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Body size allometry impacts flight-related morphology and metabolic rates in the solitary bee *Megachile rotundata*

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ABSTRACT

Body size is related to many aspects of life history, including foraging distance and pollination efficiency. In solitary bees, manipulating the amount of larval diet produces intraspecific differences in adult body size. The goal of this study was to determine how body size impacts metabolic rates, allometry, and flight-related morphometrics in the alfalfa leafcutting bee, *Megachile rotundata*. By restricting or providing excess food, we produced a range of body sizes, which allowed us to test the effect of body size on allometry, the power required for flight, and amount of energy produced, as measured indirectly through CO_2 emission. The power required during flight was predicted using the flight biomechanical formulas for wing loading and excess power index. We found larger bees had higher absolute metabolic rates at rest and during flight, but smaller bees had higher mass-specific metabolic rates at rest. During flight, bees did not have size-related differences in mass-specific metabolic rate. As bees increase in size, their thorax and abdomens become disproportionately larger, while their wings (area, and length) become disproportionately smaller. Smaller bees had more power available during flight as demonstrated by flight biomechanical formulas. Smaller body size was advantageous because of a reduced power requirement for flight with no metabolic cost.

1. Introduction

Body size is a critical determinate of performance and fitness in insects. Body size in insects correlates with aspects of flight ability, including increased foraging distance (Greenleaf et al., 2007), predator avoidance, mating ability (Sugiura, 1994), ability to carry provisions (Buchwald and Dudley, 2010), and provisioning rates (Kim, 1997; Sugiura and Maeta, 1989; Bosch and Vicens, 2006). Larger body size in solitary female bees is associated with higher rates of fecundity, production of larger oocytes (O'Neill et al., 2014), increased longevity (Sugiura and Maeta, 1989), greater overwintering survival (Tepedino and Torchio, 1989; Bosch and Vicens, 2006) and increased investment in each offspring (Kim, 1997). Despite these many advantages, larger body size also comes with costs such as higher energy requirements and reduced agility (Blanckenhorn, 2000). Thus, smaller body sizes may have an advantage for some performance measures by avoiding these costs.

As organisms grow and develop, all body parts may not grow proportionally. The scaling relationship between a morphological trait and the whole-body size of an organism is termed allometry (Huxley and Tessier, 1936). Adult body size in insects is a phenotypically plastic trait determined during the juvenile growth period (Koyama et al. 2013). Final adult size remains the same after the final molt occurs, because dimensions of most structures are fixed due to sclerotization of the cuticle (Sehnal, 1985; Nijhout et al., 2006). Nutritional conditions lead to differences in resource allocation, which can result in allometric trait growth. Examples of this are the exaggerated, hyperallometric growth of the fiddler crab claw (Huxley, 1924) and beetle horns (Emlen, 1997, 1994; Emlen et al., 2007). When morphological traits grow in proportion to whole-body size, it is termed isometry.

Allometric trait growth occurs when insects differentially invest in body parts associated with flight morphology or fecundity. Development and maintenance of flight mechanisms is energetically costly, and this investment is thought to come at a cost in egg production. This pattern

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Abbreviations: EPI, excess power index; M, body mass; TM, thorax mass; S, wing area; IT span, intergular span.

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occurs in wing-dimorphic insects (Guerra, 2011; Mole and Zera, 1993; Roff, 1986; Roff and Bradford, 1996; Tanaka and Suzuki, 1998). When resources are low some insects allocate growth to flight, exhibited by an increase in thorax size (Saastamoinen et al., 2010), or wing size which lead to increased flight performance (Tigreros et al., 2013). Typically, when resources are high, insects devote more resources toward increased body size and fecundity (Forrest, 1987; Zera and Harshman, 2001).

In solitary bees, adult bee body size is largely determined by larval provisions of pollen and nectar, which can vary widely based on environmental, and maternal conditions (Klostermeyer et al., 1973). Variation in adult body size can be generated experimentally (Wilkaniec et al., 2004; Helm et al., 2017; Seidelmann, 2018; Fischman et al., 2017) and has been observed in the field (Bosch, 2008). In the solitary bee *Megachile rotundata* (F.), when resources are high, females invest more to produce female offspring, which are larger than males and require more provisions (Kim, 1999). Diet manipulations during the larval stage have been shown to produce a wide range of adult body sizes in *Osmia lignaria*, a bee in the same family as *M. rotundata* (Megachilidae) (Helm et al., 2017). A wide range of body sizes within a bee species may have implications for performance, including metabolic rate.

Across animal species, body size is a strong predictor of metabolic rate, with larger individuals having higher metabolic rates. However, when corrected for body mass, known as mass-specific metabolic rate, the metabolic rate per gram of body tissue shows a negative trend, with larger organisms having a lower metabolic rate per gram (Rubner, 1908; Kleiber, 1947). The "mouse-to-elephant curve" demonstrates this phenomenon, in which the large elephant has a lower mass-specific basal metabolic rate than the small mouse (Brody, 1945). This trend also exists in invertebrates (Altman and Dittimer, 1968; Schmidt-Nielsen, 1984), including insects (Reinhold, 1999; Lehmann et al., 2000; Niven and Scharlemann, 2005; Coelho and Moore, 1989) and specifically bees (Casey et al., 1985; Darveau, 2005). This trend extends intraspecifically, as metabolic rate increases with mass (Billardon and Darveau, 2019; Darveau et al., 2014; Skandalis and Darveau, 2012; Greenlee and Harrison, 2005, 2004; Combes et al., 2020) and mass-specific metabolic rate shows a negative trend (Roberts et al. 2004).

For the solitary bee species *M. rotundata,* previous studies have not shown a correlation between body size and mass-specific metabolic rate (Bennett et al., 2013; Abdelrahman et al., 2014; Owings et al., 2014), although this lack of correlation could be explained by the narrow range of body masses used in those studies, which had approximately a two-fold change in mass.

Patterns of scaling are even more important for flight metabolic rate, since flight metabolic rate is energetically costly (Dudley 2002). The majority of an insect's energy allocation during flight is directed to flight muscles (Niven and Scharlemann, 2005). Flight metabolic rates in insects can vary dramatically within a species (Chown et al., 2007). However, intraspecific studies often use a narrow range of body sizes and do not represent body sizes at the extreme ends of the possible size range. Our study measured metabolic rates in bees that had a four-fold change in body size which is a larger range in body size than has previously been measured in the solitary bee *M. rotundata* (Bennett et al., 2013; Abdelrahman et al., 2014; Owings et al., 2014).

