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RESEARCH ARTICLE

Beat the heat: thermal respites and access to food associated with increased bumble bee heat tolerance

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ABSTRACT

Climate change poses a threat to organisms across the world, with cold-adapted species such as bumble bees (*Bombus* spp.) at particularly high risk. Understanding how organisms respond to extreme heat events associated with climate change as well as the factors that increase resilience or prime organisms for future stress can inform conservation actions. We investigated the effects of heat stress within different contexts (duration, periodicity, with and without access to food, and in the laboratory versus field) on bumble bee (*Bombus impatiens*) survival and heat tolerance. We found that both prolonged (5 h) heat stress and nutrition limitation were negatively correlated with worker bee survival and thermal tolerance. However, the effects of these acute stressors were not long lasting (no difference in thermal tolerance among treatment groups after 24 h). Additionally, intermittent heat stress, which more closely simulates the forager behavior of leaving and returning to the nest, was not negatively correlated with worker thermal tolerance. Thus, short respites may allow foragers to recover from thermal stress. Moreover, these results suggest there is no priming effect resulting from short- or long-duration exposure to heat – bees remained equally sensitive to heat in subsequent exposures. In field-caught bumble bees, foragers collected during warmer versus cooler conditions exhibited similar thermal tolerance after being allowed to recover in the lab for 16 h. These studies offer insight into the impacts of a key bumble bee stressor and highlight the importance of recovery duration, stressor periodicity and context on bumble bee thermal tolerance outcomes.

KEY WORDS: Nutrition, Thermal response, Climate change, Heat hardening, Acclimatization, Heat priming

INTRODUCTION

Climate change has been implicated in declines and range shifts across a diversity of taxa (Thuiller et al., 2005; Rosenberg et al., 2019; Soroye et al., 2020). Sublethal organismal responses implicate climate change as a primary threat to life in the 21st century and a catalyst for species adaptation and resilience (Altizer et al., 2013; Moritz and Agudo, 2013; Razgour et al., 2019). Preparing for and countering the detrimental effect of climate change through conservation actions requires a mechanistic understanding of the context in which these effects are most acute and the factors that can ameliorate them (Turner et al., 2020). Specifically, understanding the effects of climate change and extreme heat events on an organism requires knowledge of (1) the acute effects of heat stress, (2) the interactive effects of heat stress with other stressors, (3) the importance of duration and frequency of heat stress events on future heat tolerance, as well as (4) assessment of these effects in a field-realistic context.

Bees are one of the most important and imperiled groups; they provide the essential ecosystem service of pollination but are threatened by multiple anthropogenic factors (Potts et al., 2010; Goulson et al., 2015; Cameron and Sadd, 2020). At the population level, there is solid evidence of range constrictions, range shifts and population declines in multiple bee species (Sánchez-Bayo and Wyckhuys, 2019). Some of the best evidence for wild pollinator declines comes from bumble bees (Goulson et al., 2008; Cameron et al., 2011; Graves et al., 2020; Cameron and Sadd, 2020). Bumble bees (*Bombus* spp.) are an integrally important pollinator genus across many regions of the globe, where they are recognized as some of the most prominent, efficient pollinators that are thus central for supporting both natural ecosystems and agricultural systems (Shipp et al., 1994; Williams et al., 2014).

Global analysis of regional bumble bee declines, relative to habitat and climate change, have implicated changing climate as the leading explanatory factor for recent declines in bumble bees (Kerr et al., 2015; Soroye et al., 2020; Jackson et al., 2022). While the effects of climate change are multifaceted (e.g. rising temperatures, extreme weather, increased CO_2 levels), heat waves may be particularly detrimental to this cold-adapted genus (Maebe et al., 2021). Tolerance, or lack thereof, to temperature extremes is likely to be a species- and/or population-specific trait, reflective of local adaptations (i.e. bumble bees occupying colder habitats are more sensitive to heat waves) (Oyen et al., 2016; Pimsler et al., 2020; Martinet et al., 2021).

Beyond genetic adaptations, bumble bees have several morphological, physiological, behavioral and phenological adaptations that impact their thermal tolerance for both high and low temperatures, at the colony and individual level (reviewed in Maebe et al., 2021). At the colony level, bumble bees have behavioral adaptations that buffer them against heat stress. Bumble bee nests are often located underground, which buffers them against temperature fluctuations (Goulson, 2003), and colonies can cool the nest by fanning with their wings (Vogt, 1986). At the individual bee level, physiological and behavioral adaptations to heat stress require sufficient access to nutrition. Foragers are the most vulnerable of worker bees to extreme temperature events brought on by climate change (Perez and Aron, 2020) because they must leave the temperature-buffered nest to collect food. While bumble bees have evolved advanced endothermic mechanisms to deal with heat stress during foraging (Heinrich, 2004), including shunting heat between the thorax and abdomen and dissipating heat in the abdomen, this process has upper thermal limits and nutrition limitation may reduce bees' ability to mount these responses (Fischer et al., 2010; Dinh et al., 2016; Vanderplanck et al., 2019).

