$P \leq 0.001$). B) The temperature at which spiracular control is lost (respiratory-CTmax) differ among adult castes with drones having higher respiratory-CTmax than workers as indicated by different letters ($P=0.008$). In both panels, box plots represent the 25^{th} and 75^{th} percentiles, the error bars the range of values, the black bar across indicating the median value and the x representing the mean.

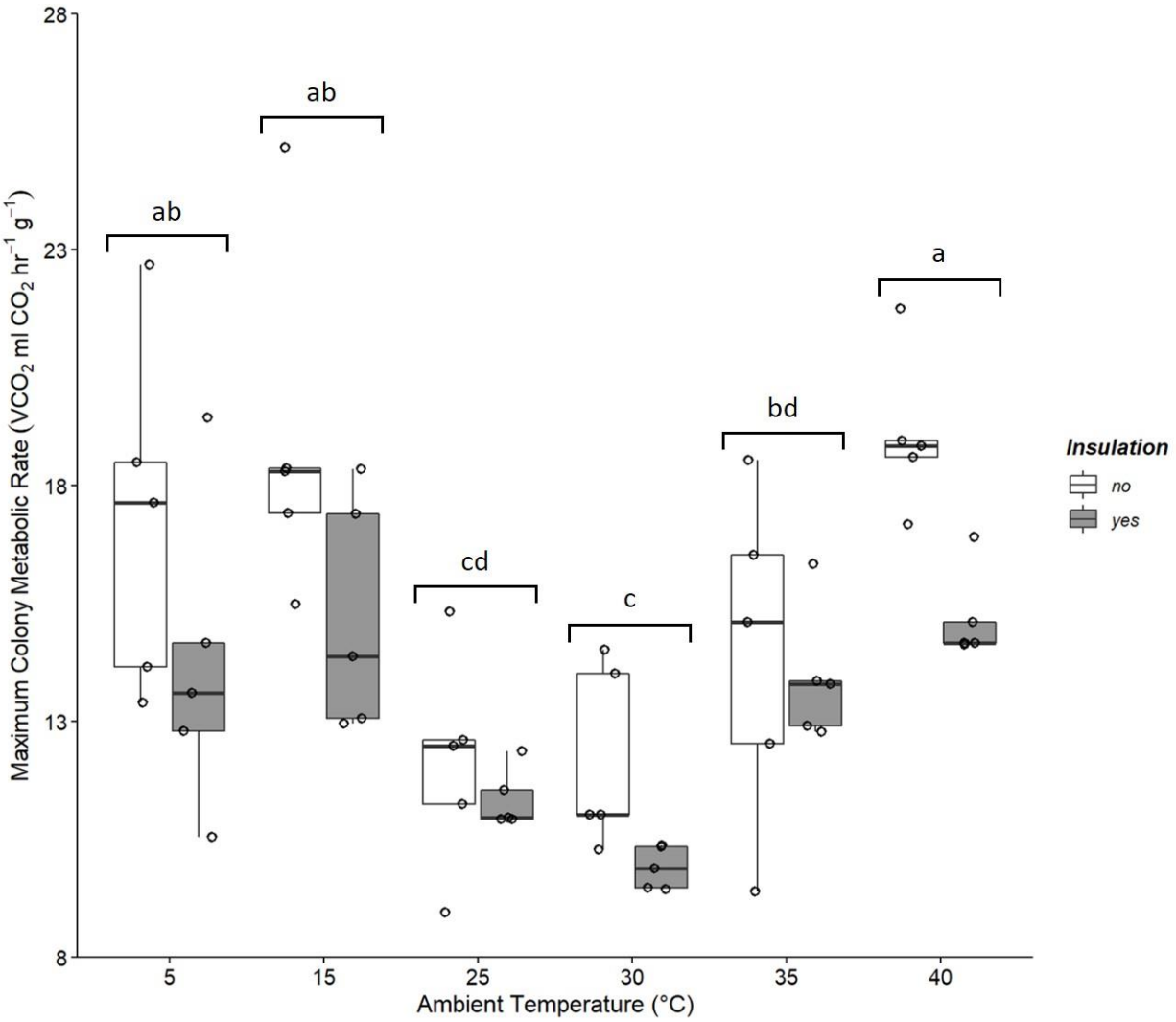


Fig. 2.2. Ambient temperature affects the colony metabolic rate of insulated and uninsulated *B. impatiens* colonies. Flow-through respirometry was used to assess the maximum metabolic rate of insulated and uninsulated bumblebee colonies during a three-hour exposure to various ambient temperatures (T_a). Colony metabolic rate increased significantly above and below Ta of 25 and 30ºC ($P \leq 0.005$). Metabolic rates differed between T_a groups (n=10) which do not share letters (a-d). Colonies with insulation have lower colony metabolic rate ($P=0.037$). Boxplots each represents percentiles, with the black bar across indicating the median value. Also present are the individual data points for each colony tested.

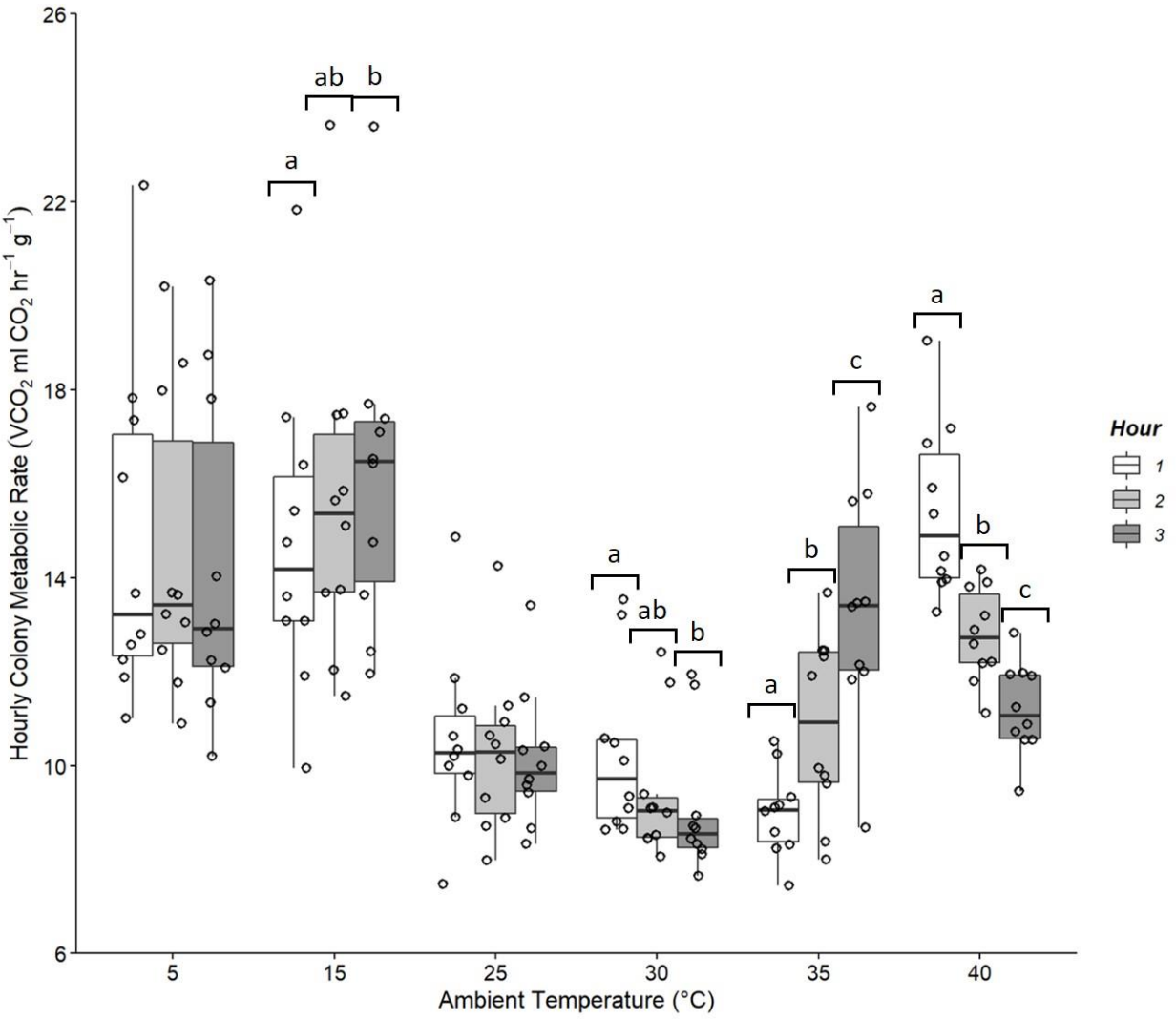


Fig. 2.3. Colony metabolic rate changes over the duration of exposure at high ambient temperature in *B. impatiens*. Flow-through respirometry was used to assess the metabolic rate of whole bumblebee colonies during a three-hour exposure to various ambient temperatures (T_a). At 30°C and below, colony metabolic rate varies little if at all over the three-hour exposure period (15°C, P=0.044; 30°C, P=0.002). At a high T_a of 35°C, colony metabolic rate increases during each hour of exposure (P<0.001), whereas at 40°C, colony metabolic rate is initially elevated but decreases after each hour of exposure (P<0.001). Significant differences between hours are presented with the letters a to c within each temperature group. Boxplots represent percentiles with the black bar across signalling the median value. Also present are the individual data points for each colony tested. A sample size of n=10 colonies was used for each group.

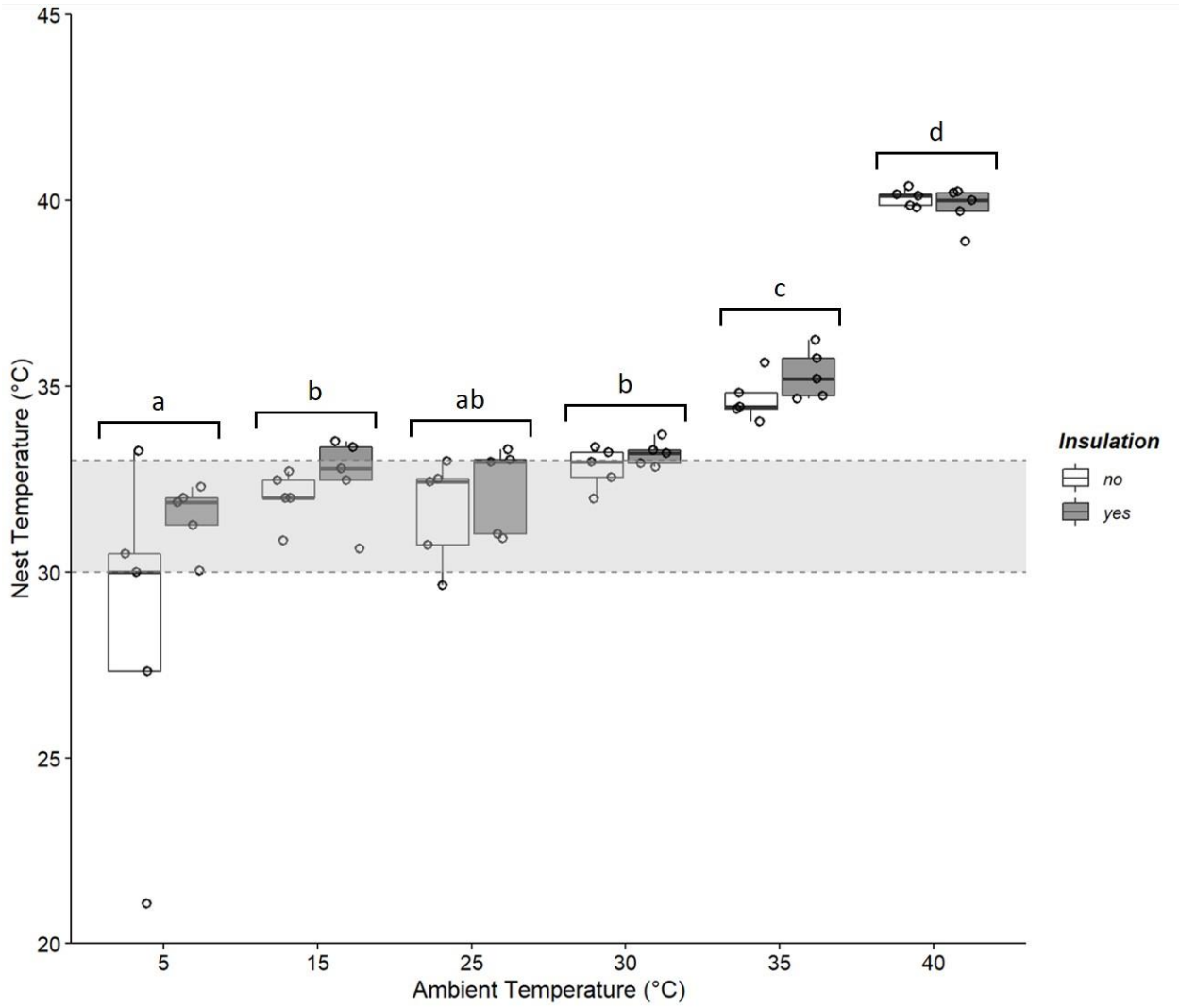


Fig. 2.4. Nest temperature of *B. impatiens* colonies, with and without insulation, exposed to various ambient temperatures. Nest temperature (T_n) represents the average temperature maintained by *B. impatiens* colonies calculated across a three-hour exposure period to various ambient temperatures (T_a) for uninsulated (n=5) and insulated (n=5) colonies. An optimal T_n of 30-33°C (see section 1), represented by the horizontal shaded area on the graph, is achieved for colonies exposed to 30°C and below. At T_a which exceed this idea range (35°C and 40°C), T_n rose to ambient, being significantly higher than T_n achieved at 30°C in both cases ($P \leq 0.003$). Insulated colony T_n was not significantly different than uninsulated T_n ($P=0.069$). Boxplots each represents percentiles, with the black bar across indicating the median value. Also present are the individual data points for each colony tested. Nest temperature means which differ between T_a groups who do not share letters (a-d).

2.8 Supplementary material

2.8.1 Larvae used for CTmax measurements

Bumblebee larvae undergo 4 instars during development (Tian and Hines, 2018). I selected larvae in later developmental stages by choosing those that originated from individual pollen cells (Fig.S1). Earlier-stage instars are contained within larger communal pollen cells (Heinrich, 2004) while pupae are found in rigid, oval-shaped cocoons (Fig. S2.1; Heinrich, 2004), thus making these developmental stages visually distinct. Furthermore, instar development can be distinguished through the mass of the larva (Cnaani et al., 2002, 2000), enabling us to identify that the larvae used in CTmax experiments corresponded to either late-stage 3^{rd} instar or 4^{th} instar of development. Larvae sampled had mean body mass of 0.262 g ± 0.020 (SE).

Figure S2.1. Selection of larvae used in thermolimit respirometry for CTmax determination. The larvae extracted for CTmax determination originated from individual larva pollen cells (IL). Early instars are found and feed within a communal cluster (LC). Individual larvae will later separate into round pollen cells (IL) where they continue to feed until spinning a rigid, oval-shaped cocoon (P) for pupation (Heinrich, 2004).

2.8.2 Individual CTmax determination

Thermolimit respirometry data were analyzed using Expedata (Sable Systems International, Las Vegas, NV, USA) using the procedure described by Lighton and Turner (2004). The absolute difference sum (ADS) was calculated first for the activity and CO_2 production. The resulting ADS were then used and regressed against time to obtain the residual values that were saved. The residual values were used to identify the inflection point that corresponds to the loss of motor control (residual ADS activity) and spiracular control (residual ADS CO_2). These inflection points were distinct for the activity that could yield clear activity CTmax (Fig. S2.2A-S2.4A). For the residuals ADS CO_2, inflection points that coincided with the individual's CTmax could sometimes be identified (Fig. S2.2B), but in many cases this approach did not yield clear inflection points despite a clear cessation of spiracular activity noticeable on the CO_2 release traces (Fig. S2.3B). This has been encountered by other authors that further used other identifiable landmarks from the respirometry traces. The cessation of spiracular activity (CSA) used by Vorhees and Bradley (2012) can be identified by enhancing the contrast of the CO_2 release traces using the 'differentiate' and 'square' function in Expedata to identify the point at which spiracular activity is lost (Fig. S2.2, S2.3). This indicator was used as criteria of CTmax, which has been shown to be indistinguishable from the residual ADS CO_2 approach in other species (Vorhees and Bradley, 2012).

Thermolimit respirometry traces obtained for larvae could be used to obtain activity CTmax using the same approach as adults (Fig. S2.4A). Traces obtained for CO_2 release rate did not, however, allow to pinpoint the cessation of spiracular activity using either the residual ADS CO_2 or the CSA point of the transformed CO_2 traces (Fig. S2.4B). Further examination of multiple traces shows a large and final CO_2 release peak (Fig. S2.5) that was interpreted as the post-mortal peak documented by Lighton and Turner (2004). In their species, Lighton and

Turner (2004) see this peak about 10 min following CTmax, which is along the same timeline as presented in other species by Vorhees and Bradley (2012) with over 10 min past their CTmax determination, and similar to the approximately 10-15mins observed for my larvae (Fig. S2.5). No other usable landmark in the CO_2 traces obtained for larvae was observed, and therefore activity CTmax was relied on solely since its value could be obtained unambiguously.

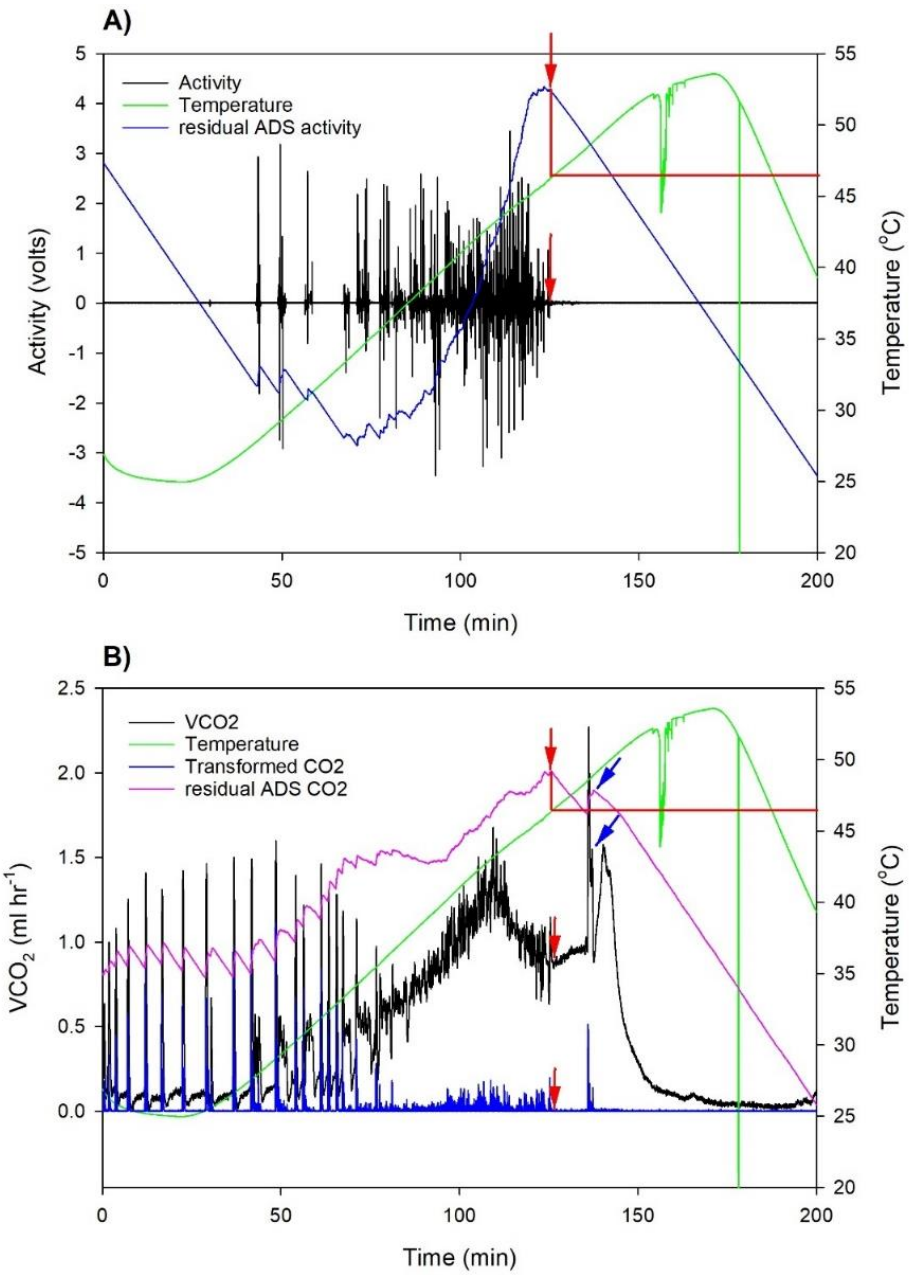


Figure S2.2. An example of a clear trace used for CTmax determination of a worker *B. impatiens*. A) Activity CTmax determined using the residual absolute difference sum (ADS) where the peak coincides with the final movement detected using infrared activity detector (red arrows). B) Respiratory CTmax where the residual ADS coincides with the cessation of spiracular activity identified by the transformed CO_2 release trace using the 'differentiate' then 'square' function in Expedata (red arrows) (see Vorhees and Bradley, 2012). The blue arrows correspond to the post-mortal peak (Lighton and Turner, 2004) where a final peak of the residual ADS is noted.

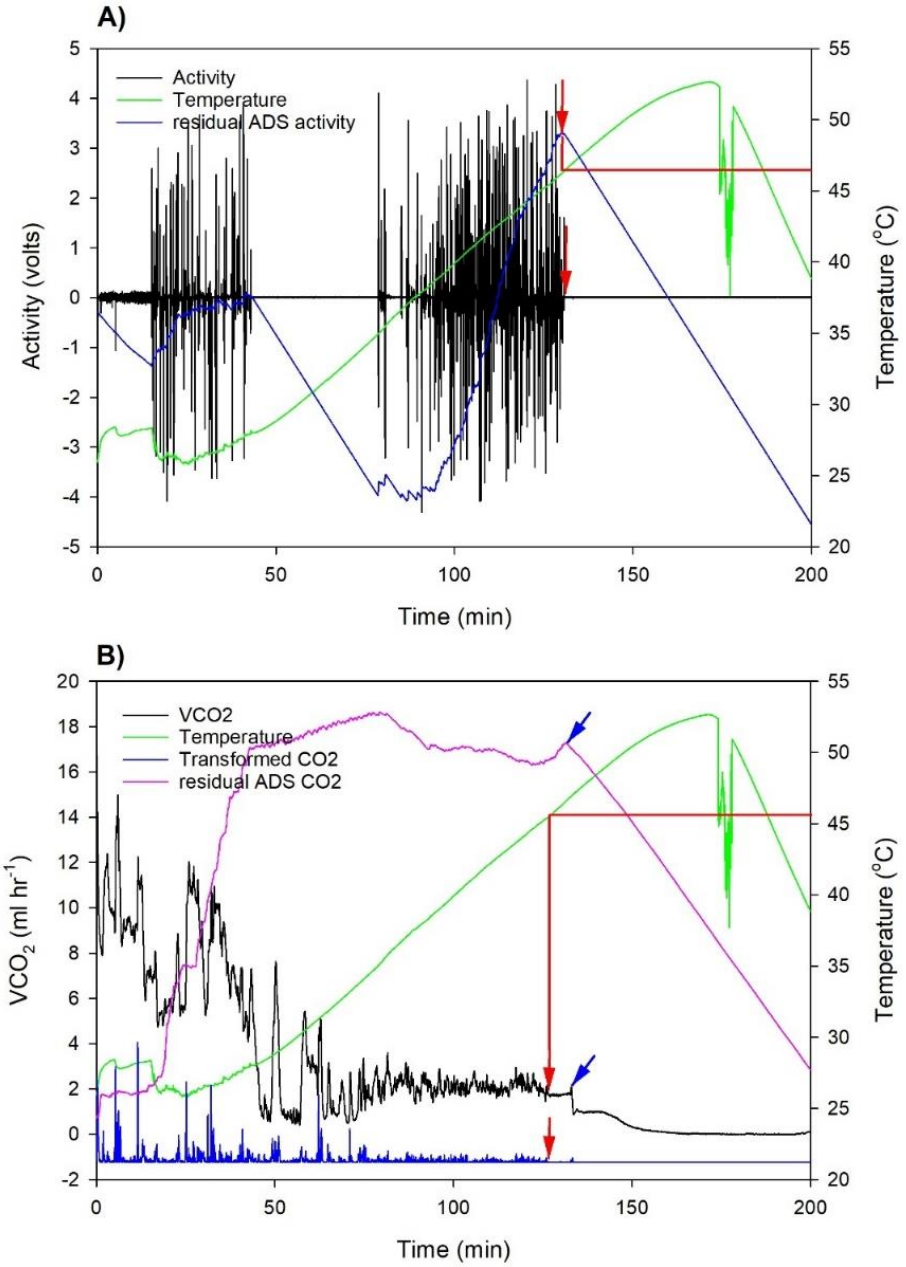


Figure S2.3. An example of an ambiguous trace used for CTmax determination of a worker *B. impatiens*. A) Activity CTmax determined using the residual absolute difference sum (ADS) where the peak coincides with the final movement detected using infrared activity detector (red arrows). B) Respiratory CTmax where the residual ADS does not yield a peak or inflection point that coincides with the cessation of spiracular activity identified by the transformed CO2 release trace using the 'differentiate' then 'square' function in Expedata (red arrows) (see Vorhees and Bradley, 2012). The blue arrows correspond to the post-mortal peak (Lighton and Turner, 2004) where a final peak of the residual ADS is noted.

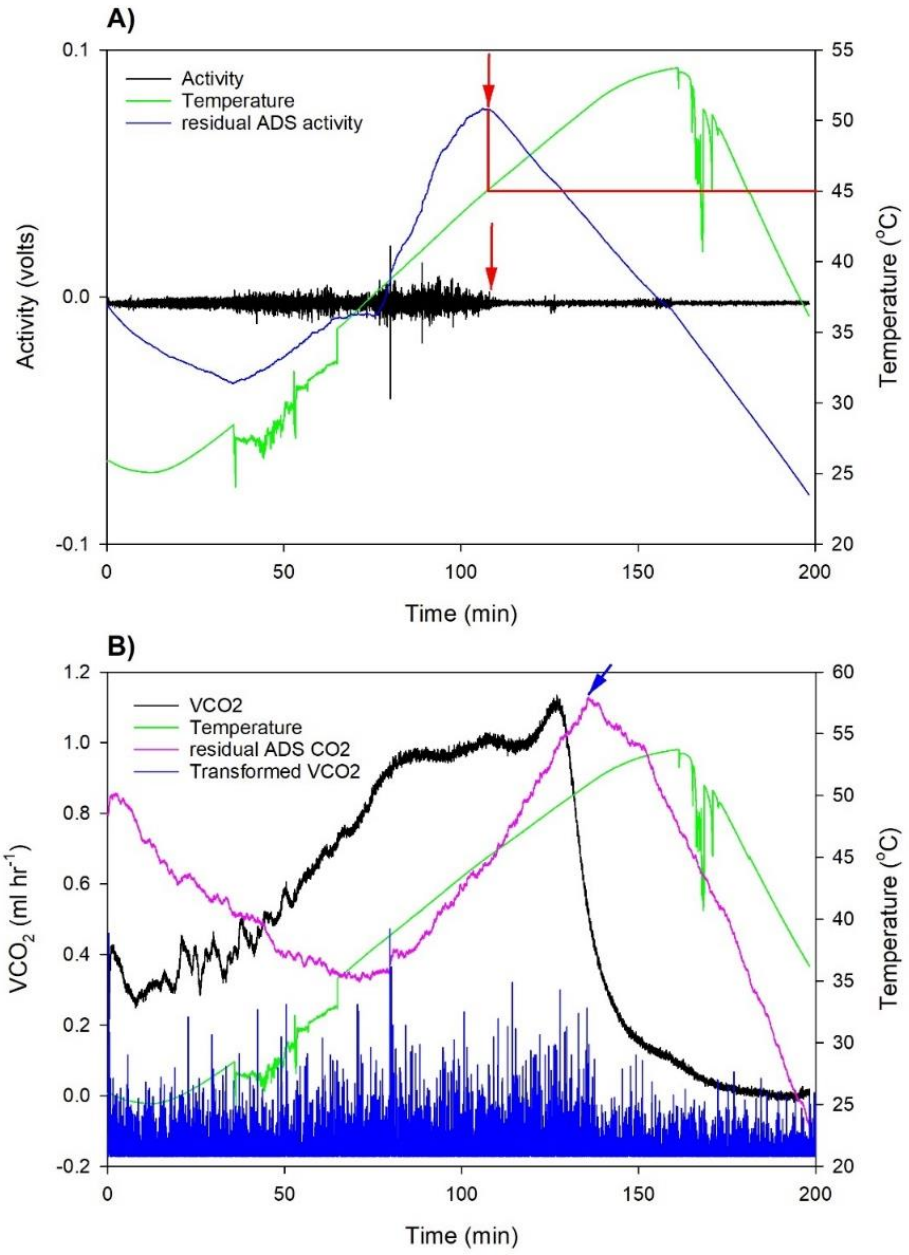


Figure S2.4. An example of trace used for CTmax determination of a *B. impatiens* larvae. A) Activity CTmax determined using the residual absolute difference sum (ADS) where the peak coincides with the final movement detected using infrared activity detector (red arrows). B) Respiratory CTmax could not be determined as the residual ADS yielded a peak after the post-mortal peak (see Figure S2.4). The cessation of spiracular activity could not be identified by the transformed CO2 release trace using the 'differentiate' then 'square' function in Expedata.

