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Hot and Cold: Assessing the Thermal Limitations of Bumble Bees in a Changing Climate

ABSTRACT

To understand bumblebee thermal limitations a controlled experiment was built and tested in different environmental conditions to determine the most stable form of conducting upper and lower thermal trials in the field. The study was conducted at field sites located at the Rocky Mountain Biological Laboratory, CO, USA. We subjected *Bombus flavifrons*, *Bombus bifarius*, *Bombus appositus*, *Bombus rufocinctus*, *Bombus californicus* to both upper and lower extreme temperatures. Preliminary trials on nonfocus organisms helped refine experimental procedures. With the data we have accumulated now there are small trends beginning to form relating to ITD and upper extreme temperature limitations.

INTRODUCTION

Temperature is a ubiquitous component in every organism's life. Throughout an organism's life span, temperature affects reproductive and metabolic rate, development and growth, migration and distribution, behavior, adaptation and much more (Some references here). Therefore, it is important to understand how organisms are affected by a range of temperatures as the concern around climate change grows. Understanding the range of tolerable thermal conditions is important, especially in organisms strongly influenced by external temperatures such as insects (Deutsch et al. 2008). The majority of insects are adapted to use different thermal cues for various stages of their life cycle. (Abarca 2019). Climate change can disrupt these life cycle patterns or restrict it from reaching certain stages due to subjecting the organism to their temperature limits. All organisms have a distinct range of temperature where they can perform at. Once an organism is introduced to their upper temperature limit "CTmax" or lower temperature limit "CTmin" they lose all fitness and performance ability. In the case of bumblebees, queens use temperature cues when nesting and hibernating, while workers use temperature for pollination activity and foraging (Maia-Silva et al. 2021). In light of ongoing and predicted changes in temperature worldwide (REFS), it is important to determine an organism's thermal limits in order to understand how they may be affected in the future (REFS). For example, bumble bees' population liveliness, range shift, and a reduction in species richness have been documented to be affected by the causes of climate change (Nooten and Rehan 2020, Gonzalez et al. 2022).

Like many insects bumble bees are sensitive to temperature, however what makes them an interesting organism to test thermal tolerance is that bumble bees are heterothermic, meaning their body temperatures can span across vast temperatures through different performance activities to regulate their body temperature (Maia-Silva et al. 2021). Bumble bees' survival relies heavily on the thermal environment they are surrounded by. More so, they are found in a broad

geographical distribution in many different climatic regions. In the Rocky Mountains bumble bees are introduced to a spectrum of temperatures across the season (Pyke et al. 2016).

There is a small, but growing body of knowledge regarding how to determine bumble bee's thermal tolerance in a controlled laboratory environment, thanks to the ease of maintaining numerous control variables (Gonzalez et al. 2022). We've learned a lot from these, but we know a lot less about CTmax/min in the wild, in part due to limited ability to run such experiments under field conditions. Therefore, the design and implementation of CTmax/min experiments, how they work in nature, and what can maximize success is important in this realm. Thus, an understanding of how each variable effects the experiment will allow for a more precise result and allow for one to find the most stable approach to conduct such experiments in the field.

The aim of this project was to minimize insect response variability while conducting thermal tolerance assays in bumblebees (both CTmax and CTmin) under field conditions. I expected that the most suitable environmental conditions for calculating CTmax/min with the most accurate insect response would be under a shaded structure. Similarly, in order to accurately measure the timing of chilled induced coma, a goal of this experiment was to stabilize the cold temperature of the experimental chamber at approximately -10° Celsius

METHODS

To determine what conditions would be the most stable to perform the thermal assays I performed multiple experiments in different environment. The different environmental conditions I ran the trials in were a complete sunlit environment, partly shaded environment, and completely shaded environment. For the partly shaded environments the thermal assays were moved out from under the shade into the sun and returned to the shade every 5 minutes. For completely shaded environments a shade canopy was used. . I conducted these experiments between June 8th and August 15th, 2023. Bumblebees of multiple species (*B. flav*, *bif*, *mixtus* etc) were collected around Gothic town area At the Rocky Mountains Biological Station, CO, USA.

