

RESEARCH ARTICLE

Operative temperature analysis of the honey bee *Apis mellifera*Stanley D. Stupski^{1,*} and Rudolf J. Schilder^{2,3}

ABSTRACT

A key challenge for linking experiments of organisms performed in a laboratory environment to their performance in more complex environments is to determine thermal differences between a laboratory and the energetically complex terrestrial ecosystem. Studies performed in the laboratory do not account for many factors that contribute to the realized temperature of an organism in its natural environment. This can lead to modelling approaches that use experimentally derived data to erroneously link the air temperature in a laboratory to air temperatures in energetically heterogeneous ecosystems. Traditional solutions to this classic problem assume that animals in an isotropic, isothermal chamber behave either as pure heterothermic ectotherms (body temperature=chamber temperature) or homeothermic endotherms (body temperature is entirely independent of chamber temperature). This approach may not be appropriate for endothermic insects which exist as an intermediate between strongly thermoregulating endotherms and purely thermoconforming species. Here, we use a heat budget modelling approach for the honey bee *Apis mellifera* to demonstrate that the unique physiology of endothermic insects may challenge many assumptions of traditional biophysical modelling approaches. We then demonstrate under modelled field-realistic scenarios that an experiment performed in a laboratory has the potential to both overestimate and underestimate the temperature of foraging bees when only air temperature is considered.

KEY WORDS: Thermal ecology, Heat budget modelling, Heat transfer, Dynamic energy budget

INTRODUCTION

One of the key challenges of thermal ecology is understanding how laboratory studies of thermal physiology relate to organisms in complex microclimates. Laboratory experiments are typically performed in isothermal, isotropic enclosures, in which animals are restricted with respect to movement. These experimental limitations have the potential to erroneously link the effects of air temperature in the laboratory to air temperatures in terrestrial ecosystems. Correcting for the fundamental differences between laboratory experiments and the complex thermal environment of the outdoors has been an intense focus of biophysical ecologists for several decades. Typically, the approach used is to model ecological processes by estimating the temperature of an animal under a given microclimate and physiological state to understand ecological

processes, rather than modelling ecological processes using only air temperatures. However, most biophysical models focus on organisms that behave as either ideal endothermic homeotherms or heterothermic ectotherms (Henwood, 1975; Natori and Porter, 2007; Bakken, 1980; Kingsolver and Moffat, 1982). This may not be an appropriate approach for groups of insects that are endothermic but not fully homeothermic. Incorporating mechanistic measurements of energy budgets from physiological measurement in climatological predictions for these insect groups will require specific modelling approaches suited for the unique thermal physiology of facultatively endothermic insects.

For example, the honey bee *Apis mellifera* uses its high flight metabolic rate to generate sufficient heat to maintain its core thorax temperature, which is often 10°C higher than the air temperature (Heinrich, 1979). In the hive environment, this heat production is used to maintain brood temperatures within a narrow thermal range. While foraging, metabolic heat production is necessary to maintain force production in the flight musculature over a broad range of air conditions (Coelho, 1991b). However, laboratory studies aiming to understand the thermal limitations of bees are often measured on individuals outside the context of their hive, and in motion-restricted spaces where flight is not possible or forward flight is constrained, thereby limiting convective cooling (Hamblin et al., 2017; Harrison et al., 1996; Kovac et al., 2014; Tomlinson et al., 2015). The discrepancy between laboratory and field dynamics is a key limitation of many high-resolution predictive models, which we will need to resolve if we are to understand how honey bees will perform in future changing environments (Tomlinson et al., 2018).

There are two primary biophysical modelling approaches to provide context for the thermal differences between laboratory derived experiments and microclimates: operative temperature (T_o) and standard operative temperature (T_{es}). Operative temperature is a general term for the corresponding temperature in a laboratory that elicits the same steady state organismal temperature as a given microclimate, after accounting for all sensible routes of heat transfer (Bakken et al., 1985). This allows physiological functions that are dependent on the temperature of the organism to be related over a variety of atmospheric conditions for which air temperatures are not the only contributor to the temperature of the organism and for which it is not feasible to measure the animal temperature under the full gamut of atmospheric possibilities. Standard operative temperature is typically used in the analysis of the thermal energetics of homeothermic endotherms. Ecological applications of standard operative temperature consider the temperature of the organism to be a static value and instead calculates the net metabolic rate needed to stabilize the organism at some predetermined body temperature. Both of these modelling approaches have proven to be useful in a wide span of contexts spanning insect behavior, mammalian exercise physiology, and avian ecology (Kingsolver and Moffat, 1982; Rockweit et al., 2012; Rowe et al., 2013; Riddell et al., 2019). However, ecological applications of both operative temperature and standard operative temperature often make

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List of symbols and abbreviations

A_{inc}	surface area incident to radiation source (m^2)
A_{surface}	surface area ($8.67 \times 10^{-5} \pm 7.66 \times 10^{-6} \text{ m}^2$)
A_w	cross-sectional area of thermocouple probe ($2.6 \times 10^{-7} \text{ m}^2$)
$C_{\text{radiation}}$	correction factor for radiative heat loss during a convective cooling trial ($\text{W } \Delta\text{K}^{-1}$; see Eqn 1)
$C_{\text{thermocouple}}$	correction factor for heat transferred to the thermocouple probe ($\text{W } \Delta\text{K}^{-1}$; see Eqn 9)
H_k	corrected convective cooling coefficient ($\text{W } \Delta\text{K}^{-1}$; see Eqn 14)
H_r	raw convective cooling coefficient ($\text{W } \Delta\text{K}^{-1}$)
I_b	intensity of beam radiation (W m^{-2})
I_d	intensity of diffuse radiation (W m^{-2})
I_n	solar power at wavelength n (W m^{-2})
$I_n^{\text{reflected}}$	power at wavelength n of reflected radiation (W m^{-2})
I_n^{solar}	power at wavelength n of solar radiation (W m^{-2})
I_r	intensity of reflected radiation (W m^{-2})
I_{source}	total irradiance of a source (W m^{-2})
K_w	conductivity of thermocouple ($16.2 \text{ W m}^{-1} \text{ K}^{-1}$)
L_w	length of thermocouple probe (0.03 m)
Q_{abs}	heat gain due to solar radiation (W)
Q_{conv}	heat loss from convection (W)
Q_{met}	heat flux from metabolism (W g^{-1})
Q_{rad}	radiative heat loss (W)
Q_{tot}	total energy flux (W)
Q_{vap}	heat flux due to water evaporative cooling (W g^{-1})
T_a	air temperature
T_{body}	internal body temperature (K)
T_{cuticle}	cuticle temperature (K; taken to be equivalent to body temperature)
T_{ground}	ground temperature (K; estimated as equivalent to air temperature)
T_{specimen}	honey bee cadaver temperature, measured by thermal camera (K)
T_{tape}	thermal camera temperature of black electrical tape (K)
v	air velocity
α_n	fraction of light absorbed at wavelength n
$\alpha_n^{\text{substrate}}$	spectral absorption at wavelength n of a substrate
$\bar{\alpha}_b$	absorptivity constant for solar beam radiation (0.903 ± 0.0159)
$\bar{\alpha}_d$	absorptivity constant for diffuse atmospheric radiation (0.903 ± 0.0159)
$\bar{\alpha}_r$	absorptivity constant for reflected solar radiation (0.874 ± 0.021)
$\bar{\alpha}_{\text{source}}$	solar absorptivity constant for a given source
$\varepsilon_{\text{cuticle}}$	emissivity of cuticle (0.96 ± 0.0058)
$\varepsilon_{\text{ground}}$	emissivity of underlying substrate (0.97)
ε_{sky}	sky emissivity (see Eqn 4)
$\varepsilon_{\text{tape}}$	emissivity of electrical tape (0.97)
λ_{max}	longest wavelength (2000 nm)
λ_{min}	shortest wavelength (250 nm)
σ	Stefan–Boltzmann constant ($5.67 \times 10^{-8} \text{ W m}^{-2} \text{ K}^{-4}$)

assumptions that do not reflect the thermal physiology of honey bees, and endothermic insects generally.

