

Characterization of thermal tolerance in sub-alpine bumblebee species

Hannah Burke

Undergraduate Student, Biological Science

Florida State University

Research Mentor: Jacquelyn Fitzgerald

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Abstract

Temperature is one of the limiting factors of life, and it influences an organism's growth, sexual reproduction, sexual determination, distribution, and metabolism. The interaction between temperature and organisms therefore an essential component of their ecology. Temperature impacts organisms in different ways, and some organisms, such as bumblebees, may be especially sensitive to temperature shifts. With the likelihood of climate change causing a gradually warmer world, it is vital to understand how bumblebees will respond. Phenology plays a large role in how an animal interacts with temperature, and I seek to evaluate if and how emergence timing influences thermal tolerance in sub-alpine bumblebee species. Critical thermal minimum (CT_{min}) and critical thermal maximum (CT_{max}) measurements were taken for five common species of sub-alpine bumblebees in the field over the course of the summer using laboratory-verified devices. We found that there was considerable difference between species type and thermal tolerance, and that earlier emerging species may have less flexibility in their thermal tolerance ranges than later emerging species. Our preliminary results suggest that phenologically earlier species may be more vulnerable to an increasing intensity and frequency of extreme temperature events under climate change.

Introduction

Temperature governs everything: from the internal chemical processes occurring in an organism to how that organism will interact with its environment. The biota of the world experiences varying temperatures in a multitude of ways. These temperatures go on to influence the ecology of species, determining the rate of growth, reproduction, and its overall survival (Clarke, 2017). Thermal ecology can be defined as the study of the interactions between an organism and temperature (Clarke, 2017). Questions surrounding thermal ecology are paramount in understanding key biological components of an organism, such as the range of environmental conditions that the organism can tolerate. One important dimension of an

organism's interaction with temperature is its thermal tolerance—the maximum and minimum temperatures it can withstand before losing basic function (Martinet et al., 2021). Thermal tolerance is a basic component of the ecology of an organism and will contribute greatly to how that organism will respond to future environmental change.

Climate change is occurring at a pronounced rate, but the effect that this will have on the biota of the world is unclear (Martinet et al., 2021; Sunday et al., 2010; Walther, 2010). An increase in the range of temperatures and unpredictable weather patterns are some of the major predicted outcomes. Many studies are concerned with how climate change will impact an organism's metabolic rate, its stages of

development, and the altitudinal gradient upon which it is naturally found (Dillon et al., 2010; Gvoždík et al., 2001; Klockmann et al., 2016). Based on this, some species will find themselves more affected by shifting temperatures and unpredictable weather patterns than others.

Bumblebees (genus *Bombus*) are a group of animals that may find themselves particularly affected by climate change, as they are generally cold-adapted and large-bodied, both of which may increase their risk of disturbance in the face of warming temperatures (Goulson et al., 2008; Martinet et al., 2021; Oyen et al., 2016). One such problem faced by bees and warming temperature presents itself as the output of metabolism. Temperature and bee metabolism are nonlinear, meaning a slight increase in temperature can lead to a very steep increase in bee metabolic rate. An increased metabolic rate leads to an increased need for food; an increased rate of water loss in dry habitats; a higher susceptibility for starvation; and possible decreased energy reserves for reproduction (Dillon et al., 2010). This is of particular concern for bumblebees which find themselves living at higher elevations and in colder temperatures, as they will not be as adapted to warmer temperatures as the species which commonly live in warm conditions. With some bumblebee species potentially being more susceptible to the changing warmer conditions than others, there lies uncertainty in how these effects will occur in a community context. Since bumblebees are an abundant and diverse pollinator for the world for wild plants as well as agriculture, studies have begun to

look at thermal tolerance in bees, with the hope that this information may provide glimpses into the persistence and stability of bumblebee populations.