Flight, and thus metabolic rate, are important traits for solitary bees because offspring success depends on the resource acquisition by a single female bee (Bosch and Vicens, 2006). Solitary bee females provision their offspring sequentially, building each offspring a brood cell and providing individual food provisions for each one. Females must collect and carry this material back to their nests, taking multiple foraging bouts to build and provision one brood cell (Pitts-Singer and Cane, 2011). *M. rotundata* uses leaf pieces to line and cap her brood cells (Pitts-Singer and Cane, 2011), and each nest requires around 120 leaf pieces, an average of 15 pieces per brood cell (Klostermeyer and Gerber, 1969; Royauté et al., 2018). Bees also carry nectar and pollen to provide nutrition for their developing offspring. Thus, an investment in the thorax as body size increases could influence reproductive fitness in solitary bees.

The weight of pollen loads carried by bees is positively correlated with body mass (Giejdasz, 1998; Ramalho et al., 1998; Goulson et al., 2002; Kerr et al., 2019) with larger females carrying heavier loads than their smaller conspecifics. The maximum load that an animal can lift during take-off flights is positively correlated with body mass (Marden, 1994). However, larger bees may be spending more of their available flight power to lift their mass and maintain hovering flight (Dillon and Dudley, 2004; Buchwald and Dudley, 2010; Seidelmann, 2014), leading to a reduction in power available for flight. Wing loading, which is body mass divided by wing area, is higher in larger species, and is linked to a higher metabolic cost (Casey, 1976; Darveau et al., 2005; Skandalis and Darveau, 2012; Dillon and Dudley, 2004). Body size also affects aerodynamics during flight. Larger individuals tend to have decreased flight maneuverability (Fry et al., 2003). Body mass and the mass of the load carried determines how effectively and efficiently an individual can fly. The thorax of insects is generally used for locomotion; it contains the legs and wings, and 95% of its mass is muscle tissue (Marden, 1987).

We manipulated provision quantity in *M. rotundata* (as previously done in Helm et al., 2017) to examine the effect of body size on metabolic rates, flight-related morphology, and growth scaling in a large range of body sizes. We measured bees with a four-fold difference in body size for metabolic rate analysis, and a 12.75-fold change in body size for allometry analysis, a larger body-size range than previously measured in *M. rotundata*. During resting metabolic rate measurements, it was observed that bees were exhibiting discontinuous gas exchange. Therefore, we measured time spent in each phase, as well as CO_2 emission. We predicted that there would be a decreasing mass-specific metabolic rate with increasing body mass, matching the general pattern observed for insects and other animals (Rubner, 1908; Kleiber, 1947; Schmidt-Nielsen, 1984; Chown et al., 2007; Niven and Scharlemann, 2005). For bees to maximize their fitness, they must invest in flight, as well as fecundity, as body size increases. When resources are high, we predicted that thorax and abdomen size would increase hyperallometrically with body size. Within the parameters of our study, we define flight performance as the predicted flight ability based on wingbeat frequency, wing loading, excess power, and metabolic rate during tethered flight. We predicted large bees would have a lower wing beat frequency, decreased lift (higher wing loading), and less flight power (lower EPI) compared with smaller bees, as determined by the wing loading and excess power index.

2. Methods

2.1 Bee Body Size and Rearing. Nests containing larval bees were collected from a polystyrene nesting board (Megablock, Beaver Plastics, Canada) throughout the summer of 2017 in Fargo, ND (46°52′17′' N 96°53′54′'W; 930 ft elevation). Adult bees used for nesting were sourced from JWM Leafcutters, Inc. of Nampa, ID, US. Each brood cell was removed from its nesting straw and housed in a 96-well plate. The brood cell cap was removed to allow access to the larva and its provision. At the fifth instar larval stage, as described by Trostle and Torchio (1994), larval food quantity was manipulated to generate adults of different body sizes (Helm et al., 2017). The feeding treatments were: starved ($n = 90$), in which the entire provision was removed from the brood cell; control ($n = 83$), in which the provision was not manipulated; and fed ($n = 109$), in which bees were given approximately double the amount of their provision. Larvae in the fed treatment were provided with leftover provisions from bees placed in the starved treatment. Once assigned a treatment, bees, along with their brood cells, were placed into 3D printed plates (125 mm × 82 mm; height 20 mm) to provide sufficient room for growth. Each plate had 77 holes (well diameter = 7 mm and volume = 770 mm^3). Bees were incubated at 29 °C in an environmental chamber to complete larval development then stored as prepupae in fluctuating overwintering temperatures (6 °C for 21 h 20 °C for one hour

- with one hour ramp up and one-hour ramp down cycle) beginning in October 2017. This fluctuating thermal regime increases survival and lowers thermal stress (Rinehart et al., 2013; Colinet et al., 2018). Bees were transferred to a 29 °C environmental chamber in July 2018 to initiate development and adult emergence. Once adults emerged, a subsample was individually placed into separate deli cups with mesh lids and fed *ad libitum* on a 50% sucrose solution for 24 hours before measuring metabolic rates. Bees that were not used in metabolic rate experiments were stored at −80 °C for subsequent morphometric measurements.

2.2 Respirometry. The effect of body size on metabolic rate during rest and flight was tested using flow-through respirometry as done previously (Bennett et al., 2013; Abdelrahman et al., 2014; Owings et al., 2014). We measured CO_2 emission as an indirect measure of metabolic rate for a subsample of bees in the starved ($n = 11$), control ($n = 13$), and fed ($n = 10$) treatments. Incurrent air was dried and scrubbed of CO_2 using a purge gas generator (Balston, Haverhill, MA). Flow rate was controlled using mass flow controller (Model 840L-OV1-SV1-D-V1-S1, Sierra Instruments, Monterey, CA). Excurrent CO_2 was measured using a CO_2 analyzer (LI-7000 CO_2/H_2O Analyzer, LI-COR Biosciences, Lincoln, NE). Voltage outputs from the CO_2 analyzer and mass flow controller were digitized and recorded using Sable Systems hardware (UI2) and software (EXPEDATA version PRO, 1.9.22, Sable Systems). This setup was used for measuring both resting and flight metabolic rates, with different chambers used for each type of measurement. We calculated the rate of CO_2 emission ($\dot{M}CO_2$) which was calculated by taking the mean parts per million CO_2 emission subtracted from the baseline average parts per million CO_2. This value was then multiplied by the flow rate (microliters per minute) and converted to micromoles using the ideal gas constant (22.4 μmol/μl).