Operative temperature is widely used to describe the realized temperature of ectotherms in the field and is primarily used for animals that do not have a sufficient metabolic rate to contribute to an elevated body temperature (Bakken, 1980). Operative temperature approaches focus on quantitative solutions to heat balance equations, or the use of physical replicas of animals placed in the field, as proxies of animal temperatures (Armbruster and Berg, 1994; Porter and Kearney, 2009; Bakken, 1992). Studies quantifying operative temperature via physical replicas or animal cadavers in the field may be informative for ectotherms that rely on

basking behaviors. However, physical replicas of bees as a proxy for actual organismal temperature may not be intuitively applicable. Bees are almost constantly in motion under a wide variety of air speeds when leaving the nest to forage, and a stationary cadaver would not be under the same convective cooling pressure. Additionally, bees have evolved a wide variety of mechanistically distinct strategies to physiologically mitigate overheating (Heinrich, 1976; Harrison et al., 1996). These thermoregulatory strategies add an extra layer of complexity to the interpretation of operative temperature models because heat dissipation with the environment is physiologically modulated.

Operative temperature models assume animals have negligible heat storage (Dzialowski, 2005; Kearney and Porter, 2009), resulting in the animal reaching instant equilibrium with its surrounding thermal environment. However, heat capacity the amount of energy necessary to raise the temperature of a material per unit mass, may have important ecological implications for the fitness of socially thermoregulating animals. Foraging honey bees leave their hives with a core thoracic temperature of approximately 35.9°C (Heinrich, 1979; Cooper et al., 1985) and the rate at which they change temperature is dependent on their physical dimensions and heat capacity. In this context, the ability to store heat can be an important trait for relating variable climates to pollinator thermal performance. Heat capacity will determine the time response of body temperature when animals are exposed to changing environmental conditions.

Standard operative temperature models consider the body temperature of an organism to be static (Dzialowski, 2005; Porter and Kearney, 2009; Natori and Porter, 2007). Standard operative temperature models then consider all of the routes of heat flux for an organism and predict the net metabolic heat production that would be necessary to stabilize the temperature of an animal at some predetermined body temperature. While this is appropriate for most mammalian and avian applications, it does not accurately reflect the endothermic nature of foraging honey bees. Honey bees are an intermediate between homeothermic and heterothermic organism, where body temperature is only partially dependent on air temperatures (Heinrich, 1980a; Roberts and Harrison, 1999; Harrison et al., 1996). Despite being more homeothermic than most insects, using a single static temperature to describe honey bees across a wide range of air temperatures is not representative of honey bees in a laboratory or in nature. Moreover, models built to reflect the thermal physiology of endotherms consider basal metabolic rate to predict thermal niches, rather than active metabolic rate (Porter and Kearney, 2009). This may be misleading for individual honey bees, which rarely rely on their basal metabolic rate without the social context of the hive, and are primarily dependent on flight metabolic rates to maintain high thoracic temperatures in the field (Heinrich, 1980a).

It is critical to be able to mechanistically link variation in bee body temperatures with their performance under projected climate scenarios (Kearney and Porter, 2004). This is particularly challenging given the experimental limitations of measuring flight energetics of endothermic insects which is typically performed in the atmospheric conditions of the laboratory environment. Here, we propose a total energy flux model to estimate the breadth of attainable temperatures of foraging honey bees in the field to explore the ways in which the laboratory microclimate differs from the thermally heterogeneous terrestrial ecosystem. Additionally, we use this model to explore the ways ecological adaptations of operative temperature may overlook important dynamics of endothermic insects. It is parameterized for five major routes of heat flux: convective heat transfer, heat gained from solar radiation, heat lost

to radiative heat transfer, heat flux due to metabolic processes and finally heat lost due to evaporative cooling, and includes previously unreported metric of cuticular spectral absorption and emissivity. We further develop the model to quantify metabolic rate and water loss as functions of internal bee temperature rather than air temperature so that energy and water budgets can be estimated under variable environmental conditions. We then extend this physiological model of heat transfer to demonstrate that in the thermal environment of a laboratory setting, the steady state temperature of honey bees equilibrates to a lower steady state temperature compared with that expected for foraging individuals at high air temperatures. We use this model to develop a mechanistic thermal niche space for which the steady state temperature of honey bees is expected to fall between physiologically relevant upper and lower threshold temperatures. We then qualitatively demonstrate the importance of flight velocity for mechanistic niche estimates of foraging insects. Finally, we demonstrate the importance of considering heat capacity as an important trait for understanding thermal limitations of foraging.

MATERIALS AND METHODS

Animals

All honey bees (*Apis mellifera ligustica* Spinola 1806) used were obtained from research hives in Centre County, PA, USA. For this study, we used only foraging individuals which were captured as they returned to their hives by netting at the hive entrances. Bees were used within 24 h of capture. Honey bees were kept overnight in a 37°C humidified incubator (Caron, Marietta, OH, USA) and had *ad libitum* access to a 50% (m/v) sucrose solution. Prior to experimentation, honey bees were anesthetized using CO₂ and killed by injection of 5.0 µl of 3.0 mol l⁻¹ KCl solution between abdominal tergites 3 and 4.

Model development

The total heat flux model for foraging honey bees is parameterized for the five major routes of heat flux: radiative heat transfer, heat gained from solar radiation, convective cooling, metabolic heat production and heat lost due to evaporative water loss (Eqn 1):

$$Q_{\text{total}} = Q_{\text{rad}} + Q_{\text{solar}} + Q_{\text{convection}} + Q_{\text{metabolism}} + Q_{\text{evaporation}} \quad (1)$$

A full list of symbols and their units can be found in the list of symbols and abbreviations. All equations, unless otherwise cited, are taken or adapted from either Gates (1980) or Campbell and Norman (1998).

Model parameters

Radiative heat transfer

Radiative heat transfer across a surface is calculated from the Stefan–Boltzmann law by Eqn 2, where all temperatures are given in Kelvin and it is necessary to experimentally determine the emissivity of *A. mellifera* cuticle, $\epsilon_{\text{cuticle}}$ and to determine the radiation surface area of honey bees A_s :

$$Q_{\text{rad}} = \sigma A_s (\epsilon_{\text{cuticle}} T_{\text{cuticle}}^4 - \frac{1}{2} \epsilon_{\text{sky}} T_a^4 - \frac{1}{2} \epsilon_{\text{ground}} T_{\text{ground}}^4) \quad (2)$$

Cuticle emissivity was determined by comparing honey bee cuticle temperature to black electrical tape, a common emissivity standard. Twelve freshly killed *A. mellifera* specimens were placed on a Styrofoam lid, aligned next to a strip of electrical tape and allowed to equilibrate to 23°C in a temperature-controlled chamber for 20 min. Infrared images were captured using a FLIR T650sc camera (with 25 deg lens; FLIR Systems, Inc. Wilsonville, OR) of

each bee specimen along with the electrical tape reference. The cuticle emissivity, $\epsilon_{\text{cuticle}}$ was then determined as:

$$\epsilon_{\text{cuticle}} = \frac{T_{\text{specimen}}}{T_{\text{tape}}} \epsilon_{\text{tape}} \quad (3)$$

where ϵ_{tape} is 0.97. Ground emissivity is taken as 0.97, which is standard for a grassy substrate. Sky emissivity is predictably dependent on cloud coverage, vapor pressure, and air temperature; for the purposes of this model, we consider the sky emissivity to be a general function of air temperature in Kelvin (Brutsaert, 2013):

$$\epsilon_{\text{sky}} = 9.2 T_a^2 \times 10^{-6} \quad (4)$$

Because honey bees have negligible thermal conduction between their thorax and their abdomen, the radiation surface area includes only the surface areas of the head and the thorax (Heinrich, 1980a). The surface area of an individual honey bee is modelled as the surface area of two separate cylinders, one representing the head and one representing the thorax. The length and diameter components of the head and thorax cylinder were determined by measuring individuals to the nearest 0.01 mm using digital calipers. Modelling the head and thorax as a single unit will cause the model to overestimate radiative heat loss because the head of a flying honey bee tends to be cooler than the thorax (Heinrich, 1980b). Additionally, cylinder models may not be the most accurate estimation of surface area for organisms compared with more advanced 3D tomographic methods. However, we expect that because of the relatively low contribution of radiative heat loss to total heat flux the error will minimally impact implications of the model.