Through the few studies of thermal tolerance that have been conducted on bumblebees, some notable patterns have been observed. It has been found that a species' thermal tolerance is most likely to be tailored to the conditions of the environment it finds itself in. For example, cold-adapted bumblebee species are more vulnerable to heat stress than temperate and Mediterranean species (Martinet et al., 2021). This makes sense, as the temperate and Mediterranean bee species would have more exposure to warm temperatures than cold-adapted bumblebees, leading to an increased heat stress resistance in those two groups. This finding poses a question for seasonality during the year: perhaps if bees spend a longer duration in warmer temperatures, the species may develop an increased thermal tolerance. Bumblebee species with a wider range have also been found to have higher thermal tolerances to extreme temperatures (Martinet et al., 2021). If a bee has a larger range, it also means it experiences a more variable climate, which would in turn lead to an increased thermal tolerance, as the bee requires a thermal adaptation to temperature extremes in order to survive in such a broad range. It is also known that thermal tolerance in organisms follows somewhat of a predictable pattern with altitude, with lower thermal minimum decreasing with latitude (Oyen et al., 2016). Studies are also seeking to draw links between thermal tolerance in bumblebees to relative body mass as well as species type

(Martinet et al., 2021; Klockmann et al., 2016; Oyen et al., 2016). However, thermal tolerance in bumblebees is still largely understudied in wild systems, and much work still needs to be done to characterize and understand thermal tolerance in bumblebees so that we may better comprehend their thermal ecology as well as make predictions for potential range shifts in the future.

I looked at five common species of bumblebees in the sub-alpine region of Western Colorado (*Bombus appositus*, *B. bifarius*, *B. californicus*, *B. flavifrons*, and *B. rufocinctus*) over the course of the summer. These species experience a large range of temperatures in an extreme mountainous environment, and I sought to understand how thermal tolerance varies among each species given its unique natural history. *B. appositus* is the largest of the species while *B. bifarius* is the smallest. Foraging activity is different across these species as well, as *B. bifarius*, *B. rufocinctus*, and *B. flavifrons* are both generalists while *B. appositus* and *B. californicus* are more specialized. It is especially important to consider these species, as they each emerge during different times in the summer, with *B. bifarius* being the first to emerge and *B. appositus* being the last. Bees that emerge early on in the summer will experience a different range of temperatures than those that emerge later in the summer, and I wish to understand if there is a connection between the phenology of the bumblebees and thermal tolerance. If *B. bifarius* is the first bee to emerge during the summer, then perhaps this species will have a lower thermal tolerance as a result of the species being more likely to encounter

colder temperatures than species that arise later in the summer, such as *B. flavifrons* and *B. appositus*.

The experiment conducted this summer focused primarily on characterizing the thermal tolerances of *B. appositus*, *B. bifarius*, and *B. flavifrons*.

Methods

Study sites and focal species

For this experiment, we captured bees from several different field sites located around RMBL. We collected data from a site up-valley from RMBL (Emerald City), a site within RMBL grounds (bill hill), and one site down-valley from RMBL (Rosy Hill) (see Fig. 1). We tested thermal tolerance limits in the most common bumblebee species in this region: *Bombus appositus*, *B. bifarius*, *B. californicus*, *B. flavifrons*, and *B. rufocinctus*. The comparison of thermal tolerance in these species resulted from data collection of several generations of worker bees of each species at each field site over the course of the summer.

Through the summer months July and August, my target sample size was to measure thermal tolerance on approximately 30 worker bees from each species.

Measurement of thermal tolerance

I recorded the upper thermal limit as critical thermal maximum (CT_{max}), lower thermal limit as critical thermal minimum (CT_{min}), recovery time for CT_{max} , and recovery time for CT_{min} for each individual bee captured.

Two devices created by Jacquelyn Fitzgerald (my research mentor) were used to test CT_{max} and CT_{min} in bumblebees this

summer. Each device will be explained more in-depth in the following sections.

As a general sequence of events, the bees were caught in the field using nets and glass vials, placed in the lower thermal tolerance device, removed from the device and allowed to recover, placed in the upper thermal tolerance device, removed from the device and allowed to recover, and then released.