Given the restrictions of performing these experiments on bumblebee queens, while awaiting bumblebee workers to emerge, I ran trials on syrphid flies and common solitary bees such as *Osmia* bees, vespids wasps, and bombyliidae flies in order to test and troubleshoot my experimental chambers. Nevertheless, these organisms are important pollinators and recording their thermal limits will contribute to the general knowledge on insect thermal tolerance. I expected these pollinators to perform the same stereotypical behaviors that bumble bees perform when they reach their thermal limits (Oyen et al. 2021).

When performing the thermal upper and lower limitation trials for bumblebees, I focused on the most abundant species at our sites: *Bombus appositus*, *Bombus flavifrons*, *Bombus rufocinctus*, *Bombus insularis*, *Bombus bifarius*. I avoided catching queens and only used workers and males for the trials. Firstly, the bumblebees were caught in the field and placed into falcon tubes where they will be introduced to the chilled induced coma assay. We recorded the time it takes to reach the chilled induced coma. Once this point was reached, their intertegular distance "ITD" will be measured, and a recording of the recovery time will be measured. It was

determined that the bumble bee had recovered when they had full control over their motor skills. Following the CT_{min} trial they will have 20 minutes to acclimate before the CT_{max} trial. Once acclimated the bumblebees were placed into glass vials and into the aluminum block. The bees will experience a ramping rate of 0.5 degrees Celsius per minute. The time it takes for the bumble bees to lose complete muscular function was recorded and then the bees were immediately removed and allowed to acclimate before being released. To account for variable ambient conditions while performing these experiments in the field, I conducted replicate CT_{min}/max measurements under shaded conditions.

Thermal maximum

The experimental chamber for testing thermal tolerance of bumblebees is a 1 ft X 2ft X 1ft Styrofoam box. The lid and a side of the box have plastic cutouts to allow viewing and recording of bumblebee physiological responses under each experimental condition. Inside the Styrofoam box is a square aluminum block with five-cylinder indents roughly an inch deep, one in each corner and one in the middle to place glass vials containing individual bumblebees. The aluminum blocks are heated up using a portable power bank at a rate of 0.5°C per minute. All cylinder indents contained glass vials with a thermal couple underneath them to track temperature ramping rates as well as the temperature of each aluminum block and viewed with the Pico Technology Software. Each glass vial has insecta slip spread all across the inside to prevent the bees from climbing up the glass vial to escape the heat from the bottom. Furthermore, the top of the vial held a damp cotton ball in order to prevent desiccation. Thermal maximum temperature was determined when individual bees lost complete function and fitness. This was determined by certain physiological behavior such as rolling over, or in some instances the tongue sticking out. Once the thermal maximum temperature was reached the glass vial would be immediately removed from the device and the bumble bees were given as much time needed to reacclimate.

Thermal minimum

The environment we used to introduce the bees into a chill induced coma is a 1ft by 1ft by 1ft Styrofoam box. The top lid of the Styrofoam box has square cut out made and a clear plastic square was placed over the hole using hot glue to allow for an observation window as described above. A mixture of ¾ cup of salt and one gallon of ice allows the mixture to reach a temperature of -12 degrees Celsius for about an hour. A thermocouple is placed inside the mixture to determine the exact temperature along with a thermocouple outside of the Styrofoam box to record the ambient temperature. There are also thermocouples placed in each falcon tube to record the temperatures that the bees are experiencing. The bees are placed inside falcon tubes which are then placed inside the mixture of salt and ice. Using ice and salt mixture allows for a freezing-point depression. When salt (sodium chloride) is introduced to water, it dissolves into separate sodium and chloride ions, which interrupt the water molecules and formation of water to ice ultimately creating a lower temperature required to freeze the mixture.