Solar absorptivity constants

Thermal flux due to solar radiation considers three major sources, i.e. direct beam radiation from the sun, radiation absorbed from substrate reflection, and diffuse sky radiation:

$$Q_{\text{solar}} = \bar{\alpha}_b A_b I_b + \bar{\alpha}_r A_r I_r + \bar{\alpha}_d A_d I_d \quad (5)$$

where $\bar{\alpha}_{\text{source}}$ is the fraction of incident radiation absorbed by a surface from beam ($\bar{\alpha}_b$), reflected ($\bar{\alpha}_r$) or diffuse radiation ($\bar{\alpha}_d$). A_b , A_r and A_d are the effective surface areas exposed to beam, reflected and diffuse radiation, respectively. I_{source} is the total irradiance from a radiation source given in W m⁻². Here, we consider A_b to be $0.25 A_s$, where 0.25 is a typical view factor for a cylindrical object exposed to beam radiation from the sun (Campbell and Norman, 1998; Gates, 1980). A_r and A_d are considered $0.5 A_s$ because reflected and diffuse radiation are homogeneously scattered radiation sources.

To determine α values, we measured the mean thoracic cuticular spectral reflectance using a Perkin-Elmer Lambda 950 UV-Vis-NIR Spectrophotometer. Using an insect pin, samples mounted in the spectrophotometer within 20 min to 2 h after euthanasia. The spectrophotometer measured the wavelength-specific reflectance across whole thorax surfaces of honey bee specimens across wavelengths varying 250–2000 nm (Fig. 1A,B). The spectral distribution of solar radiation power used to calculate α_b and α_d radiation was obtained from the Reference Solar Spectrum Irradiance (Gueymard et al., 2002) dataset hosted by the National Renewable Energy Laboratory (Fig. 1A,B). The solar absorptivity constant (Fig. 1C) for a radiative source was determined as:

$$\bar{\alpha}_{\text{source}} = \frac{\sum_{n=\lambda_{\text{min}}}^{\lambda_{\text{max}}} \alpha_n I_n}{I_{\text{source}}} \quad (6)$$

where λ_{min} and λ_{max} are the shortest and longest wavelengths measured (250 and 2000 nm), α_n is the fraction of light absorbed at

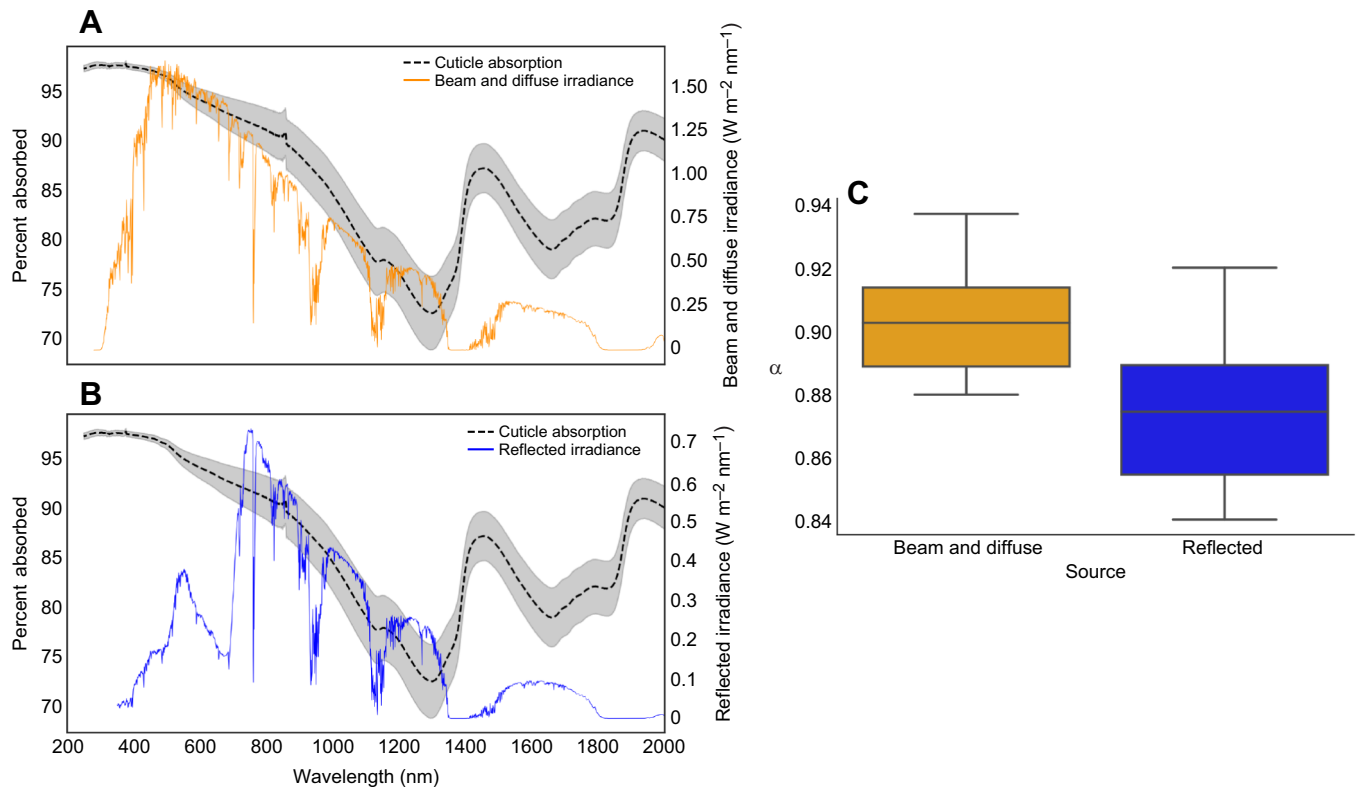


Fig. 1. Spectral properties of honey bee cuticle in relation to the irradiance spectrum of sunlight. (A,B) Left y-axis represents the fraction of absorbed light at each wavelength absorbed by the cuticle of *Apis mellifera* ($n=15$) from 250–2000 nm and corresponds to the black dashed line. Shaded regions in this curve represent 1 s.d. around the mean at each wavelength. Right y-axis in A represents the combined solar beam and diffuse irradiances based on a solar irradiance distribution standard. Right y-axis in B represents the reflected irradiance based on a solar irradiance distribution standard. (C) Box and whisker plot of absorptivity constants from beam and diffuse irradiance and reflected irradiance spectra.

the cuticle surface at wavelength n , I_n is the power of solar radiation at wavelength n , and I_{source} is the total irradiance of the source. In order to determine the spectrum of radiation reflected from the ground an absorbance spectrum for a vegetative substrate was obtained from the USGS Spectral Library sample ID: VH350 (Kokaly et al., 2017). The irradiance spectrum of reflected radiation at wavelength n (see also Fig. 1B) is here defined as:

$$I_n^{\text{reflected}} = I_n^{\text{solar}}(1 - \alpha_n^{\text{substrate}}). \quad (7)$$

Here, we consider the intensity of reflected irradiance to be $0.25I_b$, where 0.25 is a typical albedo reflection value for a vegetative substrate (Campbell and Norman, 1998). Diffuse radiation, I_d , is approximated as a constant 100 W m^{-2} (Peterson and Dirmhirn, 1981) and the absorptivity constant for diffuse radiation is assumed to be the same as for beam radiation ($\alpha_d \equiv \alpha_b$).