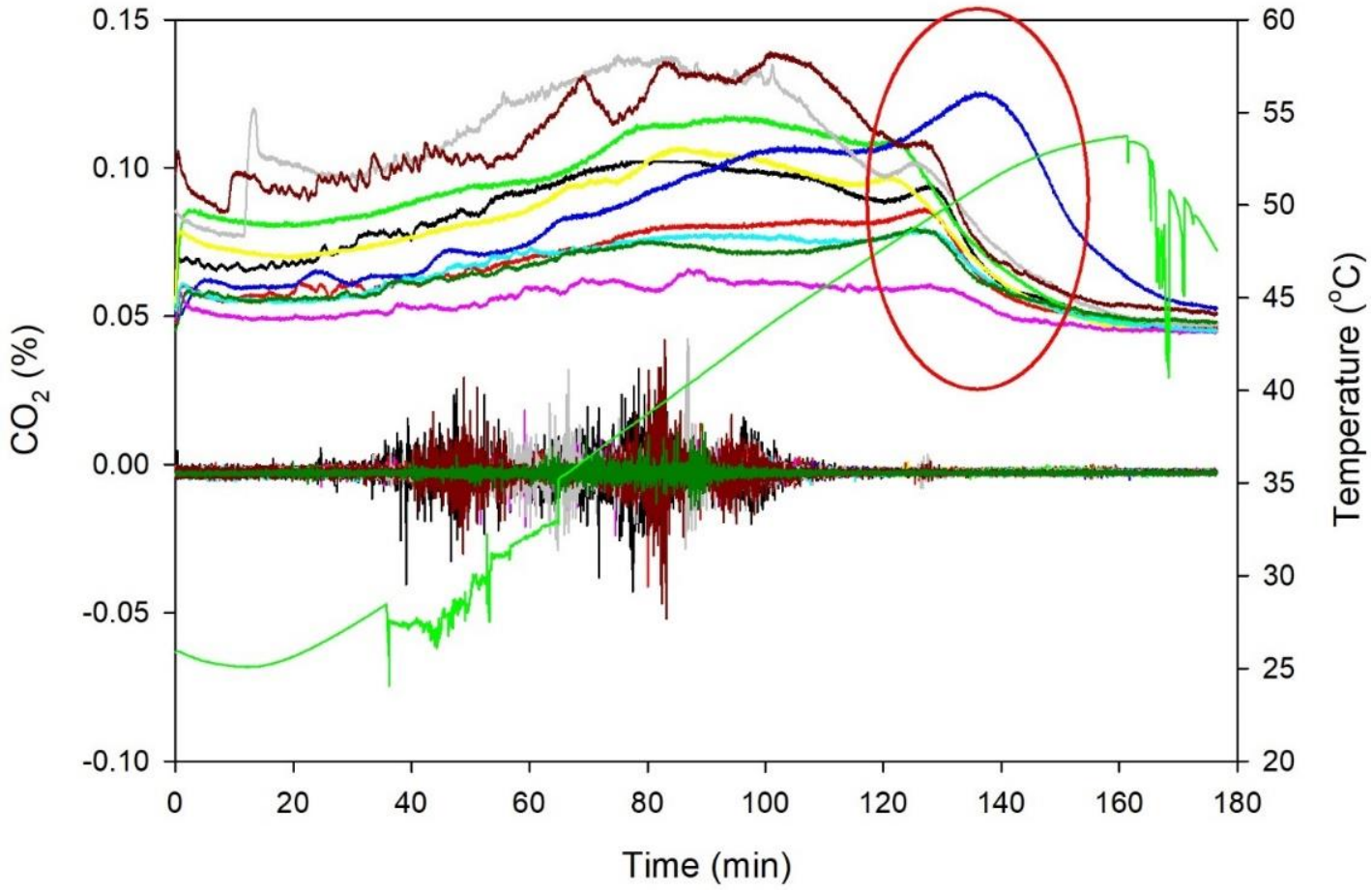


Figure S2.5. Compiled thermolimit respirometry raw traces from *B. impatiens* larvae. A final CO_2 release is noted by the circled area in red, which is interpreted as the post-mortal peak typical of such assays with corresponding timeline (Lighton and Turner, 2004; Vorhees and Bradley, 2012). No clear objective landmark could be identified for larvae (i.e., residual ADS, CSA) and therefore only relied on activity CTmax that could be determined using the ADS approach as in adults (see Fig. S2.3 for details).

2.8.3 Effect of colony on CTmax measurement

Individuals sampled for CTmax experiments originated from multiple commercial colonies that were reared within the lab. The colony of origin was not recorded for workers but the effect of colony on CTmax values obtained for the majority of queens (18/19) and drones (16/20) sampled could be determined (see Table S2.1). Using ANOVA analyses, neither activity- nor respiratory-CTmax was found to differ between colonies for queens (activity-CTmax, Colony: $F_{2,15} = 2.52$, P=0.114; respiratory-CTmax, Colony: $F_{2,15} = 1.21$, P=0.325) and drones (activity-CTmax, Colony: $F_{2,14} = 0.23$, P=0.641; respiratory-CTmax, Colony: $F_{1,14} = 1.32$, P=0.270).

Table S2.1. Mean CTmax (±SE) of queens and drones does not differ between colonies.

Caste	Colony ID	Sample Size	Activity-CTmax (°C)	Respiratory-CTmax (°C)
Queen	A	6	46.79 ± 0.48	46.14 ± 0.52
	B	5	45.38 ± 0.88	46.15 ± 0.69
	C	7	47.02 ± 0.20	46.99 ± 0.19
Drone	D	9	45.15 ± 0.52	46.41 ± 0.51
	E	7	45.56 ± 0.72	47.34 ± 0.11

Table S2. Number of workers and their mean body mass of the colonies used in the whole-colony respiration experiment.

Insulation	Colony ID	Number of Workers	Mean Worker Mass (g)
No	**1**	278	0.153 ± 0.003
	2	302	0.175 ± 0.004
	3	263	0.184 ± 0.005
	4	244	0.143 ± 0.003
	5	331	0.158 ± 0.003
Yes	**6**	359	0.126 ± 0.002
	7	369	0.137 ± 0.002
	8	334	0.159 ± 0.002
	9	404	0.141 ± 0.002
	10	274	0.143 ± 0.003

Chapter 3: Handling heatwaves: trade-offs between thermoregulation, foraging and bumblebee colony success

3.1 Abstract

Climate changes pose risks for bumblebee populations, which have declined relative to the growing frequency and severity of hot temperature extremes. Bumblebee behaviour within colonies might mitigate the effects of such extreme weather. In particular, fanning behaviour to dissipate heat is an important mechanism that could reduce exposure of thermally sensitive offspring to dangerous nest temperatures (T_n). Conversely, foraging is essential for colony-sustaining resource gathering. Colony maintenance and growth could suffer as a result of nutritional and high ambient temperature (T_a) thermal stress. Nevertheless, it remains uncertain whether a trade-off occurs between thermoregulation and foraging under chronic, sublethal heat events and how colony success is impacted as a result. This study held colonies of Bombus impatiens at constant high T_a (25, 30 or 35˚C) for two-weeks while quantifying the percentage of foragers, fanning incidence, nest temperature (T_n) and other metrics of colony success such as adult emergence and offspring production. I found that foraging and adult emergence was not significantly affected by T_a, but that thermoregulation was unsuccessful at maintaining T_n despite increased fanning at 35˚C. Furthermore, 35˚C resulted in workers abandoning the colony and fewer offspring being produced. My results imply that heatwave events that exceed 30˚C will negatively impact colony success through failed thermoregulation and reduced workforce production.

3.2 Introduction

Impacts of climate change, including current and projected increases in temperature (The Core Writing Team IPCC, 2015), are placing stress on ectotherms globally, exposing some to temperatures which equal or exceed physiological thermal limits (Sunday et al., 2014). Species inhabiting mid-latitudes, where temperature variation is greater, are likely to face greater heat stress challenges (Kingsolver et al., 2013). More frequent and intense extreme heat events are predicted in the future (Meehl and Tebaldi, 2004), leaving ectotherms, whose body temperatures respond more directly to that of the environment, especially vulnerable to increases in ambient temperature (T_a). One group of particular concern are insect pollinators, including bumblebees, who play important environmental and agricultural roles (see Klein *et al.*, 2006; Gill *et al.*, 2016). Various stressors are known to impact bumblebees, including pesticides (Alkassab and Kirchner, 2017; Bryden et al., 2013; Mommaerts et al., 2010; Whitehorn et al., 2012) and parasites (Gegear et al., 2005; Rutrecht and Brown, 2008) impacting individuals and colonies, as well as decreases in species richness linked to land use change (Vray et al., 2019). Yet, climate change also contributes to the decline of bumblebee populations (Fourcade et al., 2019; Soroye et al., 2020) and to observed range losses (Kerr et al., 2015). How the growing intensity and severity of extreme weather, such as heat waves, can alter bumblebee colony persistence is important to understanding these species' ability to cope with future alterations in global temperature.

Bumblebees are not typical ectothermic insects as they are capable of generating heat endogenously, allowing them to warm body temperatures for flight (Heinrich, 1995, 1974b, 1972b; Masson et al., 2017; Staples et al., 2004) and adults possess high critical thermal limits which reflect this heat generation (i.e., Bretzlaff et al., 2023; Heinrich, 1976; Oyen and Dillon, 2018). Moreover, bumblebees are social and workers collectively help maintain colonies through

resource gathering and nest-wide thermoregulation. Thermoregulatory abilities of the individual warm up the colony when T_a falls, and behavioural mechanisms, such as fanning, help cool the nest when conditions are hotter. Fanning has been previously described in both bumblebees (Weidenmüller, 2004) and honeybees (Cook et al., 2016) as a bee who remains in one location for period of 10s while steadily fanning with spread wings. This mechanism aids in connective and evaporative heat loss within the colony (Heinrich, 2004; Vogt, 1986a) where offspring, in particular, are vulnerable to heat. Worker bees must buffer these individuals from changes in T_a by maintaining relatively stable nest temperatures (T_n) which ranges between 30-33°C in bumblebees (Barrow and Pickard, 1985; Heinrich, 2004; Schultze-Motel, 1991; Vogt, 1986a). Deviation from optimal temperatures alter development of communication, olfactory senses and short-term memory in honeybees (Groh et al., 2004; Jones et al., 2005; Tautz et al., 2003; Wang et al., 2016) as well as hinder emergence and reduce individual longevity in honeybees (Groh et al., 2004; Medrzycki et al., 2010). Furthermore, chronic exposure to elevated temperature has been demonstrated to reduce worker size (Guiraud et al., 2021), increase wing size variation of males (Gerard et al., 2018) and hinder worker responses to stimuli (Perl et al., 2022). Collective thermoregulation at both low and high T_a incurs significant energetic costs for individuals and the colony, yet under acute heat stress, increased fanning efforts do not result in successful nest thermoregulation (Bretzlaff et al., 2023; Vogt, 1986a). Thus, high upper critical temperature limits observed among adult bumblebees may not reflect the colony's susceptibility to negative effects of high temperatures. Whole-colony responses to sublethal chronic thermal stress may be critical for understanding their susceptibility to negative effects of rapidly warming climates.

The energetic costs and the ability to succeed in nest thermoregulation are not the only challenges encountered as a result of temperature variation. Bumblebees, unlike honeybees, lack

age-caste division of labor, which partitions tasks among workers in different age classes. Instead, the tasks which help sustain a colony throughout its lifecycle, such as defense, foraging and other nest maintenance duties, are shared amongst all bumblebee workers and can be related to the size of the individual (Garófalo, 1978; Jandt and Dornhaus, 2009; Spaethe and Weidenmüller, 2002). These tasks may be prioritized based on current colony requirements (Free, 1955). For instance, about a third of a colony's workers participate in foraging (Brian, 1952; Stewart et al., 2021) and previous experiments demonstrate that colonies may divert workers from foraging to nest incubation if provided with supplementary resources (Stewart et al., 2021). However, observations under heat stress raise concerns surrounding the trade-off between thermoregulation and the maintenance of a colony since acute T_a that exceeds optimal T_n conditions induces higher incidences of fanning while simultaneously reducing the incidence of nest maintenance (Vogt, 1986a).

Colonies may be able to rebound from acute heat stress, but prolonged heat stress may pose a greater challenge. Chronic heat experiments have previously demonstrated reduced foraging (Kwon and Saeed, 2003) but that other stressors, such as the quality of pollen diet, may interact to create more pronounced negative effects. For example, a combination of heat stress and poorly suitable diets causes decreased resource procurement and colony development, with greater variation in the percent mortality for bumblebee colonies of small size with about 60 workers (Vanderplanck et al., 2019). These effects may have consequences for the long-term viability and success of a colony given that bumblebee density declines have been observed following heat waves (Rasmont and Iserbyt, 2012) with high air temperatures linked to reduced foraging trips (Couvillon et al., 2010). Therefore, there is a need to understand not only the acute

effects of high temperature stress on bumblebee colonies, but also the direct and indirect negative costs sustained if extreme heat events persist over longer periods of time.

The main objective of the present study was to test for direct and indirect effects of chronic high ambient temperature on colonies of a temperate North American species of bumblebee, *Bombus impatiens*. I subjected colonies to high T_a for two-week periods and allowed them to forage within a temperature-controlled environment. I investigated whether chronic thermal stress will induce a trade-off between foraging and thermoregulation by monitoring the proportion of workers who foraged in addition to the incidence of fanning behaviour. Furthermore, I simultaneously measured internal colony temperature to determine whether thermoregulatory efforts were successful and assessed colony success by quantifying adult emergence, mortality and offspring production, predicting that thermoregulation would be unsuccessful at high T_a and that the measures of colony success would each be negatively affected. My study provides a baseline for the impact of constant temperature exposure on the aforementioned measures of colony success.

3.3 Methods

3.3.1 Bumblebee colonies and holding conditions

Colonies of *Bombus impatiens* (Biobest Canada Ltd., Leamington, ON, Canada) were received 21 days prior to experimentation. On the day a colony arrived, the queen along with 10 workers were extracted and placed into a separate nesting box along with approximately 3 brood clumps containing larvae and pupae. The new colony was given access to BIOGLUC® sugar solution from the supplier *ad libitum* and was immediately provided with 2-3 pollen balls to facilitate nest building. Thereafter, pollen was provided twice weekly where the colony was able to build new structures and increase population size until experimentation commenced.

3.3.2 Experimental preparation

In order to monitor foraging behaviour of a colony under chronic thermal conditions, radio frequency identification (RFID) tags compatible with Microsensys technology (Microsensys GmbH, Erfurt, Germany) were fixed to worker bees as follows: Subsequent to the 21-day colony recovery period, each bee was removed from the colony and incapacitated through refrigeration. Of the individuals collected, 43 were selected at random and each was fitted with an RFID tag on the dorsal side of the thorax, anterior to the wings using a small application of cyanoacrylate glue. This position did not restrict the movement nor the function of the wings and bees were able to engage in flight. The queen was returned to the experimental colony (without a tag) along with the 43 tagged workers.

Chronic, whole-colony heat exposure experiments required a temperature-controlled environment as well as ample space to allow for foraging. Experimentation thus took place in an environmental chamber on a 12h:12:h light/dark cycle where temperature could be held constant. Many bumblebee species are known to inhabit aboveground nests, including artificial or human-made structures (Johnson et al., 2019; Liczner and Colla, 2019), thus a single experimental colony was placed in an insulating 38L Styrofoam cooler with a thickness of 1.5cm to simulate an artificial, aboveground nesting scenario. A 60x60x180cm flight cage, acting as a foraging destination for BIOGLUC® sugar solution, was connected to the colony via clear tubing with an internal diameter of 1.3cm. This pathway between colony and flight cage spanned approximately 60cm and also included two Microsensys RFID readers enabling outgoing and ingoing bee movements to be recorded (see Fig. S3.1 for experimental setup). Data collected by the readers was sent to a Microsensys iID controller where the digital information was stored. Prior to the collection of foraging data, colonies were allotted 3-5 days to discover the location of the sugar solution within the flight cage. Afterwards, the experiment was initiated by setting T_a within the

environmental chamber to 25, 30 or 35˚C for a duration of two weeks. The above procedure was repeated on 15 total colonies sorted into 3 different temperature groups (n=5 per temperature).

3.3.3 Quantification of foraging and fanning effort

The number of foraging trips gathered from RFID tag recordings over the two-week period included date and time with direction being inferred from the Microsensys reader encountered first. From this data, a "foraging trip" was chosen to be between 3.5min and 25min based on initial observations during the pre-experimental setup and the size of the flight cage (e.g., some enter and exit the flight cage a few times but do not engage in foraging, while others collect sugar solution as well as search for additional sources before returning to the colony). A bee with 10 or more logged foraging trips was considered to be a "forager" and the total number of trips taken by individuals who fall within this classification were gathered for analysis.

The incidence of individuals displaying fanning behaviour was estimated daily by capturing a 30min video of the top view of the colony covered by an acrylic transparent cover, an area corresponding to approximately 165cm^2. To estimate the number of individuals fanning, the 1st, 5th,10th,15th, 20th, 25th and 30th minute of each video was reviewed and the number of individuals fanning were counted. A "fanner" was considered as a bee who remained stationary and beat their wings continuously for 10sec in a distinct posture with abdomen raised as described in (Cook et al., 2016; Weidenmüller, 2004). The daily average number of fanners was calculated by summing the total number of fanners observed each day from a 30-minute video and using these sums to find the average across the 15 experimental days.

3.3.4 Quantification of internal colony temperature

Internal colony temperature was quantified using two thermochron iButton® digital temperature loggers (iButtonLink Technology, Whitewater, WI, USA) placed among the brood

cells with developing larvae inside the colony (see Fig. 3.1). The iButtons recorded temperature at 5min intervals for the duration of the 2-week experiment. Temperature data was extracted and averaged across the two iButtons both on a whole-trial as well as on a daily basis.

Infrared video (IR) was used to measure the maximum internal colony temperature, corresponding to bee thorax temperatures (T_{th}). Thoraces can reach as high as 45°C after engaging in flight (Heinrich, 1976) and queen T_{th} during brood incubation is maintained between 35-38°C (Heinrich, 1972a). Thus, by identifying the highest T_{th} observed, it could be determined whether there is a change in T_{th} based on different chronic thermal stressors. An IR camera (FLIR ThermaCam Ex300, 320x240 pixel resolution; FLIR Systems, Inc., Wilsonville, OR, USA) was placed above the colony to record a 30min video capture of the displayed IR image with the maximal temperature reading. The camera was inserted into a mount placed overtop an opening within the plexiglass nest box covering such that the main nest cluster could be monitored. For each video, the highest temperature observed at the 1^{st}, 5^{th},10^{th},15^{th}, 20^{th}, 25^{th} and 30^{th} minute, daily was used to calculate the mean highest maximum temperature across each experimental trial.

3.3.5 Quantification of adult emergence, mortality, abandonment and offspring production

Changes within a colony throughout an experimental trial were assessed by measuring various indicators. Adult emergence was defined as the percentage of adults which emerged from brood cells (untagged bees) during a two-week exposure period. Mortality was quantified by the percentage of total bees who died before the end of an experimental trial. Abandonment of the colony was observed by some individuals who permanently moved to the flight cage. Therefore, this measure was included in analysis by quantifying the percentage of total bees who either were found deceased within the flight cage during the experiment or, were collected within the

flight cage at the end. Finally, offspring production was assessed by counting larvae and pupae remaining within brood clumps at the end of each trial.

3.3.6 Data analysis

Statistical analyses were performed in R (R Core Team, 2014) using experimental temperature as a categorical predictor in separate one-way ANOVAs with the following dependent variables: percent foragers, daily average number of fanners, average internal colony temperature, percentage of adult emergence, percent mortality, percent abandoned, and total offspring. For the analysis of thorax temperature, the normality assumption was violated and could not be resolved using data transformations. A nonparametric Kruskal-Wallis test was conducted to test whether thorax temperature differed between experimental temperature treatments. To determine whether the internal colony temperature or the number of fanners differed across the 14-day exposure period, two-way ANOVAs were conducted for each experimental temperature (separate ANOVA for 25, 30 and 35°C) using experimental day and tested colony as categorical predictors of the daily internal colony temperature and the daily average number of fanners. Pairwise analyses were conducted using the Tukey method and values are reported as mean ± standard error of the mean.

3.4 Results

3.4.1 Effect of ambient temperature on foraging and fanning effort

On average, the percentage of tagged bees who participated in foraging was 70.09 ± 12.80% for colonies at 25°C, 79.59 ± 7.29% for colonies at 30°C and 58.08 ± 8.20% for colonies at 35°C with no significant effect of temperature between treatment groups (Fig. 3.2; $F_{2,10} = 1.71$, P=0.230). Data for 2 out of 5 colonies undergoing 25°C trials were unable to be recovered due to

data saving failures of the Microsensys iID controller, lowering the sample size for that temperature group to n=3.

The daily average number of fanners over a 15-day recording period was found to be influenced by T_a (Fig. 3.3; $F_{2,12} = 7.68$, P=0.007). The daily average number of fanners at 25°C (4.22 ± 0.91) was not different from the average at 30°C (7.19 ± 1.26; P=0.516), however, the average number of fanners at 35°C (14.25 ± 2.82) was significantly higher than at both 25°C (P=0.006) and at 30°C (P=0.049). Within experimental temperature trials, the average number of fanners did not change across the 15-day recording period for colonies at 30°C (day: $F_{14,56}$ = 1.52, P=0.134) nor at 35°C (day: $F_{14,55} = 1.15$, P=0.336). For colonies tested at 25°C, day of exposure did have a significant effect on the average number of fanners (day: $F_{14,55} = 2.46$, P=0.009) but pairwise comparisons indicate that the only significant difference occurred between day 7 and 12 (P=0.030). Within each experimental temperature analysis, the tested colony had a significant effect on the average number of fanners observed (25°C colony: $F_{4,55} = 5.24$, P=0.009; 30°C colony: $F_{4,56} = 7.37$, P<0.001; 23°C colony: $F_{4,55} = 13.21$, P<0.001).

3.4.2 Effect of ambient temperature on internal colony temperature

The internal colony temperature among the brood cells, as averaged across the two-week experimental duration, was dependent on the T_a colonies were exposed to ($F_{2,12} = 58.48$, P<0.001). The average T_n experienced by colonies at 25°C was 32.55 ± 0.23°C. Colonies at 25°C and at 30°C (average 33.22 ± 0.13°C) had internal temperatures that were not significantly different from one another (P=0.071). However, colonies who experienced 35°C had internal colony temperatures which hovered around ambient at 35.37 ± 0.20°C and were significantly higher than those at 25 and 30°C (Fig. 3.4a; P<0.001).

Daily internal colony temperature remained fairly constant overt the two-week exposure period. Within each temperature treatment group, internal colony temperature was not significantly different across the two-week exposure period for colonies at 25°C ($F_{13,52}$ = 1.12, *P*=0.368) and 35°C ($F_{13,52}$ = 1.12, *P*=0.367), with only minor differences detected at 30°C ($F_{13,52}$ = 2.17, *P*=0.025) between days 1 and 8 (*P*=0.020) and 14 (*P*=0.016). A significant effect due to the colony was also found, where each temperature treatment group had a colony with an overall slightly lower nest temperature than the others (25°C: $F_{4,52}$ = 22.30, *P*<0.001; 30°C: $F_{4,52}$ = 29.60, *P*<0.001; 35°C: $F_{4,52}$ = 85.24, *P*<0.001). The day-to-day representation of internal colony temperature is presented in Fig. 3.4b.

The mean highest T_{th} from IR camera recordings was not influenced by T_a (Fig. 3.5; Kruskal-Wallis χ^2 = 0.96, df = 2, *P*=0.619). Overall, the mean highest T_{th} observed was 41.73 ± 0.50°C at 25°C, 41.49 ± 0.46°C at 30°C and 41.51 ± 0.51°C at 35°C.

3.4.3 Effect of ambient temperature on adult emergence, mortality, abandonment and offspring production

The number of workers who hatched over the course of an experimental trial, or percentage of adult emergence, was not dependent on T_a to which colonies were exposed (Fig. 3.6; $F_{2,12}$ = 0.27, *P*=0.765). On average, colonies at 25°C grew by 61.57 ± 4.95%, 30°C by 61.98 ± 5.35% and 35°C by 66.15 ± 4.14%. No male drones were found to have emerged in any of the trials.

The percent mortality was not significantly influenced by T_a, though resulting p-values indicate a potential trend (Fig. 3.7; $F_{2,12}$ = 3.28, *P*=0.073). Pairwise comparisons show that the average mortality at 35°C (15.03 ± 3.97%; *P*=0.060) tended to be higher than at 25°C (5.23 ± 1.32%). At 30°C (9.91 ± 2.12%) however, there was no significant difference between the

percent morality at either the 25 or 35°C trials ($P \geq 0.402$). It was also found that queens died before the end of the two-week trial on four occasions; one from both the 25 and 30˚C experiments and two from the 35˚C experiments.

The proportion of bees that abandoned the colony was dependent on the T_a experienced by the colony (Fig. 3.8; $F_{2,12} = 11.4$, $P=0.002$). A greater percentage of bees were found to abandon the colony at 35°C (71.64 ± 4.92%) than at both 25°C (23.72 ± 6.60%; $P=0.001$) and 30°C (44.05 ± 9.19%; $P=0.044$), while this percentage did not differ between 25 and 30°C ($P=0.150$).

The total number of larvae and pupae offspring produced within colonies varied with T_a (Fig. 3.9; $F_{2,12} = 10.16$, $P=0.003$). On average, colonies at 25°C concluded the two-week trial with 164.20 ± 22.71 unhatched offspring, the 30°C trials had 155.20 ± 29.54 and 35°C colonies ended with only 39.20 ± 6.81 total offspring. Total offspring counts did not differ between 25 and 30°C trials ($P=0.955$), but colonies experiencing 35°C had significantly lower numbers of offspring than both 25°C ($P=0.004$) and 30°C ($P=0.007$) trials.

3.5 Discussion

Extreme heat events and recent evidence of bumblebee species declines raises concern about the effects of chronic heat on the sustainability and success of bumblebee colonies. This study assesses how chronic exposure to high ambient temperature effects various aspects of a bumblebee colony's behaviour, growth and survival. To determine whether a trade-off exists between foraging and fanning incidence and if adult emergence, mortality and nest temperature are affected by chronic heat stress, colonies underwent two-week exposures to 25, 30 and 35°C in a controlled environment. It was found that while foraging was not affected by high ambient temperature, colonies under chronic thermal stress increased thermoregulatory fanning efforts,

yet failed to reduce nest temperature. Furthermore, high temperatures resulted in increased colony abandonment as well as a reduction in the number of offspring produced under these conditions. Therefore, these results indicate that chronic high ambient temperature may pose significant risk to offspring production through reduced worker population and failed thermoregulation.

The foraging and fanning effort of colonies experiencing chronic, high ambient temperature did not result in a trade-off between the two behaviours. First, the lack of effect of ambient temperature on foraging rates (measured as the proportion of tagged workers engaging in that behaviour) may have been skewed by the presence of outlier colonies in each temperature treatment group. Without these outliers, a significant negative relationship would emerge, where the foraging activity decreases at 35°C. This trend should be interpreted with caution however, due to low sample sizes, where using a power analysis, a total of 16 colonies per temperature group are required to obtain a statistical power between 0.8-0.9. Previous research supports that higher temperatures may reduce foraging activity in social bees (Couvillon et al., 2010; Kwon and Saeed, 2003; Rami Reddy et al., 2015), including under simulated heatwaves (Hemberger et al., 2023), yet other research demonstrates that rather than influencing the foraging rates of bumblebees, temperature will instead affect the type of resources that are collected, meaning that there are differing conditions favorable for either nectar or pollen foraging (Peat and Goulson, 2005). Second, while foraging efforts were unaffected by ambient temperature, fanning effort increased under chronic exposure to 35°C. Short-term observations of bumblebee colonies under heat stress demonstrate that fanning efforts increase when ambient temperature exceeds 30°C (Vogt, 1986a; Wynants et al., 2021). For *B. impatiens* colonies, chronic exposure to high ambient temperature also leads to long-term fanning effort, which was observed to be sustained across the

full 2-week exposure period. Given that 35°C exceeds the range of optimal nest temperature preferred by bumblebees, thermoregulatory fanning under these conditions is deployed as a method to reduce nest temperature in attempt to prevent detrimental effects in unhatched offspring. In honeybees, larval contact plays a role in increasing the probability of worker fanning behaviour (Cook et al., 2016) and individual worker bumblebees possess varying thermal thresholds as a behavioural trigger, providing a graded colony response to combat increases in temperature and maintain thermal homeostasis (Weidenmüller, 2004; Weidenmüller et al., 2002).

Despite the high incidence of fanning exhibited by my tested colonies at 35°C, their efforts were unsuccessful. Internal colony temperatures rose to ambient temperature within the first day of the two-week experiments and colonies were unable to mitigate this thermal threat. At 25 and 30°C, internal colony temperature remained within the known optimal nest temperature range (Barrow and Pickard, 1985; Heinrich, 2004; Schultze-Motel, 1991; Vogt, 1986a) over the two-week period, further demonstrating that bumblebee colonies struggle to thermoregulate their nests under both chronic high ambient temperature conditions and acute thermal stress; the latter also resulting in increased energy expenditure (Bretzlaff et al., 2023; Vogt, 1986a). Fanning, which employs wing muscles within the thorax, inevitably produces heat as a result of mechanical work (Heinrich and Kammer, 1973). Thorax temperature of workers in a colony is known to increase in response to cold exposure (Macías-Macías et al., 2011) and during incubation (Heinrich, 1972a) as heat is transferred to warm the brood. During chronic ambient temperature exposure, observation of maximum thorax temperature did not yield any significant differences across temperature treatments, thus few conclusions can be drawn from

this measure regarding thermoregulatory effort. Overall, long-term fanning efforts employed by colonies are unsuccessful at regulating T_n under chronic heat stress.