2.3 Resting Metabolic Rate. Resting metabolic rate ($\dot{M}CO_2$ ($\mu molCO_2h^{-1}$)) of each bee was measured before flight trials. Adult bees inside their brood cells were placed in complete darkness in a small airtight glass chamber (~14 ml), and an activity monitor (AD-1 Activity Detector, Sable systems) was used during resting metabolic rate measurements. Air was pushed through the chamber at a flow rate of 250 ml min^{-1}. CO_2 emission was recorded for 1 hour, and the middle 20 minutes of the recording was taken as resting metabolic rate, only complete discontinuous gas exchange cycles were measured. The bee's recorded resting absolute metabolic rate ($\dot{M}CO_2$ ($\mu molCO_2h^{-1}$)) was used for analysis. Mass-specific metabolic rate ($\dot{M}CO_2$ ($\mu molCO_2g^{-1}h^{-1}$)) was calculated by dividing the resting metabolic rate by mass (g).

During rest, it was observed that bees were exhibiting discontinuous gas exchange. We measured the three phases of discontinuous gas exchange: the closed phase, the flutter phase, and the open phase, based on descriptions by Lighton (1996). The closed phase occurs when the spiracles are closed and is indicated by a near zero CO_2 emission. The flutter phase is defined as when the spiracles begin to flutter as seen by a rapidly fluctuating CO_2 emission. The open phase occurs when the spiracles release a peak of accumulated CO_2 (Lighton, 1996). We measured time spent in each phase, as well as amount of CO_2 produced during the open phase by measuring the area under the curve of resulting CO_2 peaks.

2.4 Flight Metabolic Rate. Flight metabolic rate ($\dot{M}CO_2$ ($\mu molCO_2 h^{-1}$)) was measured using tethered flight as done previously (Bennett et al., 2013). Tethered flight was used because bees refused to fly freely in the respirometry chamber. Bees were chilled at 6 °C for approximately 5 minutes, to facilitate attachment to the tether. Bees were tethered by looping a polyester string directly posterior to the head. This positioning ensured the tether would not interfere with the wings, legs or thorax during flight. Tethered bees were placed into an airtight glass jar attached to the lid (~550 ml). Air was pushed through the chamber at a flow rate of 375 ml min^{-1}. Bees were placed on a 3D printed stage (length = 4 cm) within the chamber that allowed for the bees to become acclimated to the new environment. Bees were allowed to fly for the entire flight bout duration, with 30 second rest in between each flight bout to standardize the amount of rest bees got between bouts. Bees would only fly in the presence of a heat lamp (Hotspot, I^2R, Cheltenham, PA), and temperature inside the chamber was recorded on a thermocouple (TC-2000 Thermocouple Meter, Sable systems). The average temperature with the heat lamp was 27.83 °C ± 0.2020 (min = 25.46 °C, max = 30.35 °C). For reference, the room temperature was 25.920 °C ± 0.20 (min = 24.29 °C, max = 29.873 °C). Temperature did not significantly affect flight metabolic rate ($p = 0.959$). Flight was initiated by removal of the stage from the tarsi by rotating it away from the bee, and CO_2 emissions were recorded for 30 minutes which included active flight bouts and non-flight resting periods. Each flight bout was marked at the start and end of the flight period on EXPEDATA software. The first 10 minutes were removed from the analysis to ensure the bee was acclimated to the chamber. Absolute metabolic rate was measured as the average CO_2 emission of six flight bouts, during the beginning, middle and end of the 20-minute recording period. Bees were weighed after flight metabolic rate measurements to obtain their body mass (g).

2.5 Wing beat frequency. Wingbeat frequencies were recorded during tethered flight using a high-speed camera (Sony Cyber-shot Dsc-rx10iii digital camera). Three videos were recorded during the beginning, middle and end of the 30-minute sample period for each bee during active flight. The videos were replayed in slow motion to allow for counting individual wing beats. Three 10-second measurements from each video were analyzed to obtain a measurement of wingbeats per second (Hz). The original recording speed of the videos was 960 frames per second and were reduced to 29.97 frames per second to facilitate wing beat measurements.

2.6 Morphometrics and Allometric Scaling. To evaluate the effect of body size on flight, we measured body mass (M), thorax mass (TM), wing area (S), wing length and intertegular span (IT span) of each bee. IT span is the distance between the tegulae on the dorsal side of the thorax and a common proxy for whole-bee body size (Cane, 1987). Bees were dissected into three segments: head, thorax and abdomen. All segments were dried at room temperature to a constant weight. The dry weight of the head, thorax and abdomen, as well as whole body weight, were used in analysis morphometric analysis for EPI, while wet weight was used in morphometric analysis for wing beat frequency, wing loading, and allometric analysis. Wings were dissected from the abdomen and mounted onto slides to measure wing area and wing length. Forewings were photographed under a microscope, and wing area was calculated with Image J (version 2) software. These measurements were used to determine how each body segment and wings scaled with overall body size. The linear relationship between two traits can be found when the parameters are log transformed ($\log y = \log a + b * \log x$). When comparing two morphological traits of the same dimensions, the predicted slope if there is isometric growth is 1. Thus, for the head, thorax, and abdomen mass, the expected slope is 1. Predicted slopes change when comparing different parameters. When comparing wing area and body size the expected slope is 0.667, and for wing length and IT span, the expected slope is 0.333 (Calder, 1996). These measurements were also used to calculate the wing loading ($mg\ mm^{-2}$) and excess power index (EPI) ($mg(mg^{-1})/(mm^2)^{-1}$). Wing loading measures the loaded weight the organism can carry with respect to its wing area (Marden, 1987).

Wing loading = M/S

The excess power index measures the maximum power production relative to the power required to maintain steady flight (Hepburn et al., 1998).

$$\mathbf{EPI} = \sqrt{\frac{(TM/M)^2}{M/S}}$$

2.7 Statistical Analysis. All statistical analyses were performed using R statistical software (version 3.6.1, Base R package) and graphed using the package *ggplot2* (Wickham, 2016). To determine the effect of feeding treatments (starved, control, fed) on adult body mass analysis of variance (2-way ANOVA) which included sex, and the interaction of sex and treatment, in the model. A pairwise comparison (Tukey post-hoc test) was used to determine which treatments were different from each other. Linear models were used to determine the correlation between body mass and CO_2 emission rate (absolute and mass-specific) at rest and during flight. All data were log-transformed before analysis. Analysis of covariance (ANCOVA) was used to test if the slopes of the relationship between CO_2 emission rate and body mass differed by treatment using the *car* package (Fox and Weisberg, 2019) in R. Linear regression was also used to determine the correlation between body mass and time spent in the open, closed, and flutter phases of discontinuous gas exchange at rest, as well as absolute and mass-specific CO_2 emission during the open phase. Linear regression was also used to determine the correlation between body size and flight metrics (wing beat frequency, and wing loading). For EPI, values were normalized by using the lowest and highest values (EPI = [i-min(x)]/[max(x)-min(x)]) to obtain a range from zero to one. A generalized linear model was used to test the relationship between body size and excess power index. Sex was included as a fixed effect in all the models. To determine scaling exponents of metabolic rates, EPI and wing loading, the data were log-transformed and fit to a linear relationship. To determine allometric scaling of the head, thorax, abdomen, wing length, wing area and IT span, Analysis of covariance (ANCOVA) was used to test if the slopes of the treatments were different using the *car* package (Fox and Weisberg, 2019) in R. We used the *stmatr* package (Warton et al., 2012) to test if the relationship was isometric, testing for a predicted slope of 1 (head, thorax, abdomen), 0.667 (wing area), or 0.333 (wing length, IT span).