Convective heat transfer

To quantify the relationship between a bee's heat loss due to convection across air velocities, a wind tunnel was fabricated from laser-cut wood panels. The wind tunnel was powered by a computer fan connected to a variable power supply. We outfitted the intake of the wind tunnel with a lattice of 2 cm segments of plastic straws to promote flow laminarity. Tunnel airspeed was calibrated using a hot wire anemometer (Testo 405i; Testo, Westchester, PA, USA) placed in the wind tunnel observation zone approximately 2 cm anterior to sample placement with respect to the air intake. The anemometer was removed just prior to mounting samples on the thermocouple needle probe. A freshly killed honey bee forager was heated to

approximately 45°C with an infrared heating lamp and then mounted on a Type-T Needle Microprobe (Physitemp Inc., Clifton, NJ, USA) inserted into the wind tunnel through a small hole in the floor of the test chamber. Air temperature was recorded using a small tungsten-constantan thermocouple which descended from the chamber roof, approximately 1 cm into the test chamber, and temperatures recorded 5 cm directly above the bee sample. Changes in temperature were recorded using a MC-2000 multichannel thermocouple meter (Sable Systems, Las Vegas, NV, USA) and digitized via a NI-6052E data acquisition board (National Instruments, Austin, TX, USA) data acquisition card and Spikehound software (Lott et al., 2009). Prior to experimentation in the wind tunnel, we visually inspected airflow in the tunnel by generating fog with dry ice and water. The fog was then fed into the wind tunnel intake and flow was visualized with high-speed videography at $500 \text{ frames s}^{-1}$ to check for laminarity. We did not observe any salient features of turbulence and the standard deviation air speed measurements from wind-tunnel calibrations varied less than 3% around the desired airspeed. Relative humidity in the wind tunnel was 30%.

We determined cooling rates of forager bee specimens at air velocities of 0, 0.5, 1, 2 or 4 m s^{-1} (Fig. 2). Individual bees were tested in triplicate at a single air velocity. The fresh body mass of each bee was recorded before each trial to account for any desiccation. Over the course of three trials, desiccation was usually less than 3% of total body mass and was considered negligible.

To determine the raw cooling constant H_r , we performed a partial logistic regression of the difference between the readings of the thermocouple inserted into the sample thorax and the thermocouple

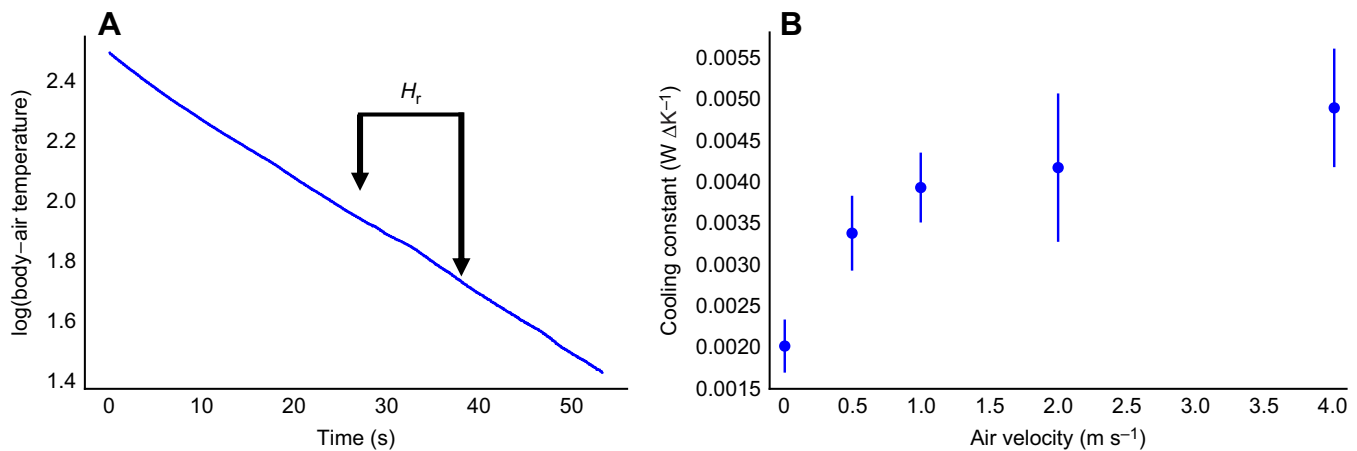


Fig. 2. Relationship between wind tunnel air velocity and average cooling constant H_k after corrections for heat conductance from the thermocouple probe and instantaneous radiative heat transfer. (A) Sample log transformed cooling curve used to calculate the raw cooling constant, H_r . (B) Corrected cooling constant H_k and its relationship to air velocity. Bars represent 1 s.d. (0 m s⁻¹, $n=14$; 0.5 m s⁻¹, $n=19$; 1 m s⁻¹, $n=20$; 2 m s⁻¹, $n=20$; 4 m s⁻¹, $n=21$).

measuring air temperature over time (Fig. 2A). H_r was reported as the average slope of the log-transformed cooling curves from each trial. We performed partial logistic regression using the SciPy Statistics library (<http://www.scipy.org>) in Python v2.3 to obtain best fit line slopes. An actualized cooling constant (H_k) was derived from the raw cooling constants by subtracting a correction factor for heat conducted through the thermocouple mount ($C_{\text{thermocouple}}$) and from radiative heat transfer ($C_{\text{radiation}}$):

$$H_k = H_r - C_{\text{thermocouple}} - C_{\text{radiation}}. \quad (8)$$

The term $C_{\text{thermocouple}}$ is estimated via the following equation (Kingsolver and Moffat, 1982):

$$C_{\text{thermocouple}} = \frac{K_w A_w}{L_w}, \quad (9)$$

where K_w , the thermal conductivity of the stainless-steel thermocouple, is taken to be 16.2 W m⁻¹ K⁻¹. A_w is the cross-sectional area of the thermocouple mount and was measured as 2.6×10^{-7} m². L_w is the length of the support structure, in this case the 3 cm stainless steel needle tip.

$C_{\text{radiation}}$ is approximated using a linearized form of the Stefan–Boltzmann law (Bakken et al., 1974):

$$C_{\text{radiation}} = 4\epsilon_{\text{cuticle}} \sigma A_s (T_a)^3, \quad (10)$$

where $\epsilon_{\text{cuticle}}$ is the sample emissivity as determined by Eqn 3, σ is the Stefan–Boltzmann constant, A_s is the radiative surface area of each specimen, and T_a is the air temperature in the wind tunnel.

On occasion, an experimental trial would display uncharacteristic spikes in heat loss, which were attributed to loss of spiracular control during experimentation. To avoid error from these anomalies, each cooling curve was visually inspected for non-linear portions and if large aberrations in linearity occurred, the trial was excluded.