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There is evidence that past heat exposure may influence future heat tolerance in bumble bees. Rapid heat hardening, i.e. heat shock protein protection from future heat stress based on pre-treatment with mild heat stress, has been demonstrated in both fruit flies (Colinet et al., 2010) and honey bees (Al-Ghzawi et al., 2022). Heat shock proteins are upregulated in bumble bees exposed to thermal stress (Pimsler et al., 2020; Blasco-Lavilla et al., 2021), suggesting they too could theoretically mount a physiological thermal response that could impact future thermal outcomes. However, Oyen and Dillon (2018) suggest that bumble bees do not acclimatize to high temperatures in the same way as other insects. Bees placed at 32°C (the upper bound of temperatures found in natural bumble bee nests) for 72 h did not differ markedly in thermal response (critical thermal maximum, CT_{max}) from those that were chilled (15°C for 72 h or 4°C for 12 h) (Oyen and Dillon, 2018). More research is needed to determine lagged effects of heat stress on future heat tolerance in bumble bees. This will help forecast the impacts that climate change could have on this important genus.

In this study, we aimed to improve understanding of how bumble bees are impacted by heat stress within different contexts (prolonged heat stress with/without nutrition stress, intermittent heat stress and heat stress in the field). Using the model bumble bee *Bombus impatiens*, we examined the role of adult nutritional limitation on survival and heat tolerance, whether prolonged periods of prior heat exposure prime individuals for future heat events, and the interaction between these variables. Heat and nutritional stress may be predicted to have synergistic negative effects on worker survival and thermal tolerance, while past heat stress, via the heat-shock response, could stimulate greater thermal tolerance (priming or rapid heat-hardening effect) after a recovery period. We also simulated intermittent heat exposure experienced by foragers as they return periodically to the nest. These short periods of exposure with respite could prime their tolerance to future heat stress through the heat-shock response, or could lead to compounded physiological stress, thus resulting in lower thermal tolerance. Finally, we assessed the thermal tolerance of wild-caught bees collected at different afternoon temperatures followed by an evening of thermal respite to determine how results from our lab assays compare with field-realistic heat exposure.

MATERIALS AND METHODS

Experiment 1: nutrition and prolonged heat stress

We used a complete block design to assess the effects of heat stress and nutrition stress across two different recovery periods for bumble bee (*Bombus impatiens* Cresson 1863) survival and heat tolerance (n=4 colonies×12 bees per colony) (Table 1). Adult bees underwent an initial period of stress (heat versus normal×starved versus fed) for 5 h. Then, we assessed survival after a recovery period of either 30 min or 24 h. We selected 24 h as the long-term recovery to represent how foragers may respond to a heat wave the next day if exposed the day prior.

We used research-grade commercial bumble bee colonies (Koppert Biological Systems, Howell, MI, USA). Colonies were kept in a dark incubator maintained at 26°C and ∼30% relative humidity (RH), were provided with Koppert's proprietary nectar solution (Koppert Biological Systems) *ad libitum*, and were fed fresh-frozen honey bee corbicular pollen pellets (sourced from Penn State research colonies) daily. For each treatment, 12 medium-sized (intertegular distance=4.01±0.02 mm; mean±s.e.m.) adult workers were removed from their parent colony and placed in Plexiglas cages (6 bees per cage×4 treatments×2 replicates).

Caged bees were exposed to heat and/or nutritional stress for a 5 h period. This represents a natural length of a mid-day heat wave (NOAA, 2022b), but is short enough to avoid mortality from starvation (Oyen and Dillon, 2018). Blasco-Lavilla et al. (2021) showed significant differential expression for heat shock genes *Hsc70* and *Aha1* in bumble bees kept at 38°C versus 9°C for 6 h and found this effect to be particularly pronounced in starved bees. We therefore used a similar duration and temperature to assess these trends at the organismal level. Because continuous exposure to high temperatures for 5 h may be unrealistic in a field setting, as bees would return to the nest between foraging bouts, we also tested bees provided with intermittent periods of recovery in the lab (see experiment 2), as well as bees collected from the field (see experiment 3).

Bees in the fed treatment had access to the fresh-frozen honey bee-collected pollen pellets in a dish, Koppert's nectar solution in a feeder (1.5 ml Eppendorf tube) and a water feeder (1.5 ml Eppendorf tube). Pollen and the nectar solution were withheld from bees in the nutrition-stressed treatment during this period, but they had access to a water feeder (1.5 ml Eppendorf tube). Heat-stressed bees were held at 36°C, ∼30% RH, and normal temperature bees were kept at 26°C, ∼30% RH. Typically, insects are primed (i.e. heat hardened) at a temperature of 5–10°C lower than the testing temperature (43°C in our case) (Chidawanyika et al., 2017). Our heat stress temperature is also consistent with what bees would experience during a heat wave; in the summer of 2022, the eastern USA experienced extreme temperatures (NOAA, 2022a; Bowman, 2022; Samenow, 2022) for several days and Pennsylvania, specifically, experienced a prolonged period (>5 h) at or above 35°C (NOAA, 2022b). Moreover, studies demonstrate that *B. impatiens* reduces foraging around 35–36°C (Couvillon et al., 2010; Hemberger et al., 2022). For additional discussion of our chosen temperatures, please see Supplementary Materials and Methods.