Results

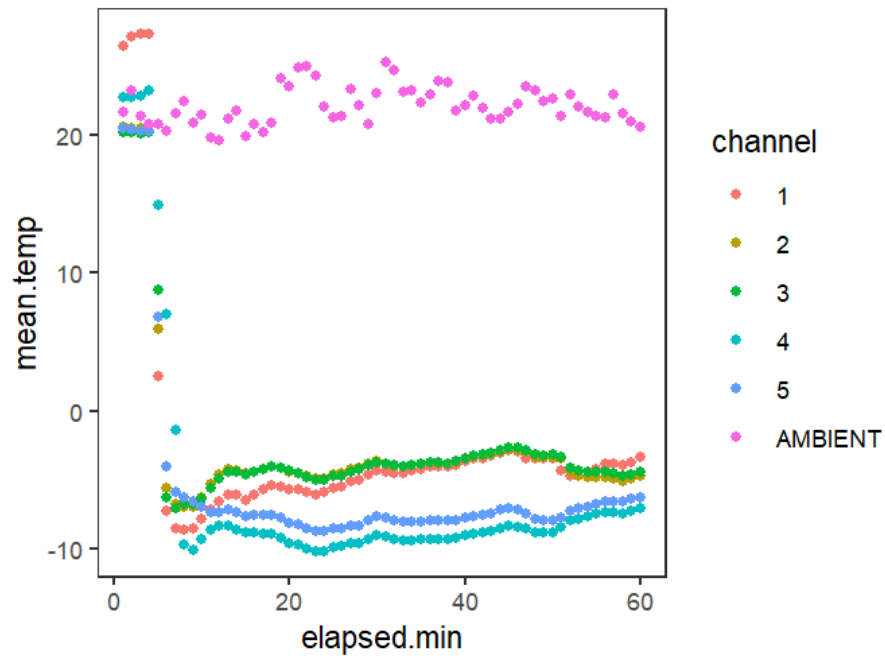
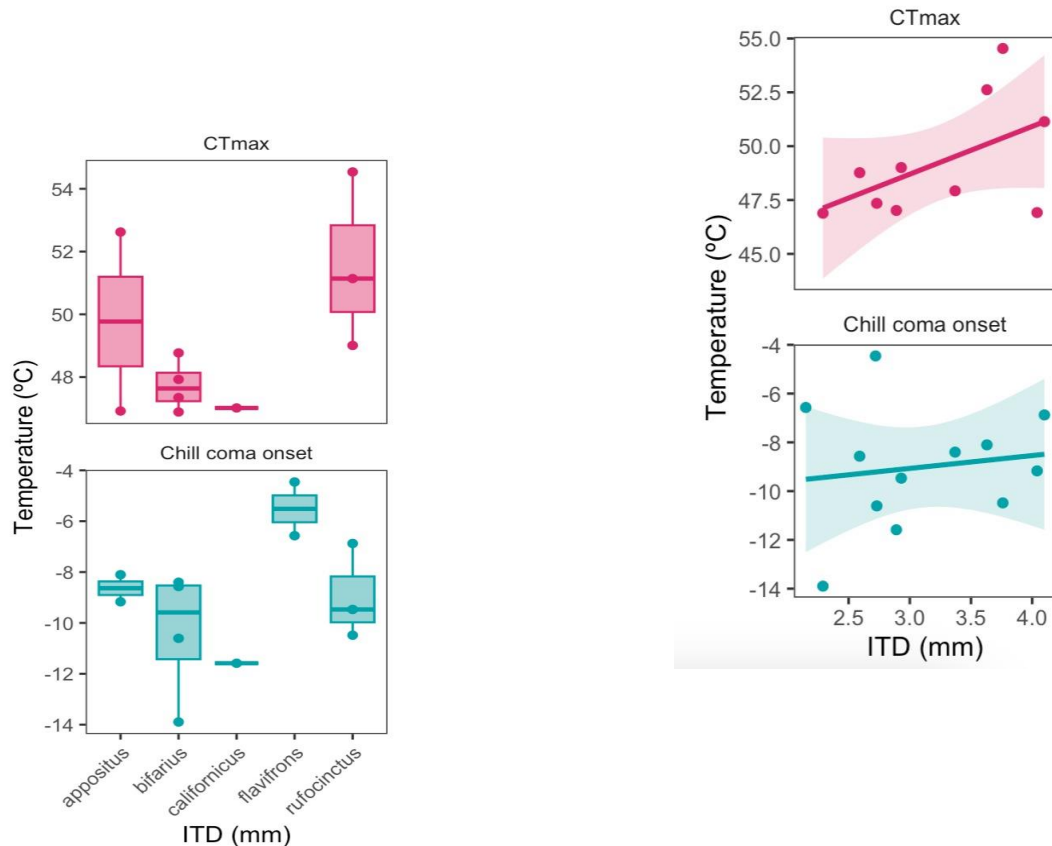