$Q_{\text{metabolism}}$ and $Q_{\text{evaporation}}$ as functions of body temperature

To quantify $Q_{\text{metabolism}}$ and $Q_{\text{evaporation}}$, our biophysical model leverages previously published data for honey bees (Roberts and Harrison, 1999). This study reported three variables of interest: (1) thoracic temperature as a function of air temperature in a respirometry chamber; (2) metabolic heat production as a function of air temperature; and (3) evaporative cooling as a function of respirometry chamber temperature. We used a pseudo-temperature

approach (Bakken, 1976; Bakken et al., 1985) and transformed metabolic heat gain and evaporative cooling into functions of bee thoracic temperature rather than a function of chamber air temperature. This is necessary to create a common temperature scale for physiological performance to compare the thermal environment of a laboratory to bees which are in field-realistic conditions. The relationship between body temperature and air temperature of honey bees in a metabolic chamber (see also Fig. 3A) from Roberts and Harrison (1999) is given in linear form by:

$$T_b = 0.388T_a + 29.65. \quad (11)$$

By extension, substituting body temperature for air temperature to approximate metabolic rate in a respirometry chamber is linearly dependent on body temperature (see also Fig. 3B) in the form:

$$Q_{\text{metabolism}} = -29.84 T_b + 1763. \quad (12)$$

Evaporative water loss, however, is exponentially dependent on body temperature (see also Fig. 3B) and is estimated as:

$$Q_{\text{evaporation}} = 0.0022e^{0.244T_b}. \quad (13)$$

For energy flux based on regression estimates, the model presented here considers the regression best fit as a mean value and the standard deviation around that mean to be the standard deviation of the residuals.

Because it is not possible to differentiate between the experimental error associated with thoracic temperature as a function of air temperature and the experimental error associated with metabolic heat production and water loss as a function of air temperature, we assume that all of variation in the residual of body temperature plotted against air temperature data propagates into the metabolic rate and water loss data. Therefore, we only include standard deviations of the residuals for metabolic heat production and water loss. The alternative – treating the error in body temperature and the error associated with respirometry parameters as entirely independent – subjects the metabolic rate and water loss estimates to a regression dilution bias, which biases best fit slope values towards 0 (Halsey and Perna, 2019).

Error propagation

We employed a Monte Carlo approach for error propagation whereby each model parameter was sampled from a random normal

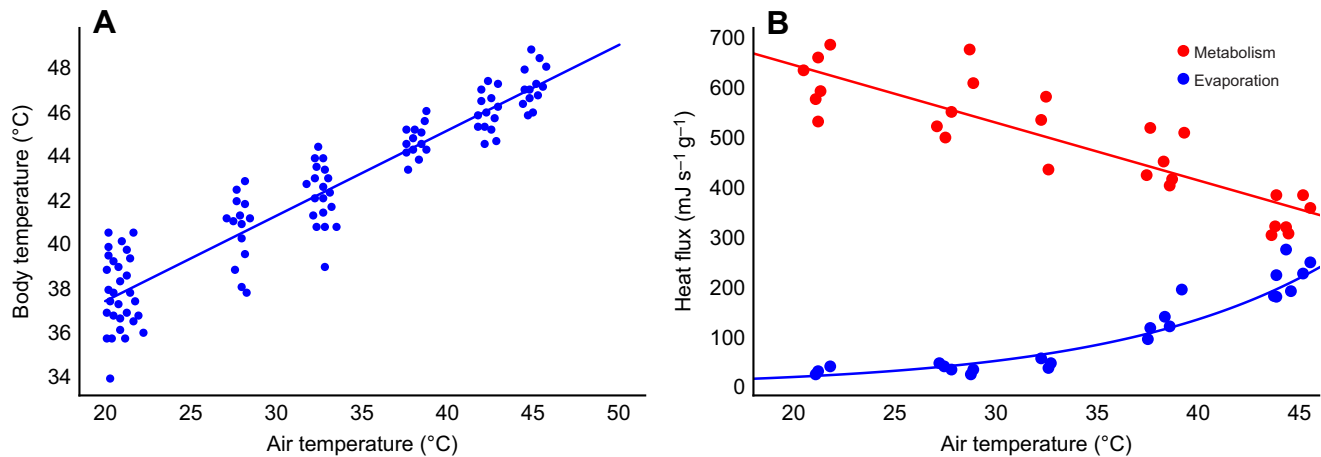


Fig. 3. Reconstructed data on body temperatures and heat flux of honey bees flying in a temperature-controlled chamber from Roberts and Harrison (1999). (A) Body temperature as a function of air temperature. (B) Heat flux due to metabolic heat gain and heat loss due to evaporative cooling of flying *A. mellifera*. Metabolic heat flux is positive, whereas evaporative heat flux indicates a negative heat flux. Data are all derived from Roberts and Harrison (1999).

distribution with mean and standard deviation either determined experimentally here or from previously published work. For each set of simulation conditions, we used 10,000 iterations to determine the mean and standard deviation of the resulting model output. All Monte Carlo analyses were performed in Python v2.3 using the SciPy OdeInt (<http://www.scipy.org>) function for integrating differential equations and random selection of parameter values under a normal distribution was generated with the Numpy random.norm function (<https://numpy.org/doc/stable/reference/random/generated/numpy.random.normal.html>) with a seed value of 1.

Model assumptions

Our model assumes that the thorax and head act as a continuous unit with identical temperatures and does not consider the abdomen to have a functional role in heat transfer dynamics. We elected to make this assumption because *A. mellifera* has a countercurrent heat exchange element at the interphase of its thorax and abdomen which makes thermal conductance between the two body parts low (Heinrich, 1980a; Coelho, 1991a). In this model, metabolic heat production and evaporative cooling act evenly throughout the mass of both the head and thorax. We additionally assumed bees have a heat capacity equivalent to water, $4.18 \text{ J g}^{-1} \text{ K}^{-1}$. Modelling the head and thorax as a single unit will cause the model to overestimate radiative heat loss, since the head of a flying honey bee tends to be cooler than the thorax (Heinrich, 1980b). However, we expected that because of the relatively low contribution of radiative heat loss to total heat flux and the small surface area of the head, the error would minimally impact the model.

For comparisons that involve dynamics of the laboratory thermal environment, laboratory conditions were identical to other model conditions with the stipulation that we excluded any thermal flux from solar, diffuse and reflected radiation. Additionally, radiative heat transfer was adjusted for the laboratory conditions by changing the sky emissivity and the ground emissivity to the emissivity of the lining of a standard temperature incubator ($\times 0.97$), as determined by the electrical tape method described above. Although it is not feasible to directly measure the exact convection contribution of induced air flow from wingbeats and motion in a small space (Sane and Jacobson, 2006) to create an exact mechanistic laboratory conditions model, the best fit regression line from Roberts and Harrison (1999), was approximately bounded by a heat budget

model using a convective cooling constant for passive convection, 0 and 0.5 m s^{-1} air flow (Fig. 4).

The initial temperature of the model for honey bees leaving the hive to forage is 35.9°C (Heinrich, 1979). For computing climate space topologies we considered the lower thermal limit for flight to be 31.4°C , the minimum temperature needed for honey bees to engage in flight behaviors in laboratory settings (Coelho, 1991b). We then used 46.1°C , the temperature that induces a regurgitation response in honey bees, as an upper thermal boundary (Coelho, 1991b).