Lower thermal tolerance

Lower thermal limits in bumblebees can be observed when the bumblebee outwardly experiences the effects of a coma chill onset, in which the muscle functions of the bee will begin to diminish and the bee will not be able to keep itself standing (Oyen et al., 2016). Coma chill onset is reversible in bees, and we did not observe any of the bees to experience harm as a result of the cold trials. To measure coma chill onset, a specific methodology created by Jacquelyn Fitzgerald was used. The bees were caught in the field using nets and glass vials. Once the bees were caught and placed in an individual glass vial, they were positioned inside an insulated cooler. Each vial contained a thermocouple probe so that we could record the exact temperature the bees experienced. The cooler contained one gallon of ice to three-fourths cup of salt which achieved temperatures around -12°C , a temperature low enough in which the bees experienced coma chill onset. The bee was then observed for signs of coma chill onset, and the time the bee was placed in the cooler and when coma chill onset was induced were recorded. When we measured this lower thermal limit in bees, we recorded the

temperature at which the bee enters coma chill onset as well as the time in seconds it takes for the bee to recover from such temperatures (characterized by the bee standing on all six legs). When the bee reached its lower thermal limit, it was removed from the ice and allowed to return to normal temperature for around 20 minutes. We recorded the coma chill recovery time as the period of time once the bee was removed from the cold chamber and when the bee was able to right itself on all of its legs. Some bees were released after chill coma recovery, but some were used in the upper thermal limit trials afterwards.

Upper thermal tolerance

Upper thermal limits in bumblebees can be observed when the bumblebee loses its motor function as a result of high temperatures, thus going into a “heat stupor” (Martinet et al., 2021). This upper limit can be lethal, and for this reason, a limited sample size of bees were used to carry out this experiment. We sampled five of each species at each site over the course of the summer. Sampling at such a low rate and a spread out time allows for lower biological impact, as we wish to make the least impact on the sites in which these bees were collected. To test upper thermal tolerance in the field, a laboratory-validated heating array designed by Jacquelyn Fitzgerald was used. The device connects to a portable power source in the field in order to work. The heating array consists of an aluminum block which has wells which the individual glass vials may be placed in. The temperature inside of the wells is able to be controlled via a

proportional-integral-derivative (PID) controller. The CT_{max} trials began at an initial temperature of 22°C held constant for 10 minutes before increasing the temperature by .5°C per minute. The increase in temperature leveled off at 65°C. The bees were removed from the heating array as soon as heat stupor was induced. The recovery time for CT_{max} began once the bee was removed from the heating array and ended once the bee was able to right itself or when the timer hit half an hour, whichever came first. For the bees which did not experience lethal effects from the heating trials, supplementary food was given during their recovery.

Measuring Intertegular Distance

During either the coma chill recovery or heat stupor recovery, each bee was measured using intertegular distance (ITD) – the distance between the two tegulas on the bees. The bees were also marked during this time to prevent running the thermal tolerance experiment on a singular bee multiple times.

Results

Species Type and Thermal Tolerance

In total, we collected 12 sub-alpine bumblebees for this experiment (4 *bifarius*, 3 *rufocinctus*, 1 *californicus*, 2 *appositus*, and 2 *flavifrons*). We measured CT_{max} for 10 bumblebees (4 *bifarius*, 3 *rufocinctus*, 1 *californicus*, and 2 *appositus*). An analysis of variance (ANOVA) was conducted to examine the effect species type has on CT_{max} ($F = 1.82$, $df = 3$, $p = .244$, Fig. 1). We measured CT_{min} for all 12 of the bees collected. We used ANOVA to examine the

effect species type has on CT_{min} ($F = 2.311$, $df = 4$, $p = .157$, Fig. 1). We did not find strong evidence of an effect of species type and CT_{max} and CT_{min} .

Emergence Date and Thermal Tolerance

We used our lab's longterm emergence data to analyze the relationship between emergence date and thermal tolerance in bumblebee species. We used ANOVA to examine the effect that emergence date has on CT_{max} ($F = .341$, $df = 1$, $p = .575$, Fig. 2). ANOVA was conducted to examine the effect that emergence date has on CT_{min} ($F = 1.856$, $df = 1$, $p = .203$, Fig. 2). We did not find strong evidence that critical thermal limits were correlated with emergence date.