3. Results

3.1 Bee Body Size: Body masses ranged from 4 to 51 mg. Females were typically larger, and weighed an average of 24.88 ± 1.06 mg. Males were typically smaller, and weighed an average of 21.85 ± 0.74 mg. Bees (both male and female) in the starved treatment had a combined average weight of 11.64 ± 0.57 mg, bees in the control treatment (both male and female) had a combined average weight of 24.30 ± 0.64 mg, and bees in the fed treatment (both male and female) had a combined average weight of 32.23 ± 0.69 mg. Feeding treatment, sex, and the interaction between feeding treatment and sex had significant effects on body size (ANOVA, Treatment $F_{2,276} = 310.09$, $p < 0.001$; Sex $F_{1,276} = 30.51$, $p < 0.001$; Interaction, $F_{2,276} = 8.95$, $p < 0.001$). Bees in the fed treatment were significantly larger than the control bees (Tukey HSD, fed & control, $p < 0.001$), which were larger than bees in the starved treatment (Tukey HSD, control & starved, $p < 0.001$; fed & starved $p < 0.001$). Overall, females were larger than males (Tukey HSD, $p < 0.001$). Although, the effect of the interaction of treatment and sex revealed control males were larger than starved females ($p < 0.001$), fed males were larger than starved females ($p < 0.001$), and there was no significant difference in size between fed males and

control females ($p < 0.001$).

3.2 Metabolic Rates: For the subsample of bees used in respirometry experiments, the starved treatment had an average weight of 16.84 ± 1.55 mg, the control treatment had an average weight of 24.93 ± 1.947 mg, and the fed treatment had an average of weight of 28.38 ± 1.479 mg. For the subsample of bees, body size varied among the feeding treatments and sex (ANOVA, Treatment $F_{2,19} = 6.666$; $p < 0.001$, Sex $F_{1,19} = 10.408$; $p = 0.005$, Interaction = F_2,19 = 1.962; $p = 0.168$). Starved bees were smaller (Tukey HSD: starved vs control, $p = 0.027$; starved vs fed; $p = 0.005$), but the control and fed treatments did not differ (Tukey HSD: $p = 0.398$). Males were smaller than females. Mass had an effect on absolute metabolic rate during flight (Table 1, Fig. 1A; Linear Model, $T_{2,25} = 5.613$; $p < 0.001$) and at rest (Table 1, Fig. 1A; Linear Model, $T_{2,26} = 3.249$; $p = 0.003$). Metabolic rates increased with larger body size. There was no significant difference in slope by treatment (starved, fed, and control) during flight (ANCOVA, $F_{3,22} = 1.170$, $p = 0.344$) and at rest (ANCOVA, $F_{2,24} = 2.551p = 0.099$). Total resting metabolic rate (slope = 0.603, all treatments) and total flight metabolic rate (slope = 1.080, all treatments) scaled differently (ANCOVA, Mass, $F_{2,56} = -24.65$; $p < 0.0001$, Sex, $F_{2,56} = -1.04$; $p = 0.303$). Mass did not affect mass-specific metabolic rate during flight (Table 1, Fig. 1B; Linear Model, $T_{2,25} = 0.419$; $p = 0.679$) but did affect metabolic rate at rest (Table 1, Fig. 1B; Linear Model, $T_{2,26} = -2.141$; $p = 0.042$), although it only explained 16.4% of the variation. This indicates that bees emitted more CO_2 as size increased, but once corrected for mass, larger bees did not have a higher flight metabolic rate. There was no significant difference in slope by treatment (starved, fed, and control) during flight (ANCOVA, $F_{3,22} = 1.170$, $p = 0.344$) and at rest (ANCOVA, $F_{2,24} = 2.551p = 0.099$).

Table 1
Results from the linear regression of metabolic rate (MR) and body size.

	Model Term	*Estimate*	*Std error*	*T value*	*Pvalue*
Absolute Flight MR	Intercept	2.443	0.314	7.788	<0.001
	Mass	1.081	0.193	5.613	<0.001
	Sex	−0.024	0.066	−0.354	0.726
Absolute Resting MR	Intercept	1.005	0.312	3.221	0.003
	Mass	0.603	0.186	3.249	0.003
	Sex	−0.013	0.060	−0.215	0.832
Mass-Specific Flight MR	Intercept	2.443	0.314	7.788	<0.001
	Mass	0.081	0.193	0.419	0.679
	Sex	0.024	0.066	0.354	0.726
Mass-Specific Resting MR	Intercept	1.005	0.312	3.221	0.003
	Mass	−0.397	0.186	− 2.141	0.042
	Sex	−0.013	0.059	−0.215	0.831

3.3 Discontinuous Gas Exchange: Bees at rest exhibited discontinuous gas exchange (Fig. 2A). There was no difference in amount of time spent in the open phase (Fig. 2B; Linear Model, Mass, $T_{2,26} = -0.275$; $p = 0.786$; Sex, $T_{2,26} = -1.349$; $p = 0.189$), closed phase (Fig. 2C; Linear Model, Mass, $T_{2,22} = 0.351$; $p = 0.729$; Sex, $T_{2,22} = -0.477$; $p = 0.639$) or the flutter phase (Fig. 2D; Linear Model, Mass, $T_{1,16} = 1.368$; $p = 0.190$; Sex, $T_{1,16} = 0.176$; $p = 0.869$) based on mass (g) or sex. As mass increased, the amount of CO_2 produced during the open phase increased (Fig. 2E; Linear Model, Mass, $T_{2,26} = 4.161$; $p < 0.001$; Sex, $T_{2,26} = -0.855$; $p = 0.400$). Once corrected for mass, there was no correlation of CO_2 emission with mass or sex (Fig. 2F; Linear Model, $T_{2,26} = -0.630$; $p = 0.534$; Sex, $T_{2,26} = -0.855$, $p = 0.400$). The slope for absolute resting metabolic rate when we averaged it across the entire sample period (Fig. 1A, Table 1, slope = 0.603) was significantly different than when we calculated the absolute resting metabolic rate only during the open phase of discontinuous gas exchange (Fig. 2E, slope = 0.869) (ANCOVA, $F_{1,61} = 6.617$; $p = 0.013$).