RESULTS

Morphological characteristics

All morphometric characteristics are derived from the set of honey bee used in convective cooling experiments ($N=91$). The fresh mass of all honey bees was $0.11 \pm 0.0023 \text{ g}$ (mean \pm s.d.; $N=91$). Head mass was $0.012 \pm 0.0019 \text{ g}$ ($N=91$). Thoracic mass was $0.034 \pm 0.0031 \text{ g}$ ($N=91$) and thoracic diameter was $3.68 \pm 0.20 \text{ mm}$

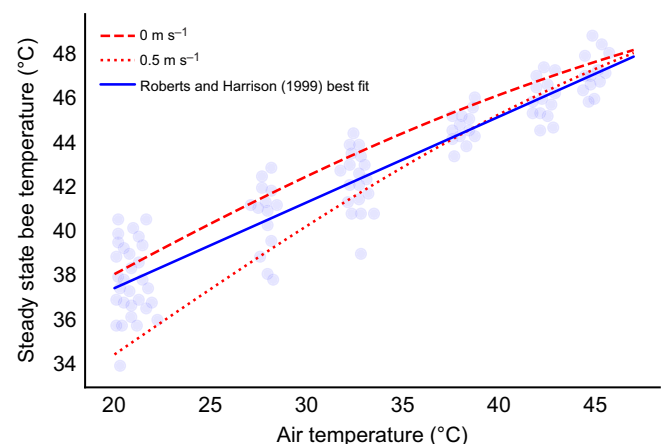


Fig. 4. Comparison of a mechanistic heat budget model for laboratory conditions with experimental data for honey bees as reported in Roberts and Harrison (1999). Model temperature indicates the steady state temperature of the head-thorax conjugate system. The dashed line represents a heat budget for which honey bees experience a convective cooling at the same rate as a cadaver in still air, the dotted line uses a convective cooling constant consistent with airflow of 0.5 m s^{-1} . The solid blue line is the least squares regression fit line for the data.

($N=91$) with cylindrical length of 3.8 ± 0.199 mm ($N=91$). Head width measured 3.68 ± 0.16 mm ($N=91$) and head cylindrical length was 1.53 ± 0.20 mm ($N=91$).

Radiative heat transfer

The cuticle emissivity mean was 0.96 ± 0.0058 ($N=12$). Total surface area for radiative heat transfer used was $8.67 \times 10^{-5} \pm 7.66 \times 10^{-6}$ m² ($N=91$).

Solar absorptivity

The beam and diffuse absorptivity constant, $\bar{\alpha}_{b+d}$, was 0.903 ± 0.0159 ($N=15$). For $\bar{\alpha}_r$, the value was 0.874 ± 0.021 ($N=15$) (Fig. 2C). The mean incident surface area for beam radiation was $2.16 \times 10^{-5} \pm 1.92 \times 10^{-6}$ m² ($N=91$) and $4.32 \times 10^{-5} \pm 3.83 \times 10^{-6}$ m² ($N=91$) for reflected and diffuse radiation, respectively. Solar absorptivity values were highly congruent with the values produced through alternative methods in Willmer and Unwin (1981) (0.90–0.92). Additionally, we did not find any systematic change in absorptivity values over the range of times used between bee euthanasia and measurement (Wald test with T -distribution, $P=0.24$).

Convective cooling

After correcting for radiative heat loss and heat flux through the thermocouple, the mean convective cooling constants for each air velocity in W ΔK^{-1} were: $2.02 \times 10^{-3} \pm 3.2 \times 10^{-4}$ ($N=14$), $3.38 \times 10^{-3} \pm 4.73 \times 10^{-4}$ ($N=19$), $3.93 \times 10^{-3} \pm 4.32 \times 10^{-4}$ ($N=20$), $4.17 \times 10^{-3} \pm 8.28 \times 10^{-4}$ ($N=20$) and $4.90 \times 10^{-3} \pm 6.80 \times 10^{-4}$ ($N=21$) for 0, 0.5, 1, 2 and 4 m s⁻¹ air velocities, respectively. The best fit relationship between air velocity (v) and convective cooling constant can be approximated as:

$$h_k = 0.0014v^{\frac{1}{2}} + 0.0023. \quad (14)$$

Metabolic rate and evaporative cooling

For metabolic heat production and evaporative water loss as functions of body temperature, the standard deviations of the residuals used were 56 and 26 mJ s⁻¹ g⁻¹, respectively.

Biophysical modeling

Our dynamic heat transfer model estimates the realized field temperatures of bees under variable atmospheric conditions. Using metabolic rate and water loss data from honey bees flown in a respirometry chamber further allowed us to estimate how changes in microclimate can affect the foraging energy and water budgets

of bees. For example, using a convective cooling constant corresponding to an air velocity of 4 m s⁻¹, the steady state temperature of the model output for a warm (30°C and 800 W m⁻² beam radiation) and a cooler (18°C and 600 W m⁻²) day were 41.2 ± 1.05 °C and 32.5 ± 1.4 °C, respectively (Fig. 5A,B).

For a comparison of the differences between the dynamic heat budget and bees flying in a laboratory, we can consider realistic conditions using a convective cooling constant corresponding to 4 m s⁻¹ air flow and 750 W m⁻² beam radiation and its relation to the linear best fit line from Roberts and Harrison (1999) (Fig. 6). Using the honey bee itself as the reference point, the field realistic heat budget reached 38°C at an air temperature of 25.0°C (1 s.d. interval 23.0–26.8°C), while bees flying in a laboratory reached the same mean steady state temperature at 21.4°C. In contrast, for the steady state temperature to reach 46.0°C, close to the upper thermal limit for flight, the dynamic energy budget corresponded to an air temperature of 38.2°C (1 s.d. interval 36.8–39.4°C) while the regression estimate was at a higher 42.1°C. This suggests that when the entire picture of heat and mass transfer are accounted for, laboratory experiments have the potential to both overestimate and underestimate the realized temperature of foraging bees.

DISCUSSION

Biophysical modeling approaches aim to link mechanistic properties of heat and mass transport of animals to the performance of organisms in their more naturalistic environments. Biophysical approaches have distinct advantages over purely correlative approaches in that they are able to give Joule-scale resolution of how climate change will affect the energetic demands of organisms (Kearney et al., 2009a,b) and can be used when the full gamut of atmospheric possibilities and physiological states of an organism cannot be directly observed. Incorporating mechanistic biophysical modelling in insect systems has spanned disease vectors (Kearney et al., 2009a,b), butterflies (Kingsolver and Moffat, 1982) and desert-dwelling beetles (Hadley, 1970). However, for insects that are facultatively endothermic, primary models make assumptions that do not reflect honey bee physiology. Here, we have presented a potential framework for incorporating biophysical heat and water budget estimation for the honey bee, *Apis mellifera*. While the study presented here is by no means the first attempt at resolving thermal energy budgets of honey bees (Cooper et al., 1985; Coelho, 1991a; Harrison et al., 1996; Roberts and Harrison, 1999), we wanted to draw specific attention to how the unique physiology of endothermic insects can impose specific challenges

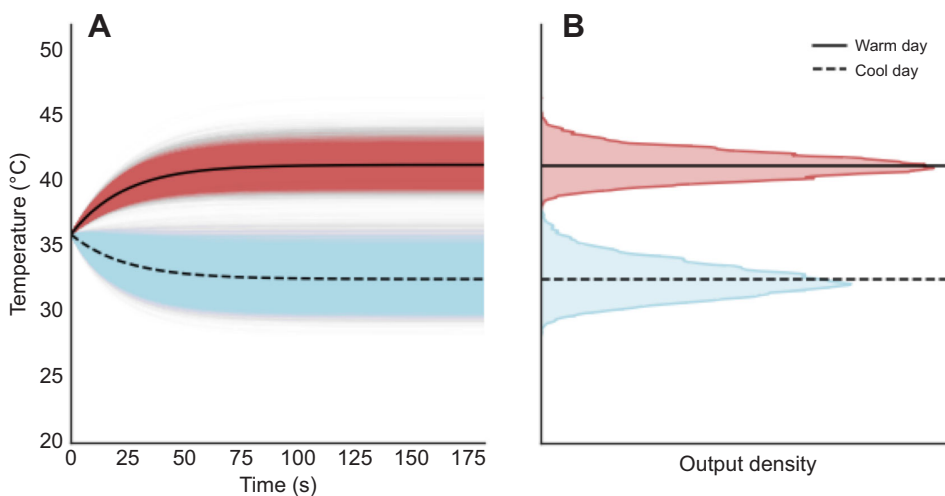


Fig. 5. A visual example of the Monte Carlo output for honey bees under two different atmospheric conditions. (A) Model considers the initial temperature of bees leaving the hive to be 35.9°C. Solid black line is Monte Carlo simulation for honey bees at 30°C with 800 W m⁻² beam radiation and uses a convective cooling constant associated with 4 m s⁻¹ air speed in a wind tunnel. The cloud surrounding the line comprises all of the 10,000 iterations. The dashed line represents a cooler less sunny day (18°C and 600 W m⁻²) but is otherwise the same. (B) Relative density of model outputs of the honey bee steady state temperatures for both Monte Carlo simulations.