Discussion

Overall, we saw much variation in thermal tolerance of different sub-alpine species as well as variation in emergence date with thermal tolerance. While we did not find strong evidence of correlation between phenology and thermal tolerance, our preliminary patterns are suggestive of species-specific responses. Our sample size was relatively low, given the constraints of the field season. This summer was an interesting season in terms of phenology and bee abundance. Later snowmelt this summer resulted in the bees emerging later than they normally do. When the bees had emerged and were at peak numbers, the vegetation and flowers were incredibly dry due to a drought the area experienced this summer. Regardless of these hurdles, we were still able to sample bees. Although our sample size is small, our data is suggestive of a

relationship between thermal tolerance, species type, and emergence date.

In the case of the effect species type has on thermal tolerance, it was found that CT_{min} was more affected by species type ($p = .157$) than CT_{max} ($p = .244$). It is probable that there is a stronger relationship between CT_{min} and species type due to low sampling this summer. More research and samples need to be taken to actually determine the strength between this relationship. The same can be said for CT_{max} and thermal tolerance: more data needs to be taken. A plausible explanation that CT_{min} may be more impacted by species type than CT_{max} is that the bee species need specific thermal minimum when living in an environment as extreme as sub-alpine conditions. It is more realistic that the bumblebee species will be exposed to temperatures close to their thermal minimum than it is their thermal maximum. It has been found that the thermal tolerance of queen bees correlates to the thermal tolerance of the workers they create, so having distinct thermal minima may be a species-specific characteristic. Something which may be of interest to consider is the cool-season sensitivity hypothesis, which states that organisms better adapted for the cold may be more sensitive to increasing temperatures during the cooler seasons. Even though queen bees are not active during the winter, they still must survive through that season to make it to the short growing season. It has been found that the strongest effects of warming in arthropods occurred during the winter and spring (Fitzgerald et al., 2021). Questions surrounding thermal tolerance in different seasons with sub-alpine bumblebees would

be a great next step for better understanding their thermal ecology. Understanding the exact science behind such will require further study and continuation of this project and new projects in the future.

Our data also suggest that there may be a relationship between emergence date and phenology. We found that emergence date more heavily influenced the CT_{min} ($p = .203$) of bee species than their CT_{max} ($p = .575$). However, this can be attributed to low sampling size. Fewer bees were samples for CT_{max} than for CT_{min} which could explain the stronger confidence levels for both species type and emergence date with CT_{min} . We simply need to sample more bees for CT_{max} . The trends observed between emergence date and thermal tolerance are of particular interest. At first, it was thought that the bees to first emerge would have a lower CT_{min} and lower CT_{max} than bees that emerged later in the summer as a response to their environment and exposure to potential colder temperatures. We found the opposite pattern, however, and see a pattern of earlier emerging bees having a higher CT_{min} and a lower CT_{max} than later emerging species. Perhaps this finding is suggestive that physical traits such as bee body size may play a greater role in thermal tolerance than environmental conditions. The bees that are earliest to emerge are typically smaller species such as *B. bifarius* and *B. flavifrons*. When an organism is larger, it has a higher surface area to volume ratio which results in greater heat retention (Clarke, 2017). This is a biological explanation to Bergmann's Principle which observes that body mass increases with colder temperatures (Bergmann, 1847). While smaller bees may

be able to handle higher temperatures better than larger bees, they are more prone to desiccation than larger bees (Burdine & McCluney, 2019). This could suggest that both larger and smaller bumblebees may be particularly affected by the warming climate in different ways. Or perhaps the earlier emerging bees don't necessarily need to have a broader thermal tolerance because perhaps the workers can take sanctuary in the hive which is warmer than the surrounding air during colder periods, and then forage for food when the temperature conditions are more suitable (Goulson, 2010; Heinrich, 1979).

