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Sperm can't take the heat: Short-term temperature exposures compromise fertility of male bumble bees (*Bombus impatiens*)

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ABSTRACT

Bumble bee (genus *Bombus*) populations are increasingly under threat from habitat fragmentation, pesticides, pathogens, and climate change. Climate change is likely a prime driver of bumble bee declines but the mechanisms by which changing climates alter local abundance, leading to shifts in geographic range are unclear. Heat tolerance is quite high in worker bumble bees (CT_{max} ~ 48–55 °C), making it unlikely for them to experience these high temperatures, even with climate warming. However, the thermal tolerance of whole organisms often exceeds that of their gametes; many insects can be sterilized by exposure to temperatures well below their upper thermal tolerance. Male bumble bees are independent from the colony and may encounter more frequent temperature extremes, but whether these exposures compromise spermatozoa is still unclear. Using commercially-reared *Bombus impatiens* colonies, males were reared in the lab and spermatozoa were exposed (*in vivo* and isolated *in vitro*) to sublethal temperatures near lower and upper thermal tolerance (CT_{min} and CT_{max}, respectively). Heat exposure (45 °C for up to 85 min) reduced spermatozoa viability both for whole males (*in vivo*; control = 79.5 %, heat exposed = 58 %, heat stupor = 57.7 %) and isolated seminal vesicles (*in vitro*; control = 85.5 %, heat exposed = 62.9 %). Whole males exposed to 4 °C for 85 min (*in vivo*; control = 79.2 %, cold = 72.4 %), isolated seminal vesicles exposed to 4 °C for 85 min (*in vitro*; control = 85.5 %, cold = 85.1 %), and whole males exposed to for 4 °C for 48 h (*in vivo*; control = 88.7 %, cold = 84.3 %) did not differ significantly in spermatozoa viability. After<85 min at 45 °C, males had significantly reduced spermatozoa viability, suggesting that short-term heat waves below CT_{max} could strongly reduce the fertility of male bumble bees with potential population-level impacts.

1. Introduction

Global climate change has played a role in the alarming declines in abundance and diversity of insects (along with other factors; Bálint et al., 2011; Wagner, 2020; Harvey et al., 2022). Increases in mean temperatures over the last century are clearly associated with shifts in distributions and timing of key life history events (Parmesan and Yohe, 2003). It has also become increasingly clear that more frequent climate extremes (Masson-Delmotte et al., 2021; Meehl and Tebaldi, 2004) may have pronounced effects on insects (Buckley and Huey, 2016; Kingsolver et al., 2013; Ma et al., 2021).

Thermal tolerance metrics have provided a compelling approach to connect direct effects of temperature on individuals to climate-associated geographic distributions of diverse organisms, including insects (Addo-Bediako et al., 2000; Sunday et al., 2011). Estimates of the minimum and maximum temperatures at which organisms can optimally function (CT_{min} and CT_{max}, respectively) have been used both to track responses to climate change (Geerts et al., 2015; Kellermann and van Heerwaarden, 2019) and to model potential long-term impacts of climate change on insect populations (Williams et al., 2015). However, insects are exposed to and are affected by temperatures well above CT_{min} and well below CT_{max} (Dillon et al., 2010; Huey et al., 2012; Kingsolver et al., 2013), suggesting that a sole focus on temperatures that lead to whole organism loss of function may be underestimating the impacts of climate change (Ma et al., 2021).

For many insects, reproductive physiology is far more sensitive to temperature than other aspects of behavior, physiology, and ecology (Walsh et al., 2019). The temperatures at which fertility, and therefore the reproductive fitness, decreases may better indicate how populations will respond to changing temperatures (Harvey et al., 2020). Several