3.4 Allometric Scaling: As body mass increased, head mass (Table 2, Fig. 3A; $p < 0.001$), thorax mass (Table 2, Fig. 3B; $p < 0.001$) and abdomen mass (Table 2, Fig. 3C; $p < 0.001$) increased. There was no difference between the slopes of the treatments (Table 2, head: $p = 0.0680$; thorax: $p = 0.492$; abdomen: $p = 0.740$), which indicates that all body parts increased with size in the same ratio across treatments. As body size increased, wing area (Table 2; Fig. 3D; $p < 0.001$;), wing length (Table 2, Fig. 3E; $p < 0.001$), and IT span increased (Table 2, Fig. 3F; $p < 0.001$). The slopes of wing traits and body mass differed by treatment (Table 2, Fig. 3D and E, wing area: $p = 0.003$; wing length: $p = 0.022$). There was no relationship between IT span and mass (Table 2, Fig. 3F; $p = 0.090$). Sex was a significant factor for head mass (Table 2, ANCOVA $p < 0.001$), thorax mass (Table 2, ANCOVA $p < 0.001$), abdomen mass (Table 2, ANCOVA, $p < 0.001$), and wing area (Table 2, ANCOVA, $p < 0.003$).

We also determined if each body segment and wings scaled isometrically to overall body size by comparing the slopes of each body segment to the expected slope. First, we measured overall scaling

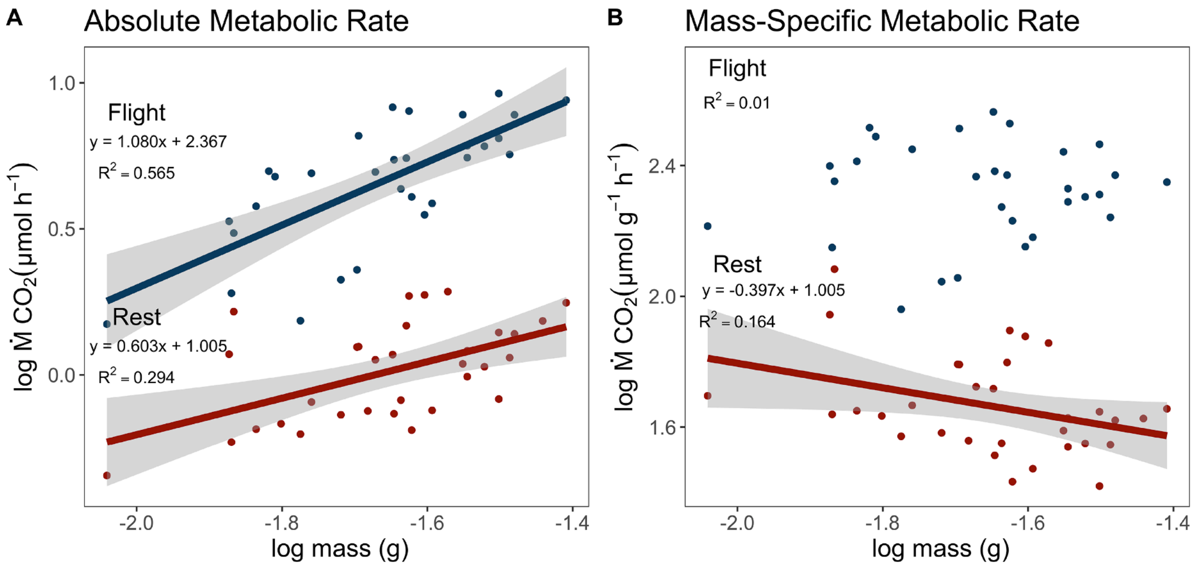

Fig 1. Metabolic rates of *M. rotundata* during rest and flight. Absolute CO_2 emission (A) during flight (blue line), and at rest (red line). Mass-specific metabolic rate (B) during flight (blue) and at rest (red line). Absolute CO_2 emission (A) of bees during flight ($p < 0.001$) and at rest ($p < 0.003$) increased with body size. Mass-specific CO_2 emission (B) did not differ with body size during flight ($p = 0.679$), but at rest CO_2 emission decreased with body size ($p = 0.042$). Grey shading in all panels indicates the 95% confidence intervals. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

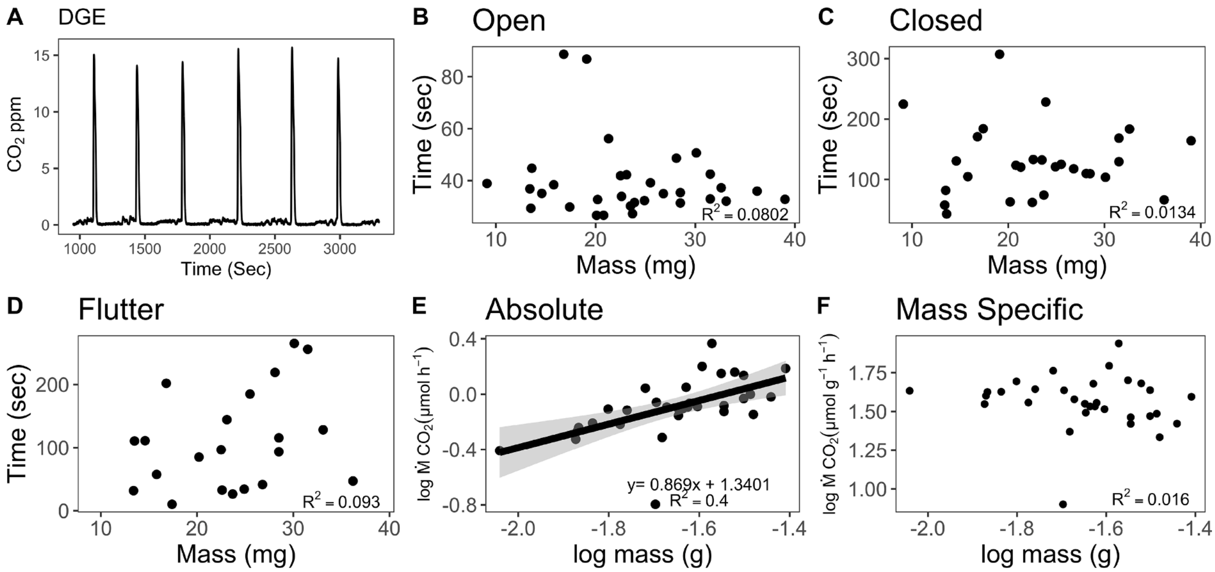

Fig. 2. The relationship between discontinuous gas exchange and body size. An example of a respirometry trace showing discontinuous gas exchange (DGE) including the closed, flutter and open phases (A). Body size does not correlate with the duration of time spent in the (B) open ($p = 0.786$), (C) closed ($p = 0.729$), or (D) flutter ($p = 0.190$) phases of discontinuous gas exchange. Larger bees had a significantly higher CO_2 emission rate (E) ($p < 0.001$) but once corrected for mass (F) there was no difference in amount of CO_2 produced ($p = 0.534$). Grey shading indicates the 95% confidence intervals.