An interesting observation occurs when emergence date vs CT_{max} and CT_{min} are plotted against each other within the same graph. When compressing the y-axis and observing the graphs close to each other, there is less space between the CT_{max} regression line and CT_{min} regression line for earlier emergence dates than for later emergence dates. This is the physical representation of the lower CT_{max} and higher CT_{min} that we observed for earlier emerging species. What this is suggestive of is that earlier emerging species do not have as broad a thermal tolerance as later emerging species. Essentially, earlier emerging bees may be more sensitive to temperature extremes than later emerging species. Perhaps this is due to the fact that later emerging species may have to deal with more variable temperature conditions, thus resulting in a broader thermal tolerance (higher CT_{max} and lower CT_{min}). It has been found in past studies that bees with a wider range (i.e. experiencing more variation in

temperature conditions) have a broader thermal tolerance (Martinet et al., 2021).

This research project is still currently underway, so by no means are the results presented in this paper the final results. As time goes on, more and more data will be collected by my research team. It will be interesting to see stronger or entirely different correlations between thermal tolerance and species type and emergence date.

Overall, we find that sub-alpine bumblebees may have species-specific variation in their thermal tolerances and that earlier emerging species may have less flexibility in their thermal tolerances than later emerging species. There are many directions that future studies can take to evaluate the thermal tolerance of sub-alpine bumblebees, and each finding and data point will work toward better understanding the thermal ecology of bumblebees.

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Tables and Figures

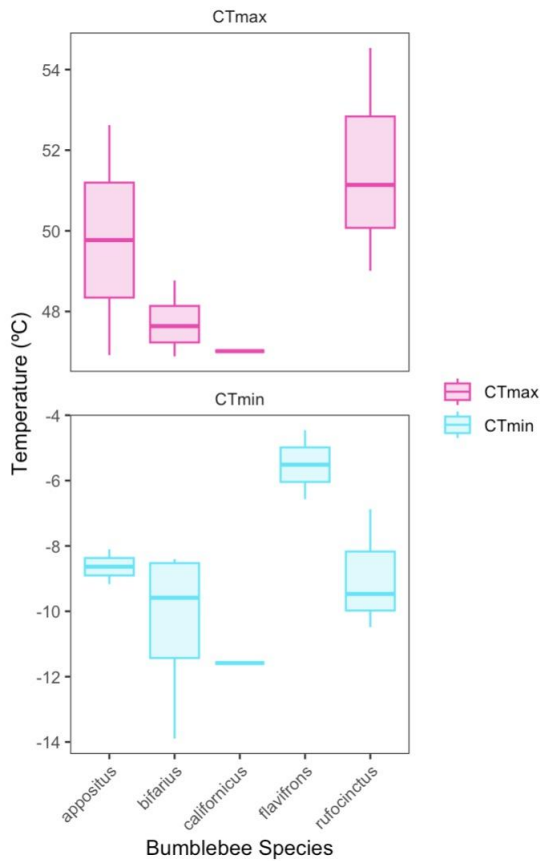


Fig. 1

Thermal tolerance as characterized by different sub-alpine bumblebee species. No statistical significance was found between species type and CT_{max} ($F = 1.82$, $df = 3$, $p = .244$) and CT_{min} ($F = 2.311$, $df = 4$, $p = .157$). However, the graph shows much variation, and the p-values are indicative that there may be a relationship between the two variables should our sample size be larger.