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studies shown that declines in fertility at temperatures below an insect's CT_{max}. In *Osmia bicornis,* males exposed to long-term exposure of 22–26 °C (warm temperatures below CT_{max}) experienced a disruption of male mating signals (Conrad et al., 2017). In queen and male *Apis mellifera* and male *Tribolium castaneum,* short-term exposure to warm temperatures below their CT_{max} led to significant decreases in spermatozoa viability (Pettis et al., 2016; Sales et al., 2018a, 2018b; Stürup et al., 2013). One way to characterize these types of responses is thermal fertility limits (TFL), or the temperatures at which a proportion of an organism's gametes become sterile. This methodology has been applied to several insect species using a variety of fertility measurements (Walsh et al., 2019). For example, the TFL in male *Drosophila* is ~ 30 °C (10° below their CT_{max}) and males experience sterility at lower temperatures compared to females (van Heerwaarden and Sgrò, 2021). Much of the literature around TFL is focused on the upper thermal limits of organisms, while the impact of exposure to lower TFL are not well understood. Assessment of TFL at upper and lower limits in insect species may explain vulnerability that CTL data has been underestimating.

Bumble bees are key native pollinators in ecosystems across the world (Goulson, 2010; Woodard, 2017) and are also managed for commercial pollination. Over the past several decades, many species have experienced striking range shifts and local extirpations that are clearly associated with changing climates (Kerr et al., 2015; Soroye et al., 2020) above and beyond other factors like land-use changes, pesticides, and pathogens (Goulson et al., 2015). However, the mechanisms by which changing global temperatures are causing declines in bumble bees is not clear (Maebe et al., 2021), particularly given that, relative to other ectotherms (Sunday et al. 2011), they appear to be quite tolerant in particular to heat and, to a lesser extent, cold (Keaveny et al., 2022; Maebe et al., 2021; Martinet et al., 2015; Oyen et al., 2016; Oyen and Dillon, 2018; Pimsler et al., 2020). Most studies have focused on the temperatures that lead to loss of function (CT_{min} and CT_{max}) in female workers (Pimsler et al., 2020; Oyen and Dillon, 2018; but see Oyen et al., 2016), with comparatively little work exploring effects of less extreme temperatures on males (Martinet et al., 2021a, 2021b).

Male bumble bees have been regarded as 'simple and small mating machines' (Tsuji, 1996). Though underappreciated, they play an important role in reproduction and therefore colony growth and population persistence (Belsky et al., 2020). Males emerge in late summer to fall when the colony produces reproductives (males and gynes). Males eclose with a fixed amount of spermatozoa that they cannot replenish as their testes become inactive once mature (Baer, 2003). Once they reach sexual maturity (5–8 days post eclosion when spermatozoa migrate to seminal vesicles; Baer, 2003), males disperse in search of mates (Belsky et al., 2020). As they spend all their time outside the thermoregulated nest, males may be more vulnerable than females to gradual shifts in environmental temperatures and to thermal extremes (Pottier et al., 2021). After mating, the queen stores the spermatozoa from one or more males (mon- or polyandrous, depending on the species; Schmid-Hempel and Schmid-Hempel, 2000) throughout the overwintering period. Production of fertilized eggs throughout the following season of colony growth therefore depends on successful storage of healthy spermatozoa, so queens that mate with males with low quality spermatozoa may be burdened with reduced reproduction the following year.

Some work suggests that fertility of male bumble bees may be reduced by heat exposure. For three species of bumble bees, males held at 40 °C until reaching heat stupor (~60–700 min.) had significantly more spermatozoa mortality and degradation of spermatozoa DNA relative to controls (Martinet et al., 2021b). While this is compelling, it is unlikely for a male to mate once experiencing heat stupor, which results in severe stress and often mortality. Whether shorter-term exposure to hot temperatures similarly affects fertility is unclear. Further, little research has explored the effects of ecologically relevant cold exposure on insect fertility (Denlinger and Lee, 1998). Bumble bees are freeze avoidant, have high survival at low temperatures near their super cooling point (SCP; Keaveny et al., 2022), and can recover from chill coma quickly (Oyen et al., 2021), but it is unknown if exposure to temperatures at or below male CT_{min} reduces fertility.