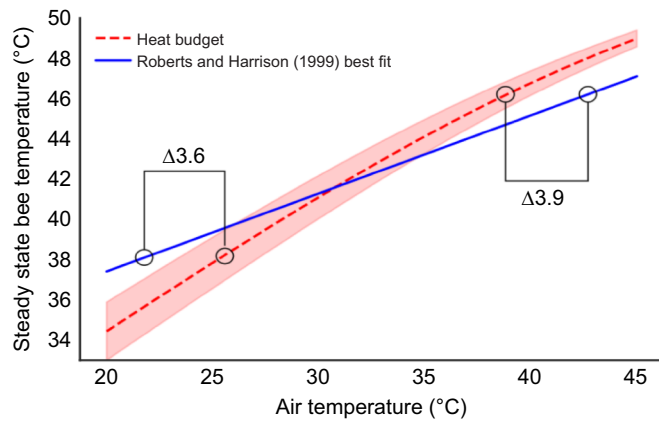


Fig. 6. Comparing a mechanistic heat budget to the least squares regression fit of bees flown in a laboratory. Blue line is from Roberts and Harrison (1999) as in Fig. 3A and Fig. 4. Red dashed line is the steady state temperature of the mechanistic heat budget presented. Shaded region around the line represents 1 s.d. of the model. A mechanistic heat budget begins to deviate from the laboratory data at both low temperatures, where convection from forward flight becomes a large source of heat loss, and at high temperatures, where convection can no longer offset the effects of incident solar radiation.

for using laboratory experiments to estimate the fitness based on projected climate scenarios. Below, we discuss the applicability of the model, the degree to which our model assumptions can influence interpretations, and potential experimental avenues to close gaps in the understanding of thermal physiology of honey bees.

Air velocity contributes to the convective cooling rates of animals and is a key driver of the realized field temperature of terrestrial organisms. For many systems assumed to be stationary, wind velocity can be interpolated from the distance from the ground (no-slip air velocity assumption; Gates, 1980). For foraging honey bees, however, flight velocity is expected to primarily drive convective cooling (Church, 1960). Since flight velocity, unlike wind, can be controlled by foraging honey bees, use of a single convective cooling coefficient may not be realistic. For cylinders and spheres, the cooling constant is proportionate to the square root of air velocity (Gates, 1980), meaning that for a flying insect, increasing air velocity comes at diminishing returns from convective cooling. Because of this, we interpret a lower flight velocity boundary of 0.5 m s^{-1} and an upper flight velocity boundary of 4 m s^{-1} in considering the likely operative temperature possibilities of foraging bees. A lower flight velocity permits the operative temperature to fall into equilibrium in favorable flight temperatures over a broader range of atmospheric conditions than a faster flight velocity (Fig. 7), but may come at a higher foraging cost by means of time spent navigating to and from floral resources.

Solar radiation can considerably contribute to the steady state temperature of animals. Although honey bees do not have pronounced features for harvesting solar radiation like butterflies or other basking insects (Tsai et al., 2020), thermal energy from the sun is still a large potential contributor to honey bee body temperature. Other thermoregulating hymenopterans may have specialized cuticle characteristics that increase their capacity to harvest sunlight as a thermal energy source (Plotkin et al., 2010). The cuticle of *Bombus impatiens* and *Dolichovespula maculata* also appears to be highly capable of harvesting solar energy (S.D.S., pers. obs.), further emphasizing the importance of solar energy to overall flight energetics of endothermic insects, even those without

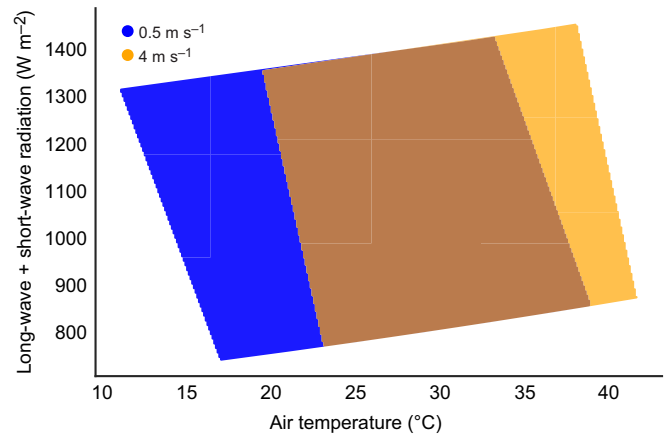


Fig. 7. Climate space models for honey bees experiencing convective cooling corresponding to 0.5 and 4 m s^{-1} . Area inside the quadrilaterals corresponds to the graphic space where the steady state temperature of a bee falls between and upper and thermal limit (31.4°C and 46.1°C). The radiation intensity includes both short-wave radiation and incident long-wave radiation.

conspicuous solar radiation harvesting structures. Solar radiation may be especially important for estimating field thermal performance and energy budgets when animals are near their upper thermal limits, given that thermal reaction norms are especially sensitive at temperatures beyond an optimum and water budgets increase exponentially with body temperature. Warming conditions often reduce vegetative shade coverage (Kearney et al., 2009a,b), and increased exposure to solar radiation may be a significant compounding factor for thermal stress of pollinator insects.

For the thermal budget model presented here, we used respirometry data previously published by Roberts and Harrison (1999). One of the challenges of relating metabolic heat production and evaporative water loss to the thermal budget of bees comes down to fundamental constraints. Because honey bees are facultative endotherms, it is necessary to relate metabolic heat production and water loss rates to the temperature of the bee itself rather than air temperatures in a laboratory setting. Experimental control over air temperature in a respirometry setting necessitates limiting the flight of honey bees to a hovering standstill. It is therefore a necessary caveat that the model presented here uses metabolic data from hovering bees. Forward flights and flights during which honey bees are carrying large loads may require a higher metabolic rate and therefore more heat production than hovering flights, which could limit the ability of bees to depress their metabolic rates under high temperatures. Metabolic rate of flying honey bees is partially dependent on flight velocity, and decreases as bees transition from hovering flight to forwards flight and begins to increase again at flight velocities over 4 m s^{-1} (Nachtigall et al., 1995).

One important caveat of this analysis is that the ability for honey bees to modulate metabolic rate in response to high air temperatures is not observed in all studies in which it has been explicitly investigated (Woods et al., 2005; Heinrich, 1980b). We opted to use data that considers metabolic rate as variable for two reasons. Firstly, the data in Roberts and Harrison (1999), report both metabolic heat production and evaporative water loss data of bees flying under a regimen of air temperatures in a single experiment. We viewed this as an ideal choice for reducing the number of separate sources of experimental data to parameterize our model.