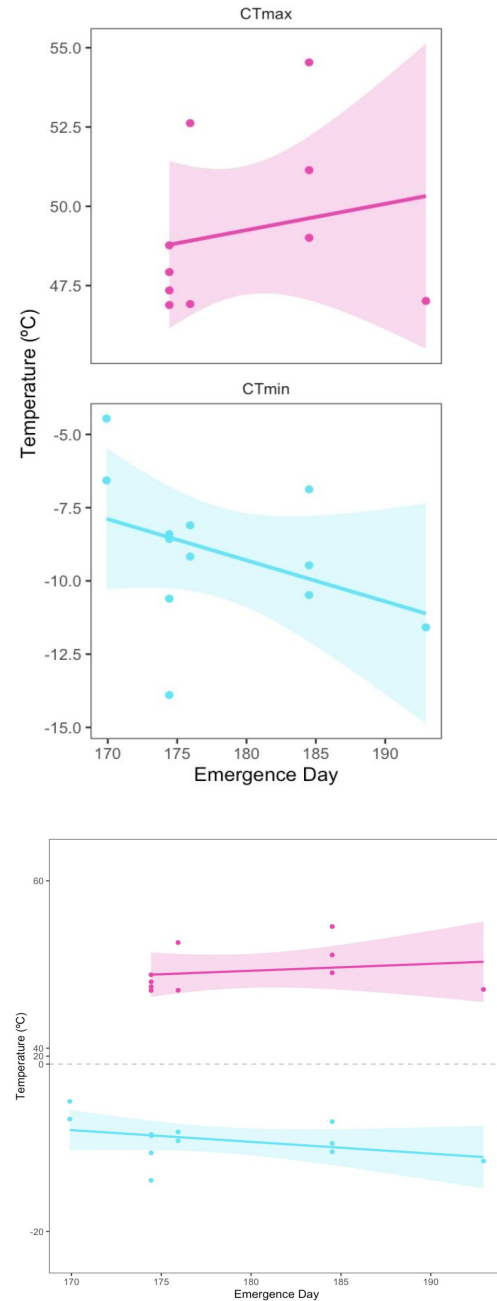
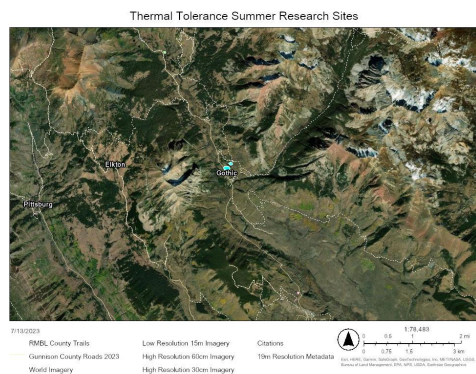
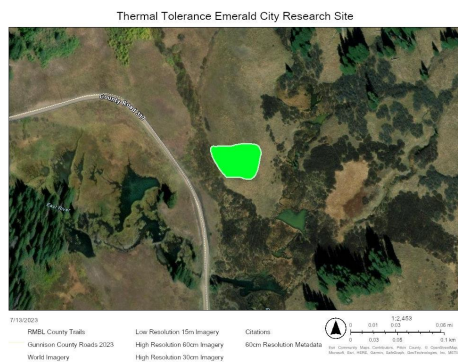


Fig. 2

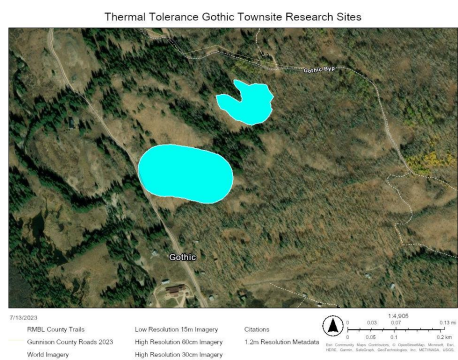
Thermal tolerance as characterized by the emergence date of worker bumblebees. No statistical significance was found between the emergence date and CT_{max} ($F = .341$, $df = 1$, $p = .575$) and CT_{min} ($F = 1.856$, $df = 1$, $p = .203$). However, the graph shows a pattern beginning to develop, and the p-values are indicative that the earlier emerging bees may have less flexibility in their thermal tolerance range than later emerging species.

**Fig. 3A**

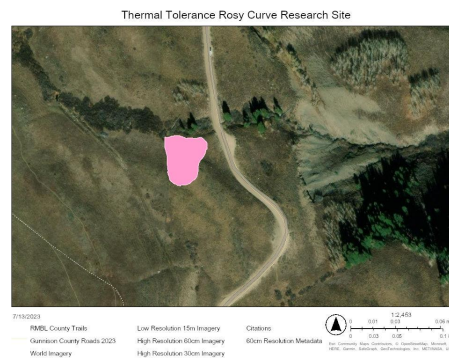
GPS mapping of the three research sites used this summer using ArcGIS.

**Fig. 3B**

GPS mapping of our up-valley site “Emerald City” using ArcGIS.

**Fig. 3C**

GPS mapping of our Gothic site “billy Hill” using ArcGIS.

**Fig. 3D**

GPS mapping of our down-valley site “Rosy Curve” using ArcGIS.