After the 5 h stressor period, the bees in the 24 h recovery treatment were given pollen and access to the nectar solution feeder, and returned to 26°C, ∼30% RH for 24 h. Bees in the 30 min recovery treatment proceeded directly to the thermal tolerance trials [bees were at room temperature (22°C) for the 30 min it took to set up the thermal tolerance trials]. Just prior to the thermal tolerance trails for both recovery periods, individual bee survival was noted.

Table 1. Experiment 1 design

Recovery treatment:	30 min	30 min	24 h	24 h
Stress treatment (5 h)	Normal temperature (26°C)	High temperature (36°C)	Normal temperature (26°C)	High temperature (36°C)
Fed	4 colonies×12 bees	4 colonies×12 bees	4 colonies×12 bees	4 colonies×12 bees
Starved	4 colonies×12 bees	4 colonies×12 bees	4 colonies×12 bees	4 colonies×12 bees

This study was a fully crossed 2×2×2 design, with the 5 h stressor treatments shown in the first column (fed versus starved) and across the top of the columns (normal versus high temperature). Recovery treatments (30 min or 24 h post-5 h stressor) are shown above the temperature treatments. The number of bumble bee colonies and worker bees per colony for each treatment is given in the corresponding cells.

To assess thermal tolerance, we used a time to heat stupor (THS) assay as outlined in Martinet et al. (2015). For the THS trial, bees were removed from their cages and placed in individual, sealed clear plastic jars (25 ml and 3 cm depth×3.5 cm height) without access to food, then placed in a 43°C, ∼15% RH incubator (Thermo Scientific, model no. 3711). THS is the time it took for bees to enter a temporary coma, indicated by the bees' inability to right themselves when flipped in the jar (Martinet et al., 2015). For details on our THS assay methods and justification, see Supplementary Materials and Methods.

All statistical analyses were carried out in R version 4.2.0. Treatment effects on bee survival and THS were analyzed using generalized linear mixed effects models (GLMM) through the lme4 package (Bates et al., 2015). To assess the effect of treatment on bee survival during the post-stressor period (but pre-THS trial), we used a binomial GLMM (Bernoulli case of 1=alive, 0=dead) with heat treatment, nutrition treatment and recovery treatment as fixed effects, and colony and treatment date as random effects (treatment date was included to account for potential day-to-day variation). Model assessments indicated good model fit. To assess the effect of treatment on THS, the interactive effects of heat treatment, nutrition treatment and recovery treatment were the fixed effects, and colony and treatment date were random effects. To investigate observed interaction effects, we used the emmeans package (https://CRAN.R-project.org/package=emmeans) to assess pairwise differences within treatment group and stratified our data by recovery period and re-ran the THS GLMM. Like the full THS model, the stratified models included an interaction of heat treatment and nutrition treatment as fixed effects and colony and treatment date as random effects. The THS data were square root transformed for analysis, and effects sizes (β) or differences (Δ) are provided on the transformed scale. To aid interpretation of the results, we also report means±s.e.m. of the untransformed THS data.

Experiment 2: intermittent heat stress

To assess the effects of intermittent heat stress on *B. impatiens* heat resistance, we exposed workers to alternating 30 min durations of heat stress (36°C) and recovery (26°C). We then assessed how 1, 2 and 3 short periods of heat exposure, alternated with periods of recovery, affected THS at 43°C (*n*=4 colonies, 3 bees per treatment per colony, with 12 bees per treatment in total). Each treatment was paired with a control group of bees from the same colony, which remained at 26°C throughout the duration of the experiment (Table 2) (*n*=4 colonies×3 bees), for a total of 72 assessed bees (4 colonies×3 bees×6 treatments+controls). In this experiment, all bees had access to pollen and nectar for the duration of the stressor period, but not during the THS trials. Thirty minutes is a conservative amount of time for foragers to be exposed to high heat (36°C). In the summer, foraging bouts for *B. impatiens* workers average 58.5 min, and foragers take 5.9 foraging trips a day, on average (Minahan and Brunet, 2018).

Three of the four colonies used in this experiment were commercially reared, research-grade colonies (Biobest, Canton, MI, USA). The fourth colony was reared from a single wild-caught *B. impatiens* queen collected on 2 May 2022 from Sproul State Forest, Clinton County, PA, USA (41°20′34″N, 77°36′03″W, 646 m elevation). The queen began producing brood in the lab on 12 May 2022, approximately 6 months before the experiment. There was no biological reason for the mix of lab-reared versus commercially reared colonies, and colony-level variation was statistically accounted for in our modeling approach (see below). All colonies were provided with a continuous supply of an artificial nectar solution which was composed of 50% sucrose and 50% invert sugar. An amino acid supplement (Amino-B Booster, Honey-B-Healthy Inc., Cumberland, MD, USA) was added to the nectar solution to provide bees with essential amino acids, and potassium sorbate was used as a preservative. The colonies were also provided with a continuous supply of honey bee corbicular pollen (sourced from Swarmbustin' Honey Bee Farm, Westgrove, PA, USA). Colonies were kept in a laboratory incubator (VWR model no. VRI20P) at 28°C, ∼65% RH prior to experiments. To ensure that all bees had a similar prior experience, about 17 h before each trial, experimental bees were removed from their parent colony (4 colonies), placed in plastic cages (3 bees per cage×6 treatments+controls for each colony), and moved into a room incubator set to 26°C, ∼65% RH. Caged bees were provisioned with *ad libitum* nectar solution (5 ml vials) and honey bee pollen. Bees from colonies 1 and 2 were treated on separate days to bees from colonies 3 and 4. To induce heat stress, we used a small incubator (Vevor, model XHC-25) set to 36°C, ∼65% RH inside the room incubator.