Here, we present our findings on the effect of sublethal heat and cold exposure on spermatozoa viability in *Bombus impatiens,* typically a polyandrous species (Bird et al., 2022; Cnaani et al., 2002) historically found across the Eastern United States and Canada and now naturalized in many areas of the western US and Canada due to its use in commercial pollination. Determining potential effects of extreme temperatures on *B. impatiens* males is an important starting point for establishing the vulnerability of other bumble bee species and could give insight into their decline, in regard to reproduction. Hence, this study measured spermatozoa viability in males before and during the stupor, as well as short- and long-term effects of being in chill coma in the seminal vesicles of whole organisms and in seminal vesicles *in vitro* to assess the thermal limits of fertility of male *B. impatiens.*

2. Methods

2.1. Study insects and rearing conditions

Males for this study came from a total of four commercial *B. impatiens* colonies (Koppert Biological Systems, Howell, MI, USA), which were housed in an incubator (Model #I-36VL, Percival Scientific, Perry, IA, USA) in 12:12 light: dark cycle at 28 ± 1 °C, 40 ± 5 % RH. Males that emerged from the main colonies, as well as those produced from newly emerged un-mated colony queens (gynes) were used in the study. Because we did not have enough males for sufficient replication across microcolony and colony sources, males from different microcolonies and colonies were distributed equally among treatments for each experiment to minimize potential bias due to the source of the males. One week after the gynes emerged, they were stimulated to induce ovarian development via CO_2 narcosis and then placed in microcolonies with 4 workers each. Exposure to CO_2 is a well-documented method to trigger queen ovary development and egg production (Amsalem and Grozinger, 2017; Röseler, 1985; Watrous et al., 2019). Microcolonies were constructed from plastic containers (16 oz Ziplock boxes) with 12 holes punched in the sides and with clear plastic lids (modified from Klinger et al., 2019). Bees were provided *ad libitum* access to ground fresh frozen pollen (Bee Pollen, Prairie River Honey Farm, Grand Island, NE, USA) and artificial nectar composed of 1 part sucrose, 1 part invert sugar, 2 parts distilled water, 0.08 % sorbic acid, 0.05 % Honey B Healthy and 0.05 % Amino Boost (Honey B Healthy, Cumberland, MD), and 0.05 % citric acid. All colonies and microcolonies were kept in the same incubator on aluminum trays (14 ½ x 20 ½ in, Nordic Ware). Newly emerged males were removed daily and kept in microcolonies with males from the same colony and emergence date and provided with nectar and pollen until they were sexually mature (7–16 days old; Amin and Kwon, 2011).

2.2. Temperature exposures for whole males

B. impatiens females have previously been shown to lose neuromuscular function and enter chill coma at ~ 4 °C (CT_{min}), from which they recover rapidly if placed back at room temperature, and 72 h later show no apparent effects of the cold exposure (Oyen and Dillon, 2018). We therefore chose 4 °C as other studies have shown males and females have similar CT_{max} and CT_{min} (Oyen et al., 2016) and this is a physiologically and ecologically relevant, but non-damaging, temperature: male bumble bees outside the nest would regularly experience temperatures below 4 °C during the cool fall nights (Oyen et al., 2021; Pimsler et al., 2020). Based on dynamic ramping assays, bumble bees do not reach CT_{max} until ~ 45–50 °C, depending on species (Oyen et al., 2016). Female *B. impatiens* tolerance limits are at the high end of those estimates, with CT_{max} ~ 52 °C. Around this temperature, they have muscular spasms prior to entering a heat coma from which they often don't recover (Oyen and Dillon, 2018). Other work has found that holding male bumble bees at 40 °C until they reach heat stupor (a static assay as opposed to the

dynamic assays used to estimate *Bombus* CT_{max}; Oyen et al., 2016) decreases male fertility for several bumble bee species (Martinet *et al.* 2021). However, it took an average of ~ 7 h and as much as 10 h for the warm-adapted species to reach heat stupor when held at 40 °C. Hence in the present study, a static 45 °C exposure was used as the high temperature treatment; this is far enough below CT_{max} to minimize chances of whole organism heat injury, but warm enough to induce heat stupor in an ecologically relevant time frame (<1.5 h).