patterns of all body segments (Fig. 3, Table 3). The head, thorax, and abdomen were predicted to scale with a reference slope of 1 in relationship to body mass. The head scaled hypometrically with body mass (Fig. 3A). The thorax and abdomen scaled hyperallometrically with body mass (Fig. 3 B and C). Wing area was predicted to scale with a 0.667 relationship to body mass, and wing length was predicted to scale with a 0.333 relationship to mass. Wing area and wing length scaled hypoallometrically with body mass (Fig. 3D and E). IT span was predicted to scale with a 0.333 relationship to mass. IT span scaled hyperallometrically with body mass (Fig. 3F). Next, we measured scaling patterns with respect to treatment (Table 3). The head scaled isometrically with body size, with the exception of bees in the control treatment which scaled hypoallometrically with body size (Table 3). Across all treatments, the thorax and abdomen both scaled hyperallometrically with body size (Table 3). Thorax width, which was measured as IT span scaled isometrically in the fed treatment. However, the control and starved treatments had relationships that were hyperallometric (Table 3). Wing length had a significantly more hypoallometric relationship with body size than expected in the fed and starved treatment but scaled hyperallometrically in the control treatment. Wing area was predicted to scale isometrically in the control and starved treatments, but the fed treatment was significantly more hypoallometric than predicted (Table 3). This suggests that as bees become larger their thoraces and abdomens get disproportionately larger, while wings become disproportionately smaller.

Wing Beat Frequency and Flight Morphometrics: Mass and sex did

Table 2
Relationship of body segments to total body mass. Results from ANCOVA.

	Model Term	Slope	Intercept	SS	DF	F value	P value
Head	Log body mass	0.907	−0.419	4.396	1	1019.948	< 0.001
	Treatment			0.023	2	2.715	0.068
	Sex			0.104	1	24.170	< 0.001
Thorax	Log body mass	1.089	−0.515	6.335	1	1865.341	< 0.001
	Treatment			0.005	2	0.712	0.492
	Sex			0.095	1	27.858	< 0.001
Abdomen	Log body mass	1.202	−0.782	7.578	1	1182.857	< 0.001
	Treatment			0.004	2	0.301	0.740
	Sex			0.162	1	25.230	< 0.001
Wing Area	Log body mass	0.408	0.168	0.853	1	306.967	< 0.001
	Treatment			0.034	2	6.069	0.003
	Sex			0.025	1	9.120	0.003
Wing Length	Log body mass	0.205	0.417	0.205	1	243.440	< 0.001
	Treatment			0.007	2	3.8850	0.022
	Sex			0.003	1	2.9996	0.085
IT Span	Log body mass	0.309	−0.055	0.398	1	290.319	< 0.001
	Treatment			0.007	2	2.432	0.090
	Sex			0.002	1	1.644	0.201

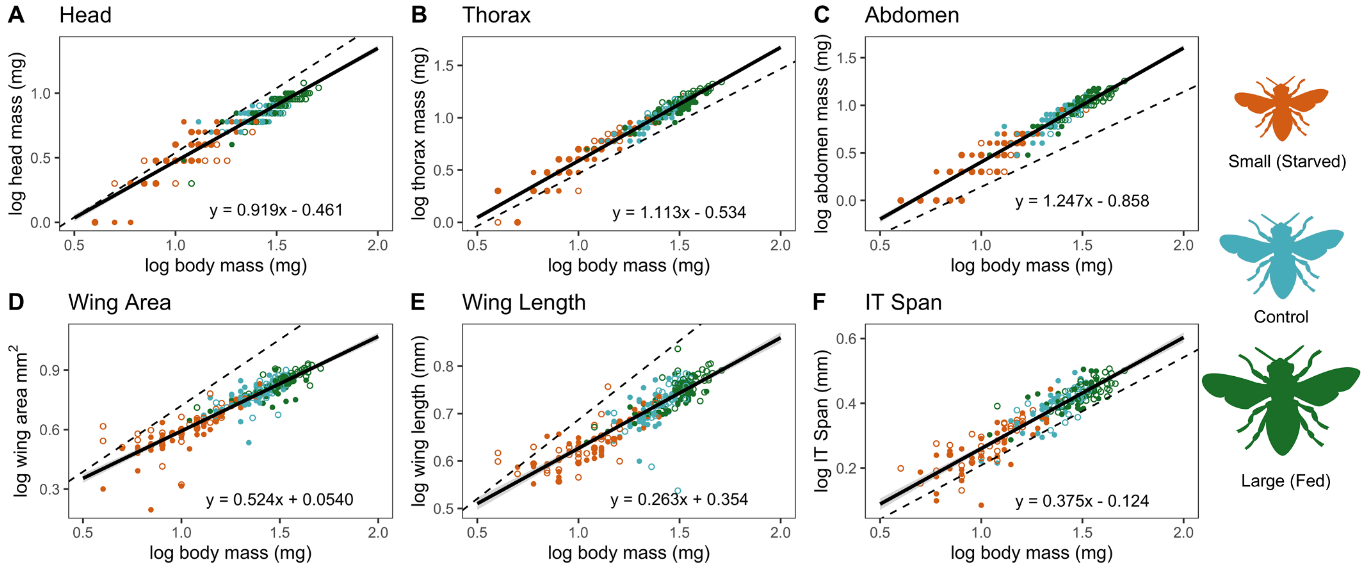

Fig. 3. Scaling relationship between body size, body segments and wings. Allometric scaling relationships for head mass (A), thorax mass (B), abdomen mass (C), wing area (D), wing length (E), and IT span (F). Open circles represent males, and closed circles represent females. The predicted isometric slope is represented by the dashed line. Predicted slope for head mass, thorax mass, and abdomen mass (A-C) is 1, predicted slope for wing area (D) is 0.667, and the predicted slope for wing length and IT span (E & F) is 0.333. Head mass scaled isometrically with body mass. Thorax mass, abdomen mass, and IT span scaled hyperallometrically with body mass. Wing area and wing length scale hypoallometrically with body mass.

not have an effect on the number of wing beats (Fig. 4A; Linear Model, Mass, $T_{2,23} = 0.070$; $p = 0.945$, Sex, $T_{2,23} = 0.687$; $p = 0.499$). Mass and sex have a significant effect on wing loading (Fig. 4B; Linear Model, Mass, $T_{2,210} = 19.402$; $p < 0.001$; Sex, $T_{2,210} = 2.375$; $p = 0.0184$) and EPI (Fig. 4C; Generalized Linear Model, Mass, $T_{7,210} = -12.978$; $p < 0.001$; Sex, $T_{7,210} = -3.573$; $p = 0.000$). Larger bees had higher wing loading, but lower EPI. Wing loading scaled with mass$^{0.515}$, and EPI scaled with mass$^{-0.474}$.