Secondly, the experimental evidence that metabolic heat production varies with air temperature is not limited to solely honey bees. (Harrison et al., 1996; Roberts et al., 1998) but exists as a heat mitigation strategy in other bee species as well (Borrell and Medeiros, 2004). We believe that modelling the metabolic rate in this way may prove more broadly adaptable for other systems.

The use of evaporative water loss rates determined by flow-through respirometry also must be interpreted with nuance. Typically, respirometry measurements require a desiccating pre-treatment of air flowing into respirometry chambers. This allows water to evaporate from the surface of an organism at a maximized rate compared to air with higher water content. In reality, a humid environment would likely reduce the efficacy of utilizing evaporative cooling as a thermoregulatory mechanism. However, the effects of humidity on the flight energetics and evaporative cooling capacity of bees have not been investigated in detail. Additionally, because cooling calculations are based on recovered water from a respirometry chamber, the heat budget analysis here assumes that the cooling rates of this behavior are maximally efficient, whereas the efficiency of this thermoregulatory behavior has not been explicitly measured.

Traditional biophysical modelling methods neglect the potential for an organism to store heat. Thermal storage could have large implications in the interpreting biophysical models of honey bee foraging. Honey bees can engage in forward flight until they reach an internal temperature that is too low to allow powered flight, at which point they begin shivering (Heinrich, 2013). The time between shivering stops will be dependent on the heat capacity and body mass of honey bees. Although exact dynamics have not been addressed in detail, anecdotally, honey bees foraging at an air temperature of 4.5°C have been observed to stop and heat up every 10 s (Heinrich, 2013). Our model predicts 10.6 s (1 s.d. interval 8.4–13.6 s) for a bee foraging at 4 m s⁻¹ with a field-realistic beam solar irradiance value of 500 W m⁻² (Fig. 8). While this is not a rigorous validation of the model presented, it serves to demonstrate the importance of thermal capacitance in linking biophysical modeling approaches of heat transfer with in-field behavioral observations.

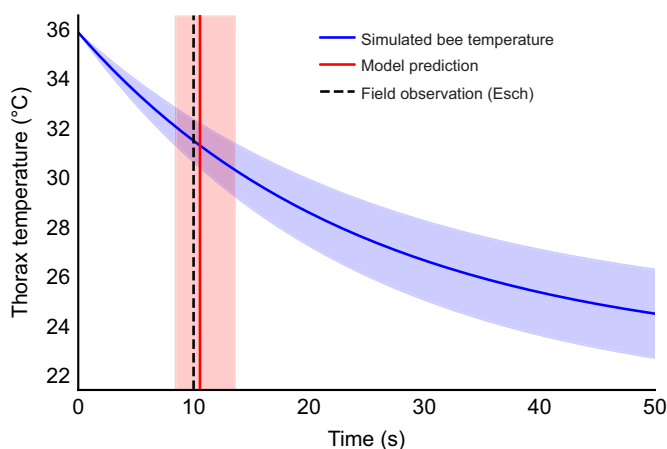


Fig. 8. Predicted shivering timing at 4.5°C and 500 W m⁻² beam radiation for a honey bee experiencing convective cooling at 4 m s⁻¹. Model considers initial conditions to be 35.9°C and minimum flight temperature to be 31.4°C (see model assumptions for rationale). Under this scenario, honey bees have 10.6 s before reaching the minimum flight temperature (shaded region is 1 s.d. around the model). Black dashed line represents the field note by Esch that bees flying at 4.5°C stop approximately every 10 s.

Hymenopterans display a wide variety of thermoregulatory strategies to mitigate overheating while either foraging or cooling their hive and nest structures. Although there is a wealth of information on these strategies (Heinrich, 1976), most studies are functionally qualitative. This means that developing similar operative temperature models for other key endothermic pollinating species will require models with their own nuance. For example, many species of *Bombus* can dynamically shunt thoracic heat into their abdomen. While this phenomenon has been described and experimentally studied in detail, little is known about precisely how much heat this behavior can dissipate under variable atmospheric conditions, and what the energetic costs are. Additionally, honey bees disperse heat in their hive environments by fanning the entrance, bringing cool outside air in one side of the hive entrance and driving the hot hive air out of the other side (Peters et al., 2019). Understanding how well this behavior can protect a hive and developing brood from thermal stress will require more in-depth experimentation, especially as it relates to the fluid dynamics of this behavior. It is critical as physiologists, to not only experimentally describe the ways in which organisms are able to control their internal thermal environment, but also quantify their biophysical efficiency in ways that can be readily incorporated into models.

Plasticity in traits associated with heat and mass transfer offer specific adaptive modalities for organisms threatened by climate change. One challenge with this is that many of the traits, although modelled independently here, are biophysically dependent on each other. Body size is a highly plastic trait in many hymenopterans and directly contributes to changes in convective cooling, thermal storage capacity, metabolic rate and flight velocity. Across a wide range of hymenopteran species, body size is positively correlated with higher thoracic temperatures (Rodríguez et al., 2018), and even for a single species, body size of foragers can be a highly plastic trait. While there tends to be little body size plasticity in honey bees, for other social, thermoregulating hymenopterans such as *Bombus* sp., body size can vary dramatically among colony individuals (Chole et al., 2019). This may represent a colony-wide thermal adaptation given the potential for body size to influence nearly every heat transfer parameter and the evidence that air temperatures can modulate the heterogeneity of worker sizes in *Bombus* (Kelemen and Dornhaus, 2018). A key future challenge will be to understand body size plasticity in the context of thermal adaptation.

The range of temperatures that maximize water and calorie yield for foraging honey bees may be entirely dependent on the landscape. The metabolic cost of foraging may be lower at higher temperatures when bees need to produce less heat for flight. However, the cost to water budgets is also high at these temperatures. Conversely, at low temperatures the cost of metabolic heat production is high but water budget costs are low. The former case may have higher foraging yields in landscapes with high water availability but reduced nutritional density whereas the latter case may have highest yields in landscapes with high floral resources with sparse water availability. Additionally, under high air temperatures, exposure to solar radiation can have large implications on the interpreted water budgets of foragers. Interpreting the thermal optimum of honey bee foraging as simply the temperature at which their basal metabolic rate is highest (Tomlinson et al., 2018) does not capture the energetic complexity of landscape interactions with the resources needed for foragers to maintain thoracic temperatures in flight under variable atmospheric conditions. A biophysical model of these processes has the potential to shed mechanistic light on studies aiming to connect landscapes to bee performance that correlative studies alone may miss.

The unique thermal physiology of many social insects complicates mechanistic linkage of microclimatic variables with thermal performance. Nonetheless, studies that aim to use high resolution microclimatic data to link landscapes to pollinator thermal performance should take into consideration the thermal caveats of their research subjects. While honey bees themselves may not be a primary conservation target, we expect that collating the known thermal physiology of a representative hymenopteran to generate operative temperature analyses will be broadly informative for a wider range of insects. At present, many of the operative temperature modelling approaches that have been used to link experimental data to field ecology have focused on sets of organisms that are relatively stationary and fit neatly into an endothermic or ectothermic category. While honey bees are arguably the most-studied hymenopteran with respect to its thermal physiology, there are still wide gaps in our understanding of fundamental aspects of their physiology that are necessary to build more informed mechanistic models linking climatic data to dynamic energy and water budgets.

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Competing interests

The authors declare no competing or financial interests.

Author contributions

Conceptualization: S.D.S.; Methodology: S.D.S., R.J.S.; Formal analysis: S.D.S.; Investigation: S.D.S.; Resources: R.J.S.; Data curation: S.D.S.; Writing - original draft: S.D.S., R.J.S.; Writing - review & editing: S.D.S., R.J.S.; Visualization: S.D.S.; Funding acquisition: R.J.S.

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Data availability

Relevant code for this model can be found at https://github.com/dstupski/operative_temp_api

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