For both cold (4 °C) and hot (45 °C) treatments, we used a water bath (Isotemp Refrigerated Circulator Model 901, Thermo Scientific DC 10 Immersion Circulator) to maintain exposure temperatures. For each experiment, 7–16 day old males were taken from microcolonies, weighed, and placed in individual glass tubes (2 cm diameter, 5 cm long). A cotton ball covered the top of the glass tube. Males of similar mass were represented in both the control and treatment groups. Males in their individual glass vials were placed in a plastic tray to keep the vials in place and then were immersed in the water bath. Control males were kept on the bench top (~22 °C) in individual glass vials next to the water bath but otherwise handled identically. To minimize potential effects of holding times before and after dissection and of colony-level differences, experiments were done in batches, with control animals taken from the same colonies and at the same time as those assigned to treatments. For cold exposure, males were kept in the bath for 85 min (with all animals entering chill coma within 10 min of the cold exposure) or 48 h (simulating a late-season cold snap). For heat exposure, males immersed in the water bath were checked every minute for signs of heat stupor such as inability to turn upright when laying on their back, loss of reflexes, or extended proboscis. Once a male in the treatment group was noted to be in heat stupor (complete loss of physiological movement), a male of similar weight that had not yet reached heat stupor was removed from the water bath and the time since immersion was recorded. This allowed for subsequent comparison of effects on spermatozoa viability after high temperature exposure, even if males had not reached heat stupor. Seminal vesicles were dissected from males immediately after both hot and cold thermal exposures.

2.3. Spermatozoa extraction

Spermatozoa were extracted for all males using a method adapted from Campion et al. (2021), and other studies have used a similar approach to estimate sperm viability in hymenoptera (Hunter and Birkhead, 2002; Martinet et al., 2021b; McAfee et al., 2021; Rajamohan et al., 2020; Stürup et al., 2013; Zhao et al., 2021). Males were secured (ventral side up) to wax in a glass dissection dish with a pin through the ventral side of the thorax. The most distal 3–4 abdominal sternites were cut medially, followed by a lateral cut to sternite 3 or 4, making a "T" shape. This created two sternal flaps that could be pinned back to reveal the male reproductive organs. Using fine forceps, the pair of seminal vesicles were dissected from the rest of the genitalia and transferred into 30 μl of phosphate buffered saline (PBS) solution (pH = 7.4) at room temperature in a 1.5 ml Eppendorf tube. The seminal vesicles were then gently crushed with a glass rod to release the spermatozoa. Twenty-five microliters of the sample was then transferred into a new Eppendorf, leaving behind the tissue debris.

2.4. Temperature exposure for isolated seminal vesicles

To assess whether males can physiologically alter the effects of temperature exposure on spermatozoa viability, seminal vesicles were dissected out of males and exposed directly to thermal treatments. As described above, 7–16 day old males were collected and sorted by weight to ensure each treatment group had a similar mass distribution. Male seminal vesicles were dissected and placed in 30 μl of PBS medium in a 1.5 ml Eppendorf. Once all males were dissected, the Eppendorf vials containing the seminal vesicles were placed at 4 °C, 45 °C, or on the benchtop (22 °C control) for 85 min. The vials were then removed from the treatment conditions and spermatozoa were extracted as described above.

2.5. Spermatozoa viability assessment

We estimated spermatozoa viability under the conservative assumption that living sperm were not compromised in any other way (e.g. same mobility, capacity for fertilization). Viability was assessed using the LIVE/DEAD^{TM} Sperm Viability Kit (L-7011, Molecular Probes) which consists of a membrane-permeable nucleic acid stain (SYBR14; diluted 1:50; emission maxima 516 nm, green) and a dead-cell stain [propidium iodide (PI); emission maxima 617 nm, red]. For this study, 1.5 μl of 25 mM of SYBR was added to the spermatozoa suspension and gently mixed. The mixture was then incubated for 10 min in the dark. Thereafter, 1.5 μl of 240 mM propidium iodide was admixed and incubated for another 5 min in the dark. Twenty microliters of the sample was loaded onto a Cellometer SD100 slide and assessed using a cell viability counter (Cellometer K2, Nexcelom Bioscience, MA, USA). Each slide was imaged three times in different focal regions and the mean spermatozoa viability from the three images was used for subsequent analyses. This automated cell counter counts all live (green SYBR14 stained) and dead (red PI stained) nuclei in a section of the slide and takes into account the total volume of the slide to estimate the total concentration, such that the spermatozoa viability could be estimated using the total number of live spermatozoa cells divided by the total number of cells counted. Manual counts on a subset of images were consistent with automated counts.