4. Discussion

Across insect species, as body size increases, metabolic rate increases (Coelho and Moore, 1989; Bartholomew and Casey, 1978; Darveau et al., 2005; Fielding and DeFoliart, 2008), and there is a negative correlation between body size and mass-specific metabolic rate and body size (Reinhold, 1999; Casey et al., 1985). In contrast, we found that although larger bees had a higher absolute metabolic rate, once corrected for mass there was a correlation between metabolic rate and body size at rest and no correlation during flight. Previous work in *M. rotundata* found the same trend across a two-fold range of body sizes (Bennett et al., 2013) and our study found this trend across a four-fold range of body sizes, which is a larger range of body sizes than previously seen in studies looking at metabolic rate in *M. rotundata*. This suggests both large and small bees are using the same amount of energy per gram during fight, but at rest small bees expend more energy per gram. Other studies on intraspecific metabolic rates have also found no differences in mass-specific metabolic rates based on body size (Darveau et al., 2014, 2005; Dillon and Dudley, 2004; Bennett et al., 2013), but intraspecific correlations between body size and absolute metabolic rate have been found (Billardon and Darveau, 2019; Darveau et al., 2014; Skandalis and Darveau, 2012).

Interspecific scaling exponents of flight metabolic rate range from 0.63 to 0.94 in insects (Harrison and Roberts, 2000). Intraspecific trends in some insect species have scaling exponents higher than 1 (Chappell and Morgan, 1987; Morgan et al., 1985; Harrison and Roberts, 2000). We calculated a scaling exponent of mass$^{1.080}$ for absolute flight

Table 3
Allometric scaling relationships to body size. Results from the test of isometry comparing the slopes of the head, thorax, and abdomen and treatment to a slope of 1, 0.667 in the case of wing area, and 0.333 for wing length and IT span using the 'smatr' package in R.

	Treatment	Slope	Lower Limit	Upper Limit	P value	Allometry	Reference Slope
Head	Total	0.919	0.887	0.953	<0.001	Hypoallometric	1
	Fed	0.965	0.887	1.050	0.407	Isometric	1
	Control	0.789	0.698	0.893	<0.001	Hypoallometric	1
	Starved	1.072	0.973	1.182	0.158	Isometric	1
Thorax	Total	1.113	1.084	1.143	<0.001	Hyperallometric	1
	Fed	1.085	1.016	1.158	0.0160	Hyperallometric	1
	Control	1.168	1.089	1.252	<0.001	Hyperallometric	1
	Starved	1.252	1.158	1.354	<0.001	Hyperallometric	1
Abdomen	Total	1.247	1.207	1.288	<0.001	Hyperallometric	1
	Fed	1.282	1.195	1.375	<0.001	Hyperallometric	1
	Control	1.208	1.085	1.345	0.001	Hyperallometric	1
	Starved	1.346	1.218	1.487	<0.001	Hyperallometric	1
Wing Area	Total	0.524	0.498	0.552	<0.001	Hypoallometric	0.667
	Fed	0.479	0.424	0.543	<0.001	Hypoallometric	0.667
	Control	0.595	0.508	0.697	0.155	Isometric	0.667
	Starved	0.581	0.504	0.669	0.056	Isometric	0.667
Wing Length	Total	0.263	0.248	0.279	<0.001	Hypoallometric	0.333
	Fed	0.282	0.248	0.321	0.012	Hypoallometric	0.333
	Control	0.412	0.346	0.491	0.018	Hyperallometric	0.333
	Starved	0.242	0.210	0.278	<0.001	Hypoallometric	0.333
IT Span	Total	0.375	0.355	0.396	<0.001	Hyperallometric	0.333
	Fed	0.335	0.288	0.391	0.925	Isometric	0.333
	Control	0.466	0.396	0.549	<0.001	Hyperallometric	0.333
	Starved	0.414	0.359	0.479	0.003	Hyperallometric	0.333

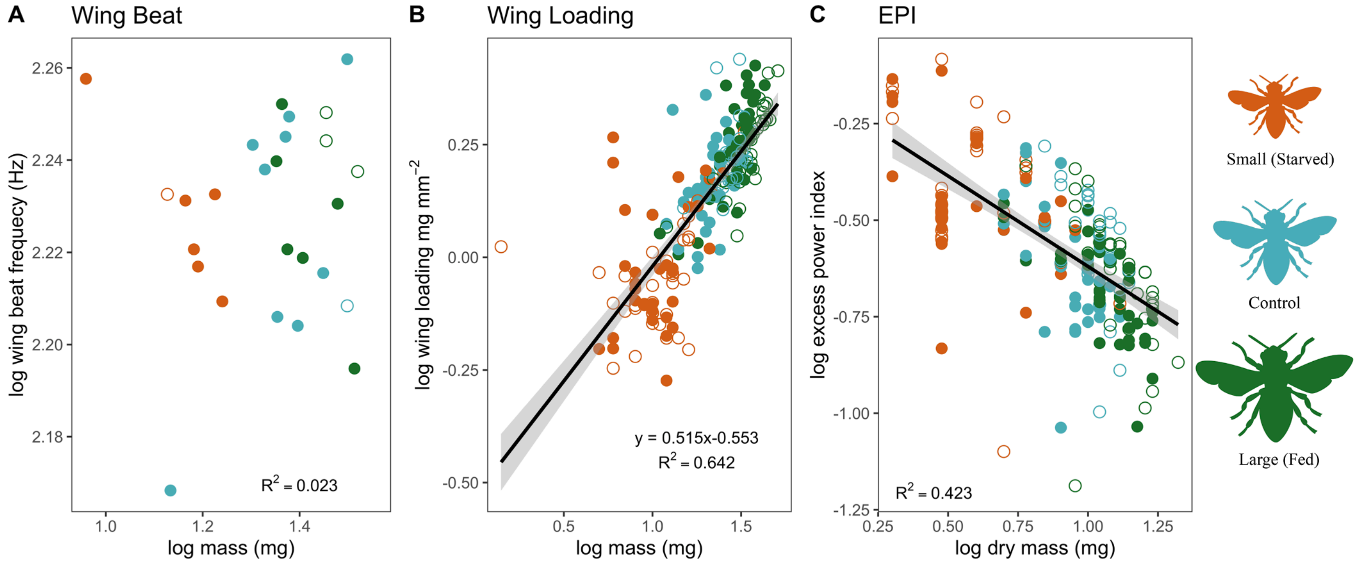

Fig. 4. Flight performance and body size. There is no correlation between body size and wing beat frequency (Hz) ($p = 0.070$) (A) Smaller bees had a lower wing loading ($p < 0.001$) (B) and higher excess power index ($p < 0.001$) (C) than larger bees. Mass is shown as wet body mass (mg) in panels A and B, and as dry body mass in panel C. Grey shading in all panels indicates the 95% confidence intervals). Open circles represent males, and closed circles represent females.