At the end of the last 30 min recovery period, both treatment and control bees began the THS trial. Bees were immediately transferred into individual preheated (43°C) glass vials and placed inside the water bath incubator (43°C; Benchmark, MyBath 4 L, model H2004). Cotton stoppers were used to plug the tops of the vials to limit the bees' mobility, retain heat inside the vial and allow gas exchange between the inside and the outside of the vial, thereby limiting the accumulation of CO_2 inside the vial caused by respiration. Humidity inside the vial was ∼90%, which differed from the 15% humidity in THS incubators for experiment 1. Once placed in the water bath, bees were assessed every 5 min for heat stupor. A hot water bath was chosen for this experiment to enable more controlled humidity during THS in the face of natural weather fluctuations during the times of the experiment (mid-November).

Treatment effects on THS were analyzed using a GLM, with duration (1, 2 or 3 alternating 30 min sessions), treatment/control, the interaction of duration and treatment/control group, and colony (four levels) as fixed effects. We also analyzed a subset of our data (just the three commercially reared colonies) to determine experimental group and colony-level effects.

Experiment 3: field-collected bees

To assess in a field setting whether bees exposed to greater heat stress on a given day show signs of heat stress the following day, on both 25 July and 28 July 2023, 12 foraging worker bumble bees (*B. impatiens*) (24 bees total) were hand netted off flowers between 15:30 h and 16:30 h from the Arboretum at Penn State. These days were chosen to target naturally varying temperature conditions; collection temperatures were 27.3°C and 31.7°C, based on temperature data inferred from a temperature/humidity data logger (Drop D2, Kestrel Instruments, Boothwyn, PA, USA). Collection days likewise varied in diel mean temperature (21.3 versus 25.6°C) and 3 day average temperature (22.4 versus 25.3°C) (https://centre.weatherstem.com/data) (Table S1). Couvillon et al. (2010) describe foraging for *B. impatiens* between 16 and 36°C, finding that foraging peaks around 21°C and decreases at higher temperatures. Peat et al. (2005) similarly describe foraging temperatures between 18 and 33°C for *Bombus terrestris*. Therefore, our chosen sampling days reflect a day of near-ideal foraging conditions compared with a day where we would expect reduced foraging activity due to elevated temperatures. Because we collected bees in the late afternoon, our field data reflect our lab experiments, as the bees had several hours to forage throughout the day (experiment 1), likely

Table 2. Experiment 2 design

Treatment	30 min	30 min	30 min/**THS**	30 min	30 min/**THS**	30 min	**THS**
Treatment 1	36°C	26°C	**43°C**				
Control 1	26°C	26°C	**43°C**				
Treatment 2	36°C	26°C	36°C	26°C	**43°C**		
Control 2	26°C	26°C	26°C	26°C	**43°C**		
Treatment 3	36°C	26°C	36°C	26°C	36°C	26°C	**43°C**
Control 3	26°C	26°C	26°C	26°C	26°C	26°C	**43°C**

Three bumble bee workers each from 4 colonies were exposed to 3 different intermittent heat stress treatments, each paired with a control treatment before entering the time to heat stupor (THS) trial. Treatments involved alternating 30 min bouts of heat stress (36°C) and recovery (26°C); control bees were held at a constant, low-stress temperature (26°C) for an equivalent period. After the treatment duration, bees proceeded to THS trails at 43°C until they reached heat stupor.

experiencing intermittent heat stress as they traveled between the field and their nest (experiment 2).

Bees were transported back to the lab in a cooler with ice packs to maintain them at ∼7°C, which slowed their activity without inducing torpor (<10 min transport time). Once in the lab, bees were provided with artificial nectar and honey bee pollen and kept in a room incubator at 28°C, ∼65% RH for 16 h; thus, this experiment tested whether bees experiencing different heat levels show signs of this stress the following day. After acclimatizing in the lab, each bee was subject to a THS trial using a water bath incubator, as described in experiment 2. We used analysis of variance (ANOVA) to compare the THS distributions of these two groups.

RESULTS

Experiment 1: nutrition and prolonged heat stress

In total, 37 of the 384 bees (10%) died before the THS trials. Heat stress had the greatest negative effect on bee survival ($\beta=-2.44$, $z=-4.46$, $P<0.01$), followed by a negative effect of nutrition stress ($\beta=-1.27$, $z=-2.83$, $P<0.01$) and a negative effect of amount of recovery time ($\beta=-0.96$, $z=-2.35$, $P=0.02$; the longer the experiment, the greater the number of bees that died) (Fig. 1). We were unable to assess interactive effects on survival; because of the small proportion of dead bees, the more complex model caused convergence issues.