2.6. Statistical analyses

All data were analyzed in R 4.1.2 (R Core Team, 2022), using the RStudio IDE (RStudio Team, 2020) and packages *ggplot2* (Wickham and Grolemund, 2016), and *glmmTMB* (Brooks et al., 2017). We compared male mass among treatments using ANOVA. We used generalized linear models with a beta distribution (to account for the proportional response variable) to assess the effects of treatment (heat or cold exposure) on spermatozoa viability, with mass included as a covariate. For heat exposure, we also included treatment type (heat stupor vs heat exposure) and exposure time in the models.

3. Results

Spermatozoa viability was measured in 149 males with an average mass of 109 ± 35 mg (range 41–209 mg). The spermatozoa cell count within whole animals was highly variable among males (mean: 185,000 ± 147,000), ranging from<5,000 to over 700,000 total detected spermatozoa cells (live or dead). No control or treatment bees died in any of the experiments.

3.1. Effects of cold exposure on spermatozoa viability

For males exposed to 4 °C for 85 min, spermatozoa viability tended to be reduced (72.4 % ± 13.9, N = 26) relative to controls (79.2 % ± 8.3, N = 16), but this effect was only borderline significant ($P = 0.055$; mass did not significantly affect post-treatment spermatozoa viability, $P = 0.102$) (Fig. 1**A**). This may have been driven by just four animals that had spermatozoa viability<57 % after cold exposure whereas most had viability closer to 75 %. Viability of spermatozoa from isolated seminal vesicles exposed to 4 °C for 85 min (85.1 % ± 6.2, N = 10) was not significantly different from spermatozoa viability for controls (85.5 % ± 8.82, N = 10; isolated seminal vesicles kept on the bench top at ~ 22 °C; $P = 0.632$, no significant effect of mass, $P = 0.915$) (Fig. 1**C**).

Males exposed to 4 °C for 48 h had significantly reduced spermatozoa viability (84.3 % ± 5.82, N = 13) relative to controls (88.7 % ± 6.21, N = 16; $P = 0.0375$; no significant effect of mass, $P = 0.8142$) (Fig. 1**B**).