Warm conditions, where fanning behaviour provides little benefit to thermoregulation, put colony growth at risk by affecting offspring production and survival. Heat stress has been found to decrease nest mass growth and slow brood production, especially in bumblebee colonies experiencing nutritional stress as well (Vanderplanck et al., 2019). In contrast, I found that the number of bees which emerged during a two-week exposure period was relatively unchanged by ambient temperature. Given that each colony tested began an experimental trial with similar brood sizes, my findings for adult emergence suggest that the eclosion of existing pupae is not significantly impacted by chronic high ambient temperature. Adult emergence, however, had low power and would require a sample size of n=88 colonies to achieve a statistical power of 0.8-0.9. Instead, prolonged exposure to high ambient temperature may have greater potential to increase the mortality rates of adult colony members as indicated by observed increases between 25 and 35°C which approached borderline significance. My results are likely also nuanced by the low sample size used, where instead, n=8 colonies per temperature treatment would improve the statistical power of my analyses. Furthermore, the number of unhatched larvae and pupae collected after a two-week exposure period was dramatically reduced at 35°C, implying that chronic heat stress imposes negative effects on either egg laying or the early instar development of new offspring. Previous works further emphasize the direct effects of chronic exposure to high T_a during rearing on bee survival and development, such as lowering the colony's investment in offspring production in bumblebees (Vanderplanck et al., 2019), reducing or preventing pupal emergence in honeybees (Groh et al., 2004), decreasing adult longevity in honeybees and Megachilidae bees (CaraDonna et al., 2018) as well as causing morphological deformities in

honeybees (Groh et al., 2004; Medina et al., 2018). Unfortunately, humidity was not controlled within the environmental chamber. Previous work on honeybees explains the importance of relative humidity for egg hatching and that low relative humidity can result in reduced worker survival (see Abou-Shaara et al., 2017). Thus, the lack of humidity control within my experiment may act as a confounding variable, influencing results such as offspring production and adult mortality. Bumblebees have also been shown to preferentially forage for pollen when humidity is low and nectar when humidity is high (Peat et al., 2005). In my study, colonies were supplied with pollen in the nest box, while the percentage of nectar foragers was found not to be significantly different among temperature groups. Finally, high temperature also leads to increased abandonment of the colony by workers. One limitation in my measurement of abandonment is that bees which died foraging within the flight cage and those which were out foraging at the end of the two-week experiment could not be distinguished from bees who had fully abandoned the colony. Yet, the overall significant increase in the percentage of workers who were found outside of the colony at 35°C suggests that chronic heat stress drives individuals to leave the colony, but the permanence of such a phenomenon remains in question once the heat stress is removed. Abandonment may thus further impact colony success by reducing the number of individuals available to perform behaviours which are essential to colony function such as foraging, thermoregulation and nest maintenance. Impairments to colony function, as a result a sublethal environmental stressors, are linked with reduced colony success (Bryden et al., 2013), therefore, combined increases in worker abandonment and reduced offspring production may act to have the greatest impact on bumblebee colony success under chronic heat stress.

Our study also incurred some limitations based on experimental design. Through my methods, I simulated above ground nesting by housing colonies within an insulated container.

Previous work indicate that underground nest sites are one of the most frequently observed nesting strategies across multiple bumblebee species, with one such species being *B. impatiens* (Colla et al., 2014). However, aboveground nest sites also appear as frequent choices for natural and artificial nest locations (Liczner and Colla, 2019). Aboveground temperatures can cause wide fluctuations in nest temperature as indicated by experiments on empty nest boxes where temperatures inside can be seen to vary by as much as 24°C throughout the day (Mullan, 2022). Bumblebee colonies in aboveground nest boxes can also respond in different ways to environmental temperature variation. For example, some species will permit brood temperature to fluctuate during day, reaching temperatures above 35°C and tightly regulate it at night, while other species will maintain relatively stable brood temperature over extended periods of time (Gradišek et al., 2023). Underground temperatures fluctuate as little as 0.3°C during the day (Mullan, 2022), yet the frequent occurrence of aboveground nesting sites, as well as commercially available bumblebee colonies, like *B. impatiens*, for garden and greenhouse pollination (Velthuis and Van Doorn, 2006), provide further relevance to my study's methods and results.

3.6 Conclusions

Increasing global temperature and more frequent occurrences of heat wave events are well documented to have negative broad-scale impacts on bumblebee species' ranges and richness (Kerr et al., 2015; Soroye et al., 2020), as well as abundance (Rasmont and Iserbyt, 2012), respectively. The underlying causes of such impacts, however, remain poorly understood, especially when considering the effects of prolonged heat exposure on colony success. My study thus investigated the baseline effects of constant high temperature exposure on bumblebee colonies and demonstrated that chronic exposure to high ambient temperature imposes both

direct and indirect effects to colony success. Temperatures which exceed optimal thermal nest conditions result in elevated nest temperature that cannot be mitigated by the increased fanning efforts observed. Additionally, increases in heat-related mortality in existing workers and unhatched larvae and pupae may either be a direct effect of exposure to high ambient temperature or a combination of indirect consequences of prolonged exposure. While the number of foragers did not significantly decline at high ambient temperature, the slight reduction in foragers observed at 35°C leaves fewer individuals to carry out tasks such as fanning and nest maintenance which are energetically expensive (Vogt, 1986a). Nutrient-deficient colonies of bumblebees both produce fewer individuals (Vanderplanck et al., 2019; Vaudo et al., 2018) and may cause conflicts between thermoregulatory and foraging task allocation (Stewart et al., 2021). If unable to provide the necessary resources through foraging, and if coupled with increased abandonment of workers, chronic high ambient temperature ultimately results in fewer individuals to care for the current brood, at least under experimental conditions. Those individuals who do remain must then carry out thermoregulatory and maintenance tasks on limited resources. As such, decreases in adult emergence and hindered success at high ambient temperature may be explained by a combination of the above direct and indirect effects of chronic thermal stress, allowing a possible explanation for observed declines to date. My study provides a starting point to investigate chronic heat stress on whole bumblebee colonies and I propose that future work incorporate daily temperature fluctuations to the exposure regime in order to mimic environmental conditions to further determine how wild bumblebees cope with extreme heat events like heatwaves.

3.7 Figures

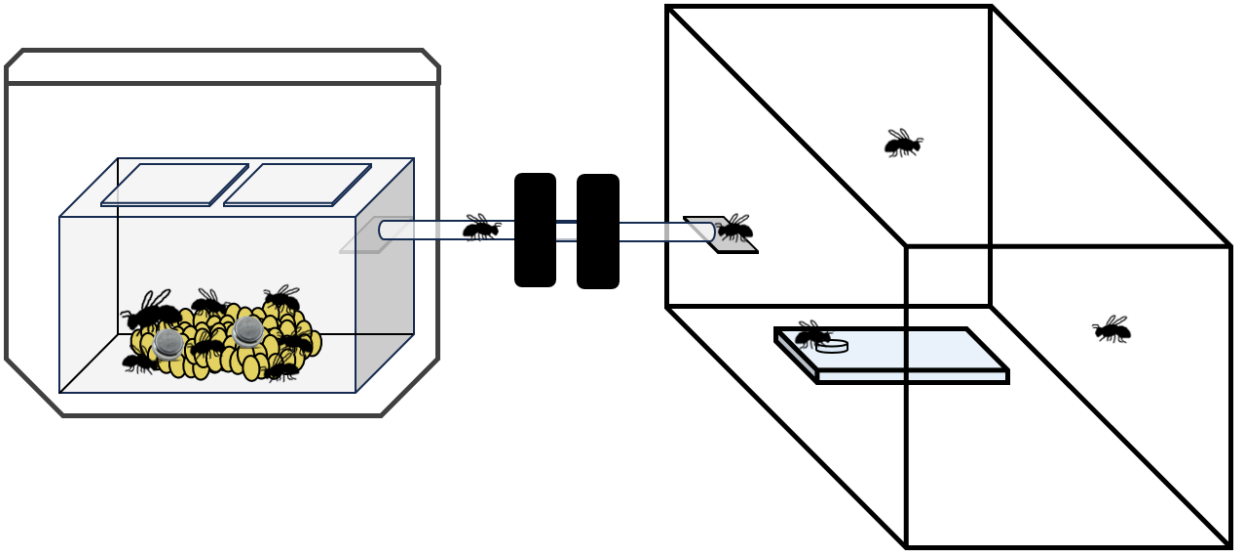


Fig. 1. Colony setup of chronic foraging experiment. Bumblebee (*Bombus impatiens*) colonies within their nest box were placed within a 38L Styrofoam cooler of 1.5cm thickness and sealed with the corresponding Styrofoam lid. The colony entrance was fitted with tubing of an internal diameter approximately 1.30cm wide. Tubing was run to two RFID reader boxes and then connected to an entrance in a large flight cage (60x60x180cm). This permitted the movements of the 43 RFID tagged workers to be recorded as they exited and re-entered the colony. The total distance between the colony and the flight cage measured approximately 60cm. Within the flight cage, a 2kg container of BIOGLUC® solution was placed near the entrance allowing bees to forage for a nectar-like solution. Bumblebee colonies were exposed to 25, 30 and 35°C (n=5 colonies per temperature) for two-weeks. Two thermochron iButton® digital temperature loggers were placed among the brood cells containing developing larvae to monitor internal colony temperature. A plexiglass cover was fit overtop the colony with two holes cut into it for access to administer pollen balls every second day and to film the colony for the purposes of quantifying fanning incidence. When not filming, glass coverings were kept over the holes in the plexiglass and the Styrofoam lid remained in place.

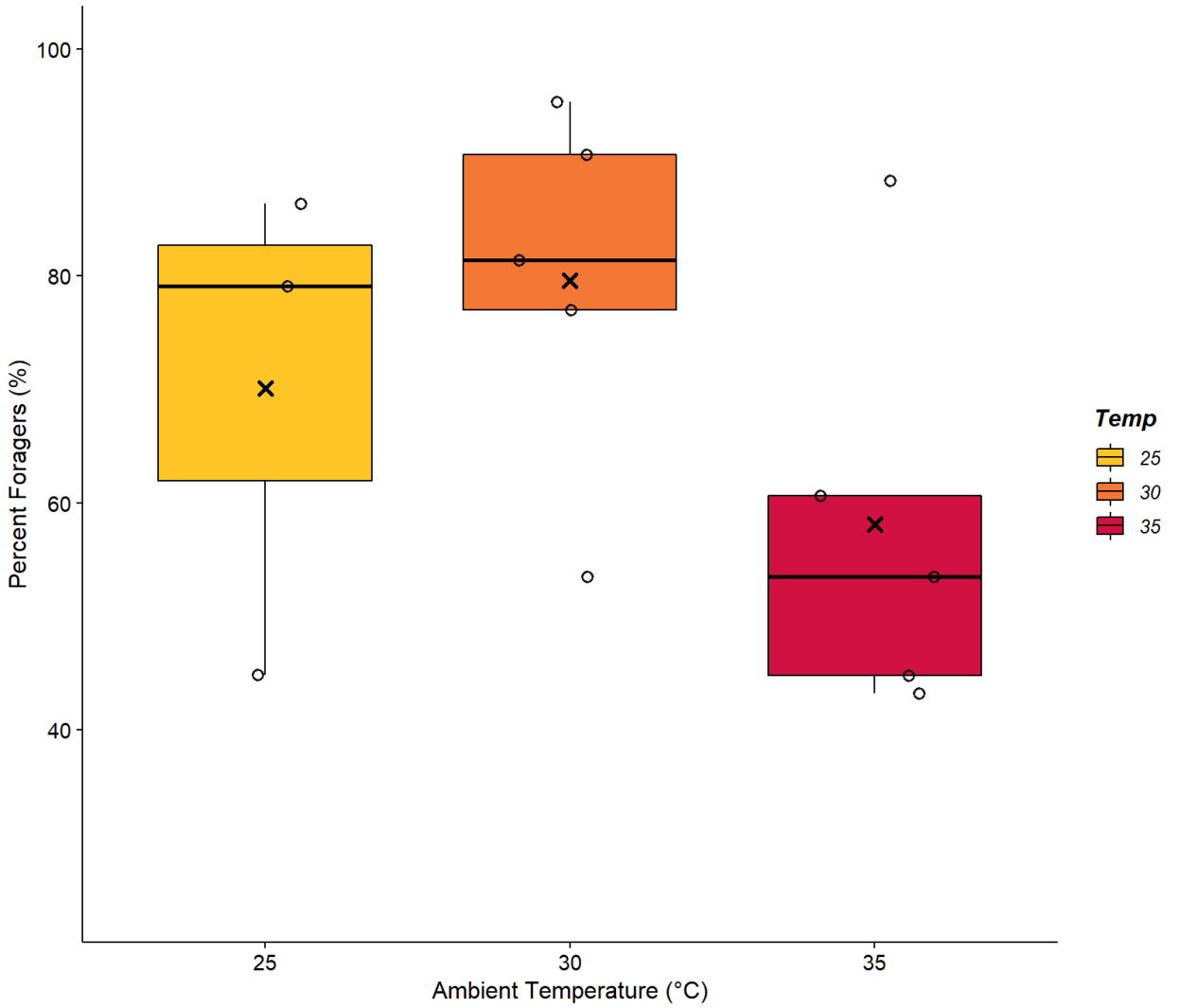


Fig. 3.2. The percentage of *B. impatiens* workers that forage shows no clear trend. Bumblebee colonies of 43 workers were exposed to 25°C (yellow), 30°C (orange) or 35°C (red) for two-week periods (25°C, n=3 colonies; 30, 35°C, n=5 colonies). The percentage of tagged workers who engaged in foraging (10+ trips total) did not differ across T_a treatments (P=0.230). Box plots represent percentiles with the black bar across equalling the median value. Also present are the individual data points to show the spread of the colonies tested. The average value is denoted by an (x) within each box.

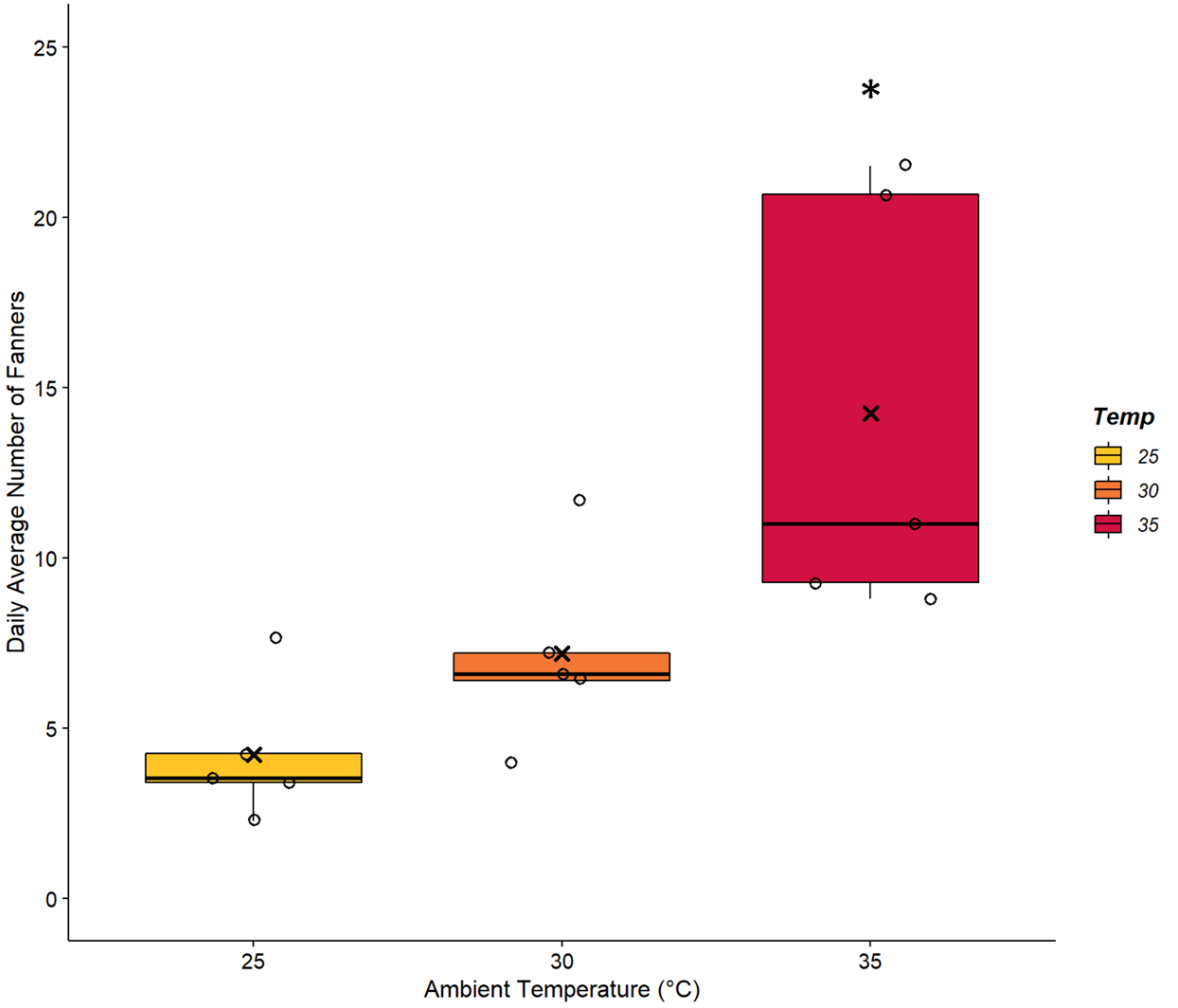
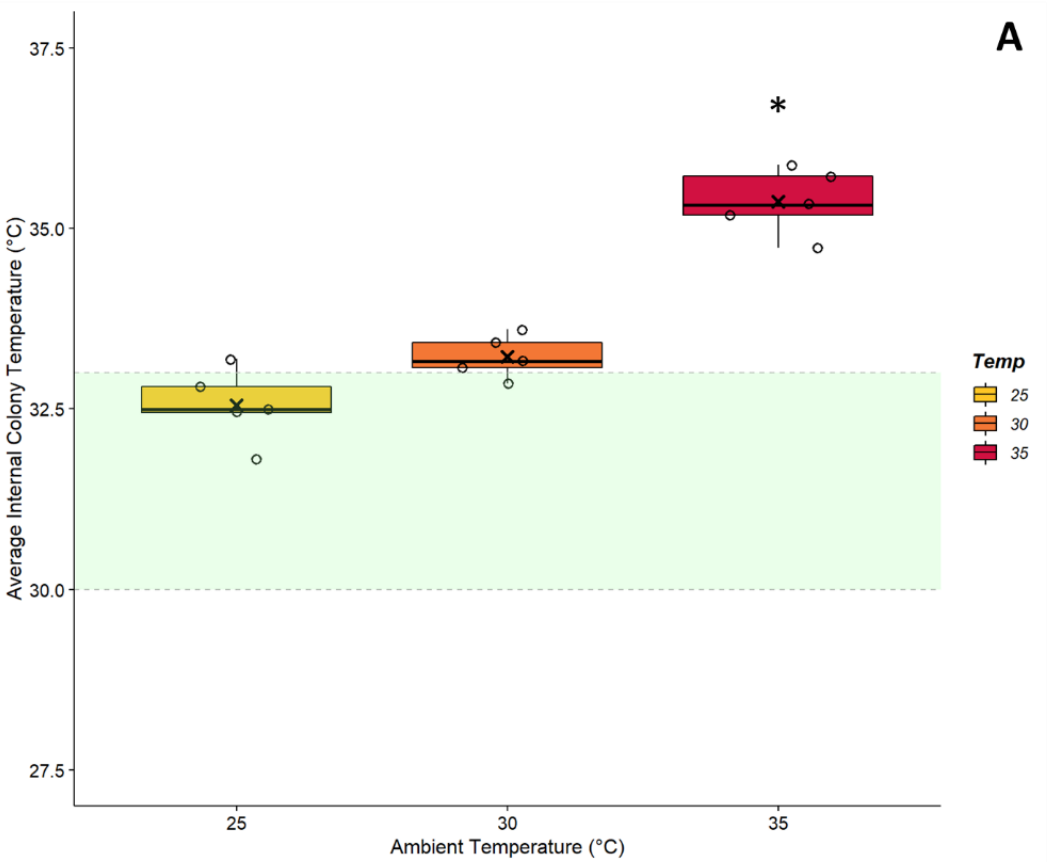


Fig. 3.3. Fanning incidence of *B. impatiens* workers increases under chronic heat stress. Bumblebee colonies of 43 workers were exposed to 25°C (yellow), 30°C (orange) or 35°C (red) for two-week periods (n=5 colonies per ambient temperature, T_a). The incidence of fanning was quantified daily by counting workers engaged wing fanning for 10+ seconds within a 165cm^2 area overtop the brood. The daily totals were averaged across each T_a trial. Fanning incidence at 35°C was significantly higher than at lower T_a (*$P \leq 0.049$). Box plots represent percentiles with the black bar across equalling the median value. Also present are the individual data points to show the spread of the colonies tested. The average value is denoted by an (x) within each box.


A
37.5
35.0
32.5
30.0
27.5
Average Internal Colony Temperature (°C)
25
30
35
Ambient Temperature (°C)
Temp
25
30
35

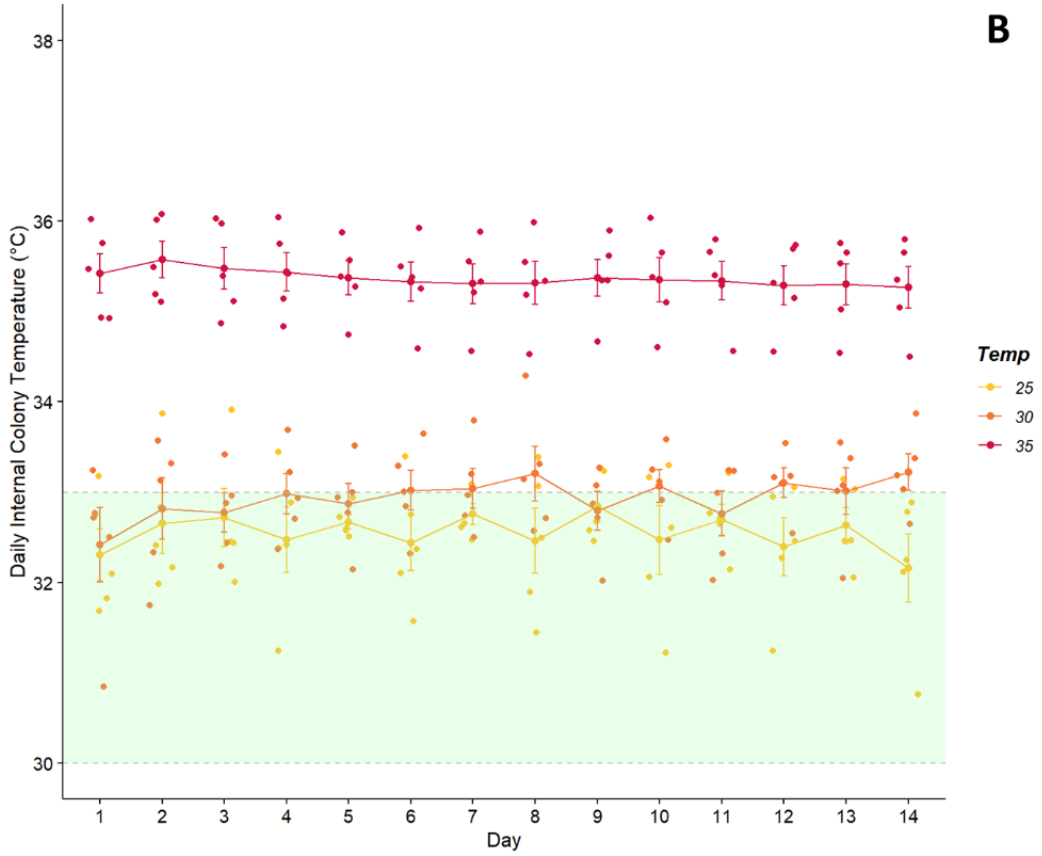

B
38
36
34
32
30
Daily Internal Colony Temperature (°C)
1
2
3
4
5
6
7
8
9
10
11
12
13
14
Day
Temp
25
30
35

Fig. 3.4. Internal colony temperature cannot be maintained under chronic heat stress. Bumblebee colonies (*B. impatiens*) of 43 workers were exposed to 25°C (yellow), 30°C (orange) or 35°C (red) for two-week periods (n=5 colonies per ambient temperature, T_a). Internal colony temperature was determined using iButtons placed within colony brood clumps. Optimal nest temperature (Barrow and Pickard, 1985; Heinrich, 2004; Schultze-Motel, 1991; Vogt, 1986a) is represented by the green shaded area on each panel. **A)** Average internal colony temperature, calculated across each T_a trial, was significantly higher at 35°C than at lower T_a (*P<0.001). Box plots represent percentiles with the black bar across equalling the median value. Also present are the individual data points to show the spread of the colonies tested. The average value is denoted by an (x) within each box. **B)** The daily average internal colony temperature, calculated as the mean on each experimental day, generally did not differ on a daily basis across two-week expose periods with the exception of an increase on days 8 and 14 at 30°C (P≤0.016). Individual data points are scattered about the mean line and standard error points for each colony tested.

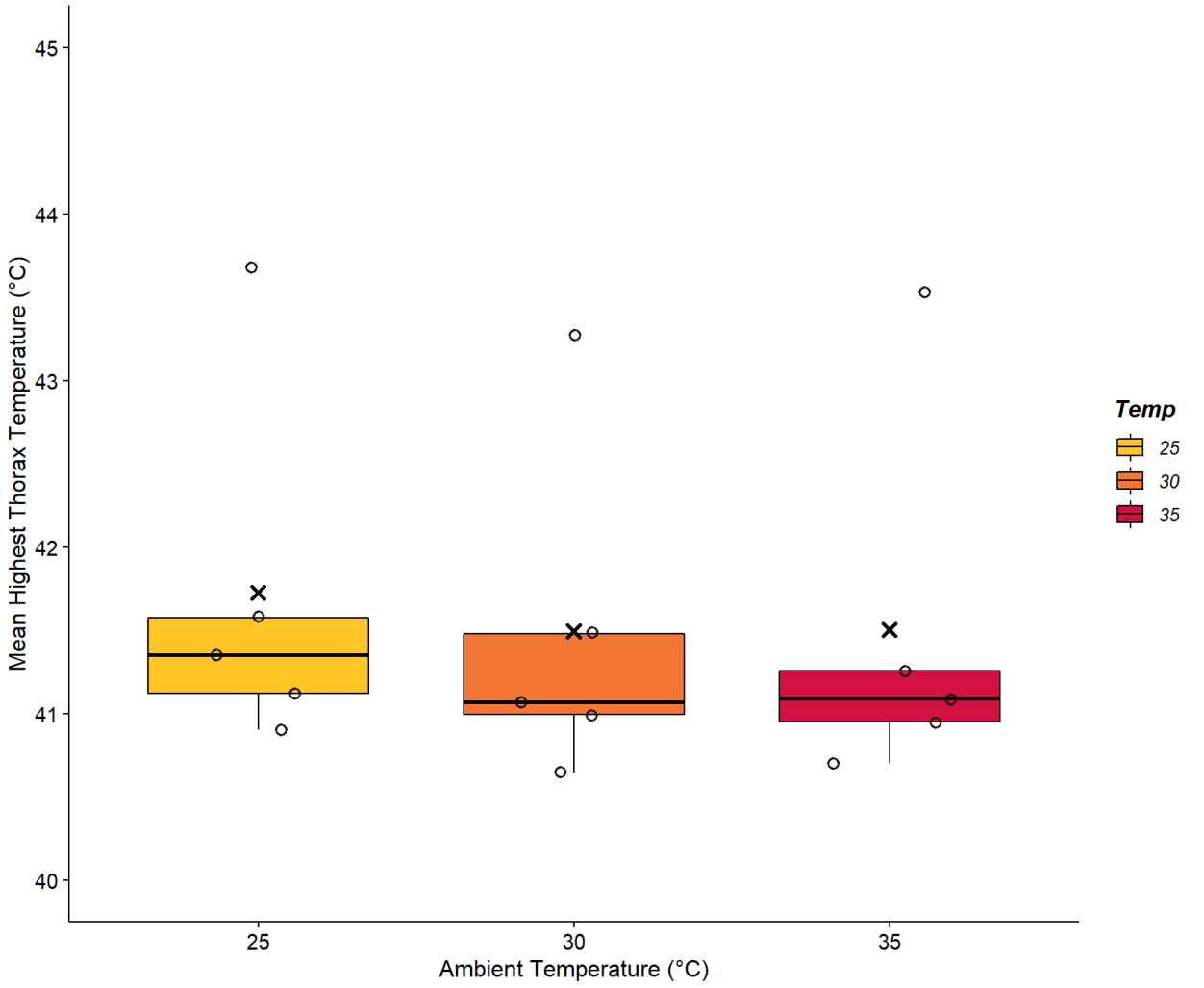


Fig. 3.5. The mean highest thorax temperature observed within *B. impatiens* colonies is not altered by chronic heat stress. Bumblebee colonies of 43 workers were exposed to 25°C (yellow), 30°C (orange) or 35°C (red) for two-week periods (n=5 colonies per ambient temperature, T_a). An infrared (IR) digital camera recorded the maximum thorax temperature (T_{th}) within a 165cm^2 field of view, daily. Daily totals were averaged across each experimental trial where mean values for a given T_a were not significantly different (P=0.312). Box plots represent percentiles with the black bar across equalling the median value. Also present are the individual data points to show the spread of the colonies tested. The average value is denoted by an (x) within each box.