Among the survivors, the average THS was 58±2 min, with bees from one colony (68±3 min) lasting significantly longer than bees from the three other colonies (54±2 min) (1.23–0.81 Δmaximum–Δminimum, $F_{3,343}=6.24$, $P<0.01$). We found a significant effect of nutrition treatment ($\beta=-1.40$, $F_{1,16.04}=4.82$, $P=0.04$), whereby, on average, the fed bees (64±2 min) lasted 13 min longer before heat stupor than the starved bees (51±2 min) (Fig. 2). We also observed a significant effect of recovery treatment ($\beta=-0.77$, $F_{1,240.85}=4.35$, $P=0.04$), and an interaction between recovery treatment and nutrition treatment ($\beta=1.64$, $F_{1,230.32}=6.66$, $P=0.01$) and between recovery treatment and heat treatment ($\beta=1.31$, $F_{1,328.75}=3.96$, $P=0.05$). While these interactions make it difficult to directly interpret the model estimates (β) of recovery treatment, our raw data suggest that bees in the 24 h recovery group (60±3 min) lasted approximately 4 min longer than those in the 30 min recovery group (56±2 min) on average.

To investigate the significant interactions, we assessed pairwise comparisons separately for each treatment group. Pairwise comparisons indicate that starved bees ($t=2.93$, d.f.=24.4, $P=0.01$) and heat-stressed bees ($t=2.00$, d.f.=330, $P=0.05$) only had lower thermal tolerance immediately after the stressor period, but not after 24 h of recovery (nutrition stress: $t=0.45$, d.f.=41.2, $P=0.65$; heat stress: $t=-0.83$, d.f.=330, $P=0.41$). We also found some evidence of a significant negative effect of starvation among the heat-stressed bees ($t=2.11$, d.f.=34, $P=0.04$) but not the control bees ($t=1.45$, d.f.=30, $P=0.16$). However, this interaction was not significant in the overall model ($F_{1,329.3}=0.66$, $P=0.42$).

We then stratified our data into separate models to better understand these interactions. Within the 30 min recovery data, there was a significant effect of both heat treatment ($\beta=-0.66$, $F_{1,168.40}=5.91$, $P=0.02$) and nutrition treatment ($\beta=-1.69$, $F_{1,5.58}=7.52$, $P=0.04$), whereby the effect of nutrition stress on THS was more than twice that of heat stress (Fig. 2). However, there was not an interaction between these two effects ($\beta=0.11$, $F_{1,168.33}=0.05$, $P=0.83$). After the 24 h recovery, there was no effect of the prior heat stress ($\beta=0.17$, $F_{1,155.38}=0.90$, $P=0.34$), nutrition stress ($\beta=0.10$, $F_{1,11.47}=0.00$, $P=0.96$) or the interaction term ($\beta=-0.19$, $F_{1,155.60}=1.40$, $P=0.24$) on the THS data (log-transformed for this model only) (Fig. 2).

Experiment 2: intermittent heat stress

Among bees in our intermittent heat stress trial, the mean±s.e.m. THS of all bees was 72.6±19.4 min, with a range of 30–135 min. For bees in the heat-stressed treatments, THS averaged 72.2 min, and for bees in the control treatments, THS averaged

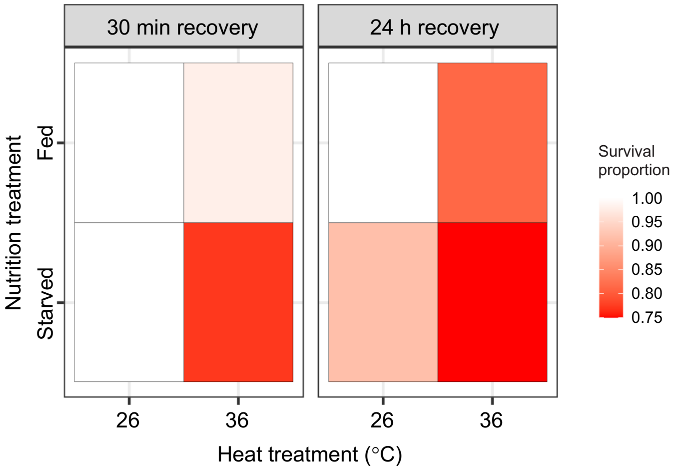

Fig. 1. Bumble bee survival in different heat, nutrition and recovery treatments. Survival immediately after each treatment was assessed across the 48 bumble bees (*Bombus impatiens*) per treatment (4 colonies×12 bees, 2 nutrition treatments×2 heat treatments×2 recovery lags), and survival proportion was calculated and plotted. Of those bees that died, 12 were from the starved/heat/24 h treatment, 11 from the starved/heat/30 min treatment, 9 from the fed/heat/24 h treatment, 4 from the starved/normal/24 h treatment and 1 from the fed/heat/30 min treatment, and no bees died in the starved/normal/30 min, fed/normal/30 min or fed/normal/24 h treatments. Binary survival (alive=1, dead=0) was assessed using a generalized linear mixed effects model (GLMM), and the results indicate that heat treatment ($\beta=-2.44$, $z=-4.46$, $P<0.01$), nutrition treatment ($\beta=-1.27$, $z=-2.83$, $P<0.01$) and recovery treatment ($\beta=-0.96$, $z=-2.35$, $P=0.02$) were each significantly associated with survival.