Figure 1. A chilled induced coma essay under a shaded environment over the course of an hour. The x axis represents the elapsed time, and the y axis represents the mean temperature. Each dot represents the time the data was recorded. The purple dots represent the ambient temperature in the environment. While all other colors represent a falcon tube inside the mixture.

During the summer I ran trials under the sun, trials under partial shade, and trials under complete shade when trying to determine what the best conditions to perform the thermal limitation essays in the field. Performing the assays under complete shade seemed to provide the most stable conditions. Even with the ambient temperature fluctuating the shaded environment allowed the mixture to remain the same temperature for a minimum of an hour, and the falcon tubes to remain the same temperature for the chilled induced coma trials. Similarly, performing



the upper thermal limitation essay under a shaded environment protects the vials and aluminum block from being affected by the sun such as unnecessary and unwanted heat fluctuations.

Figure 2A. displays the different species of bumble bees and their mean CTmax on top and mean chilled coma onset on the bottom. Figure 2B displays the bees CTmax on top and chill coma onset on the bottom in relation to their ITD.

Throughout the summer we were able to get data points across five different species: *Bombus flavifrons*, *Bombus bifarius*, *Bombus appositus*, *Bombus rufocinctus*, *Bombus californicus*. Currently, we have collected 10 data points for our upper thermal limitation essays and 12 data points for our lower thermal limitation assays. To determine if there are any trends amongst the CTmax and CTmin between castes, size, or species we need to continue collecting data which is in the works. As of now there is a small relationship between larger Intertegular distance and higher CTmax temperatures, however this could change as we collect more data. For the lower thermal limitations there are no clear trends or relationships between intertegular distance and critical thermal minimum temperature.

Furthermore, there is not an even amount of data collected from the five focus species. With what we do have it is clear that *Bombus appositus*, and *Bombus rufocinctus* have higher upper thermal limitation temperatures than the other species collected. As for the lower thermal limitation trials *Bombus flavifrons* had to the highest CTmin.

Discussion

Due to the late winter snow melt and unusually dry summer our sample size was much smaller than we anticipated. In order to truly see any trends with ITD and CTmax/CTmin we need to achieve more data collection, which is currently in the process. Furthermore, we currently have an uneven amount of data from each species, and we would like to get the same sample size across all five species. Another aspect we will investigate is differences in thermal limitations amongst castes, species, and ITD sizes. Nevertheless, our data suggests certain trends regarding thermal tolerance of bumblebees and other local pollinating insects.

My data displays that there is small relationship between larger Intertegular distance and higher CTmax temperatures, which could suggest that larger bumble bees have a greater heat tolerance, whereas smaller bees may be more susceptible to hotter temperature fluctuations. This could be due to larger organisms having higher mass and volume resulting in greater thermal inertia. For the lower thermal limitation trials *Bombus flavifrons* had the highest CTmin mean value “warmer temperature” than other species. Which could suggest that they are less resistant to colder temperatures. If this is true, this could help explain why we did not see as many *Bombus flavifrons* as there was a later snow melt season and they organisms were introduced to cold temperatures for a longer period.

In the midst of climate change bumble bees are experiencing new temperatures so it is important to understand their limitations. This could help any conservation efforts fight the declining bumble bee populations. With the data I have obtained now there are a few trends we can see are beginning to form. However, with more data the trends may become more confined and or change.

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