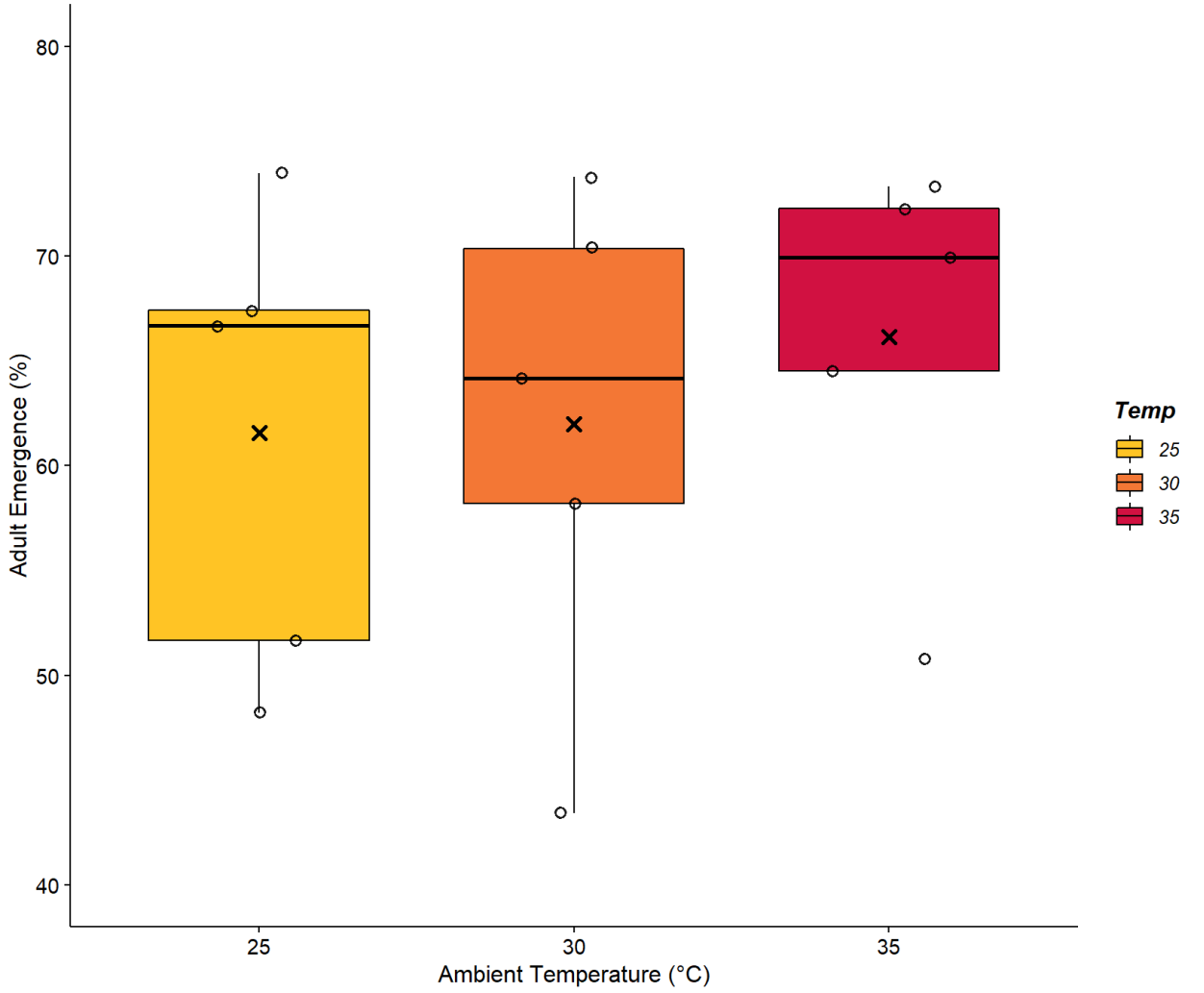


Fig. 3.6. Chronic heat stress does not alter the percent of adult emergence of *B. impatiens* colonies. Bumblebee colonies of 43 workers were exposed to 25°C (yellow), 30°C (orange) or 35°C (red) for two-week periods (n=5 colonies per ambient temperature, T_a). The percentage of workers that emerged during an experimental trial was calculated to determine adult emergence, which ultimately did not depend on T_a (P=0.765). Box plots represent percentiles with the black bar corresponding with the median value. Also present are the individual data points to show the spread of the colonies tested. The average value is denoted by an (x) within each box.

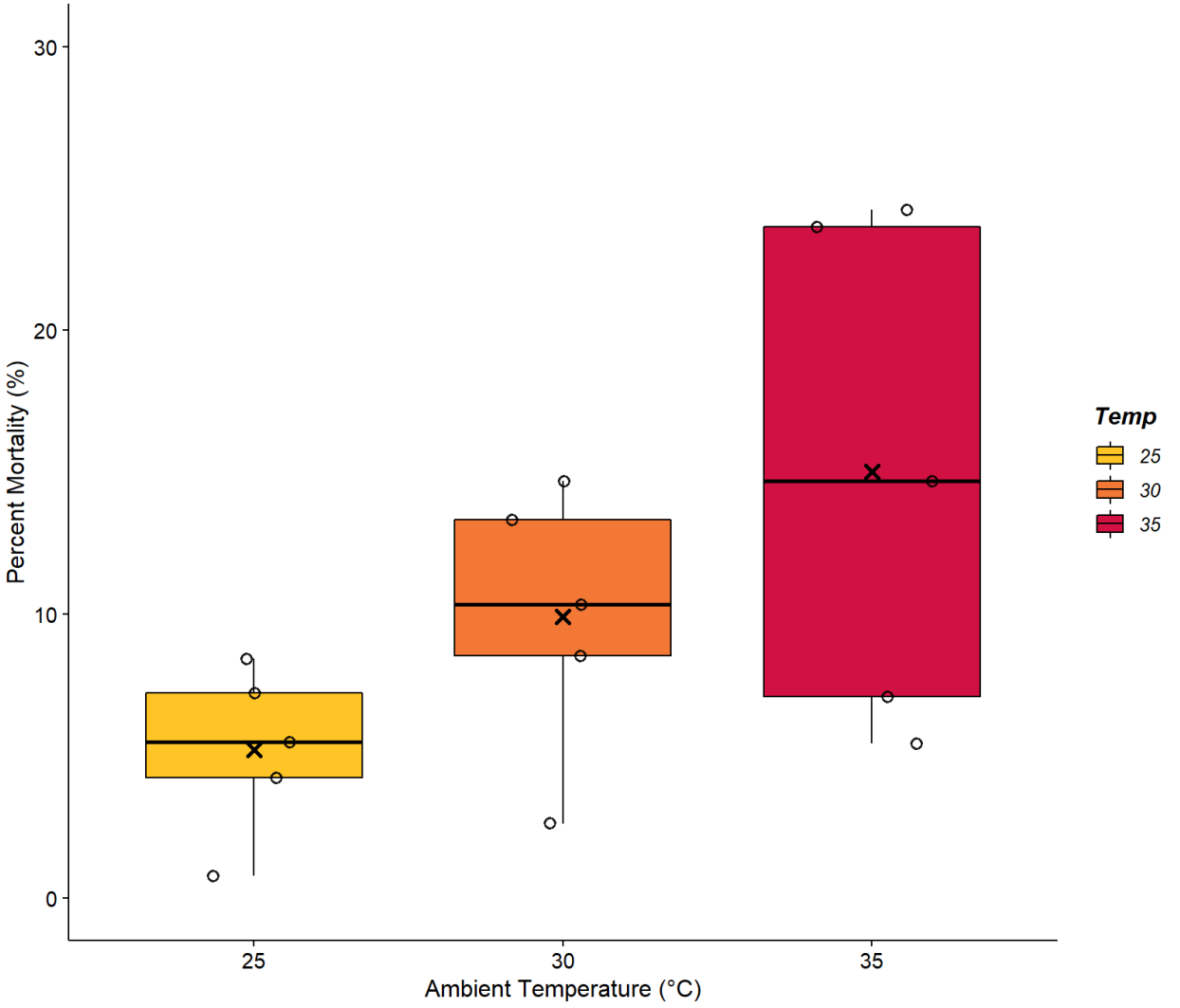


Fig. 3.7. Chronic heat stress does not change the percentage of mortality in *B. impatiens* colonies. Bumblebee colonies of 43 workers were exposed to 25°C (yellow), 30°C (orange) or 35°C (red) for two-week periods (n=5 colonies per ambient temperature, T_a). The percentage of bees who were found deceased during or at the end of each experimental trial represents mortality rate. No significant differences were found between tested values of ambient temperature, T_a (P=0.073). Box plots represent percentiles with the black bar across equalling the median value. Also present are the individual data points to show the spread of the colonies tested. The average value is denoted by an (x) within each box.

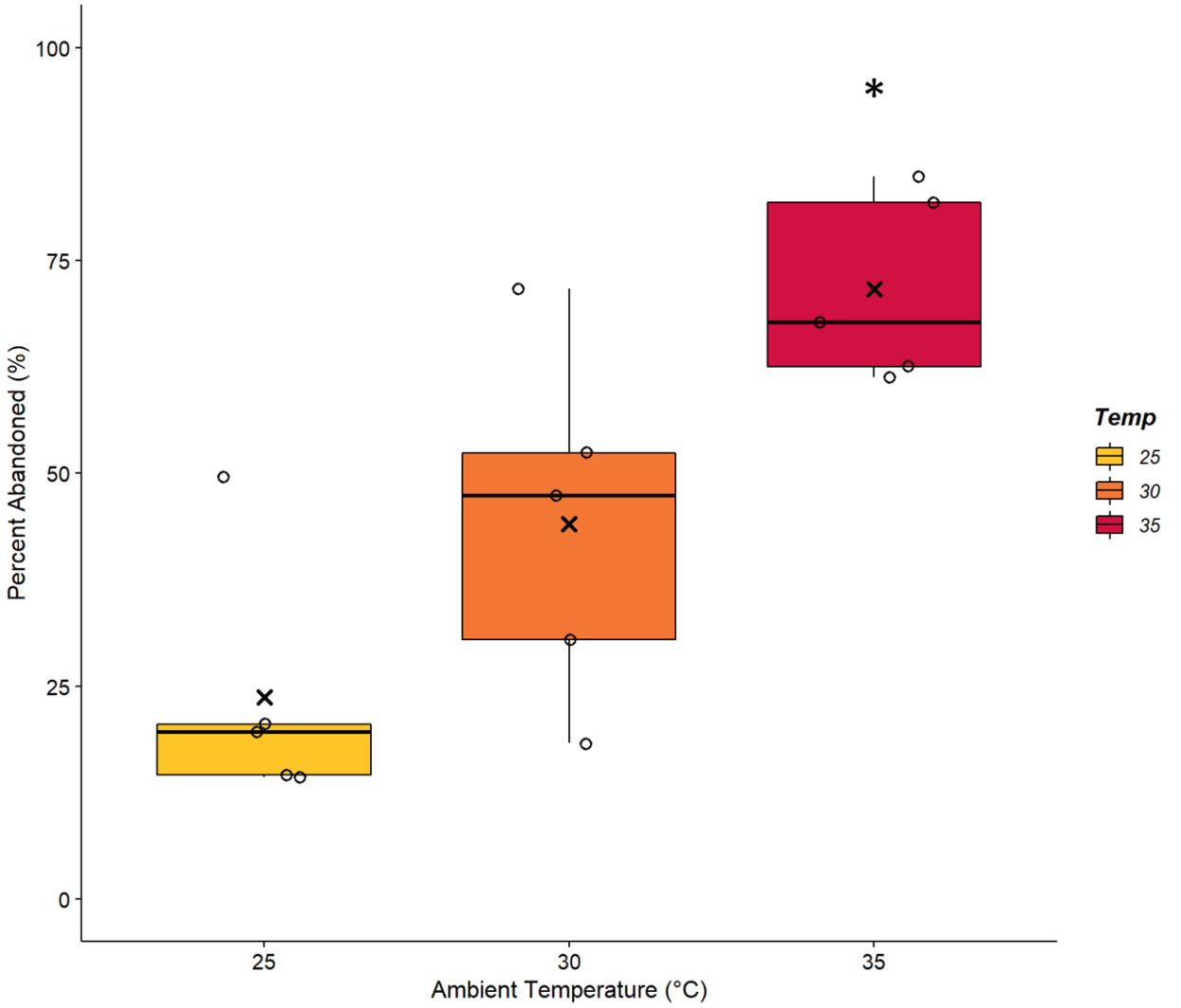


Fig. 3.8. Chronic heat stress results in a higher percentage of *B. impatiens* workers abandoning their colony. Bumblebee colonies of 43 workers were exposed to 25°C (yellow), 30°C (orange) or 35°C (red) for two-week periods (n=5 colonies per ambient temperature, T_a). The percentage of workers who were found outside the colony and in the flight cage either deceased during or alive at the end of each experimental trial was used to quantify percent abandonment. This value was significantly higher at 35°C than at lower T_a (*$P \leq 0.044$). Box plots represent percentiles with the black bar across equalling the median value. Also present are the individual data points to show the spread of the colonies tested. The average value is denoted by an (x) within each box.

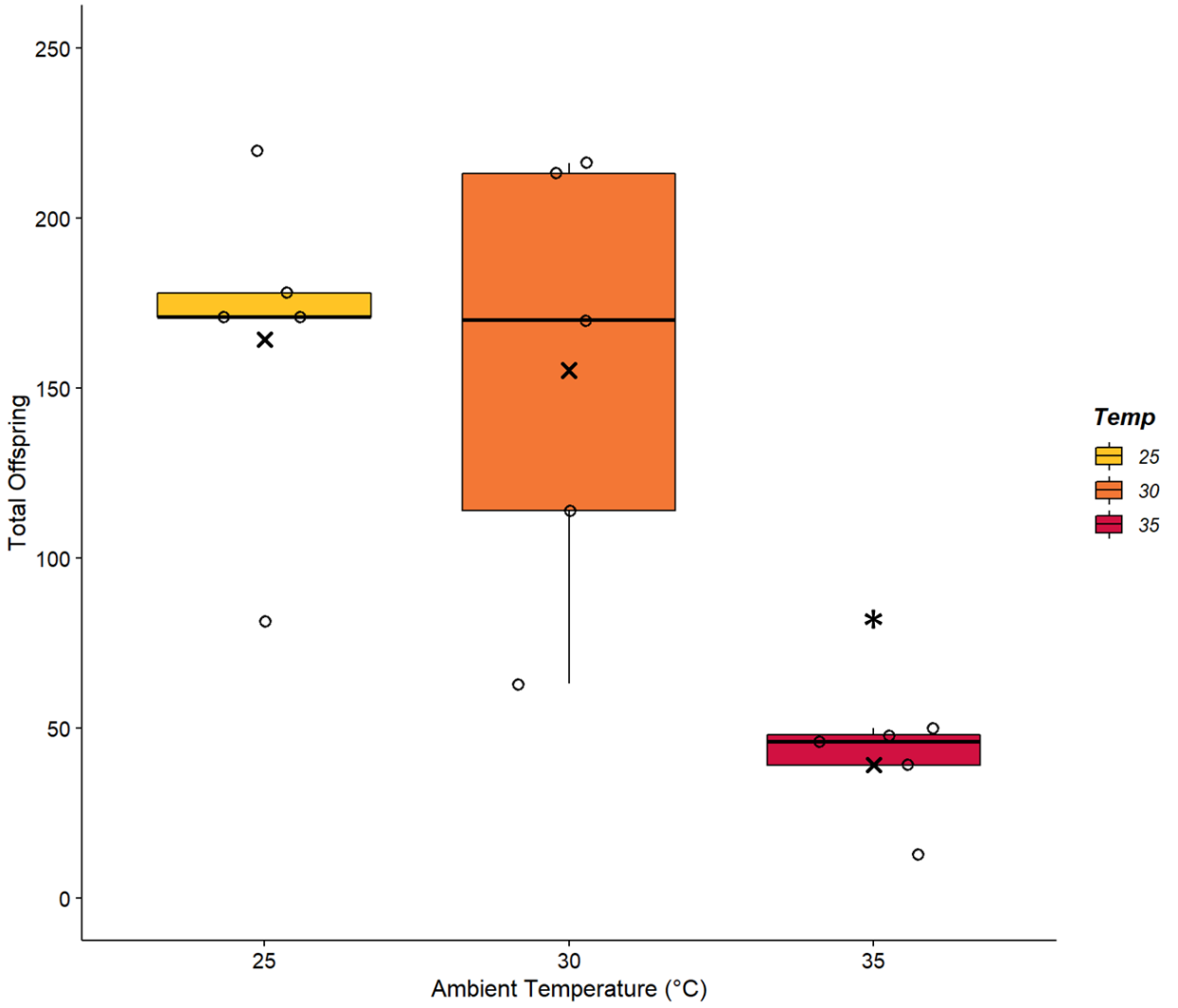


Fig. 3.9. Offspring production is negatively impacted by chronic heat stress. Bumblebee colonies (*B. impatiens*) of 43 workers were exposed to 25°C (yellow), 30°C (orange) or 35°C (red) for two-week periods (n=5 colonies per ambient temperature, T_a). The total number of offspring present was dependent on T_a (P=0.0026). The number of larvae and pupae present in dissected broods following the end of each experimental trial quantifies offspring production. Significantly fewer offspring were found at 35°C when compared to lower T_a (*$P \leq 0.007$). Box plots represent percentiles with the black bar across equalling the median value. Also present are the individual data points to show the spread of the colonies tested. The average value is denoted by an (x) within each box.

Chapter 4: The effects of an insulative pile layer at high ambient temperature on the flight metabolic rate of bumblebees

4.1 Abstract

Flight is an essential behaviour for adult bumblebees, allowing for the dispersion of reproductive queens and drones, as well as enabling workers to forage for resources. Temperate bumblebees possess heat-conserving adaptations which permit them to achieve flight, including an insulative layer of fur, known as pile. While these adaptations prove useful in cool, temperate environments, higher ambient temperatures (T_a) can cause overheating as well as reduce individual flight performance, such as endurance. It is unknown however, whether adaptations to prevent heat dissipation at cool T_a will instead impede this process at high T_a, especially under future projected increases in global temperature. This study thus investigated whether the adaptation of pile may be a source of hindered flight performance at high T_a for a temperate species of bumblebee, *Bombus impatiens*. I assessed the metabolic outputs during flight of both workers, queens and drones both with their pile intact and removed at 25, 30 and 35°C. The absence of pile did not alter the flight metabolic rates of bumblebees at any given temperature, suggesting that pile does not increase nor decrease the energetic requirements of flight under warm conditions. Future direction would be to continue to assess whether pile affects other flight performance measures, enhancing our understanding of what may act to limit bumblebees within their thermal environment.

4.2 Introduction

Rising global temperatures (The Core Writing Team IPCC, 2015) place urgency on determining how species cope physiologically to temperature, especially in the case of ectotherms, where body temperature primarily conforms to that of the environmental ambient temperature, T_a (Angilletta et al., 2010; Clarke, 2017). In general, the lower thermal limits of ectothermic insects can be predicted by latitudinal gradients, yet their heat tolerances are not as predictable (e.g., Addo-Bediako et al., 2000; Calosi et al., 2010; Oyen et al., 2016; Sunday et al., 2019). Moreover, some terrestrial ectotherms already live at or beyond their physiological upper thermal limits (Sunday et al., 2011), especially in direct exposure to sunlight (Pinsky et al., 2019). Flying insect pollinators, such as bees, are a group of particular concern with regards to climate change. Not only do they play a crucial role in our ecosystems and agriculture (Gill et al., 2016; Klein et al., 2006) but have experienced range shrinkages (Kerr et al., 2015), as well as species (Soroye et al., 2020) and observed density declines (Rasmont and Iserbyt, 2012). Therefore, it is important to understand how vulnerable species will respond to temperature and whether essential behaviours will become constrained under future increases in global temperature.

A starting point for understanding the ecological effects of temperature and climate change is to assess whole-animal performance (reviewed in Sinclair et al. 2016). Between upper and lower thermal limits lie a range of temperatures over which individual performance varies. Plotted as a thermal performance curve (Huey and Stevenson, 1979), performance – usually a quantification of metabolism, growth, locomotion, reproduction, etc. – increases with temperature towards an optimum where it will subsequently decline with further temperature elevation (Huey and Stevenson, 1979; Schulte et al., 2011). In the case of bees, the understanding of their response to T_a is complicated by their ability to generate heat for flight.

Flight muscles within the thorax require a minimum of 30˚C to achieve flight (Heinrich, 2004) which is generated primarily through wing shivering. Flight and associated thermogenesis however, impose both energetic and thermal costs on the individual.

The first cost of flight is the overall energetic expense of the behaviour. Flight increases insect metabolic rates by upwards of and exceeding 50 times that of rest (Billardon and Darveau, 2019; reviewed in Ellington, 1985), making it one of the most energetically demanding activities. This energy expenditure however, can lead to a secondary flight cost wherein approximately 90% of that energy is converted to heat within the flight muscles (Heinrich, 2004). Bees are the second hairiest class of insect pollinators with bumblebees having the longest pile (or fur) lengths within bee genera (Roquer-Beni et al., 2020). Species inhabiting cool climates tend to have longer pile lengths versus those from warm climates (Peat and Goulson, 2005). This is a positive adaption since excess heat generation, in addition to an insulative layer of pile to reduce heat loss, allows flight muscle temperature to be maintained when T_a is below the minimum necessary for flight (see Heinrich 1974a, 2004). These adaptations however, may not always be beneficial. For example, increases in thorax temperature (T_{th}) from metabolic heat may be in excess of surface related rates of convective and evaporative cooling (stingless bees; Unwin and Corbet 1984), which stresses the need for heat-dissipating behavioural and physiological adaptations. For instance, tongue lashing – the manipulation of a regurgitated fluid droplet – promotes evaporative cooling in bumblebees (i.e. Heinrich 1976) and honeybees (see Jones and Oldroyd 2006), while in bumblebees, heated blood from the thorax is actively transferred to the abdomen for convective heat loss (Heinrich, 1976) under high T_a. Though, previous works describe how feathers and fur constrain heat loss in endotherms (Speakman and Król, 2010) and that seasonal moults may help lower body temperature by increasing thermal conductance (Zhao

and Cao, 2009), moulting is not possible for endothermic insects. Furthermore, research demonstrates that longer pile lengths decrease rates of cooling for euthanized bumblebees (Parsons, 2017), meaning that the ability of these species to dissipate excess heat under high T_a, and the resultant consequences on performance, comes into question, especially for temperate adapted species with insulative layers of pile.

Flight is one of the most important behaviours for social bees, permitting them to procure energy rich resources to sustain colony growth and reproduction (Heinrich, 2004; Vaudo et al., 2018) and thus it is essential to determine what constraints high T_a may impose on an individual's flight performance. It has been previously determined that high T_a reduces flight endurance (bumblebees; Kenna et al. 2021), time spent in flight (honeybees; Woods et al. 2005) and can also reduce the number of foraging trips taken (bumblebees; Couvillon et al. 2010). Optimal T_a for flight endurance peaks at 25˚C (bumblebees; Kenna et al. 2021) and force production, as well as flight metabolic rate (FMR), are maximized at a T_{th} of 39˚C (Coelho, 1991; Glass and Harrison, 2022), but bumblebee queens appear to overheat when flown at a T_a of 35˚C which corresponds to a T_{th} above 40˚C (Heinrich, 1975). Therefore, understanding what aspects of bee physiology set and constrain flight performance within these species is paramount in determining whether the essential behaviour of flight will be limited under climate change.

The present study aimed to examine whether the temperate adaptation of pile imposes any such limitations to flight at high T_a by examining performance through FMR on a species of temperate bumblebee, *Bombus impatiens*. Energy expenditure during voluntary free-flight is known to be independent of T_a for honeybees and bumblebees (Heinrich, 1980, 1975; Woods et al., 2005) but bumblebees flown above 30˚C, for instance, appear unable to prevent T_{th} from rising above the preferred range of 36-41˚C (Heinrich, 1975), values which encroach on critical

thermal limits (Hamblin et al., 2017; Oyen and Dillon, 2018; Chapt. 2). When T_{th} is elevated, abdominal pumping rates increase in bees both at rest (Heinrich, 1977; Dzialowski et al., 2014) and during flight (Heinrich and Buchmann, 1986). This phenomenon coincides with increased abdominal temperature, supporting the active heat transfer of excess heat from the thorax to the abdomen in an attempt to prevent overheating (Heinrich, 1976). The lack of change in FMR across T_a for bumblebees implies that this mechanism of active heat transfer is also employed to prevent overheating during flight (Heinrich, 1975). However, given that overheating appears to occur when T_{th} exceeds 40 ˚C in flight (Heinrich, 1975), I sought to determine whether the absence of pile would lower the energetic costs of active thermoregulation during flight, given that insulative pile may otherwise hinder excess heat dissipation from the thorax and abdomen of a bee. In order to test this prediction, I compared the FMR of bees with and without pile at 25, 30 and 35˚C. Additionally, each adult caste partakes in flight, whether they are foraging workers or drones and queens dispersing for reproduction. Thus, I also included each of these castes within my study. Finally, methodological differences to experimentally promote flight, such as agitation, are documented to induce an inverse relationship between T_a and FMR (Harrison et al., 1996a; Roberts and Harrison, 1998; Woods et al., 2005). Furthermore, given that T_{th} can approach lethal values at high T_a, I used a subset of workers both with pile intact and removed to test for the effects of agitation on FMR and to quantify T_{th} at each T_a.

4.3 Methods

4.3.1 Bumblebee colonies and holding conditions

Colonies of *Bombus impatiens* (Biobest Canada Ltd., Leamington, ON, Canada) were contained in the supplier's housing boxes within a room maintained at approximately 25˚C on a 12h:12h light:dark photoperiod. BIOGLUC® sugar solution from the supplier was available *ad*

libitum and pollen was provided twice weekly. Individual workers and drones were randomly sampled from four colonies while new queens emerged from two colonies.

In order to test whether the presence or absence of pile altered a bee's metabolic rate during flight, bees were first divided into two treatment groups: pile intact and removed. A day prior to flight testing, bees were collected from one parent colony at random. Half of these individuals were chosen randomly to have their pile removed. While kept incapacitated on a cold-pack, small forceps were used to gently remove pile from the dorsal and lateral sides of the thorax and a razor blade was used to carefully remove pile from the dorsal side of the abdomen. Preliminary testing revealed that bees would not fly if pile removal occurred on the same day as flight testing. As such, individuals with both pile intact and removed were placed into a separate housing box with access to only BIOGLUC® sugar solution until testing the following day.

4.3.2 Ambient temperature, flight metabolic rate and thorax temperature

To determine the effect of temperature on the FMR of workers, drones and queens, both individuals with pile intact and removed from each caste were flown at three different ambient temperatures: 25, 30 and 35°C. Temperature was controlled using a PELT-5 (Peltier Effect Temperature Controller, Sable Systems International, Las Vegas, NV, USA) connected to a Peltier Effect Temperature-Controlled Portable Cabinet (PTC-1; SSI). The PTC-1 was fitted with a plexiglass lid, housing a rubber glove insert. Within the PTC-1 a 2L narrow-mouth, glass jar with hose connector outlet was fitted with inflow and outflow tubing to measure the CO_2 output of a bee during flight. A FOXBOX Respirometry System (Field Gas Analysis System; SSI) was used to push air, scrubbed free of water using a Drierite (W A Hammond Drierite Co Ltd, Xenia, OH, USA) column, at a rate of 800-900 ml hr^{-1} and a copper coil equilibrated incurrent air temperature with that of the PTC-1. Temperature within the jar was confirmed using a

thermochron iButton® (iButtonLink Technology, Whitewater, WI, USA) digital temperature logger. Data on the percent CO_2 production and flow rate was directly sent to Expedata Analysis Software (SSI) where it was graphed real-time.

Individual bees vary in their willingness to fly, and previous methods have established that agitation will impact the relationship between FMR and temperature for honeybees (Woods et al., 2005). As such, both agitated and non-agitated flight was performed to assess these patterns for *B. impatiens*. For both agitated and non-agitated flight trials, individuals collected the previous day were transferred to the glass respirometry jar, one bee per trial. UV and white-light flashlights were placed at the top of the PTC-1 to stimulate flight. Metabolic measurements from these individuals were recorded until bees were able to maintain a constant flight period resulting in 30 seconds of stable CO_2 production. This data was extracted using the "flattest" function in Expedata and converted into VCO_2 (ml CO_2 hr^{-1}) to represent FMR.

Agitated flight involved a gentle yet constant shaking motion of the jar. A total of 12-13 workers were used for each pile and temperature treatment group. For non-agitated flight, I considered all bees who required minor or no agitation to resume flight (see Skandalis and Darveau 2012) and divided them into two grades of flight quality: A-quality flight, consisting of bees who needed little to no agitation and who did not use the walls of the jar to fly; and B-quality flight, consisting of bees who required minor agitation and would occasionally fly against the jar walls. Free-flight in queens could not be elicited and I instead defined their flight quality as two separate grading categories: C+ quality flight, consisting of queens who typically flew against the walls with constant wing beats and required infrequent jar tilting or agitation to maintain this behaviour; and C-quality flight, consisting of queens who were less motivated, requiring slightly more agitation or jar tilting to encourage them to fly against the walls of the

jar. All other bees who either required heavy agitation or refused to fly, were not considered for analysis. Within the considered grade categories, a total of 17-25 workers, 8-13 drones, and 6-12 queens comprised the usable samples for each pile × temperature treatment group. The mass of each bee flown was recorded post-flight.

Finally, to investigate the effect of T_a and pile on T_{th}, infrared (IR) photographs were taken for a subset of workers in the agitated flight trials (4-8 bees for each pile and temperature group combination). Following FMR data collection, bees were transferred into a tube with a wire mesh stopper on one end. A syringe plunger was forced into the tube to trap the bee and press it against the mesh wire. An IR camera (FLIR Systems, Inc., Wilsonville, OR, USA) set up beside the PTC-1 was used to take a photograph of the bee where maximum temperature within the photo window was recorded representing the thorax of the individual.

4.3.3 Data analysis

Separate analyses were conducted for agitated flight and non-agitated flight of workers, drones and queens as well as for T_{th} of agitated flight using general linear models in R (R Core Team, 2014). FMR or T_{th} was considered the dependent variable with mass as a covariate. The effects of ambient temperature, pile, colony of origin and grade (for non-agitated flight) were independent variables in these models. For non-agitated worker, drone and queen flight, I was unable to compute Type III sum of squares for my saturated models due to warnings of collinearity. As such, I initially attempted to use data transformations as well as mass-corrected FMR (residual values obtained from the regression between FMR and mass) in my models to solve the issue. Neither of these options alone resolved the warnings and thus, individual regressions were carried out between FMR and single categorical variables to determine if one could be eliminated from the saturated model due to non-significance. For workers, colony was

non-significant, removed from the saturated model and a new model was able to be computed using mass-corrected FMR as the dependent variable. For drones, colony was also insignificant, and a new model with colony removed was able to be computed using FMR as the dependent variable and mass as a covariate. For queens, flight grade was non-significant, removed from the saturated model, and similar to drones, FMR was used as the dependent variable with mass as a covariate. All final model choices were determined by removing non-significant terms and verified using AIC. Pairwise analyses were conducted using the Tukey method.