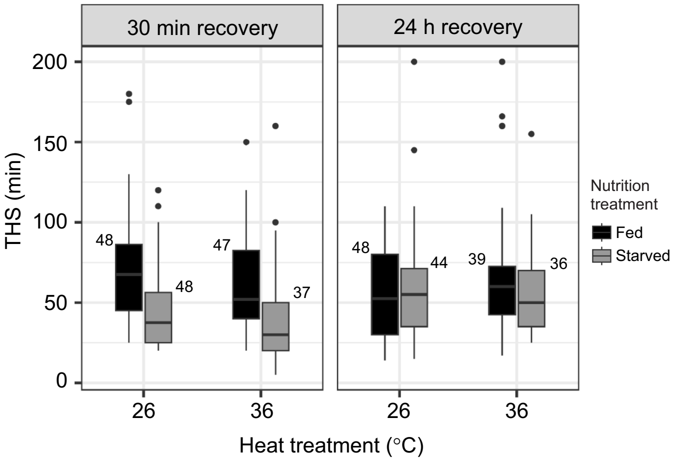

Fig. 2. Association between heat and nutrition stress and bumble bee worker time to heat stupor (THS). Using a fully crossed design, bees (*B. impatiens*) were exposed to the heat and nutrition treatment for 5 h, then their THS was assessed either 30 min or 24 h later. Data are plotted as traditional boxplots, showing median, quartiles, minimum, maximum and outliers. Significant differences were determined using a GLMM of the interactive effects of these three treatments. Overall, there was a significant effect of nutrition treatment (β=−1.40, $F_{1,16.04}$=4.82, P=0.04) and recovery treatment (β=−0.77, $F_{1,240.85}$=4.35, P=0.04), and a significant interaction between recovery treatment and nutrition treatment (β=1.64, $F_{1,230.32}$=6.66, P=0.01) and recovery treatment and heat treatment (β=1.31, $F_{1,328.75}$=3.96, P=0.05). When stratified by lag time, we observed a significant effect of heat treatment (β=−0.66, $F_{1,168.40}$=5.91, P=0.02) and nutrition treatment (β=−1.69, $F_{1,5.58}$=7.52, P=0.04) after 30 min, but no effect of either stressor after 24 h. Only bees that survived the initial 5 h stressor period were subjected to THS trials; the number of bees tested per treatment is given beside each box.

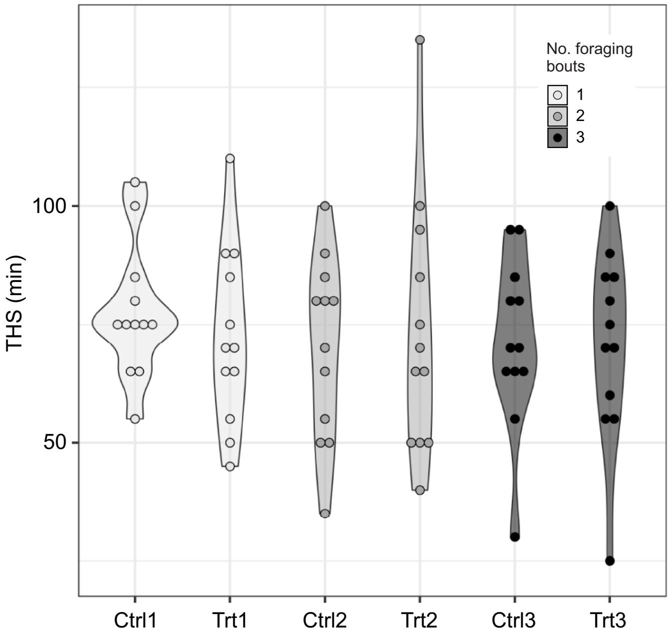

Fig. 3. Comparison of the effects of intermittent heat stress/recovery treatments and corresponding controls across differing numbers of foraging bouts for bumble bee worker THS. Bees (*B. impatiens*, n=12 bees per treatment; 3 bees per colony×4 colonies) were subject to increasing numbers of alternating 30 min periods of heat stress (36°C) and recovery (26°C), representing increasing numbers of foraging bouts (Trt1–3) – corresponding controls were held at 26°C for an equivalent duration (see Table 2 for details). After finishing the last recovery period, bees entered the THS water bath trial (43°C). There was no significant (α=0.05) difference among treatments and controls, number of foraging bouts, or an interaction between these factors based on GLMMs. Individual data points are plotted over violin plots to illustrate the distribution of the data.

72.9 min. There was no significant difference overall in THS among the different durations of the experiment ($F_{2,63}$=0.27, P=0.76), no difference between control or heat-stressed bees ($F_{1,63}$=0.02, P=0.88), and no interaction between duration and treatment/control ($F_{2,63}$=0.26, P=0.77) (Fig. 3), such that the heat treatment was not different from control at any time point. There were also no significant differences among colonies ($F_{3,63}$=0.68, P=0.57). Likewise, when only commercially reared colonies were analyzed (3 colonies), there were still no significant duration ($F_{2,46}$=0.96, P=0.39), treatment/control ($F_{1,46}$= 0.02, P=0.88), interaction ($F_{2,46}$=1.09, P=0.34), or colony-level ($F_{2,46}$=1.03, P=0.37) effects.