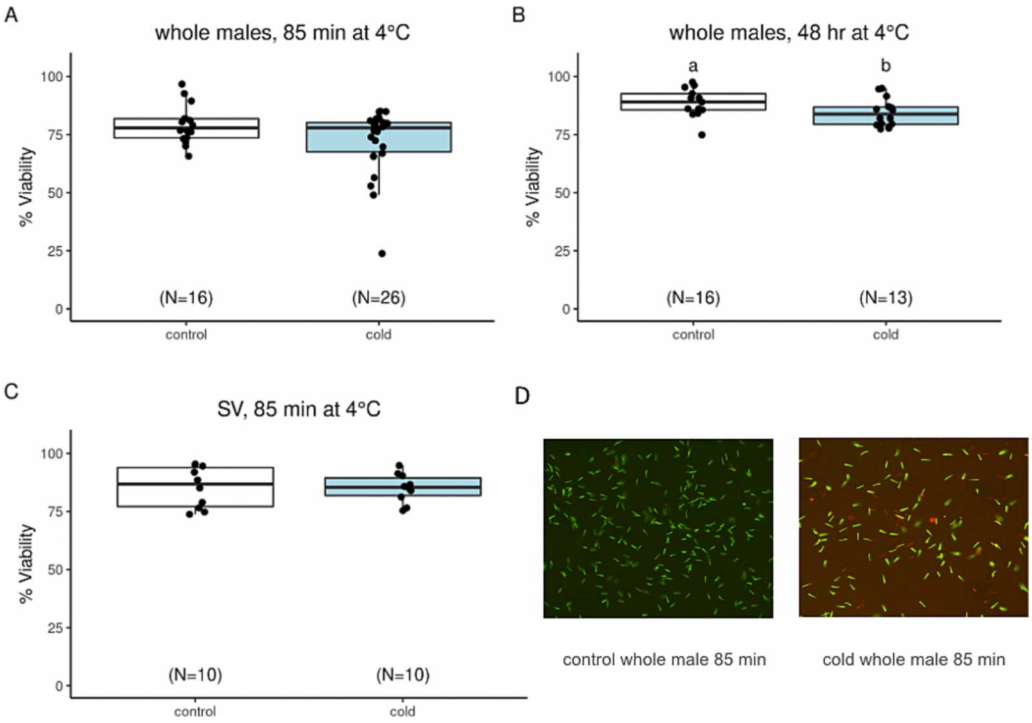

Fig. 1. Cold exposure had little impact on spermatozoa viability. A. Assessment of spermatozoa viability after short-term cold exposure in whole male *B. impatiens* to 4 °C for 85 min. B. Viability of spermatozoa cells in whole males after long-term cold exposure of 4 °C for 48 h. C. Spermatozoa viability in seminal vesicles after short-term exposure cold exposure of 4 °C for 85 min. D. Spermatozoa stained using LIVE/DEAD differential stains in control 85 min whole male (left) and whole male exposed to 4 °C for 85 min (right). Letters indicate significantly different groups (see text for details).

3.2. *Effects of heat exposure on spermatozoa viability*

Males exposed to 45 °C for up to 85 min had significantly decreased spermatozoa viability relative to controls (79.5 % ± 6.44, N = 9) whether they entered heat stupor (57.7 % ± 15.4, N = 15; $P < 0.018$) or did not ("heat exposed"; 58.4 % ± 17.3, N = 15; $P < 0.021$). Mass did not significantly affect spermatozoa viability ($P = 0.623$). Spermatozoa viability was not significantly different between heat exposed and heat stupor males ($P = 0.650$) (Fig. 2**A**). All males reached heat stupor within 28–85 min of being exposed to 45 °C, but time of exposure did not significantly affect spermatozoa viability ($P = 0.799$) (Fig. 2**B**).

Isolated seminal vesicles exposed to 45 °C for up to 85 min had significantly lower spermatozoa viability (62.9 ± 13.6 %, N = 11) than control seminal vesicle samples (85.5 ± 8.8 %, N = 10; $P < 0.001$) Mass of the male from which the seminal vesicles were extracted significantly affected spermatozoa viability ($P = 0.0103$; Fig. 2**C**), with seminal vesicles extracted from larger males having slightly lower viability after heat exposure.

4. Discussion

This study gives insight into the impacts of sublethal temperature exposure on fertility in male *Bombus impatiens*. Understanding thermal fertility limits of organisms gives further insight into an individual's ability to cope with stressful temperatures. In reference to the temperatures that cause neuromuscular failure of *B. impatiens* females (CT_{min}: ~4 °C, CT_{max}: ~52 °C; Oyen and Dillon, 2018), and to the temperatures which cause loss of righting response for both males and females of other bumble bee species (~7–10 °C and ~ 40–45 °C; Oyen et al., 2016), we assessed the spermatozoa viability of whole males (*in vivo*) and seminal vesicles (*in vitro*) before and during heat stupor, as well as short- and long-term effects of chill coma.

While cold exposure had modest effects on spermatozoa viability and only after 48 h, short-term exposure to 45 °C significantly decreased spermatozoa viability, regardless of whether males had entered heat stupor or not. Accumulation of damaged spermatozoa occurred in as little as 30 min (Fig. 2B). Similar results were shown in seminal vesicles that were exposed to 45 °C for 85 min. This supports the premise that the thermal tolerance of spermatozoa *in vitro* is similar to spermatozoa *in vivo*. While this study was focused on determining spermatozoa cell viability, some possible explanations for the declines in viability could be attributed to increased oxidative stress and/or denatured seminal fluid proteins leading to the increase in damaged cell membranes (Avila et al., 2011; Baer et al., 2009; Bisconti et al., 2021). Cell viability is just one mechanism commonly used to determine if damage to cell membranes has occurred, but additional assays like motility, DNA integrity, and mitochondrial function should be explored to assess damage beyond the cell membrane.