4.4 Results

4.4.1 The effect of agitation, ambient temperature and pile on flight metabolic rate and thorax temperature

The flight metabolic rate of workers experiencing agitated flight was independent of ambient temperature (Fig. 4.1). Ambient temperature was not a significant predictor of FMR (T_a: $F_{2,68} = 0.382$, $P=0.684$) and was subsequently excluded from the final model. Analysis through a final model of best fit revealed that both mass and pile, as well as the interaction between the two, significantly influenced FMR (mass: $F_{1,70} = 33.59$, $P<0.001$; pile: $F_{1,70} = 4.38$, $P=0.031$; mass × pile: $F_{1,70} = 5.93$, $P=0.017$). The relationship between mass and FMR produced different slopes depending on whether or not a worker had its pile removed (Fig. 4.2).

Thorax temperature for the subset of agitated flight workers was found to only be dependent on T_a (T_a: $F_{2,31} = 4.32$, $P=0.022$) with bees flown at 25˚C having a higher T_{th} (35.01 ± 0.65˚C, SE) than bees flown at 30˚C (31.82 ± 0.97˚C, SE; $P=0.017$) but not compared to those at 35˚C (33.34 ± 0.59˚C, SE; $P=0.280$). Thoracic temperatures of bees flown at 30˚C and 35˚C were neither significantly different from one another ($P=0.251$).

4.4.2 The effect of ambient temperature and pile on flight metabolic rate during free-flight

The mass of a worker undergoing non-agitated flight was found to be a significant predictor of FMR (Fig. 4.3; mass: $F_{1,123} = 659.87$, $P<0.001$). When accounting for mass through residual values from the regression with body mass, mass-corrected FMR was independent of temperature and of pile removal treatment (Fig. 4.4) but dependent on flight quality and the interaction between flight quality and T_a (T_a: $F_{2,118} = 0.18$, $P=0.838$; pile: $F_{1,118} = 3.37$; $P=0.069$; grade: $F_{1,118} = 15.55$, $P<0.001$; $T_a \times$ grade: $F_{2,118} = 3.36$, $P=0.038$). Within flight-quality groupings, FMR remained independent of T_a ($P\geq0.076$). Only when comparing A-quality fliers and B-quality fliers did differences in FMR emerge ($P\leq0.046$) being that A-quality fliers had higher mean FMR values compared to B-quality fliers at all temperatures, except at 35˚C ($P=0.155$).

For drones who underwent non-agitated flight, FMR was found to only be influenced by the mass of the individual (Fig. 4.5) and neither ambient temperature nor the presence or absence of pile affected FMR (Fig. 4.6; mass: $F_{1,58} = 20.70$, $P<0.001$; T_a: $F_{2,58} = 1.30$, $P=0.279$; pile: $F_{1,58} = 1.63$, $P=0.207$).

Like workers and drones, non-agitated queens, also produced FMR values that were dependent on the mass of the queen (Fig. 4.7; mass: $F_{1,42} = 27.69$, $P<0.001$). Pile treatment, colony of origin and the interaction between T_a and pile each had a significant effect on FMR, whereas T_a alone did not (pile: $F_{1,42} = 5.84$, $P=0.020$; colony: $F_{1,42} = 8.43$, $P=0.006$; T_a: $F_{2,42} = 1.33$, $P=0.274$; $T_a \times$ pile: $F_{2,42} = 3.34$, $P=0.045$; $T_a \times$ colony: $F_{2,42} = 2.82$, $P=0.071$). Pairwise comparisons determined that FMR did not differ across T_a for bees with pile removed ($P\geq0.207$) nor intact ($P\geq0.612$) and neither for any other $T_a \times$ pile combinations (Fig. 4.8; $P\geq0.162$). Queen FMR neither varied across T_a within either of the two colonies of origin used for queens ($P\geq0.483$). Differences only emerged when comparing mean FMR between these two colonies at

various temperatures: the first colony had lower FMR values versus the second colony at each T_a ($P \leq 0.002$), except when comparing the first colony at 25˚C with the second colony at 25 and 30˚C ($P \geq 0.061$).

4.5 Discussion

Flight is an essential behaviour for bees and their colonies which enables foraging and dispersal of reproductive castes, but the limitations that high T_a imposes on flight performance is still unclear. To assess limitations of high T_a and whether the temperate adaptation of pile is a hinderance to performance under these conditions, I measured the FMR of *B. impatiens* at 25, 30 and 35℃ using individuals with either their pile intact or removed. The FMR of all adult castes, including workers, drones and queens, were tested using non-agitated flight methods. Additionally, agitated flight was assessed for workers to compare with previous findings indicating that the relationship between T_a and FMR differs depending on whether bees are engaged in voluntary or experimentally agitated flight. Overall, the absence of pile did not lower the metabolic output during flight at high T_a for *B. impatiens* adults. This finding implies that the energetic costs associated with active heat dissipation during flight are not impeded by an insulating layer of pile. Future testing of the impact of pile on other flight measures may reveal whether physiological mechanisms which promote heat dissipation are unable to sufficiently prevent overheating, resulting in previously observed declines to flight performance.

Body mass was found to be a significant predictor of FMR where the metabolic output of hovering flight will be greater for larger queens, drones and workers engaged in both agitated and non-agitated flight. Allometric scaling of FMR previously documented in *B. impatiens* workers, queens and drones (Billardon and Darveau, 2019; Darveau et al., 2014; Skandalis and Darveau, 2012), demonstrates the importance of accounting for body mass in FMR analyses.

Increased metabolic heat production is necessary for thermoregulation at cool temperatures to offset heat loss and, during flight, this reduction in metabolic rate is thought to correspond with increases in T_a (Moffatt, 2001). Such inverse relationships between FMR and T_a have been observed for honeybees (Harrison et al., 1996a) and bumblebee queens (Silvola, 1984), but these results may be due to the methodological application of agitation to promote flight in these studies. Woods et al. (2005) tested such an effect on honeybee flight, finding that without agitation the relationship disappears. The Woods et al. study also offers a potential explanation for observed inverse relationships between FMR and T_a, being that when not in flight, bees ready themselves for the behaviour by shivering to maintain flight muscle temperature. Warm-up processes increase metabolic costs at low T_a but as T_a approaches the T_{th} necessary for flight, these costs decrease (Heinrich, 1993, 1975; Stone, 1993). Therefore, flight measurements which also capture non-flight behaviours (Harrison et al., 1996a; Moffatt, 2001) likely discover inverse relationships due to the reduced costs of warm-up under warmer conditions (Woods et al., 2005). In contrast, results from my study show that there was no effect of T_a on FMR for agitated worker flight. However, FMR analyses was uniform across all bees flown, in that the 30 seconds which best represented free flight (plateau), was used. Thus, primarily voluntary flight would have been represented instead of FMR data which also included the energetic outputs for warm-up. Future testing to include agitation could thus focus on the metabolic costs and subsequent flight of bees with and without pile present.

When comparing my FMR values for non-agitated flight of *B. impatiens* workers and drones to previous work, I find that they are within a similar range found for workers of the same species (Billardon and Darveau, 2019; Darveau et al., 2014; Skandalis and Darveau, 2012) but slightly lower than that for drones (Darveau et al., 2014). Additionally, when examining the

effect of T_a alone, my findings of FMR across T_a are consistent with previous studies on the non-agitated flight of honeybee workers (Heinrich, 1980; Woods et al., 2005) and bumblebee queens (Heinrich, 1975), showing independence between the two variables over a similar range of T_a. The independence between metabolic output and T_a which emerges for voluntary flight is explained by Woods et al. (2005) where previous work on honeybees indicates that the thermal optimum for FMR occurs when T_{th} is equal to 39˚C. Both bumblebees (Heinrich, 1975) and honeybees (Woods et al., 2005) appear to have an optimal in-flight T_{th} of 36-41˚C and in both works, when flown under a similar T_a range as in my study, bees achieved T_{th} within this optimal range. As such, the independence of FMR from T_a observed in my study coincides with FMR measures within a similar T_a range.

Additional sources of variation in free-flight FMR were the flight-quality of workers and the colony of origin of queens. Flight quality in *B. impatiens* is known to alter FMR (Skandalis and Darveau, 2012), with agitation elevating these measures for honeybees (Harrison and Fewell, 2002). In contrast, A-quality flier bees within my study generally had higher FMR compared to lower quality B-fliers at any given temperature. One possible explanation for this occurrence may be that B-quality fliers had reduced flight effort and willingness to partake in hovering flight. Secondly, colony relatedness has previously been found to influence FMR (Billardon and Darveau, 2019; Harrison et al., 1996b). As such, for the two colonies I tested, queens demonstrated similar intra-colonial FMR. However, overall, I did not find that FMR varied across T_a, neither within flight-quality grades of workers nor within colony of origin of queens, reinforcing the main finding that for the thermal conditions tested, FMR is independent of T_a.

The effect an insulating pile layer on flight performance at high T_a was one of the main points of interest within this study. Pile is considered to have thermoregulatory benefits, reducing the rate of convective heat loss (Heinrich, 1974a). Observed variations in pile length, possibly relate to heat retention (Hines et al., 2022), especially given previous associations with climate (Peat et al., 2005) and thermal activity (Peters et al., 2016). At high T_a when T_{th} approaches values which threaten overheating, heat-conserving counter-current exchange mechanisms are effectively "shut off", enabling heated hemolymph from the thorax to pass into the abdomen and facilitate convective heat loss (Heinrich, 1976). Elevated rates of abdominal pumping enables this process of active heat dissipation to occur (Heinrich, 1977; Heinrich and Buchmann, 1986). Therefore, with pile acting as an insulator, its removal should then promote passive heat loss at high T_a and thus reduce the overall energetic costs associated with flight at these temperatures. My results, however, do not support such a reduction in metabolic costs as a result of pile removal at high T_a for free-flying workers, drones or queens; FMR remained unchanged across T_a for pile treatment groups. In contrast, I did find that there is a significant interaction between mass and pile for workers undergoing agitated flight, where smaller, bees without pile had higher FMR values than bees with their pile intact of similar size. To understand this observation, it is important to note that the minimum T_{th} required for flight is approximately 30˚C and that heat, as a biproduct of flight muscle exercise, helps to achieve and maintain this temperature. Furthermore, with pile present as an insulator, bumblebees can attain T_{th} that are 65-75% higher than that of other insects of similar size and metabolic rates (Heinrich, 2004). Given that small bees with larger surface area to volume ratios warm up and lose heat more rapidly (stingless bees; Pereboom and Biesmeijer 2003), the adaptation of pile would be an essential component in a bee's ability to maintain this optimal T_{th} for flight. This concept likely explains why smaller

bees without pile display higher FMR values than their counterparts with pile still intact, since they would be more subjected to higher rates of passive heat loss without an insulative layer of pile. Alternatively, bees with pile intact would be better able to retain heat generated for flight, lowering their FMR. In contrast, large, bees with intact pile had higher FMR values than large, bees with their pile removed. With already higher metabolic rates per their size, larger bees may experience overall higher body temperatures resulting from an intact insulative pile layer further augmenting their metabolic rates.

Thorax temperature plays a key role in flight, where sufficiently high muscle temperatures are required to achieve and sustain the behaviour, and excess heat is a consequence of muscle activity (Heinrich, 1974a). Previously, queens, whose T_{th} is indistinguishable from workers over T_a ranging from 18-35˚C, possessed T_{th} of 36-45 ˚C when flown at 2-35˚C (Heinrich, 1975). Over T_a values of 25-35˚C, a range corresponding with my study, queen T_{th} increases with T_a to values between 41-45˚C (Heinrich, 1975). A similar positive relationship emerges for honeybees when T_a exceeds 25˚C (Roberts and Harrison, 1999; Woods et al., 2005) and for bumblebee workers (Heinrich, 1972c). Previous work on my study species depict mean worker T_{th} as 37.4˚C when measured 2-3 seconds after flight (Skandalis and Darveau, 2012). The discrepancies between previous works and my measures depict that I underestimated true post-flight T_{th}. Unfortunately, the time between removing the bee and capturing T_{th} was not consistent across individuals and could not be completed within a 30 second window. Since FMR data was prioritized, bees may have already ceased flight prior to being captured and photographed. Previous methods for measuring T_{th} post-flight, such as the “grab and stab” technique, occurs within a few seconds after flight to prevent heat loss from the thorax (see Heinrich 2004). Time delays thus introduced error within my measures of T_{th}. Reducing this delay between flight

termination and IR photography, in addition to increasing sample size, would improve my collection of post-flight T_{th}.

Understanding T_{th} during flight can thus lead to determining an optimal T_{th} where performance is maximized and at which temperature values performance declines are expected. This value for maximal honeybee FMR is 39˚C (Glass and Harrison, 2022). In bumblebees, flight endurance was examined where an optimal T_a for this measure of performance was 24.7˚C (Kenna et al., 2021). Furthermore, estimates of queen T_{th} at 25˚C from Heinrich (1975) are 40-41˚C, which reinforce that optimal flight metabolism and endurance occur at approximately 25˚C. It has also been shown that at high T_a, honeybees reduce their percentage of time spent in flight (Woods et al., 2005) and bumblebees are observed to take fewer foraging trips (Couvillon et al., 2010). These findings together indicate that if T_a surpasses what is optimal for flight, there is some physiological limitation on these performance metrics. While removing the insulative layer of pile did not lower the metabolic output of free-flight in my tested bees, the question still remains as to what constrains flight performance at high T_a in these species. Heinrich (1975) described that queens are able to fly at T_a as high as 35˚C but overheat as T_{th} neared lethal limits at 45˚C. Kenna et al. (2021) proposed that reductions in flight endurance at temperatures above 25˚C may be due to an inability to effectively dissipate heat, and the thermoregulatory mechanisms of bumblebees also appear to have limited capacities. For example, foragers are unable to stabilize T_{th} when T_a rises between 26-31˚C (Heinrich, 1972b) and tongue lashing behaviour is only able to reduce T_{th} by 0.5˚C at high T_a (Heinrich, 1976). Moreover, when foraging by walking between nearby floral resources, convective heat loss from blood shunted to the abdomen is reduced (Heinrich, 1972b). These findings therefore leave room for additional investigation into the effects of pile on other aspects of flight performance, such as endurance

and foraging, and whether this temperate adaptation is a hinderance to performance at high T_a by preventing heat dissipation.

4.6 Conclusions

The present study investigated whether the temperate adaptation of pile would impact the flight metabolic rates of bumblebees at different ambient temperatures. I did not find the absence of pile to have an effect on metabolic output at high T_a, though FMR is only one aspect of flight performance that can be assessed. Other performance aspects, such as flight duration and distance and resulting T_{th}, should be tested to determine whether an insulating pile layer hinders heat dissipation at high T_a. Though Kenna et al. (2021) describe how increases in T_a as a result of climate change may push bees closer to their thermal optimum for flight endurance, they also caution that extreme heat events such as heat waves, may negatively impact colony fitness as flight performance declines when optimum temperature is surpassed. Additionally, in 2008, Deutsch et al. reported that many temperate insect ectotherms would have a thermal tolerance that is 10-20°C higher than experienced during summer conditions (Deutsch et al., 2008). Johansson et al. (Johansson et al., 2020), however, re-assessed these findings using temperatures corresponding only to the active season of various temperate insect species and found that the thermal safety margin is not as expansive as originally predicted, with 9% of the species examined thought to be vulnerable to future warming. Experimental manipulation of pile presents a novel method in assessing the limitations of heat dissipation during flight for temperate-adapted species of bumblebees. Such exploration can thus aid in determining which potential factors impede performance and contribute to observed species declines (Soroye et al., 2020), ultimately furthering our understanding of how these important pollinators will respond to future thermal challenges as a result of climate change.

4.7 Figures

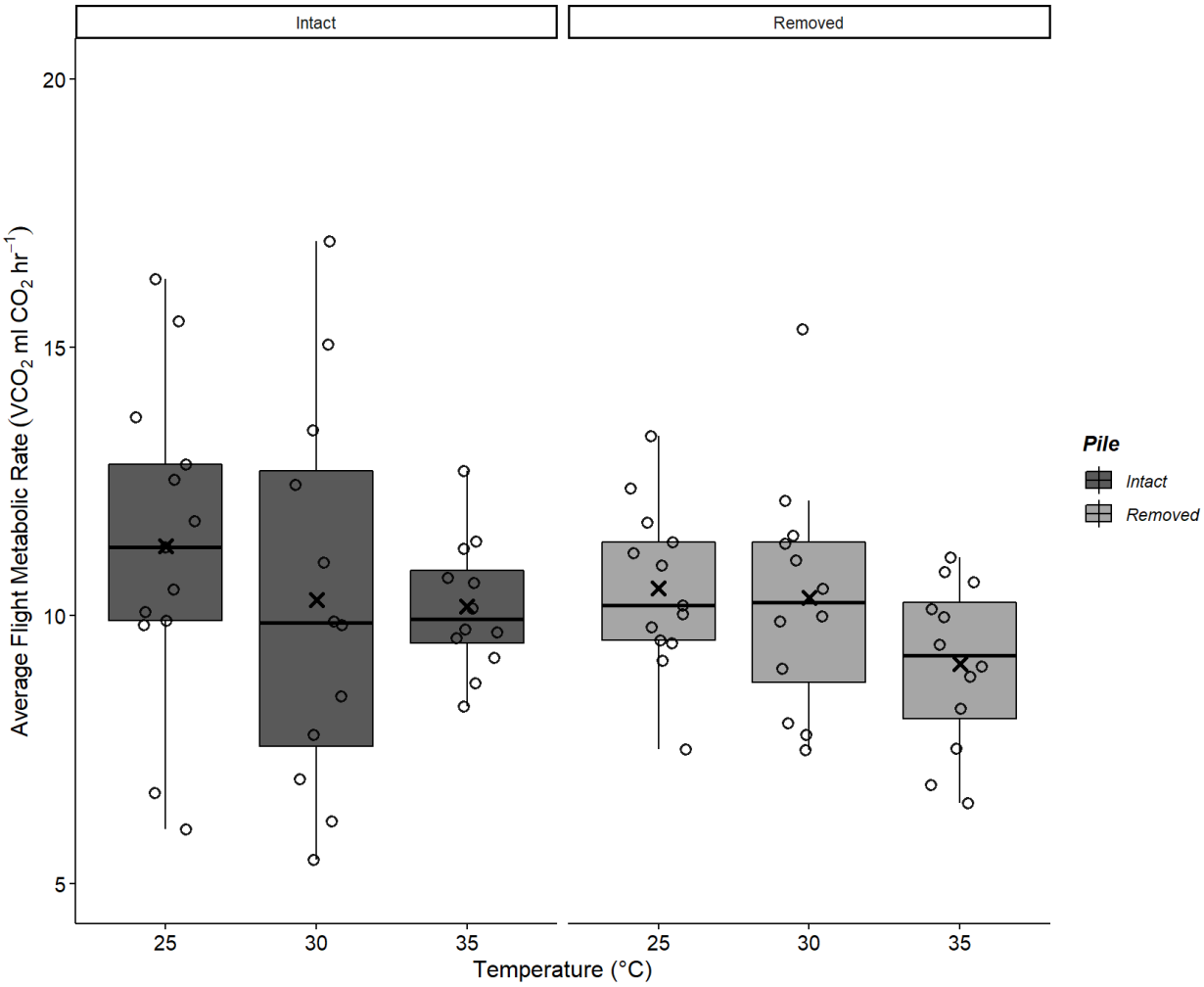


Fig. 4.1. The absence of pile does not alter the metabolic output across high ambient temperature for *B. impatiens* workers during agitated flight. Worker with their pile removed (light grey) and intact (dark grey) were flown under constant agitation at 25, 30 and 35°C to obtain their CO_2 output. Ambient temperature was not a significant predictor of flight metabolic rate (FMR; P=0.684). Box plots represent percentiles with the black bar across signalling the median value. Also present are the individual data points indicating the distribution of FMR values across individual workers. The average FMR value within each temperature group is indicated by an (x).

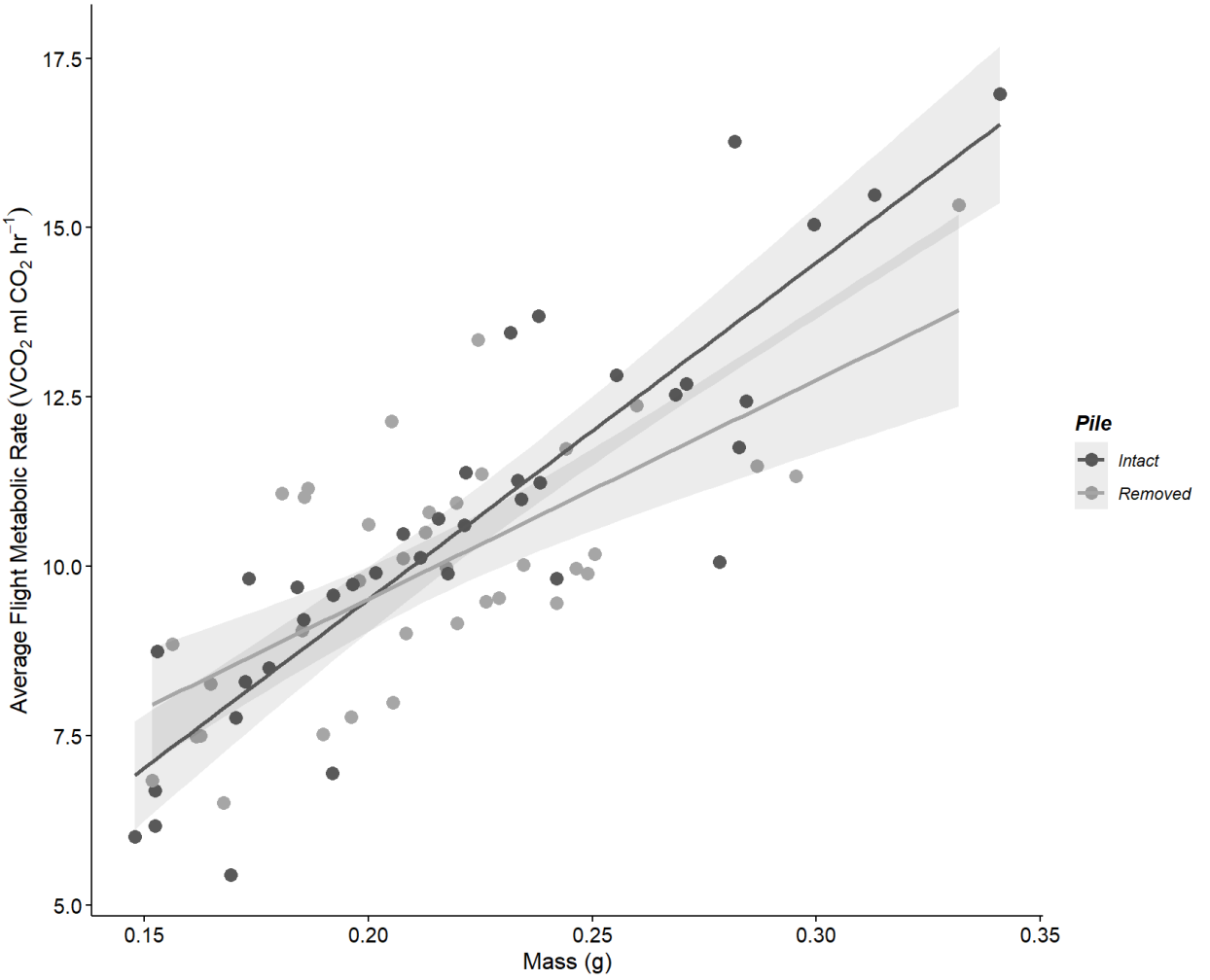


Fig. 4.2. Pile differentially affected the metabolic output of large and small *B. impatiens* workers during agitated flight. Workers with pile removed (light grey) and intact (dark grey) were flown under constant agitation at 25, 30 and 35°C to obtain their CO_2 output. The regression lines with 95% confidence intervals of pile removed and intact bees are represented by grey shading. The interaction between mass and pile was significant (P=0.017), producing different slopes between pile treatments (removed: $FMR = 32.34(mass) + 3.05$; intact: $FMR = 49.76(mass) - 0.44$).

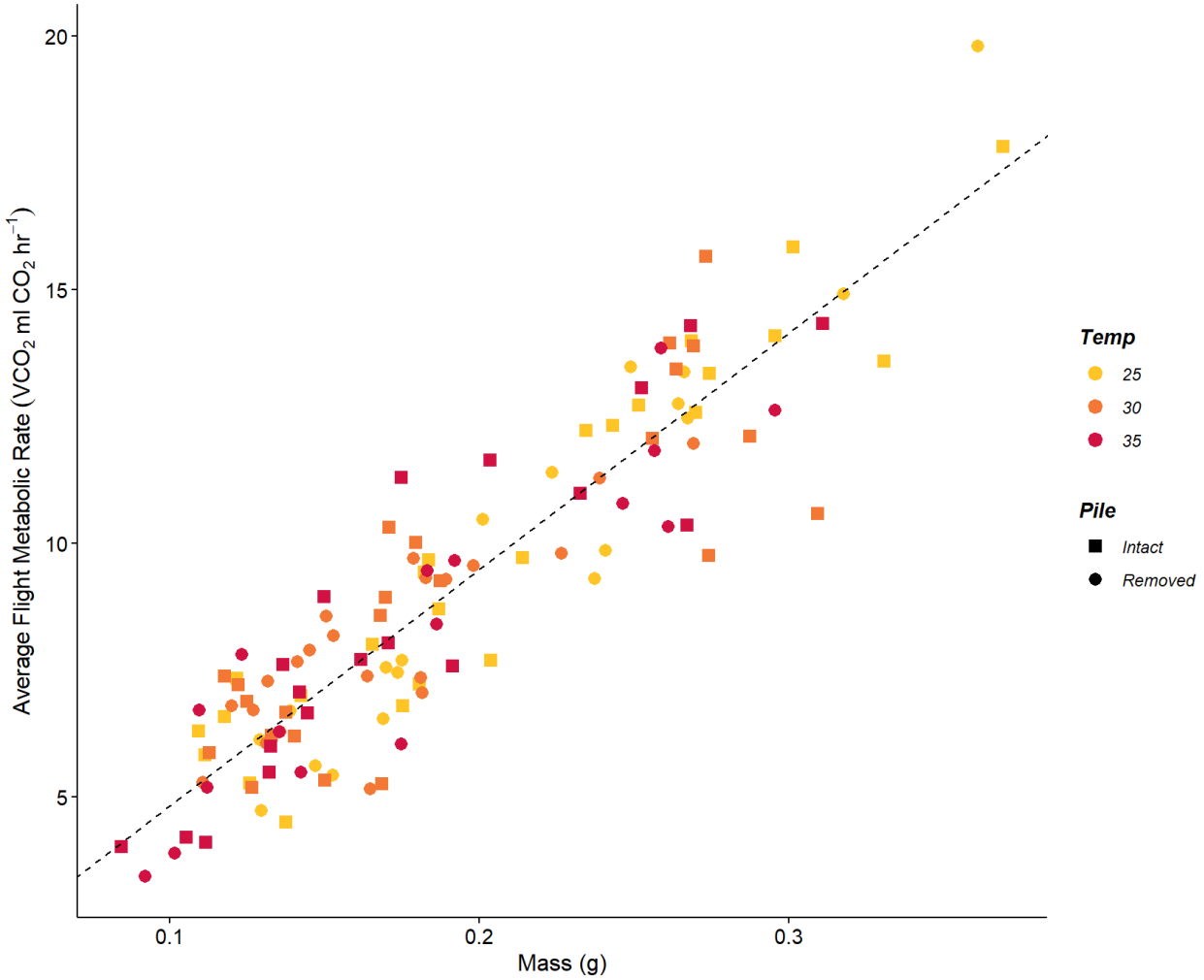


Fig. 4.3. *B. impatiens* worker mass predicts their metabolic output during free flight. Workers with pile removed (●) and intact (■) were flown without constant agitation at 25°C (yellow), 30°C (orange) and 35°C (red) to obtain their CO_2 output (flight metabolic rate, FMR). The regression between FMR and mass was significant (P<0.0001; Adj R^2=0.84; slope: $FMR = 46.65(mass) + 0.15$).