Experiment 3: field-collected bees

The field-collected bees sampled on more ideal versus hotter foraging days showed no significant difference in heat tolerance after a 16 h reprieve ($F_{1,22}$=1.08, P=0.31) (Fig. 4), further supporting a lack of priming and negative effects after prior heat exposure under field-realistic conditions. For further, data-driven justification of our THS methods across all three experiments, see Table S2 and Fig. S1.

DISCUSSION

Contrary to our expectations, given that bumble bees are known to mount a heat shock response, we found no evidence of heat priming – an increased ability to tolerate heat after prior heat exposure. There was no evidence of improvements in heat tolerance 24 h after prior prolonged heat stress, no improvement in heat tolerance when bees had short, intermittent exposure to heat, as they would when foraging in the field, and no difference among our field-collected treatment groups. It is possible that our chosen temperatures, recovery periods and/or assessment timing may have impeded the bees' ability to mount a response and/or our ability to detect heat priming. Future studies aimed at examining additional time points and temperatures could provide additional nuance.

Any differences we observed among bees following heat stress (immediately after prolonged heat exposure and in field-collected bees) showed lowered thermal tolerance, rather than increased tolerance, suggesting negative consequences of prior heat exposure. This lowered tolerance, however, seems highly context dependent; it depends on recovery time as well as the periodicity of heat exposure. Our data from experiment 2 suggest that 30 min was a long enough recovery period to enable normal levels of heat tolerance after a short (30 min) heat exposure, whereas the results from experiment 1 suggest a longer recovery time (24 h) was required to recover from a longer bout of heat exposure (5 h). Our field data support this recovery period as bees exposed to high heat levels showed no differing effects in their ability to tolerate heat after an overnight reprieve. A negative association between recent and intense stress and thermal tolerance without much respite is perhaps to be expected, as the THS trial essentially prolonged the period of heat exposure and starvation. Oyen and Dillon (2018) found that ramping rate was negatively correlated with CT_{max} in *B. impatiens*, which the authors attribute to a longer duration of heat stress. These results suggest that periods of respite, overnight and between short foraging bouts, enable recovery and renewed ability to tolerate future heat stress in these bees. For experiment 2, while we found no

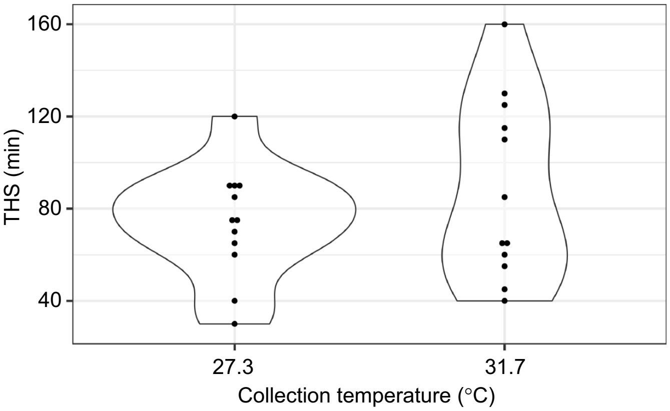

Fig. 4. Comparison of THS for wild-caught bumble bees collected at different temperatures. Bumble bees (*B. impatiens*) were collected from the Arboretum at Penn State on a cooler (27.3°C; *n*=12 bees) and warmer (31.7°C; *n*=12 bees) day in late July 2023. Bees were then transported back to the lab and subjected to THS trials. There was no significant difference between the two treatment groups at (α=0.05) based on analysis of variance. Individual data points are plotted over violin plots to illustrate the distribution of the data.

evidence for even a weak trend, limited sample size could have led to difficulties in detecting a smaller effect. Further studies across more time points could help elucidate the exact duration of recovery necessary for bees under different heat stress conditions.

Evidence for priming is theoretically supported by other insect systems, including bees (Sørensen et al., 2013; Al-Ghzawi et al., 2022), and it is still possible that there are priming effects occurring at different time scales that we did not capture with our study. Blasco-Lavilla et al. (2021) found that heat shock proteins were elevated at 2, 6 and 12 h of heat exposure (a range which our trials fell within), but that by 24 h of continuous heat exposure, these proteins no longer differed from those of bees that were not heat treated. Future research could examine a more graded response to different durations of exposure and periods of respite, to better understand both priming and needs for recovery. It is possible, if heat shock response is rapid (within a few minutes), that the bees in our study were already showing maximal heat tolerance during THS experiments. If this is the case, the heat shock response may not be able to improve future outcomes but may instead be degraded through the physiological strain of prolonged heat exposure. Given research by Al-Ghzawi et al. (2022), which found that pre-heat treatment on honey bee larvae conveys heat tolerance to adult worker honey bees, priming effects may also be better conferred from exposure to earlier life stages.