In *Bombus,* it has been suggested that thermal sensitivity of male bumble bee spermatozoa is species-specific, and that reaching heat stupor (which is often lethal and can take as long as 7–10 h) causes significant declines in spermatozoa viability (Martinet et al., 2021b). Our findings suggest that, at least in *B. impatiens*, the decline in spermatozoa viability can occur before a male enters heat stupor. Further assessment of the lower fertility thermal limit is needed as spermatozoa viability was only slightly reduced after 48 h at 4 °C. Male *B. impatiens* freeze (and die) at −10.0 ± 1.9, which is significantly lower than the freezing points of workers or queens (Keaveny et al., 2022). The increased cold tolerance of males could reflect their life history, as bumble bee males spend a significant portion of their life outside the colony searching for mates and would regularly be exposed to cold nighttime temperatures. Future work exploring the effect of colder temperatures, or repeated cold exposures, on male fertility will help clarify lower fertility thermal limits for male bumble bees.

In both experiments (cold and hot exposure), seminal vesicle samples overall had higher spermatozoa viability than whole male samples. A possible explanation is the general variability in an individual's spermatozoa count, viability, and response to temperature. However, variability in our estimates of spermatozoa viability were comparable to variability documented in other studies (Baer, 2003; Campion et al., 2021; Tasei et al., 1998). We also did not find any correlation between sperm viability and mass, but as this study was done with commercially reared colonies, wild caught species may show that mass plays a role in male success.

In the context of the bumble bee mating system, significant declines in spermatozoa viability in response to short-term heat exposure is