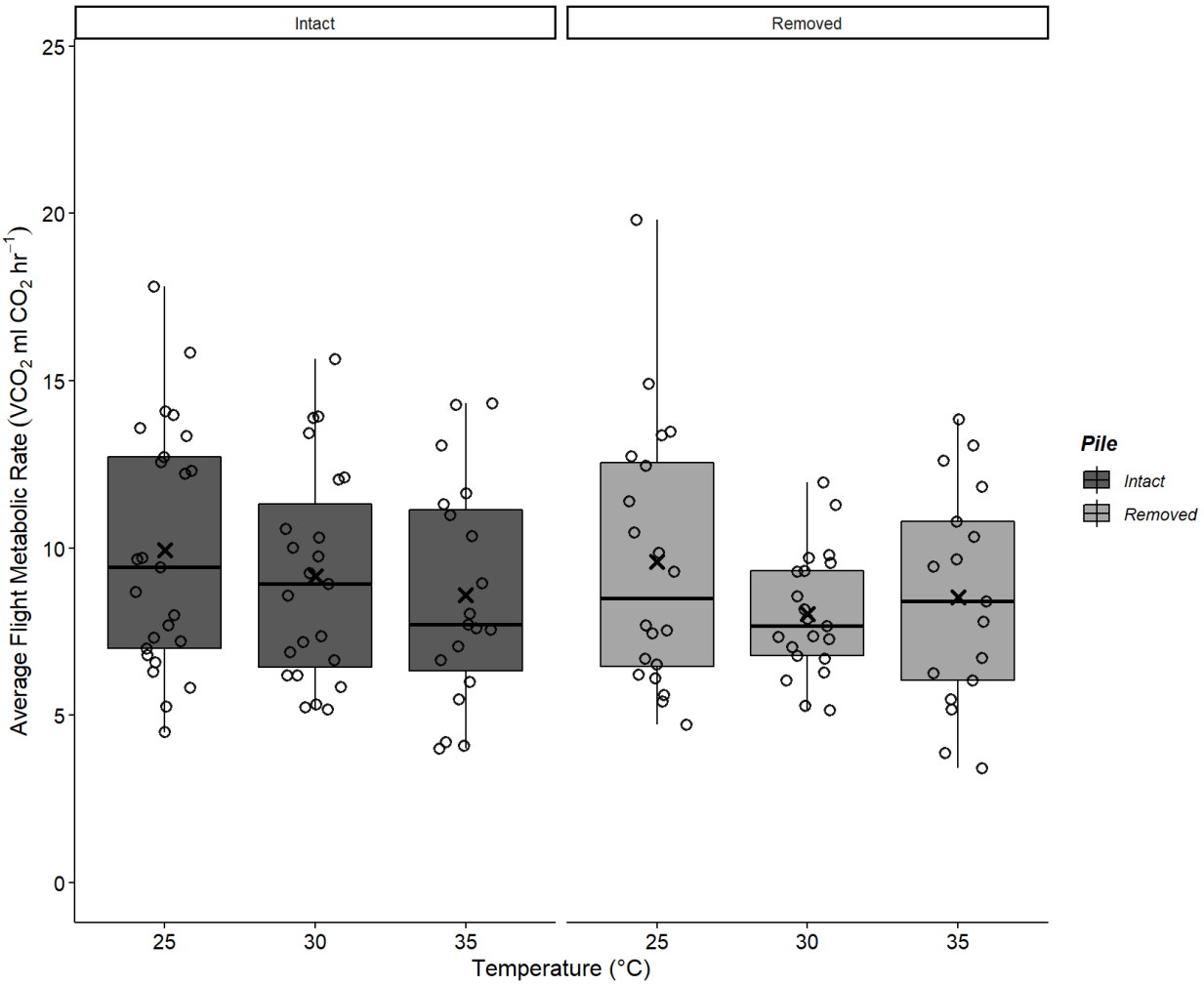


Fig. 4.4. Neither pile nor ambient temperature affects the metabolic output of *B. impatiens* workers during free flight. Workers with their pile removed (light grey) and intact (dark grey) were flown without constant agitation at 25, 30 and 35°C to obtain their CO_2 output. Flight metabolic rate (FMR) did not vary across ambient temperatures (P=0.838) and neither was it significantly different between pile treatments (P=0.069). Box plots represent percentiles with the black bar across signalling the median value. Also present are the individual data points indicating the distribution of FMR values across individual workers. The average FMR value within each temperature group is indicated by an (x).

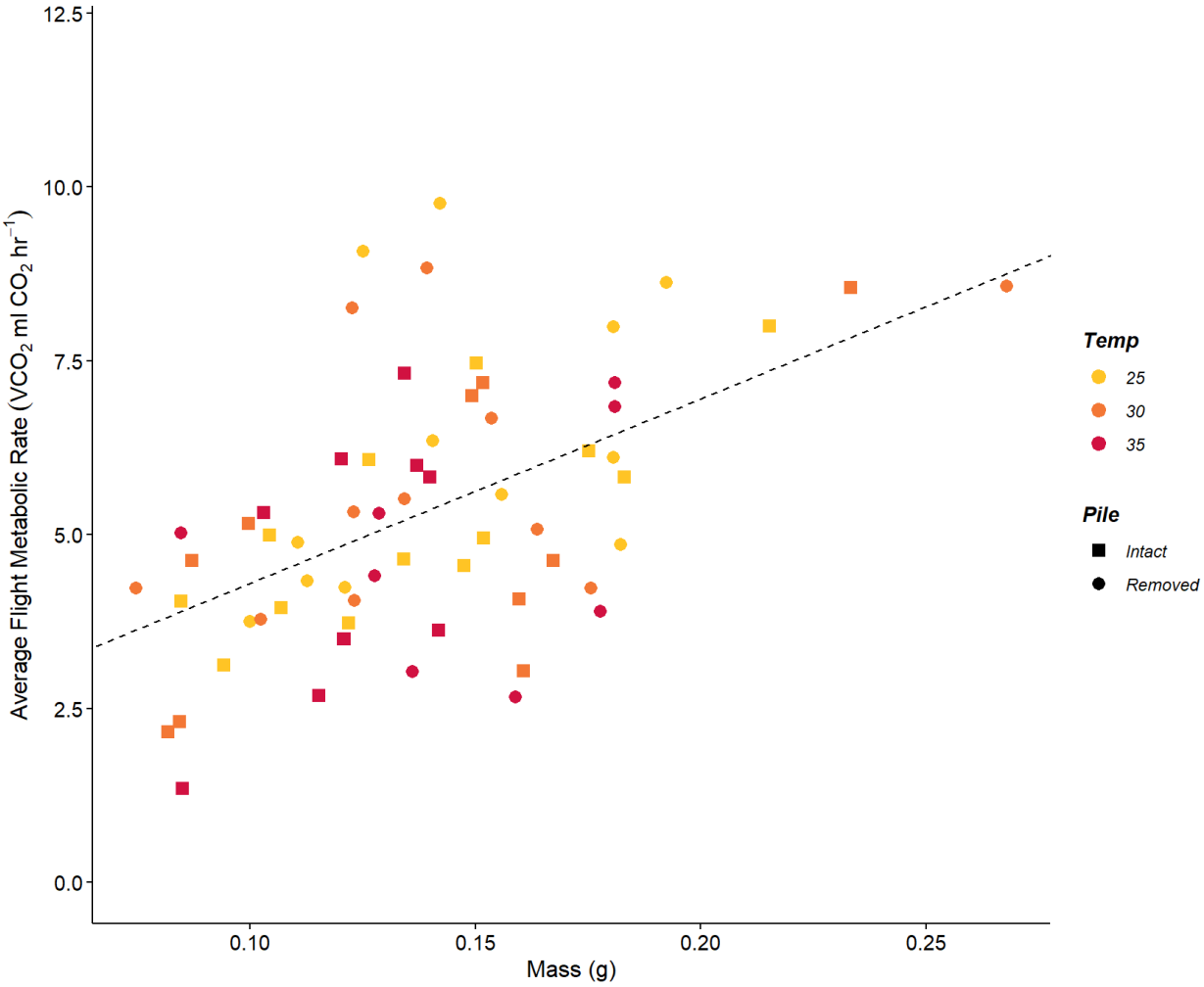


Fig. 4.5. *B. impatiens* drone mass predicts their metabolic output during free flight. Drones with pile removed (●) and intact (■) were flown without constant agitation at 25°C (yellow), 30°C (orange) and 35°C (red) to obtain their CO_2 output (flight metabolic rate, FMR). The regression between FMR and mass was significant (P<0.0001; Adj R^2=0.84; slope: $FMR = 46.65(mass) + 0.15$).

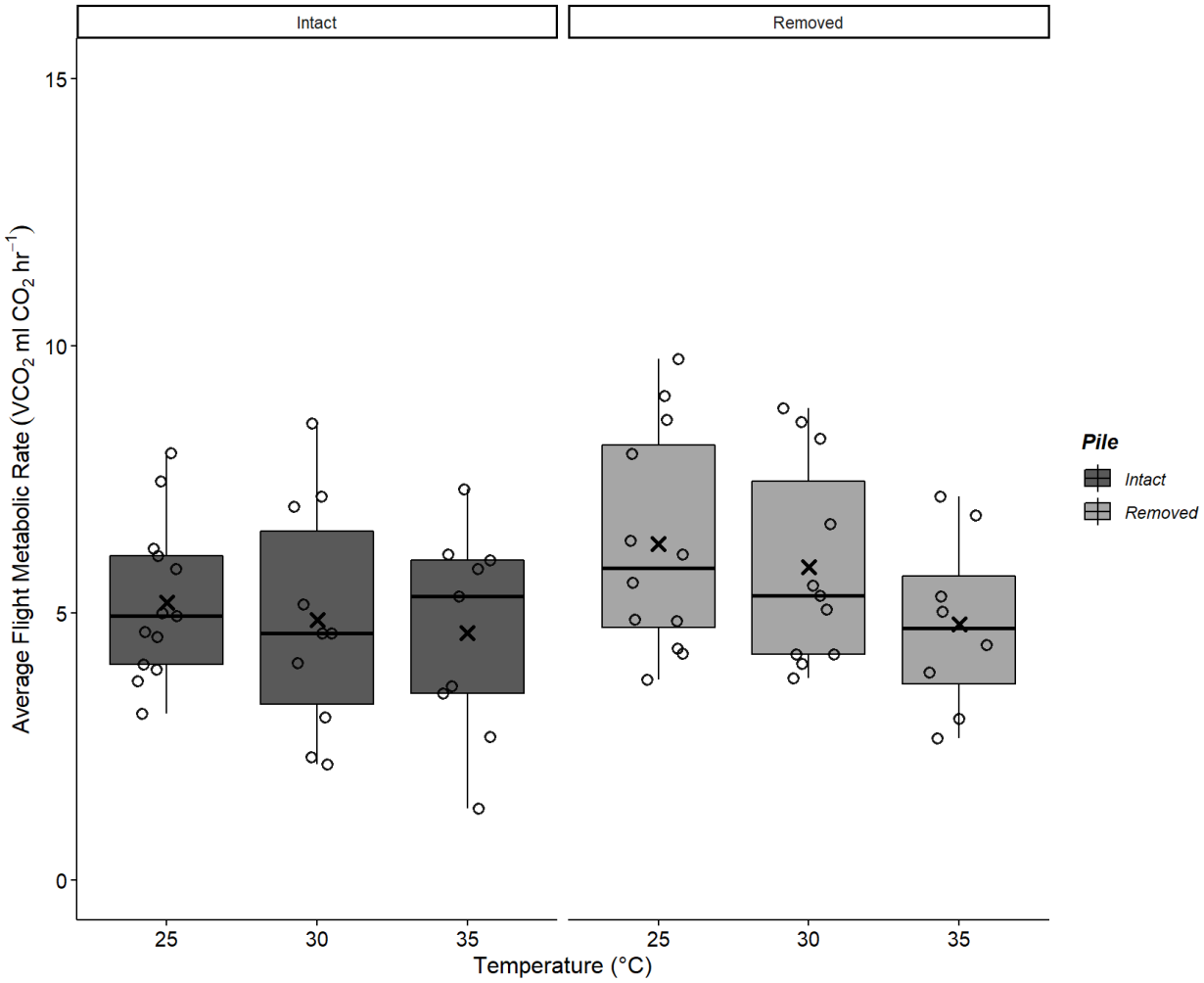


Fig. 4.6. Neither pile nor ambient temperature affects the metabolic output of *B. impatiens* drones during free flight. Drones with pile removed (light grey) and intact (dark grey) were flown without constant agitation at 25, 30 and 35°C to obtain their CO_2 output. Flight metabolic rate (FMR) did not vary across ambient temperatures (P=0.279) and neither was it significantly different between pile treatments (P=0.207). Box plots represent percentiles with the black bar across signalling the median value. Also present are the individual data points indicating the distribution of FMR values across individual drones. The average FMR value within each temperature group is indicated by an (x).

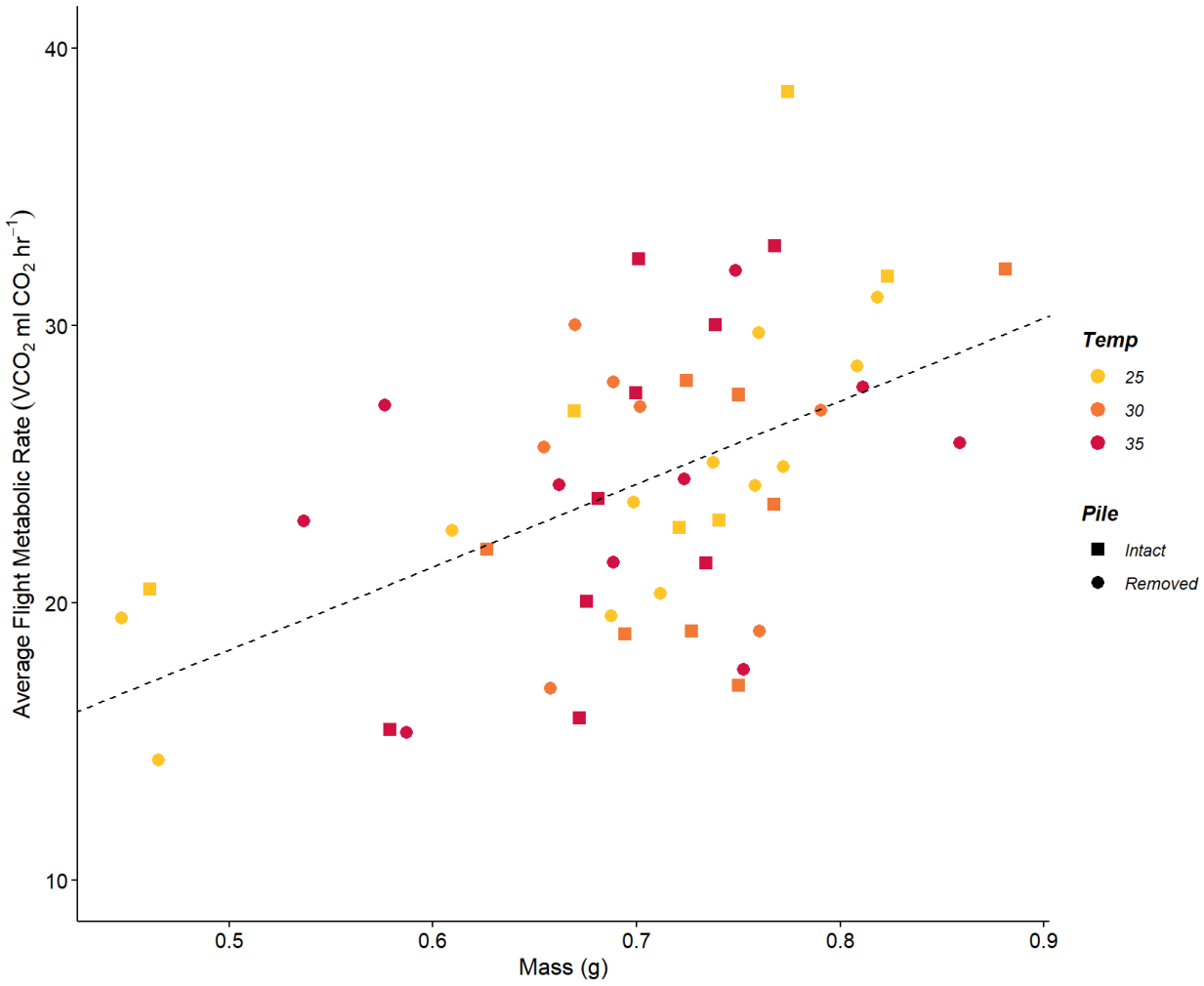


Fig. 4.7. *B. impatiens* queen mass predicts their metabolic output during free flight. Queens with pile removed (●) and intact (■) were flown without constant agitation at 25°C (yellow), 30°C (orange) and 35°C (red) to obtain their CO_2 output (flight metabolic rate, FMR). The regression between FMR and mass was significant (P<0.0001; Adj R^2=0.25; slope: $FMR = 29.89(mass) + 3.37$).

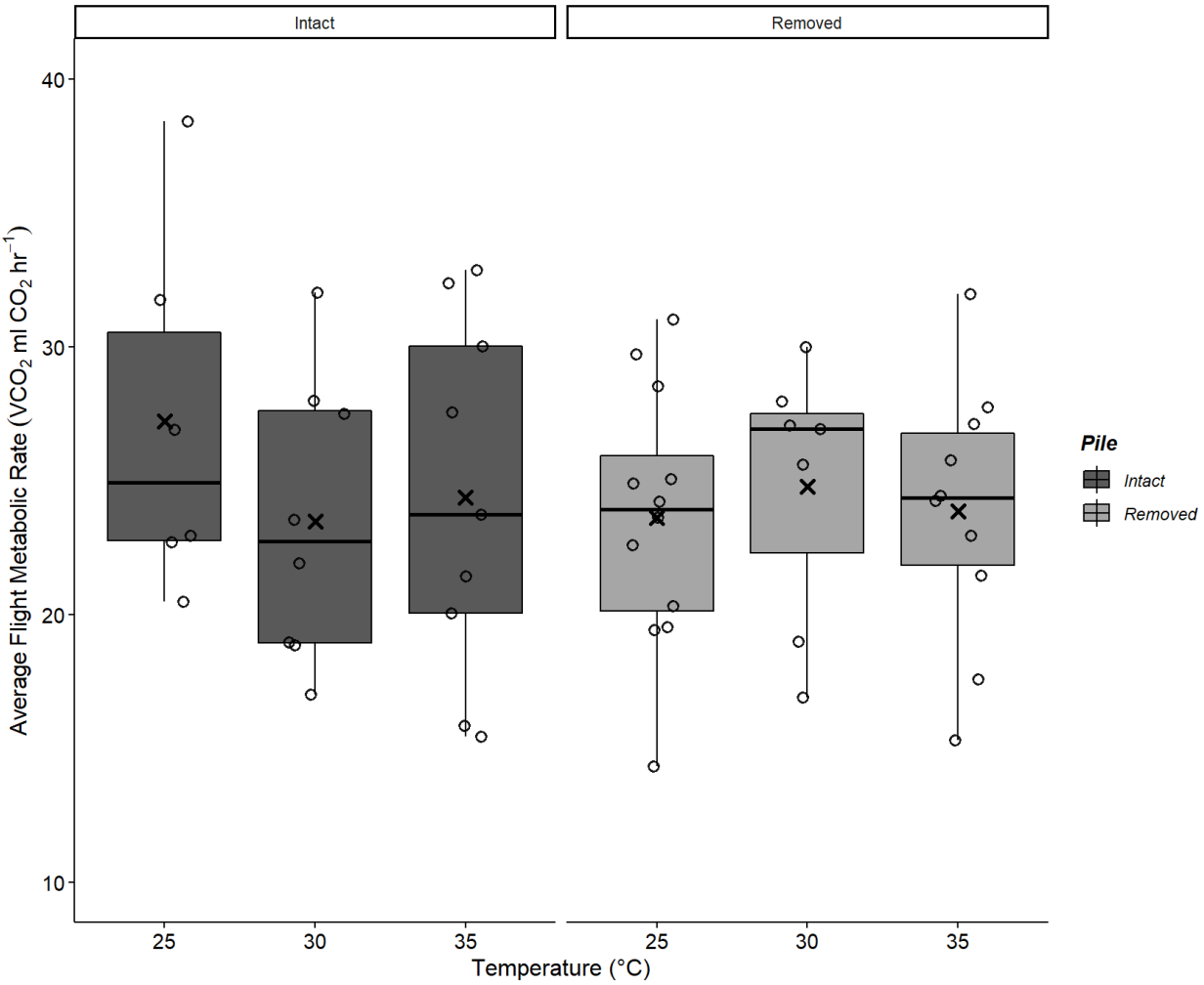


Fig. 4.8. The absence of pile does not alter the metabolic output of queens under high T_a. Queens with pile removed (light grey) and intact (dark grey) were flown without constant agitation at 25, 30 and 35°C to obtain their CO_2 output. There was an interactive effect of T_a and pile on flight metabolic rate (FMR; P=0.045) but no combination of T_a and pile produced significant differences in FMR ($P \geq 0.162$, pairwise analysis). Thus, FMR neither varied across T_a nor between bees with pile removed or intact. Box plots represent percentiles with the black bar across signalling the median value. Also present are the individual data points indicating the distribution of FMR values across individual queens. The average FMR value within each temperature group is indicated by an (x).

Chapter 5: General Conclusion

Increasing global temperature (The Core Writing Team IPCC, 2015) emphasizes the need to understand the effects that ambient temperature (T_a) has on species around the world. Studies on species distribution (e.g. Herrando-Pérez et al., 2020; Sunday et al., 2019) demonstrate that predicting where animals, such as ectotherms, may thrive successfully cannot be simplified by merely examining patterns of thermal limits. Instead, there needs to be a broad understanding of how temperature effects individual performance across many systems of biological organization (Morash et al., 2018; Pörtner et al., 2017; Sinclair et al., 2016).

Current literature supports that both tropical (Deutsch et al., 2008; Pinsky et al., 2019; Sunday et al., 2014) and temperate (Johansson et al., 2020) ectotherms are susceptible to increases in temperature by pushing these organisms closer towards or beyond their upper thermal limits (UTLs). In the specific case of bees, alarming declines in these valuable pollinators (Biesmeijer et al., 2006; Fourcade et al., 2019; Iserbyt and Rasmont, 2012; Jacobson et al., 2018; Powney et al., 2019; Soroye et al., 2020; Vray et al., 2019) warn that temperature increases, as a result of climate change, are closely linked to these occurrences (Soroye et al., 2020) and to constricting their distributions (Kerr et al., 2015; Sirois-Delisle and Kerr, 2018). As such, it is imperative that the physiological limitations that high temperature poses on such species are thoroughly examined to determine how they will respond to and cope with current and future thermal conditions.

Through this thesis, I sought to determine the physiological limitations on both individuals and colonies of a temperate species of bumblebee, *Bombus impatiens*. In order to accomplish this, I first determined which colonial caste is most susceptible to heat by establishing the UTL of the species. Being eusocial, bumblebees act collectively to maintain their hive, thermoregulating the internal climate of this environment to buffer their most sensitive

caste members from changes in T_a. Thus, I determined the energetic costs associated with thermoregulation at the colonial level and at which temperatures a colony's energetic inputs best contribute to maintaining thermal homeostasis within the nest. However, if challenging temperatures are sustained for long periods of time, such as in a heat wave, it leads to question whether colonial thermoregulation is necessarily prioritized over resource procurement. I therefore evaluated thermoregulatory and foraging efforts in addition to measuring various metrics of colony success for colonies undergoing chronic heat exposure. Finally, given that foraging – and therefore flight – is essential for ensuring colony growth and success, I investigated how flight performance is impacted by high T_a and whether a temperate adaptation present in individual bumblebees, such as an insulative layer of pile, would act as a potential limitation under such thermal conditions.

5.1 Chapter summaries

5.1.1 Chapter 2

Previous work introduced the "weak link" hypothesis, where colony workers with the weakest UTL would ultimately limit colony performance (Baudier and O'Donnell, 2017). However, thermal tolerance is known to be affected by ontogeny (Bowler and Terblanche, 2008) and deviations from optimal rearing temperature cause subsequent developmental defects in bees (Groh et al., 2004; Heinrich, 2004; Jones et al., 2005; Medrzycki et al., 2010; Tautz et al., 2003; Wang et al., 2016). Therefore, expanding upon the "weak link" concept to include castes, I hypothesized that bumblebee offspring, specifically the larvae, would be the most thermally sensitive individuals when compared to adults within a bumblebee colony. I used thermolimit respirometry to quantify the upper thermal limit (by way of critical thermal maxima, CTmax) of each caste of bee, including workers, queens, drones and larvae; the evaluation of the latter being

a novel contribution for my study species. I found that my hypothesis was supported and that larvae were the most susceptible to heat, with an UTL nearly 3°C lower than their adult counterparts.

Given that larvae are more thermally sensitive and that they may sustain developmental defects from deviations in optimal rearing temperature (Groh et al., 2004; Heinrich, 2004; Jones et al., 2005; Medrzycki et al., 2010; Tautz et al., 2003; Wang et al., 2016), the second part of Chapter 2 worked to underscore the importance of colony thermoregulation as well as the energetic costs incurred by the colony, especially considering the high energetic costs associated with it (Heinrich, 2004; Kronenberg and Heller, 1982; Vogt, 1986a). The energetic costs of thermoregulation for large colonies of bumblebees are not as well studied as they are in smaller ones (Vogt, 1986a) and thus, using colony sizes of over 200 workers, the second hypothesis I tested was that as T_a deviates away from the optimal nest temperature (T_n) preferred by bumblebees, it would pose a greater challenge for large colonies to maintain thermal homeostasis, including elevating the energetic costs associated with thermoregulation. I evaluated colonies under a wide range of acute thermal stressors in three main ways: first, I found the maximum energetic expenditure using flow-through respirometry to quantify metabolic output; second, I determined the change in those energetic costs over each hour of the exposure duration; and third, I measured the internal T_n to evaluate thermoregulatory success. Additionally, I investigated whether the presence of insulation within a colony would help to buffer the nest from changes in T_a and reduce the overall costs of thermoregulation. Previously, the effect of insulation had only been studied in small colonies under cool thermal conditions (Vogt, 1986b).

I found support for my hypothesis wherein T_a that is greater or less than that of optimal T_n results in increases to the metabolic outputs of colonies. Increased energy expenditure is therefore indicative of increased thermoregulatory efforts as colonies work to maintain thermal homeostasis within the nest. Furthermore, I discovered that *B. impatiens* colonies are exceptional at thermoregulating under acute thermal stress as low as 5°C. Acute heat stress however, poses significant challenges. Firstly, the metabolic output of the colony increases when T_a equals or exceeds 35°C, yet colonies still fail to regulate T_n within the optimal range. Thus, increased thermoregulatory efforts, as are implied by elevated metabolic rates, do not result in thermoregulatory success under acute heat stress. I also found that T_n rose to equal T_a. These findings can be further broken down within high T_a groupings. At 35°C, colonies exhibited a continual elevation in metabolic output over each hour of the exposure period. In contrast, at 40°C, metabolic output is initially elevated but continually decreased over time. In the former scenario, colonies appeared to be vigorously engaged in thermoregulatory fanning, but at 40°C, they were instead agitated and seeking escape. Therefore, it is implied that acute exposure to high T_a differentially affects colonies over time depending on the severity of the heat stress yet, regardless of that temperature, energetic costs remain high without achieving thermal homeostasis. Additionally, insulation acted to reduce the overall energetic costs of colony thermoregulation.

This chapter reveals that thermally sensitive members within a colony, such as larvae, drive social insects to invest greater amounts of energy into thermoregulation when the thermal homeostasis of the nest microclimate deviates away from optimal. While previous literature highlights the robust ability of bumblebees to cope with cold temperatures, my research helps reveal the challenge that high temperatures pose to these species. Ineffective thermoregulation at

ambient temperatures above optimal not only pose energetic costs, but also place thermally sensitive larvae and pupae at risk of developmental defects. The elevated costs associated with high ambient temperature and the inability to successfully thermoregulate, may place colonies at risk, especially if these conditions persist over longer periods of time.

5.1.2 Chapter 3

Heatwave frequency and intensity is predicted to increase in the future (Meehl and Tebaldi, 2004) with previous works cautioning that sublethal, yet prolonged exposure to thermal stress can result in negative effects for the organism (Pörtner et al., 2017; Rasmont and Iserbyt, 2012). In bees, exposure duration has been linked with a reduction in UTL (Maia-Silva et al., 2021) as well as increased mortality (Cane and Neff, 2011). Moreover, colony energy budgets are tightly controlled between energy expenditure for various nest maintenance behaviours and energy procurement via foraging (see Heinrich, 2004). Therefore, given that combined nutritional and thermal stress negatively impacts colony growth (Vanderplanck et al., 2019), questions arise as to how colonies balance thermoregulation and resource procurement under chronic, high thermal stress. Thus, with the knowledge that high T_a elevates the energetic costs for colonies as they attempt to buffer against changes in T_n (Chapt. 2), in Chapter 3 I hypothesized that bumblebee colonies would then face a trade-off between thermoregulation and resource procurement if that thermal stress was sustained over longer periods of time. It was predicted that thermoregulation would be unsuccessful under chronic high T_a and that the measures of colony success would each be negatively affected. I subjected medium-sized colonies composed of 43 *B. impatiens* workers to two weeks of sustained high T_a. Foraging activity was monitored to quantify the percentage of workers who engaged in syrup gathering while simultaneously measuring the incidence of thermoregulatory fanning behaviour. This

ultimately permitted the exploration of a trade-off between the two behaviours under chronic thermal stress. Internal T_n was also measured during this time frame to assess thermoregulatory success. Additionally, metrics of colony success were measured such as adult emergence, percent mortality, percent abandonment of the colony and the total number of offspring remaining at the end of the chronic exposure period. I did not find direct evidence to support my hypothesis that a trade-off between foraging and thermoregulation occurs during chronic high T_a exposure. The percentage of foraging workers was not significantly different across ambient temperatures, yet I found a higher incidence of fanning at 35°C. Similar to acute exposure experiments, internal T_n rose to match T_a at this temperature as well. I neither could support that adult emergence or mortality was negatively affected by chronic high T_a stress, but did find that more workers abandoned the colony and fewer offspring were produced. Therefore, my results are a starting point from which to build our knowledge of chronic, colony-level effects of high temperature and contribute that chronic high T_a may pose the greatest risk to offspring production through reduced worker population and failed thermoregulation.

5.1.3 Chapter 4

The resources procured from foraging leads further to colony success by way of offspring production; where large colonies are seen to produce more reproductive individuals (see Heinrich, 2004). The foraging examined in Chapter 3 is thus reliant on flight of individual worker bees. Being temperate species, historical adaptations, such as an insulative layer of pile, have allowed bumblebees to remain active even at cooler temperatures (Corbet et al., 1993; Heinrich, 2004). Given that pile helps retain heat but dissipation may hinder flight performance at high T_a (Kenna et al., 2021), with Chapter 4 I aimed to examine how flight performance may be impacted by high T_a for bee species who have historically adapted to temperate climates. It is

unclear, however, whether this temperate adaptation would prevent excess heat dissipation, elevating the energetic costs associated with active thermoregulation under high T_a during flight. Thus, I hypothesized that the absence of pile would lower the metabolic output during flight at high T_a when compared to bumblebees with their pile intact. I assessed the performance metric of flight metabolic rate (FMR) using the novel approach of comparing *B. impatiens* workers, drones and queens, both with and without their pile removed when flown at high T_a. Additionally, I tested whether methodological differences in promoting flight in bees would alter FMR-T_a relationships for *B. impatiens* workers as has been previously demonstrated for other bee species (Woods et al., 2005). My results do not support that pile removal reduces the energetic costs of flight at high T_a for workers, drones or queens. I also found opposing results to that of previous works for the effects of methodology on FMR assessment. Agitation, used to encourage flight, typically produces a negative relationship between T_a and FMR, yet I found no relationship; a result similar to my flight assessments without agitation. Additionally, I found a significant interaction between the size of an individual and the presence/absence of pile. This signifies that irrespective of T_a, the presence or absence of an insulative layer of pile will affect the metabolic output of flight depending on how large or small an individual worker is. It remains unclear however, whether aspects of flight performance, aside from FMR, are hindered by the presence of an insulative layer of pile under heat stress, allowing further research into the limitations of active heat dissipation mechanisms of bumblebees.