In our prolonged heat and nutrition stress experiment (experiment 1), bees that were heat stressed had the lowest survival, while bees that were starved and survived tended to have lower heat tolerance. This suggests that our heat stress conditions may have been more of an acute stressor, while access to nutrition was important for longer-term thermal stress resistance. Previous literature supports the importance of nutrition to heat resistance (Vanderplanck et al., 2019; Blasco-Lavilla et al., 2021; Maia-Silva et al., 2021). Access to food, particularly sugar water, may provide energy to fuel behavioral adaptations, such as bumble bees' ability to shunt heat from their thorax to their abdomen (Heinrich, 1976) or to achieve evaporative cooling through the action of the spiracles or perhaps orally (Heinrich and Buchmann, 1986). The bees in our experiment showed full recovery in thermal tolerance a day after nutritional stress, lending support to the importance of shorter-term access to floral resources on heat tolerance. Our results differ, however, from those of Oyen and Dillon (2018), who found that access to sugar water did not alter CT_{max}. This discordance may be due to the different metrics used between studies (THS versus CT_{max}) or the fact that bees in our study also had access to pollen. Future work on the role of sugar water and pollen could help clarify this relationship.

Our nutritional results point to the need to further consider the impact of the feedback loop of heat stress on nutrition, plant–pollinator interactions and pollination services more broadly. Hot days prevent foraging (Couvillon et al., 2010; Vanderplanck et al., 2019; Hemberger et al., 2022); Vanderplanck et al. (2019) found that small colonies collected less pollen and syrup (a proxy for pollination visits) as the duration of heat stress intensified. Fewer floral visits increases the likelihood of starvation, which our results and those of others show decreases thermal tolerance (Hemberger et al., 2022). Thermal stress may also impact the temperature of the colony itself and colonies of bumble bees maintained at higher ambient temperatures have been found to be less effective pollinators (Greenop et al., 2020). Finally, weather and climate affect flowering plant communities, plant health and floral resources (Mu et al., 2015; Ziska et al., 2016; Hemberger et al., 2022), which adds an additional dimension to this system. Field studies aimed at examining how intermittent thermal stress influences foraging and pollination effectiveness will be important to forming a holistic understanding of the risks climate change poses to plant–pollinator systems.

We observed a significant difference in thermal tolerance for one colony in our prolonged heat and nutrition stress experiment (experiment 1). The magnitude of the colony-level difference was greater than the effect of past heat stress on future heat tolerance. Martinet et al. (2021) and Pimsler et al. (2020) have each shown that there can be appreciable intraspecies variation in critical thermal limits, which may help explain this colony-level variation. Anecdotally, the colony with higher thermal tolerance did appear to be more mature (it had a larger population size and started producing gynes during the experiment); thus, the bees from this colony could be in a different age and physiological state. Perhaps in larger colonies, more workers were available to fan and feed larvae (Vanderplanck et al., 2019), thereby improving the mean health status of individual workers, or perhaps pheromone signals differ with colony age/demography and influence worker physiology (Amsalem et al., 2015). Future research should assess the role of colony characteristics and other extraneous factors on individual bee thermal tolerance in the lab and in the wild.

Our lab studies suggest that bees may recover from heat events between foraging or, if exposed to longer periods of stress, after several hours of recovery, such as would be experienced by resting in a cool nest or through a cooling period overnight. In the field, bees collected at different temperatures did not show any difference in THS after being allowed to recover for 16 h. Similarly, recent work by Sepúlveda and Goulson (2023) found that under simulated heat wave conditions, *B. terrestris* shows no acclimation. Because of the unreliable nature of extreme heat, the weather conditions we captured with our field treatments were not as extreme as our lab assays, which may have contributed to the lack of significance. Furthermore, field collections can introduce several unknown and potentially confounding factors including nutritional status, disease status, genetic variation and bee age. We attempted to minimize these factors by collecting from a single site (theoretically similar bee populations with similar access to nutrition and disease pressure) over a short period (3 days). Future studies aimed at collecting a wider range of field data could help elucidate the role of landscape factors on thermal tolerance.

We show that prolonged heat and nutrition stress are negatively associated with worker bee heat tolerance. The lack of increased heat tolerance 24 h after prolonged heat stress and 30 min after periodic heat stress suggests that prior exposure does not prime adult bees on a day-to-day scale or within the same day, and that stressors can be considered much more proximally. It should be noted that *B. impatiens* is a common species that has been relatively resilient to declines and range shifts (Hemberger et al., 2021; Jackson et al., 2022). It is unclear whether other species, or different populations that are perhaps less resilient to climate change, would be just as resilient to stress events (Oyen et al., 2016). It is therefore important to examine these effects in several other, more imperiled species. As called for in a recent review (González-Tokman et al., 2020), studies – such as ours – that provide information on organismal responses to environmental stressors are important for ecological modeling of species distributions under various habitat and climate change scenarios.

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Author contributions
Conceptualization: H.M.H., C.M.G.; Methodology: G.M.Q., C.F., H.M.H., C.M.G.; Validation: C.F., G.M.Q., H.M.H., C.M.G.; Formal analysis: G.M.Q., C.F.; Investigation: G.M.Q., C.F.; Resources: H.M.H., C.M.G.; Data curation: G.M.Q., C.F.; Writing - original draft: G.M.Q.; Writing - review & editing: G.M.Q., C.F., H.M.H., C.M.G.; Visualization: G.M.Q., C.F.; Supervision: H.M.H., C.M.G.; Project administration: H.M.H., C.M.G.; Funding acquisition: G.M.Q., H.M.H., C.M.G.

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Data availability
All relevant data can be found within the article and its supplementary information.

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