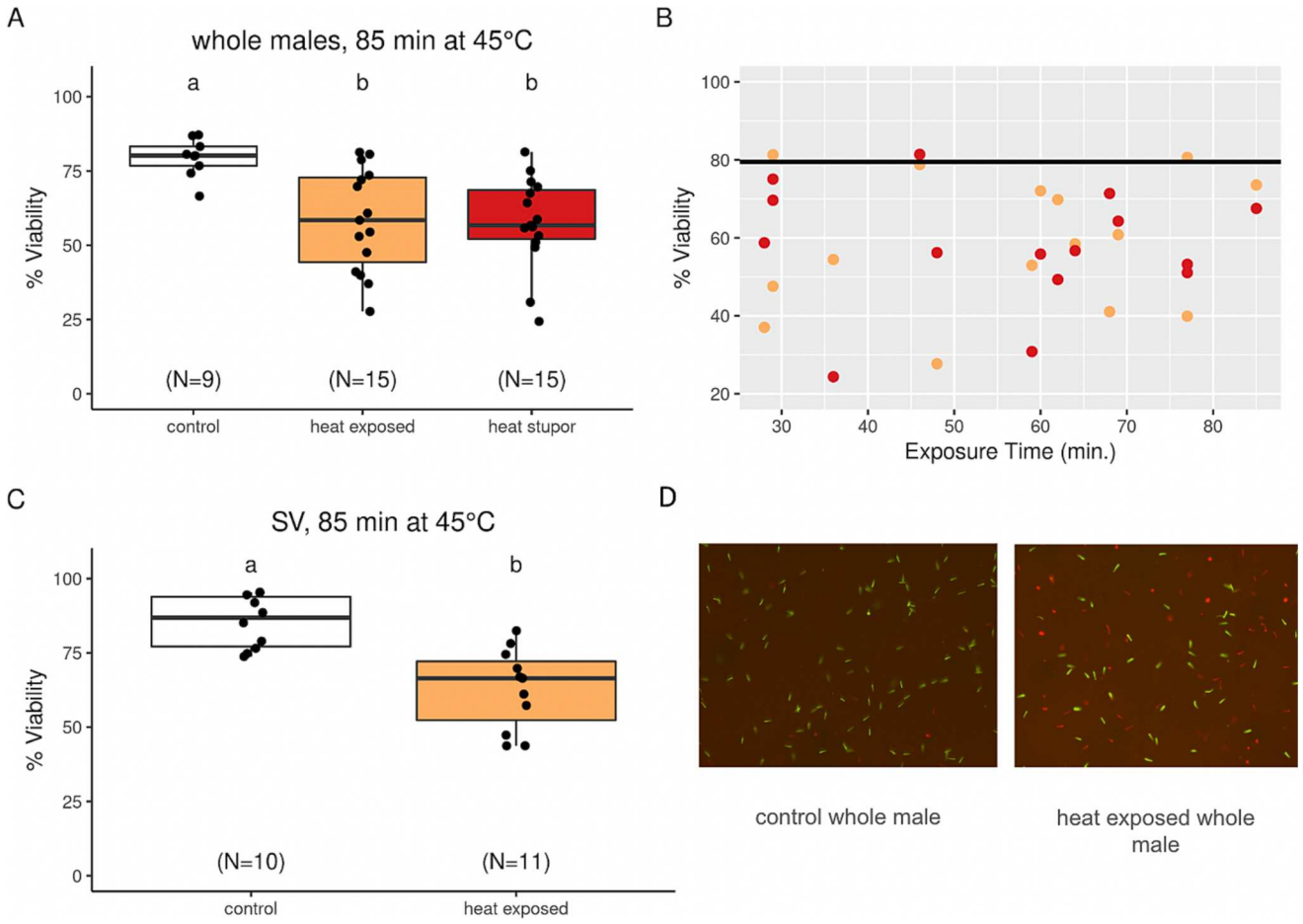

Fig. 2. Heat exposure significantly reduced sperm viability, regardless of whether males reached heat stupor. A. Spermatozoa viability estimates in whole control (white), heat exposed (yellow) and heat stupor (red) males after being held at 45 °C for up to 85 min. B. Spermatozoa viability of males exposed to 45 °C and the length (in minutes) of heat exposure (yellow) or time till heat stupor (red), with average control spermatozoa viability indicated with black line (79.5 %). C. Percentage of viable spermatozoa cells after exposure of isolated seminal vesicles to 45 °C for 85 min. D. Representative images of stained spermatozoa (green – live; red – dead) from a control whole male (left) and a heat-exposed whole male (right). Letters indicate significantly different groups (see text for details).

concerning as the queen typically mates (with one or more males depending on the species; Schmid-Hempel and Schmid-Hempel, 2000) and stores between 40,000 and 60,000 spermatozoa cells in the spermatheca (Gosterit and Gurel, 2016). Our findings indicate that a short-term heat exposure leaves males with ~ 58 % of their viable sperm. Queens mating with heat-exposed males could therefore suffer declines in reproductive output due to sperm shortage, but the degree to which *Bombus* queens are limited by quantity or quality of sperm is unclear. Future studies should assess thermal fertility limits for spermatozoa stored by bumble bee queens to better establish how winter temperature exposure might compromise reproduction during the following growing season. This is particularly relevant given increases in mean winter temperatures and increasingly more common and extreme weather events (Masson-Delmotte et al., 2021). In *Apis mellifera* queens, short-term exposure to 40 °C and 8 °C at the beginning of the growing season caused a significant decrease in spermatozoa viability (McAfee et al., 2020; Pettis et al., 2016). This could be explained by only live sperm migrating to the spermatheca during mating and a threshold number of live sperm being necessary for queens to lay fertilized eggs (Collins, 2000). Further studies should be done to determine this threshold in bumble bees to better understand the effects of decreased sperm viability on queen fecundity.

The impact of temperature on the reproductive fitness and physiology in bumble bees is an important issue to address as global temperatures increase and extreme weather events, like heat waves and cold snaps, become more frequent. Establishing the impacts of sublethal temperatures on spermatozoa viability across *Bombus* species will facilitate a better understanding of how climate change may affect male reproduction. In addition, further TFL studies need to include wild caught species (Martinet et al., 2021a) to understand the effects of changing climates on the abundance, distribution, and diversity of bumble bees.

CRediT authorship contribution statement

Claire Campion: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Project administration, Resources, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing. **Arun Rajamohan:** Conceptualization, Investigation, Methodology, Validation, Writing – review & editing. **Michael E. Dillon:** Conceptualization, Funding acquisition, Resources, Formal analysis, Supervision, Validation, Writing – review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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