5.2 Limitations and future directions

5.2.1 Upper thermal limits

The study of UTLs often receive criticism concerning their environmental relevance. Aside from studies which demonstrate that T_a in particular areas already meet or exceeded the

UTL of some species (e.g., Pinsky et al., 2019; Sunday et al., 2014), these measures can also help us determine an organism's susceptibility to climate extremes or predict thermal vulnerability for various traits (Hoffmann and Sgrò, 2018). Furthermore, UTLs can be used to understand why organisms avoid certain extreme temperatures or the adaptations which may arise as a result of exposure to such conditions (Huey and Stevenson, 1979). We can clearly understand the relevance of UTLs when considering bumblebees. Not only do adult UTLs reflect the thorax temperatures (T_{th}) reached during flight (Heinrich, 1975; Oyen and Dillon, 2018; Chapt. 2), but the increased heat susceptibility of larvae found in Chapter 2 emphasizes the importance of keeping T_n closely regulated. Therefore, the contextual relevance of UTLs becomes apparent and their usefulness reaches beyond mere numerical values to allow comparisons across and within populations and species.

Future work regarding the UTLs of bumblebees could further explore intra-colonial differences. While adult bumblebee UTLs are invariant according to age (Oyen and Dillon, 2018), works on other insects demonstrate that this variance exists between juvenile stages (Bowler and Terblanche, 2008; Davison, 1969). Therefore, I could further expand UTL investigations to include the pupae and younger instar larvae and thus parse out which stage of bumblebee development is most susceptible to heat.

5.2.2 Whole-colony thermoregulation

Acute colony responses to thermal stress tested in Chapter 2 were limited in some capacity by the laboratory setting. Colonies were contained within a respirometry chamber during acute temperature exposure, restricting individual bee movements compared to colonies located in an open environment. Honeybees, for example, are known to exit the hive to reduce metabolic heat within the nest under high T_a (Stabentheiner et al., 2021) and fanning has been

observed at the hive entrance of bumblebee colonies (e.g., Weidenmüller, 2004). Both behaviours are naturally employed to facilitate the reduction and movement of excess heat away from the internal nest environment in times of heat stress. In my experimental setup, these two behaviours could not be accommodated in favor of capturing metabolic rates. To circumvent these limitations, future works could study colonies which are permitted free movement to and from the nest, placing digital temperature loggers inside to monitor T_n. A controlled environment could be utilized such that it would still simulate aboveground nesting but also incorporate the immediate area outside of the nest as well. Given that high T_a elevates the energetic costs to a colony, quantifying nest traffic would ultimately determine if fanning entrances and/or reducing the number of individuals contributing metabolic heat actually aids in the thermoregulation of T_n under high T_a stress.

Another potential limitation deals with the incorporation of insulation into my methods. *B. impatiens* is typically classified as an underground nester (Colla et al., 2014) where the below-ground temperature does not experience large daily fluctuations in T_a (Mullan, 2022). Many species of bumblebees, however, do nest aboveground, using opportunistic locations such as human-made nest boxes or spaces within walls (Liczner and Colla, 2019) to found their colonies. Furthermore, Biobest Canada Ltd., the company from which experimental colonies were purchased, advertises *B. impatiens* for outdoor pollination in some areas of Canada and the USA. Each come with a layer of insulative cotton batten within the colony which I either left in place or removed for insulated or uninsulated trials, respectively. Thus, my experimental design is relevant for commercial scenarios where *Bombus* species are purchased for crop or garden pollination and for species which nest aboveground.

Addressing the energetic costs and thermoregulatory success of underground nesting *B. impatiens* would prove more difficult. One potential step forward would be to locate wild underground nests in early spring, just as a colony is being established, and place temperature loggers within. Internal colony temperature could be monitored not only on a daily basis, but also across an entire growing season and subsequently compared with local weather conditions. While thermoregulatory behaviours themselves could not be quantified during this period, detecting deviations in T_n from its optimal thermal window would allow for thermoregulatory success to be inferred. Furthermore, and if possible, studying nests which are in exposed areas vs in sheltered areas (e.g. canopy cover) would also provide insight into how the passive thermoregulatory mechanism of nest site selection (Jones and Oldroyd, 2006) impacts a colony's ability to maintain thermal homeostasis within the nest.

5.2.3 Chronic heat stress and trade-offs of colony success

Heatwaves can be difficult to simulate in a laboratory setting because environmental temperatures are rarely static in nature, fluctuating temporally according to the time of day. One method to address this challenge is to manipulate experimental T_a such that it is reduced at night and increased during the day. Such methods have been successfully accomplished in other studies on bees (Bordier et al., 2017; Greenop et al., 2020). The environmental chamber that was available for my use in Chapter 2 did not accommodate dynamic temperatures and thus I was limited to using static temperature treatments, similar to previous works (e.g., Vanderplanck et al., 2019). Despite this limitation, my study acts as a starting point from which to assess the affects of chronic heat stress on a colony of bumblebees. Understanding the outcomes of prolonged heat exposure on a colony can establish a baseline of the negative effects experienced

without the added variable of daily temperature fluctuation. From these findings, future works can then simulate more natural heatwaves by fluctuating daily temperature.

Another limitation of Chapter 3 was sample size. I was only able to gather data from 5 colonies per temperature treatment, and only 3 of 5 colonies at 25°C had recoverable foraging data. Each experimental trial took a substantial amount of time to complete and the environmental chamber capacity only accommodated one colony per temperature trial. With more time and fewer equipment failures, I would have ideally increased my sample size for each tested temperature (e.g., 9 large colonies per temperature treatment; Vanderplanck et al., 2019). Perhaps more pronounced trends in foraging, adult emergence and mortality would have emerged across tested temperatures.

Another area for improvement would be to examine foraging data in more depth. Tracking the foraging incidence across the duration of an experimental trial, such as on a daily basis, would perhaps permit us to determine if there is a particular point during a simulated heatwave where foraging effort begins to decrease. Given that high T_a results in a reduction in foraging activity among other bees (Couvillon et al., 2010; Kwon and Saeed, 2003; Rami Reddy et al., 2015), a broader view of foraging activity may identify a critical temperature where a trade-off between foraging and thermoregulation occurs during chronic heat stress. Allowing colonies to recover post-chronic exposure would also determine if it is possible for them to rebound following a heatwave event. On a similar note, increasing the number of temperatures tested in both Chapters 2 and 3, could parse out the thermal conditions where a colony first begins to experience negative effects at an acute (Chapt. 2) and chronic (Chapt. 3) level. In turn, it could provide valuable insight into how colonies respond to heat events in the future. Interest in the effects of heatwaves continues to grow, including a recent study investigating the

interaction between nectar production of flowers and foraging of bumblebees under such conditions (Hemberger et al., 2023), ultimately highlighting the importance of understanding how these essential pollinators will cope to a changing thermal environment.

5.2.4 Flight performance

The main concept surrounding Chapter 4 was that a temperate adaptation to permit retention of heat (pile) may actually hinder excess heat dissipation during flight in bumblebees. My study assessed the performance metric of flight metabolic rate as a first step into this investigation, but the overall lack of significant findings also allows room for future methodological improvements and research.

The main limitation of Chapter 4 involved the sampling of T_{th}. Previous works studied T_{th} during flight, finding that FMR peaked when thoraces reached 39°C (Glass and Harrison, 2022) and that queens appear to overheat when T_{th} encroaches upon 45°C under high T_a (Heinrich, 1975). The methods used in the aforementioned studies involved inserting a thermal probe into the thorax to immediately capture T_{th} post-flight, whereas I attempted to use infrared imaging. Unfortunately, the delay between bee capture and T_{th} measurement was too great to provide accurate estimations. As such, future studies could utilize the aforementioned "grab and stab" method or a faster capture of IR imaging to measure the T_{th} post-flight of bees with and without pile under various high T_a conditions. This would help us better understand if the removal of pile facilitates heat dissipation at these temperatures.

Complementary to this approach, other metrics of flight performance could be assessed as well. The findings that flight endurance decreases at T_a exceeding 25°C (Kenna et al., 2021) and that full abdominal syrup loads increase T_{th} (Heinrich, 1975) in bumblebees leaves the door open to investigate whether it is pile that hinders endurance and/or load lifting by preventing heat

dissipation at high T_a. The ability to disperse for foraging and the capacity to return with nectar and pollen loads to the nest are key components of resource collection and ultimately colony growth (see Heinrich, 2004). Creating a thermal performance curve for bumblebees with their pile removed and intact which relates T_{th} and T_a to various measures of flight performance for these individuals, would allow us to discern if overheating is a risk during flight and whether a historic temperate adaptation like pile is a limitation of bumblebees in our modern climate.

5.3 Overall conclusions

Observed declines in bumblebees linked to climate change emphasize the need to understand the underlying physiological effects which act to limit these important pollinators within their thermal environment. My thesis investigated the effect of temperature on individuals as well as colonies, both at an acute and chronic level. I found that larvae are the most thermally sensitive bumblebee caste which underscores the importance of colony thermoregulation. At high T_a however, colonies struggle to buffer these larvae from changes in T_n despite elevated energetic inputs. With high energetic costs associated with thermoregulation when encountering high T_a, a colony's ability to cope with heat-wave like conditions becomes of utmost concern; particularly determining whether they will favor thermoregulation at the expense of foraging. While no conclusive evidence of such a trade-off emerged, I found that colony work forces could be negatively impacted by chronic heat events due to worker abandonment and reduced offspring production. Foraging behaviours which enable colonies to procure resources rely on flight, but temperate species of bumblebees have evolved to thrive in cool climates. The adaptation of an insulative layer of pile thus came into question as a potential limitation of bee flight under heat stress. Though the metabolic output of bumblebees at various high T_a remained unaltered by the presence or absence of pile, it still remains to be seen if this historic adaptation would hinder

other aspects of flight performance. Together my findings illustrate that the thermal response of bumblebees is complex, encompassing both individuals and colonies. Recent authors highlight the importance of investigating physiological traits in addition to ecological and population-level data in order to best contribute to bumblebee conservation efforts (Leroy et al., 2023). My research adds individual- and colony-level physiological effects of acute and chronic high temperature to the growing evidence on the thermal limitations of bumblebees. In turn, these findings help scaffold our comprehension of the potential factors which underlie macroecological patterns of observed bee declines and act as a starting point from which to help predict how climate change will impact bumblebee species in the future.

References

Abou-Shaara, H.F., Owayss, A.A., Ibrahim, Y.Y., Basuny, N.K., 2017. A review of impacts of temperature and relative humidity on various activities of honey bees. Insectes Soc. 64, 455–463. https://doi.org/10.1007/s00040-017-0573-8

Addo-Bediako, A., Chown, S.L., Gaston, K.J., 2000. Thermal tolerance, climatic variability and latitude. Proc. R. Soc. B Biol. Sci. 267, 739–745. https://doi.org/10.1098/rspb.2000.1065

Alkassab, A.T., Kirchner, W.H., 2017. Sublethal exposure to neonicotinoids and related side effects on insect pollinators: honeybees, bumblebees, and solitary bees. J. Plant Dis. Prot. https://doi.org/10.1007/s41348-016-0041-0

Amundrud, S.L., Srivastava, D.S., 2020. Thermal tolerances and species interactions determine the elevational distributions of insects. Glob. Ecol. Biogeogr. 29, 1315–1327. https://doi.org/10.1111/geb.13106

Angilletta, M.J., Huey, R.B., Frazier, M.R., 2010. Thermodynamic effects on organismal performance: Is hotter better? Physiol. Biochem. Zool. 83, 197–206. https://doi.org/10.1086/648567

Barrow, D.A., Pickard, R.S., 1985. Larval Temperature in Brood Clumps of Bombus Pascuorum (SCOP.). J. Apic. Res. 24, 69–75. https://doi.org/10.1080/00218839.1985.11100651

Bates, D., Mächler, M., Bolker, B.M., Walker, S.C., 2015. Fitting linear mixed-effects models using lme4. J. Stat. Softw. 67, 1–48. https://doi.org/10.18637/jss.v067.i01

Baudier, K.M., O'Donnell, S., 2017. Weak links: how colonies counter the social costs of individual variation in thermal physiology. Curr. Opin. Insect Sci. 22, 85–91. https://doi.org/10.1016/j.cois.2017.06.004

Berrigan, D., Hoffmann, A.A., 1998. Correlations between measures of heat resistance and acclimation in two species of Drosophila and their hybrids. Biol. J. Linn. Soc. 64, 449–462.

https://doi.org/10.1006/bijl.1998.0232

Biesmeijer, J.C., Roberts, S.P.M., Reemer, M., Ohlemüller, R., Edwards, M., Peeters, T., Schaffers, A.P., Potts, S.G., Kleukers, R., Thomas, C.D., Settele, J., Kunin, W.E., 2006. Parallel declines in pollinators and insect-pollinated plants in Britain and the Netherlands. Science (80-.). 313, 351–354. https://doi.org/10.1126/science.1127863

Billardon, F., Darveau, C.A., 2019. Flight energetics, caste dimorphism and scaling properties in the bumblebee, Bombus impatiens. J. Exp. Biol. 222, 187807. https://doi.org/10.1242/jeb.187807

Bordier, C., Dechatre, H., Suchail, S., Peruzzi, M., Soubeyrand, S., Pioz, M., Pélissier, M., Crauser, D., Conte, Y. Le, Alaux, C., 2017. Colony adaptive response to simulated heat waves and consequences at the individual level in honeybees (Apis mellifera). Sci. Rep. 7, 3760. https://doi.org/10.1038/s41598-017-03944-x

Bowler, K., Terblanche, J.S., 2008. Insect thermal tolerance: What is the role of ontogeny, ageing and senescence? Biol. Rev. 83, 339–355. https://doi.org/10.1111/j.1469-185X.2008.00046.x

Bozinovic, F., Bastías, D.A., Boher, F., Clavijo-Baquet, S., Estay, S.A., Angilletta, M.J., 2011. The mean and variance of environmental temperature interact to determine physiological tolerance and fitness. Physiol. Biochem. Zool. 84, 543–552. https://doi.org/10.1086/662551

Bretzlaff, T., Kerr, J.T., Darveau, C.A., 2023. High temperature sensitivity of bumblebee castes and the colony-level costs of thermoregulation in Bombus impatiens. J. Therm. Biol. 117, 103710. https://doi.org/10.1016/j.jtherbio.2023.103710

Brian, A.D., 1952. Division of Labour and Foraging in Bombus agrorum Fabricius. J. Anim. Ecol. 21, 223–240. https://doi.org/10.2307/1959

Bryden, J., Gill, R.J., Mitton, R.A.A., Raine, N.E., Jansen, V.A.A., 2013. Chronic sublethal stress causes bee colony failure. Ecol. Lett. 16, 1463–1469. https://doi.org/10.1111/ele.12188

Burdine, J.D., McCluney, K.E., 2019. Differential sensitivity of bees to urbanization-driven changes in body temperature and water content. Sci. Rep. 9, 1–10. https://doi.org/10.1038/s41598-018-38338-0

Calosi, P., Bilton, D.T., Spicer, J.I., Votier, S.C., Atfield, A., 2010. What determines a species' geographical range? Thermal biology and latitudinal range size relationships in European diving beetles (Coleoptera: Dytiscidae). J. Anim. Ecol. 79, 194–204. https://doi.org/10.1111/j.1365-2656.2009.01611.x

Cane, J.H., Neff, J.L., 2011. Predicted fates of ground-nesting bees in soil heated by wildfire: Thermal tolerances of life stages and a survey of nesting depths. Biol. Conserv. 144, 2631–2636. https://doi.org/10.1016/j.biocon.2011.07.019

CaraDonna, P.J., Cunningham, J.L., Iler, A.M., 2018. Experimental warming in the field delays phenology and reduces body mass, fat content and survival: Implications for the persistence of a pollinator under climate change. Funct. Ecol. 32, 2345–2356. https://doi.org/10.1111/1365-2435.13151

Clarke, A., 2017. Principles of Thermal Ecology: Temperature, Energy and Life. Oxford University Press.

Clusella-Trullas, S., Blackburn, T.M., Chown, S.L., 2011. Climatic predictors of temperature performance curve parameters in ectotherms imply complex responses to climate change. Am. Nat. 177, 738–751. https://doi.org/10.1086/660021

Cnaani, J., Robinson, G.E., Hefetz, A., 2000. The critical period for caste determination in

Bombus terrestris and its juvenile hormone correlates. J. Comp. Physiol. - A Sensory, Neural, Behav. Physiol. 186, 1089–1094. https://doi.org/10.1007/s003590000163

Cnaani, J., Schmid-Hempel, R., Schmidt, J.O., 2002. Colony development, larval development and worker reproduction in Bombus impatiens Cresson. Insectes Soc. 49, 164–170. https://doi.org/10.1007/s00040-002-8297-8

Coelho, J.R., 1991. The Effect of Thorax Temperature on Force Production during Tethered Flight in Honeybee (Apis mellifera) Drones, Workers, and Queens. Physiol. Zool. 64, 823–835. https://doi.org/10.1086/physzool.64.3.30158209

Colla, S.R., Williams, P.H., Thorp, R.W., Richardson, L.L., 2014. Bumble Bees of North America, Bumble Bees of North America. Princeton University Press. https://doi.org/10.1515/9781400851188

Cook, C.N., Durzi, S., Scheckel, K.J., Breed, M.D., 2016. Larvae influence thermoregulatory fanning behavior in honeybees (Apis mellifera L.). Insectes Soc. 63, 271–278. https://doi.org/10.1007/s00040-016-0463-5

Cooley, N.L., Emlen, D.J., Woods, H.A., 2016. Self-heating by large insect larvae? J. Therm. Biol. 62, 76–83. https://doi.org/10.1016/j.jtherbio.2016.10.002

Corbet, S.A., Fussell, M., Ake, R., Fraser, A., Gunson, C., Savage, A., Smith, K., 1993. Temperature and the pollinating activity of social bees. Ecol. Entomol. 18, 17–30. https://doi.org/10.1111/j.1365-2311.1993.tb01075.x

Couvillon, M.J., Fitzpatrick, G., Dornhaus, A., 2010. Ambient air temperature does not predict whether small or large workers forage in bumble bees (Bombus impatiens). Psyche (London) 2010. https://doi.org/10.1155/2010/536430

Cruz, F.B., Fitzgerald, L.A., Espinoza, R.E., Schulte, J.A., 2005. The importance of phylogenetic

scale in tests of Bergmann's and Rapoport's rules: Lessons from a clade of South American lizards. J. Evol. Biol. 18, 1559–1574. https://doi.org/10.1111/j.1420-9101.2005.00936.x

Darveau, C.A., Billardon, F., Belanger, K., 2014. Intraspecific variation in flight metabolic rate in the bumblebee Bombus impatiens: Repeatability and functional determinants in workers and drones. J. Exp. Biol. 217, 536–544. https://doi.org/10.1242/jeb.091892

Davison, T.F., 1969. Changes in temperature tolerance during the life cycle of Calliphora erythrocephala. J. Insect Physiol. 15, 977–988. https://doi.org/10.1016/0022-1910(69)90138-3

Deutsch, C.A., Tewksbury, J.J., Huey, R.B., Sheldon, K.S., Ghalambor, C.K., Haak, D.C., Martin, P.R., 2008. Impacts of climate warming on terrestrial ectotherms across latitude. Proc. Natl. Acad. Sci. U. S. A. 105, 6668–6672. https://doi.org/10.1073/pnas.0709472105

Diamond, S.E., Nichols, L.M., McCoy, N., Hirsch, C., Pelini, S.L., Sanders, N.J., Ellison, A.M., Gotelli, N.J., Dunn, R.R., 2012. A physiological trait-based approach to predicting the responses of species to experimental climate warming. Ecology 93, 2305–2312. https://doi.org/10.1890/11-2296.1

Dzialowski, E.M., Tattersall, G.J., Nicol, S.C., Frappell, P.B., 2014. Fluctuations in oxygen influence facultative endothermy in Bumblebees. J. Exp. Biol. 217, 3834–3842. https://doi.org/10.1242/jeb.107052

Ellington, C.P., 1985. Power and efficiency of insect flight muscle. J. Exp. Biol. 115, 293–304. https://doi.org/10.1242/jeb.115.1.293

Fourcade, Y., Åström, S., Öckinger, E., 2019. Climate and land-cover change alter bumblebee species richness and community composition in subalpine areas. Biodivers. Conserv. 28, 639–653. https://doi.org/10.1007/s10531-018-1680-1

Free, J.B., 1955. The division of labour within bumblebee Colonies. Insectes Soc. 2, 195–212. https://doi.org/10.1007/BF02224381

Garófalo, C.A., 1978. Bionomics of bombus (fervidobombus) morio 2. Body size and length of life of workers. J. Apic. Res. 17, 130–136. https://doi.org/10.1080/00218839.1978.11099918

Gegear, R.J., Otterstatter, M.C., Thomson, J.D., 2005. Does parasitic infection impair the ability of bumblebees to learn flower-handling techniques? Anim. Behav. 70, 209–215. https://doi.org/10.1016/j.anbehav.2004.09.025

Gerard, M., Michez, D., Debat, V., Fullgrabe, L., Meeus, I., Piot, N., Sculfort, O., Vastrade, M., Smagghe, G., Vanderplanck, M., 2018. Stressful conditions reveal decrease in size, modification of shape but relatively stable asymmetry in bumblebee wings. Sci. Rep. 8, 15169. https://doi.org/10.1038/s41598-018-33429-4

Gill, R.J., Baldock, K.C.R., Brown, M.J.F., Cresswell, J.E., Dicks, L. V., Fountain, M.T., Garratt, M.P.D., Gough, L.A., Heard, M.S., Holland, J.M., Ollerton, J., Stone, G.N., Tang, C.Q., Vanbergen, A.J., Vogler, A.P., Woodward, G., Arce, A.N., Boatman, N.D., Brand-Hardy, R., Breeze, T.D., Green, M., Hartfield, C.M., O'Connor, R.S., Osborne, J.L., Phillips, J., Sutton, P.B., Potts, S.G., 2016. Protecting an Ecosystem Service: Approaches to Understanding and Mitigating Threats to Wild Insect Pollinators. Adv. Ecol. Res. 54, 135–206. https://doi.org/10.1016/BS.AECR.2015.10.007

Glass, J.R., Harrison, J.F., 2022. The thermal performance curve for aerobic metabolism of a flying endotherm, in: Proceedings of the Royal Society B: Biological Sciences. p. 20220298. https://doi.org/10.1098/rspb.2022.0298

Gonzalez, V.H., Hranitz, J.M., Percival, C.R., Pulley, K.L., Tapsak, S.T., Tscheulin, T.,

Petanidou, T., Barthell, J.F., 2020. Thermal tolerance varies with dim-light foraging and elevation in large carpenter bees (Hymenoptera: Apidae: Xylocopini). Ecol. Entomol. 45, 688–696. https://doi.org/10.1111/een.12842

Gonzalez, V.H., Oyen, K., Ávila, O., Ospina, R., 2022. Thermal limits of Africanized honey bees are influenced by temperature ramping rate but not by other experimental conditions. J. Therm. Biol. 110, 103369. https://doi.org/10.1016/j.jtherbio.2022.103369

Gradišek, A., Bizjak, J., Popovski, A., Grad, J., 2023. Bumble bee nest thermoregulation: a field study. J. Apic. Res. https://doi.org/10.1080/00218839.2022.2164651

Greenop, A., Mica-Hawkyard, N., Walkington, S., Wilby, A., Cook, S.M., Pywell, R.F., Woodcock, B.A., 2020. Equivocal evidence for colony level stress effects on bumble bee pollination services. Insects 11, 191. https://doi.org/10.3390/insects11030191

Groh, C., Tautz, J., Rössler, W., 2004. Synaptic organization in the adult honey bee brain is influenced by brood-temperature control during pupal development. Proc. Natl. Acad. Sci. U. S. A. 101, 4268–4273. https://doi.org/10.1073/pnas.0400773101

Guiraud, M., Cariou, B., Henrion, M., Baird, E., Gérard, M., 2021. Higher developmental temperature increases queen production and decreases worker body size in the bumblebee Bombus terrestris. J. Hymenopt. Res. 88 39-49 88, 39–49. https://doi.org/10.3897/JHR.88.73532

Guo, F., Guénard, B., Economo, E.P., Deutsch, C.A., Bonebrake, T.C., 2020. Activity niches outperform thermal physiological limits in predicting global ant distributions. J. Biogeogr. 47, 829–842. https://doi.org/10.1111/jbi.13799

Guzman, L.M., Johnson, S.A., Mooers, A.O., M'gonigle, L.K., 2021. Using historical data to estimate bumble bee occurrence: Variable trends across species provide little support for

community-level declines. Biol. Conserv. 257, 109141. https://doi.org/10.1016/j.biocon.2021.109141

Hamblin, A.L., Youngsteadt, E., López-Uribe, M.M., Frank, S.D., 2017. Physiological thermal limits predict differential responses of bees to urban heat-island effects. Biol. Lett. 13, 20170125. https://doi.org/10.1098/rsbl.2017.0125

Harrison, J.F., Fewell, J.H., 2002. Environmental and genetic influences on flight metabolic rate in the honey bee, Apis mellifera, in: Comparative Biochemistry and Physiology - A Molecular and Integrative Physiology. Elsevier Inc., pp. 323–333. https://doi.org/10.1016/S1095-6433(02)00163-0

Harrison, J.F., Fewell, J.H., Roberts, S.P., Hall, H.G., 1996a. Achievement of thermal stability by varying metabolic heat production in flying honeybees. Science (80-.). 274, 88–90. https://doi.org/10.1126/science.274.5284.88

Harrison, J.F., Nielsen, D.I., Page, R.E., 1996b. Malate Dehydrogenase Phenotype, Temperature and Colony Effects on Flight Metabolic Rate in the Honey-Bee, Apis mellifera. Funct. Ecol. 10, 81–88. https://doi.org/10.2307/2390265

Harrison, J.F., Woods, H.A., Roberts, S.P., 2012. Ecological and Environmental Physiology of Insects, Ecological and Environmental Physiology of Insects. Oxford University Press. https://doi.org/10.1093/acprof:oso/9780199225941.001.0001

Heinrich, B., 2004. Bumblebee economics. Harvard University Press.

Heinrich, B., 1995. Insect thermoregulation. Endeavour 19, 28–33. https://doi.org/10.1016/0160-9327(95)98891-I

Heinrich, B., 1993. The Hot-Blooded Insects: Strategies and Mechanisms of Thermoregulation. Springer-Verlag Berlin Heidelberg. https://doi.org/10.1007/978-3-662-10340-

Heinrich, B., 1980. Mechanisms of Body-Temperature Regulation in Honeybees, Apis Mellifera II. Regulation of Thoracic Temperature at High Air Temperatures. J. Exp. Biol. 85, 73–87. https://doi.org/10.1242/jeb.85.1.73

Heinrich, B., 1977. The Physiology of Exercise in the Bumblebee: Bumblebees are limited in their superb athletic performance by extremes of air temperature, but by regulating their body temperature they achieve considerable control on JSTOR. Am. Sci. 65, 455–465.

Heinrich, B., 1976. Heat exchange in relation to blood flow between thorax and abdomen in bumblebees. J. Exp. Biol. 64, 561–585. https://doi.org/10.1242/jeb.64.3.561

Heinrich, B., 1975. Thermoregulation in bumblebees - II. Energetics of warm-up and free flight. J. Comp. Physiol. 96, 155–166. https://doi.org/10.1007/BF00706595

Heinrich, B., 1974a. Thermoregulation in Endothermic Insects, Science. https://doi.org/10.1126/science.185.4153.747

Heinrich, B., 1974b. Thermoregulation in bumblebees - I. Brood incubation by Bombus vosnesenskii queens. J. Comp. Physiol. 88, 129–140. https://doi.org/10.1007/BF00695404

Heinrich, B., 1972a. Physiology of brood incubation in the bumblebee queen, Bombus vosnesenskii. Nature 239, 223–225. https://doi.org/10.1038/239223a0

Heinrich, B., 1972b. Temperature regulation in the bumblebee bombus vagans: a field study. Science (80-.). 175, 185–187. https://doi.org/10.1126/science.175.4018.185

Heinrich, B., 1972c. Patterns of endothermy in bumblebee queens, drones and workers. J. Comp. Physiol. 77, 65–79. https://doi.org/10.1007/BF00696520

Heinrich, B., 1971. Temperature regulation of the sphinx moth, Manduca sexta. I. Flight energetics and body temperature during free and tethered flight. J. Exp. Biol. 54, 141–152. https://doi.org/https://doi.org/10.1242/jeb.54.1.141

Heinrich, B., Buchmann, S.L., 1986. Thermoregulatory physiology of the carpenter bee, Xylocopa varipuncta. J. Comp. Physiol. B 156, 557–562. https://doi.org/10.1007/BF00691042

Heinrich, B., Kammer, A.E., 1973. Activation of the fibrillar muscles in the bumblebee during warm up, stabilization of thoracic temperature and flight. J. Exp. Biol. 58, 677–688. https://doi.org/10.1242/jeb.58.3.677

Hemberger, J.A., Rosenberger, N.M., Williams, N.M., 2023. Experimental heatwaves disrupt bumblebee foraging through direct heat effects and reduced nectar production. Funct. Ecol. 37, 591–601. https://doi.org/10.1111/1365-2435.14241

Herrando-Pérez, S., Monasterio, C., Beukema, W., Gomes, V., Ferri-Yáñez, F., Vieites, D.R., Buckley, L.B., Araújo, M.B., 2020. Heat tolerance is more variable than cold tolerance across species of Iberian lizards after controlling for intraspecific variation. Funct. Ecol. 34, 631–645. https://doi.org/10.1111/1365-2435.13507

Hines, H.M., Kilpatrick, S.K., Mikó, I., Snellings, D., López-Uribe, M.M., Tian, L., 2022. The diversity, evolution, and development of setal morphologies in bumble bees (Hymenoptera: Apidae: Bombus spp.). PeerJ 10, e14555. https://doi.org/10.7717/peerj.14555

Hoffmann, A.A., Sgrò, C.M., 2018. Comparative studies of critical physiological limits and vulnerability to environmental extremes in small ectotherms: How much environmental control is needed? Integr. Zool. 13, 355–371. https://doi.org/10.1111/1749-4877.12297

Huey, R.B., Deutsch, C.A., Tewksbury, J.J., Vitt, L.J., Hertz, P.E., Pérez, H.J.Á., Garland, T., 2009. Why tropical forest lizards are vulnerable to climate warming. Proc. R. Soc. B Biol. Sci. 276, 1939–1948. https://doi.org/10.1098/rspb.2008.1957

Huey, R.B., Kearney, M.R., Krockenberger, A., Holtum, J.A.M., Jess, M., Williams, S.E., 2012.

Predicting organismal vulnerability to climate warming: roles of behaviour, physiology and adaptation. Philos. Trans. R. Soc. B Biol. Sci. https://doi.org/10.1098/rstb.2012.0005

Huey, R.B., Stevenson, R.D., 1979. Integrating thermal physiology and ecology of ectotherms: A discussion of approaches. Am. Zool. 19, 357–366. https://doi.org/10.1093/icb/19.1.357

Iserbyt, S., Rasmont, P., 2012. The effect of climatic variation on abundance and diversity of bumblebees: A ten years survey in a mountain hotspot. Ann. la Soc. Entomol. Fr. 48, 261–273. https://doi.org/10.1080/00379271.2012.10697775

Jackson, H.M., Johnson, S.A., Morandin, L.A., Richardson, L.L., Guzman, L.M., M'Gonigle, L.K., 2022. Climate change winners and losers among North American bumblebees. Biol. Lett. 18, 20210551. https://doi.org/10.1098/RSBL.2021.0551

Jacobson, M.M., Tucker, E.M., Mathiasson, M.E., Rehan, S.M., 2018. Decline of bumble bees in northeastern North America, with special focus on Bombus terricola. Biol. Conserv. 217, 437–445. https://doi.org/10.1016/j.biocon.2017.11.026

Jandt, J.M., Dornhaus, A., 2014. Bumblebee response thresholds and body size: Does worker diversity increase colony performance? Anim. Behav. 87, 97–106. https://doi.org/10.1016/j.anbehav.2013.10.017

Jandt, J.M., Dornhaus, A., 2009. Spatial organization and division of labour in the bumblebee Bombus impatiens. Anim. Behav. 77, 641–651. https://doi.org/10.1016/j.anbehav.2008.11.019

Johansson, F., Orizaola, G., Nilsson-Örtman, V., 2020. Temperate insects with narrow seasonal activity periods can be as vulnerable to climate change as tropical insect species. Sci. Reports 2020 101 10, 8822. https://doi.org/10.1038/s41598-020-65608-7

Johnson, S.A., Tompkins, M.M., Tompkins, H., Colla, S.R., 2019. Artificial Domicile Use by

Bumble Bees (Bombus; Hymenoptera: Apidae) in Ontario, Canada. J. Insect Sci. 19. https://doi.org/10.1093/JISESA/IEY139

Jones, J.C., Helliwell, P., Beekman, M., Maleszka, R., Oldroyd, B.P., 2005. The effects of rearing temperature on developmental stability and learning and memory in the honey bee, Apis mellifera. J. Comp. Physiol. A. Neuroethol. Sens. Neural. Behav. Physiol. 191, 1121–1129. https://doi.org/10.1007/s00359-005-0035-z

Jones, J.C., Oldroyd, B.P., 2006. Nest Thermoregulation in Social Insects. Adv. In Insect Phys. https://doi.org/10.1016/S0065-2806(06)33003-2

Kellermann, V., Overgaard, J., Hoffmann, A.A., Fljøgaard, C., Svenning, J.C., Loeschcke, V., 2012. Upper thermal limits of Drosophila are linked to species distributions and strongly constrained phylogenetically. Proc. Natl. Acad. Sci. U. S. A. 109, 16228–16233. https://doi.org/10.1073/pnas.1207553109

Kenna, D., Pawar, S., Gill, R.J., 2021. Thermal flight performance reveals impact of warming on bumblebee foraging potential. Funct. Ecol. 35, 2508–2522. https://doi.org/10.1111/1365-2435.13887

Kerr, J.T., Pindar, A., Galpern, P., Packer, L., Potts, S.G., Roberts, S.M., Rasmont, P., Schweiger, O., Colla, S.R., Richardson, L.L., Wagner, D.L., Gall, L.F., Sikes, D.S., Pantoja, A., 2015. Climate change impacts on bumblebees converge across continents. Science (80-.). 349, 177–180. https://doi.org/10.1126/science.aaa7031

Kingsolver, J.G., Diamond, S.E., Buckley, L.B., 2013. Heat stress and the fitness consequences of climate change for terrestrial ectotherms. Funct. Ecol. 27, 1415–1423. https://doi.org/10.1111/1365-2435.12145

Klein, A.M., Vaissière, B.E., Cane, J.H., Steffan-Dewenter, I., Cunningham, S.A., Kremen, C.,

Tscharntke, T., 2006. Importance of pollinators in changing landscapes for world crops. Proc. R. Soc. B Biol. Sci. 274, 303–313. https://doi.org/10.1098/RSPB.2006.3721

Klok, C.J., Chown, S.L., 2001. Critical thermal limits, temperature tolerance and water balance of a sub-Antarctic kelp fly, Paractora dreuxi (Diptera: Helcomyzidae). J. Insect Physiol. 47, 95–109. https://doi.org/10.1016/S0022-1910(00)00087-1

Klok, C.J., Sinclair, B.J., Chown, S.L., 2004. Upper thermal tolerance and oxygen limitation in terrestrial arthropods. J. Exp. Biol. 207, 2361–2370. https://doi.org/10.1242/jeb.01023

Kovac, H., Käfer, H., Stabentheiner, A., Costa, C., 2014. Metabolism and upper thermal limits of Apis mellifera carnica and A. m. ligustica. Apidologie 45, 664–677. https://doi.org/10.1007/s13592-014-0284-3

Kovac, H., Stabentheiner, A., Hetz, S.K., Petz, M., Crailsheim, K., 2007. Respiration of resting honeybees. J. Insect Physiol. 53, 1250. https://doi.org/10.1016/J.JINSPHYS.2007.06.019

Kronenberg, F., Heller, H.C., 1982. Colonial thermoregulation in honey bees (Apis mellifera). J. Comp. Physiol. B. 148, 65–76. https://doi.org/10.1007/BF00688889

Kuznetsova, A., Brockhoff, P.B., Christensen, R.H.B., 2017. lmerTest Package: Tests in Linear Mixed Effects Models. J. Stat. Softw. 82, 1–26. https://doi.org/10.18637/JSS.V082.I13

Kwon, Y.J., Saeed, S., 2003. Effect of temperature on the foraging activity of Bombus terrestris L. (Hymenoptera: Apidae) on greenhouse hot pepper (Capsicum annuum L.). Appl. Entomol. Zool. 38, 275–280. https://doi.org/10.1303/aez.2003.275

Leroy, C., Brunet, J.-L., Henry, M., Alaux, C., Cooke, S., 2023. Perspective Using physiology to better support wild bee conservation 11. https://doi.org/10.1093/conphys/coac076

Li, C., Wang, L., Li, J., Gao, C., Luo, Y., Ren, L., 2019. Thermal survival limits of larvae and adults of Sirex noctilio (Hymenoptera: Siricidae) in China. PLoS One 14, e0218888.

https://doi.org/10.1371/JOURNAL.PONE.0218888

Liczner, A.R., Colla, S.R., 2019. A systematic review of the nesting and overwintering habitat of bumble bees globally. J. Insect Conserv. 23, 787–801. https://doi.org/10.1007/s10841-019-00173-7

Lighton, J.R.B., Turner, R.J., 2004. Thermolimit respirometry: An objective assessment of critical thermal maxima in two sympatric desert harvester ants, Pogonomyrmex rugosus and P. californicus. J. Exp. Biol. 207, 1903–1913. https://doi.org/10.1242/jeb.00970

Macías-Macías, J.O., Quezada-Euán, J.J.G., Contreras-Escareño, F., Tapia-Gonzalez, J.M., Moo-Valle, H., Ayala, R., 2011. Comparative temperature tolerance in stingless bee species from tropical highlands and lowlands of Mexico and implications for their conservation (Hymenoptera: Apidae: Meliponini). Apidologie 42, 679–689. https://doi.org/10.1007/s13592-011-0074-0

MacMillan, H.A., Williams, C.M., Staples, J.F., Sinclair, B.J., 2012. Metabolism and energy supply below the critical thermal minimum of a chill-susceptible insect. J. Exp. Biol. 215, 1366–1372. https://doi.org/10.1242/jeb.066381

Maebe, K., De Baets, A., Vandamme, P., Vereecken, N.J., Michez, D., Smagghe, G., 2021. Impact of intraspecific variation on measurements of thermal tolerance in bumble bees. J. Therm. Biol. 99, 103002. https://doi.org/10.1016/j.jtherbio.2021.103002

Maia-Silva, C., da Silva Pereira, J., Freitas, B.M., Hrncir, M., 2021. Don’t stay out too long! Thermal tolerance of the stingless bees Melipona subnitida decreases with increasing exposure time to elevated temperatures. Apidologie 52, 218–229. https://doi.org/10.1007/s13592-020-00811-z

Masson, S.W.C., Hedges, C.P., Devaux, J.B.L., James, C.S., Hickey, A.J.R., 2017.

Mitochondrial glycerol 3-phosphate facilitates bumblebee pre-flight thermogenesis. Sci. Rep. 7, 13107. https://doi.org/10.1038/s41598-017-13454-5

McCue, M.D., De Los Santos, R., 2013. Upper thermal limits of insects are not the result of insufficient oxygen delivery. Physiol. Biochem. Zool. 86, 257–265. https://doi.org/10.1086/669932

Medina, R.G., Paxton, R.J., De Luna, E., Fleites-Ayil, F.A., Medina Medina, L.A., Quezada-Euán, J.J.G., 2018. Developmental stability, age at onset of foraging and longevity of Africanized honey bees (Apis mellifera L.) under heat stress (Hymenoptera: Apidae). J. Therm. Biol. 74, 214–225. https://doi.org/10.1016/j.jtherbio.2018.04.003

Medrzycki, P., Sgolastra, F., Bortolotti, L., Bogo, G., Tosi, S., Padovani, E., Porrini, C., Sabatini, A.G., 2010. Influence of brood rearing temperature on honey bee development and susceptibility to poisoning by pesticides. J. Apic. Res. 49, 52–59. https://doi.org/10.3896/IBRA.1.49.1.07

Meehl, G.A., Tebaldi, C., 2004. More Intense, More Frequent, and Longer Lasting Heat Waves in the 21st Century. Science (80-.). 305, 994–997. https://doi.org/10.1126/science.1098704

Mitchell, J.D., Hewitt, P.H., van der Linde, T.C. d. K., 1993. Critical thermal limits and temperature tolerance in the harvester termite Hodotermes mossambicus (Hagen). J. Insect Physiol. 39, 523–528. https://doi.org/10.1016/0022-1910(93)90085-6

Moffatt, L., 2001. Metabolic rate and thermal stability during honeybee foraging at different reward rates. J. Exp. Biol. 204, 759–766. https://doi.org/10.1242/jeb.204.4.759

Mommaerts, V., Reynders, S., Boulet, J., Besard, L., Sterk, G., Smagghe, G., 2010. Risk assessment for side-effects of neonicotinoids against bumblebees with and without impairing foraging behavior. Ecotoxicology 19, 207–215. https://doi.org/10.1007/s10646-

009-0406-2

Morash, A.J., Neufeld, C., MacCormack, T.J., Currie, S., 2018. The importance of incorporating natural thermal variation when evaluating physiological performance in wild species. J. Exp. Biol. https://doi.org/10.1242/JEB.164673

Mullan, F., 2022. Connecting above-and belowground effects of climate warming on bumble bee health. Kennesaw State University.

Naumchik, M., Youngsteadt, E., 2023. Larger pollen loads increase risk of heat stress in foraging bumblebees. Biol. Lett. 19, 12596. https://doi.org/10.1098/rsbl.2022.0581

Neven, L.G., 2000. Physiological responses of insects to heat. Postharvest Biol. Technol. 21, 103–111. https://doi.org/10.1016/S0925-5214(00)00169-1

Oyen, K.J., Dillon, M.E., 2018. Critical thermal limits of bumblebees (Bombus impatiens) are marked by stereotypical behaviors and are unchanged by acclimation, age or feeding status. J. Exp. Biol. 221. https://doi.org/10.1242/jeb.165589

Oyen, K.J., Giri, S., Dillon, M.E., 2016. Altitudinal variation in bumble bee (Bombus) critical thermal limits. J. Therm. Biol. 59, 52–57. https://doi.org/10.1016/j.jtherbio.2016.04.015

Parsons, Z., 2017. The influence of pile length and body size on rates of heat loss in the bumble bee, Bombus vosnesenskii. University of Wyoming.

Peat, J., Darvill, B., Ellis, J., Goulson, D., 2005. Effects of climate on intra- and interspecific size variation in bumble-bees. Funct. Ecol. 19, 145–151. https://doi.org/10.1111/j.0269-8463.2005.00946.x

Peat, J., Goulson, D., 2005. Effects of experience and weather on foraging rate and pollen versus nectar collection in the bumblebee, Bombus terrestris. Behav. Ecol. Sociobiol. 58, 152–156. https://doi.org/10.1007/s00265-005-0916-8

Pereboom, J.J.M., Biesmeijer, J.C., 2003. Thermal constraints for stingless bee foragers: The importance of body size and coloration. Oecologia 137, 42–50. https://doi.org/10.1007/S00442-003-1324-2/FIGURES/4

Perl, C.D., Johansen, Z.B., Moradinour, Z., Guiraud, M., Restrepo, C.E., Wen Jie, V., Miettinen, A., Baird, E., 2022. Heatwave-Like Events During Development Are Sufficient to Impair Bumblebee Worker Responses to Sensory Stimuli. Front. Ecol. Evol. 9, 776830. https://doi.org/10.3389/FEVO.2021.776830/BIBTEX

Peters, M.K., Peisker, J., Steffan-Dewenter, I., Hoiss, B., 2016. Morphological traits are linked to the cold performance and distribution of bees along elevational gradients. J. Biogeogr. 43, 2040–2049. https://doi.org/10.1111/JBI.12768

Pimsler, M.L., Oyen, K.J., Herndon, J.D., Jackson, J.M., Strange, J.P., Dillon, M.E., Lozier, J.D., 2020. Biogeographic parallels in thermal tolerance and gene expression variation under temperature stress in a widespread bumble bee. Sci. Rep. 10, 17063. https://doi.org/10.1038/s41598-020-73391-8

Pinsky, M.L., Eikeset, A.M., McCauley, D.J., Payne, J.L., Sunday, J.M., 2019. Greater vulnerability to warming of marine versus terrestrial ectotherms. Nature 569, 108–111. https://doi.org/10.1038/s41586-019-1132-4

Pörtner, H.-O., Bock, C., Mark, F.C., 2017. Oxygen- and capacity-limited thermal tolerance: bridging ecology and physiology. J. Exp. Biol. 220, 2685–2696. https://doi.org/10.1242/jeb.134585

Pörtner, H., 2001. Climate change and temperature-dependent biogeography: Oxygen limitation of thermal tolerance in animals. Naturwissenschaften. https://doi.org/10.1007/s001140100216

Potts, S.G., Willmer, P., 1997. Abiotic and biotic factors influencing nest-site selection by Halictus rubicundus, a ground-nesting halictine bee. Ecol. Entomol. 22, 319–328. https://doi.org/10.1046/J.1365-2311.1997.00071.X

Powney, G.D., Carvell, C., Edwards, M., Morris, R.K.A., Roy, H.E., Woodcock, B.A., Isaac, N.J.B., 2019. Widespread losses of pollinating insects in Britain. Nat. Commun. 10, 1018. https://doi.org/10.1038/s41467-019-08974-9

Rami Reddy, P. V., Rashmi, T., Verghese, A., 2015. Foraging activity of Indian honey bee, Apis cerana in relation to ambient climate variables under tropical conditions. J. Environ. Biol. 36, 577–581.

Rasmont, P., Iserbyt, S., 2012. The bumblebees scarcity syndrome: Are heat waves leading to local extinctions of bumblebees (Hymenoptera: Apidae: Bombus)? Ann. la Soc. Entomol. Fr. 48, 275–280. https://doi.org/10.1080/00379271.2012.10697776

Richards, K.W., 1973. Biology of Bombus polaris Curtis and Bombus hyperboreus Schonherr at Lake Hazen, Northwest Territories (Hymenoptera: Bombini). Quaest. Entomol. 9, 115–158.

Rivière, B., 2012. Phenotypic plasticity and population-level variation in thermal physiology of the bumblebee Bombus impatiens. University of Ottawa.

Roberts, S.P., Harrison, J.F., 1999. Mechanisms of thermal stability during flight in the honeybee Apis mellifera. J. Exp. Biol. 202, 1523–1533. https://doi.org/10.1242/jeb.202.11.1523

Roberts, S.P., Harrison, J.F., 1998. Mechanisms of Thermoregulation in Flying Bees. Am. Zool. 38, 492–502. https://doi.org/10.1093/icb/38.3.492

Roquer-Beni, L., Rodrigo, A., Arnan, X., Klein, A.M., Fornoff, F., Boreux, V., Bosch, J., 2020. A novel method to measure hairiness in bees and other insect pollinators. Ecol. Evol. 10, 2979–2990. https://doi.org/10.1002/ece3.6112

Rutrecht, S.T., Brown, M.J.F., 2008. The life-history impact and implications of multiple parasites for bumble bee queens. Int. J. Parasitol. 38, 799–808. https://doi.org/10.1016/j.ijpara.2007.11.004

Scharf, I., Wexler, Y., Macmillan, H.A., Presman, S., Simson, E., Rosenstein, S., 2016. The negative effect of starvation and the positive effect of mild thermal stress on thermal tolerance of the red flour beetle, Tribolium castaneum. Sci. Nat. 103, 1–10. https://doi.org/10.1007/s00114-016-1344-5

Schulte, P.M., Healy, T.M., Fangue, N.A., 2011. Thermal performance curves, phenotypic plasticity, and the time scales of temperature exposure. Integr. Comp. Biol. 51, 691–702. https://doi.org/10.1093/icb/icr097

Schultze-Motel, P., 1991. Heat loss and thermoregulation in a nest of the bumblebee Bombus lapidarius(hymenoptera, apidae). Thermochim. Acta 193, 57–66. https://doi.org/10.1016/0040-6031(91)80174-H

Silvola, J., 1984. Respiration and energetics of the bumblebee Bombus terrestris queen. Ecography (Cop.). 7, 177–181. https://doi.org/10.1111/j.1600-0587.1984.tb01119.x

Sinclair, B.J., Marshall, K.E., Sewell, M.A., Levesque, D.L., Willett, C.S., Slotsbo, S., Dong, Y., Harley, C.D.G., Marshall, D.J., Helmuth, B.S., Huey, R.B., 2016. Can we predict ectotherm responses to climate change using thermal performance curves and body temperatures? Ecol. Lett. https://doi.org/10.1111/ele.12686

Sirois-Delisle, C., Kerr, J.T., 2018. Climate change-driven range losses among bumblebee species are poised to accelerate. Sci. Rep. 8, 14464. https://doi.org/10.1038/s41598-018-32665-y

Skandalis, D.A., Darveau, C.A., 2012. Morphological and physiological idiosyncrasies lead to

interindividual variation in flight metabolic rate in worker bumblebees (bombus impatiens). Physiol. Biochem. Zool. 85, 657–670. https://doi.org/10.1086/665568

Snyder, G.K., Weathers, W.W., 1975. Temperature adaptations in amphibians. Am. Nat. 109, 93–101. https://doi.org/10.1086/282976

Soroye, P., Newbold, T., Kerr, J., 2020. Climate change contributes to widespread declines among bumble bees across continents. Science (80-.). 367, 685–688. https://doi.org/10.1126/science.aax8591

Spaethe, J., Weidenmüller, A., 2002. Size variation and foraging rate in bumblebees (Bombus terrestris). Insectes Soc. 49, 142–146. https://doi.org/10.1007/s00040-002-8293-z

Speakman, J.R., Król, E., 2010. Maximal heat dissipation capacity and hyperthermia risk: Neglected key factors in the ecology of endotherms. J. Anim. Ecol. 79, 726–746. https://doi.org/10.1111/j.1365-2656.2010.01689.x

Stabentheiner, A., Kovac, H., Brodschneider, R., 2010. Honeybee colony thermoregulation - Regulatory mechanisms and contribution of individuals in dependence on age, location and thermal stress. PLoS One 5. https://doi.org/10.1371/journal.pone.0008967

Stabentheiner, A., Kovac, H., Mandl, M., Käfer, H., 2021. Coping with the cold and fighting the heat: thermal homeostasis of a superorganism, the honeybee colony. J. Comp. Physiol. A Neuroethol. Sensory, Neural, Behav. Physiol. 207, 337–351. https://doi.org/10.1007/s00359-021-01464-8

Staples, J.F., Koen, E.L., Laverty, T.M., 2004. “Futile cycle” enzymes in the flight muscles of North American bumblebees. J. Exp. Biol. 207, 749–754. https://doi.org/10.1242/jeb.00825

Stewart, T., Bolton-Patel, N., Cresswell, J.E., 2021. Eating versus heating: a study of the allocation of workers between foraging and nest incubation in bumble bees. Ecol. Entomol.

46, 844–855. https://doi.org/10.1111/een.13021

Stone, G.N., 1993. Endothermy in the Solitary Bee Anthophora Plumipes : Independent Measures of Thermoregulatory Ability, Costs of Warm-Up and the Role of Body Size. J. Exp. Biol. 174, 299–320. https://doi.org/10.1242/jeb.174.1.299

Sunday, J., Bennett, J.M., Calosi, P., Clusella-Trullas, S., Gravel, S., Hargreaves, A.L., Leiva, F.P., Verberk, W.C.E.P., Olalla-Tárraga, M.Á., Morales-Castilla, I., 2019. Thermal tolerance patterns across latitude and elevation. Philos. Trans. R. Soc. B Biol. Sci. 374. https://doi.org/10.1098/rstb.2019.0036

Sunday, J.M., Bates, A.E., Dulvy, N.K., 2012. Thermal tolerance and the global redistribution of animals. Nat. Clim. Chang. 2, 686–690. https://doi.org/10.1038/nclimate1539

Sunday, J.M., Bates, A.E., Dulvy, N.K., 2011. Global analysis of thermal tolerance and latitude in ectotherms. Proc. R. Soc. B Biol. Sci. 278, 1823–1830. https://doi.org/10.1098/rspb.2010.1295

Sunday, J.M., Bates, A.E., Kearney, M.R., Colwell, R.K., Dulvy, N.K., Longino, J.T., Huey, R.B., 2014. Thermal-safety margins and the necessity of thermoregulatory behavior across latitude and elevation. Proc. Natl. Acad. Sci. U. S. A. 111, 5610–5615. https://doi.org/10.1073/pnas.1316145111

Tautz, J., Maier, S., Groh, C., Rössler, W., Brockmann, A., 2003. Behavioral performance in adult honey bees is influenced by the temperature experienced during their pupal development. Proc. Natl. Acad. Sci. U. S. A. 100, 7343–7347. https://doi.org/10.1073/pnas.1232346100

Terblanche, J.S., Deere, J.A., Clusella-Trullas, S., Janion, C., Chown, S.L., 2007. Critical thermal limits depend on methodological context. Proc. R. Soc. B Biol. Sci.

https://doi.org/10.1098/rspb.2007.0985

Tewksbury, J.J., Huey, R.B., Deutsch, C.A., 2008. Ecology: Putting the heat on tropical animals. Science (80-.). 320, 1296–1297. https://doi.org/10.1126/science.1159328

The Core Writing Team IPCC, 2015. Climate Change 2014: Synthesis Report. Contribution of Working Groups I, II and III to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change, Ipcc. Gian-Kasper Plattner.

Tian, L., Hines, H.M., 2018. Morphological characterization and staging of bumble bee pupae. PeerJ 2018, e6089. https://doi.org/10.7717/PEERJ.6089/SUPP-6

Tremblay, P., MacMillan, H.A., Kharouba, H.M., 2021. Autumn larval cold tolerance does not predict the northern range limit of a widespread butterfly species. Ecol. Evol. 11, 8332–8346. https://doi.org/10.1002/ece3.7663

Unwin, D.M., Corbet, S.A., 1984. Wingbeat frequency, temperature and body size in bees and flies. Physiol. Entomol. 9, 115–121. https://doi.org/10.1111/j.1365-3032.1984.tb00687.x

Van Berkum, F.H., 1988. Latitudinal patterns of the thermal sensitivity of sprint speed in lizards. Am. Nat. 132, 327–343. https://doi.org/10.1086/284856

Vanderplanck, M., Martinet, B., Carvalheiro, L.G., Rasmont, P., Barraud, A., Renaudeau, C., Michez, D., 2019. Ensuring access to high-quality resources reduces the impacts of heat stress on bees. Sci. Rep. 9, 12596. https://doi.org/10.1038/s41598-019-49025-z

Vaudo, A.D., Farrell, L.M., Patch, H.M., Grozinger, C.M., Tooker, J.F., 2018. Consistent pollen nutritional intake drives bumble bee (Bombus impatiens) colony growth and reproduction across different habitats. Ecol. Evol. 8, 5765–5776. https://doi.org/10.1002/ece3.4115

Velthuis, H.H.W., Van Doorn, A., 2006. A century of advances in bumblebee domestication and the economic and environmental aspects of its commercialization for pollination.

Apidologie. https://doi.org/10.1051/apido:2006019

Vickruck, J.L., Richards, M.H., 2012. Niche partitioning based on nest site selection in the small carpenter bees Ceratina mikmaqi and C. calcarata. Anim. Behav. 83, 1083–1089. https://doi.org/10.1016/j.anbehav.2012.01.039

Vogt, F.D., 1986a. Thermoregulation in Bumblebee Colonies. I. Thermoregulatory versus Brood-Maintenance Behaviors during Acute Changes in Ambient Temperature. Physiol. Zool. 59, 55–59. https://doi.org/10.1086/physzool.59.1.30156090

Vogt, F.D., 1986b. Thermoregulation in Bumblebee Colonies. II. Behavioral and Demographic Variation throughout the Colony Cycle. Physiol. Zool. 59, 60–68. https://doi.org/10.1086/physzool.59.1.30156091

Vorhees, A.S., Bradley, T.J., 2012. Differences in critical thermal maxima and mortality across life stages of the mealworm beetle Tenebrio molitor. J. Exp. Biol. 215, 2319–2326. https://doi.org/10.1242/JEB.070342

Vorhees, A.S., Gray, E.M., Bradley, T.J., 2013. Thermal Resistance and Performance Correlate with Climate in Populations of a Widespread Mosquito. Physiol. Biochem. Zool. 86, 73–81. https://doi.org/10.1086/668851

Vray, S., Rollin, O., Rasmont, P., Dufrêne, M., Michez, D., Dendoncker, N., 2019. A century of local changes in bumblebee communities and landscape composition in Belgium. J. Insect Conserv. 23, 489–501. https://doi.org/10.1007/s10841-019-00139-9

Wang, Q., Xu, X., Zhu, X., Chen, L., Zhou, S., Huang, Z.Y., Zhou, B., 2016. Low-temperature stress during capped brood stage increases pupal mortality, misorientation and adult mortality in honey bees. PLoS One 11, e0154547. https://doi.org/10.1371/journal.pone.0154547

Weidenmüller, A., 2004. The control of nest climate in bumblebee (Bombus terrestris) colonies: Interindividual variability and self reinforcement in fanning response. Behav. Ecol. 15, 120–128. https://doi.org/10.1093/beheco/arg101

Weidenmüller, A., Kleineidam, C., Tautz, J., 2002. Collective control of nest climate parameters in bumblebee colonies. Anim. Behav. 63, 1065–1071. https://doi.org/10.1006/anbe.2002.3020

Whitehorn, P.R., O'Connor, S., Wackers, F.L., Goulson, D., 2012. Neonicotinoid pesticide reduces bumble bee colony growth and queen production. Science (80-.). 336, 351–352. https://doi.org/10.1126/science.1215025

Woods, W.A., Heinrich, B., Stevenson, R.D., 2005. Honeybee flight metabolic rate: does it depend upon air temperature? J. Exp. Biol. 208, 1161–1173. https://doi.org/10.1242/jeb.01510

Wuellner, C.T., 1999. Nest site preference and success in a gregarious, ground-nesting bee Dieunomia triangulifera. Ecol. Entomol. 24, 471–479. https://doi.org/10.1046/J.1365-2311.1999.00215.X

Wynants, E., Lenaerts, N., Wäckers, F., Van Oystaeyen, A., 2021. Thermoregulation dynamics in commercially reared colonies of the bumblebee Bombus terrestris. Physiol. Entomol. 46, 110–118. https://doi.org/10.1111/phen.12350

Zhao, Z.J., Cao, J., 2009. Effect of fur removal on the thermal conductance and energy budget in lactating Swiss mice. J. Exp. Biol. 212, 2541–2549. https://doi.org/10.1242/jeb.029603