metabolic rate, higher than previously seen in bees (Euglossine bees, 0.64; Casey et al., 1985) (Euglossine bees 0.69; Darveau et al., 2005) (Honeybees, 0.63; Wolf et al.1989) (*M. rotundata,* 0.92; Bennett et al., 2013) (Worker bumblebees, 0.829; Billardon and Darveau 2019), although a scaling exponent above 1 has been documented in worker bumblebees (1.35; Darveau et al., 2014). Differences in metabolic rate scaling in our study may be due to use of tethering or short flight bouts. Bees were tethered to measure flight metabolic rates, because they would not fly freely in the metabolic chamber. Tethered flight has been successfully used to measure flight metabolic rate in other instances (Brodschneider et al., 2009; Bennett et al. 2013). Bees flew for <30 seconds per flight bout which may be a consequence of the environment of the room in which metabolic rates were measured, including artificial light. However, the wing beat frequencies were similar to other insects in the order Hymenoptera, which range from 87 to 230 Hz (Tercel et al., 2018). The range of wingbeats found in our study was 147.3–182.7 Hz, suggesting that bees were exhibiting flight behavior, supporting our use of tethered flight.

Resting metabolic rate in insects scales as mass$^{0.75}$, once corrected for phylogeny (Harrison and Roberts, 2000). Intraspecific scaling exponents are more variable and typically range from 0.67 to 1.0 (Chown et al 2007, Glazier, 2005). Our results show a scaling exponent of mass$^{0.603}$ at rest. Differences in scaling exponents are likely due to physiological, taxonomic, or environmental factors (Glazier 2005). Differences in resting metabolic rate within and between species may be due to body size (Speakman et al., 2004; Chown et al., 2007), growth rates (Sadowska et al., 2009), and availability of food resources (Sadowska et al., 2009).

Metabolic scaling of bees at rest and during flight differed. In our study, the scaling exponent for flight metabolic rate was mass$^{1.080}$, and the scaling exponent for resting metabolic rate was mass$^{0.603}$. Our results are similar to data collected for a meta-analysis (Niven and Scharlemann, 2005), which found flight metabolic rate in insects scaled with body mass$^{1.10}$ and resting metabolic rates scaled with body mass$^{0.66}$. Differences in metabolic scaling may be due to how the insect partitions its metabolic energy. During flight, most of the insect's metabolism will be consumed by flight muscles (Dudley, 2002), while metabolism at rest is allocated to function and maintenance (Weibel, 2002; Glazier, 2005).

At rest, adult bees exhibited discontinuous gas exchange (DGE). We measured the amount of time spent in each phase (closed, open, flutter), as well as the amount of CO_2 produced during the open phase. Patterns of cyclic gas exchange in which spiracles do not entirely close have been observed during the pupal (Yocum et al., 2011) and adult stages in *M. rotundata* (Bennett et al., 2013). We found that there was no correlation between body size and the amount of time spent in each phase. Yocum et al. (2011) showed similar results in pupal *M. rotundata,* where mass had no effect on respiratory patterns, although, that study measured CO_2 emission patterns differently than our study. Larger bees produced more CO_2 during the open phase, but once corrected for mass, there was no correlation between body size and CO_2 production. Studies have found no correlation between body mass and the frequency and duration of the phases of discontinuous gas exchange (Davis et al., 1999, Chappell and Rogowitz, 2000), although a meta-analysis by Terblanche et al. (2008) found the frequency of the phases in discontinuous gas exchange decreases with mass across species. We found the slope for total absolute resting metabolic rate was different than absolute resting metabolic rate measured during DGE because only the open phase was used in the calculation of DGE. The reason for this is unclear, but this may be due to the influence of the flutter phase, which was only included in the analysis of the total absolute resting metabolic rate.

We also analyzed the scaling relationships between body segments and wings to determine whether the relationships were proportional as body size increased. Our study measured bees that had a 12.75-fold change in body size. As bees got larger their thorax and abdomen were larger than expected based on body size. Head size scaled isometrically with body size. Forewing length, and wing area, in the fed treatment, were smaller than expected based on body size. As bees became larger, their thoraces grew proportionately larger, while their wings grew proportionately smaller. There was also a sex effect in which females had larger heads, thoraces, abdomens, and wing area than similarly-sized males. Resource allocation to somatic or reproductive growth can lead to differences in the size of each body segment (Nijhout and Wheeler, 1996). The hyperallometric scaling of the abdomen may reflect an increased investment in reproduction as body size increases. While these scaling relationships were statistically significant, the slopes and confidence intervals for the thorax and abdomen are very close to 1 and may not be biologically significantly different than 1 for the range of body sizes in this study.

Our study showed that wings grew smaller than expected in response to body size. Differential scaling of wings may occur because final wing size is determined by imaginal disc growth, which is separate but interrelated to overall body growth (Nijhout and Callier, 2015). Wings complete growth after the body has stopped growing, potentially leading to allometric response to size. In response to starvation, in *Manduca sexta* (L.) and *Drosophila melanogaster* (M.), wings reduced proportionally to body size (Nijhout and Grunert, 2010; Shingleton et al., 2008). Here, we saw a different overall pattern where wings grew smaller than expected in response to body size.

The regulation of wing growth throughout development has been well documented in insects that have a critical weight as a cue for metamorphosis, such as *M. sexta,* and *D. melanogaster.* This cue is an important regulator for imaginal disc growth. Once the larval insect reaches the critical size, a hormonal cascade is initiated influencing imaginal disc growth. Models describing wing imaginal disc growth, and final size are based upon a critical weight (Shingleton et al., 2008). However, there is a different growth dynamic in solitary bees, which may explain the hypoallometric scaling of wing size in bees. The solitary bee, *Osmia lignaria*, a bee in the same family as *M. rotundata* (Family: Megachilidae), does not have a critical weight, but rather rely on the absence of food as the cue for metamorphosis (Helm et al. 2017). The reliance on nutrition as a cue for development, which is not the case for insects such as *D. melanogaster* (De Moed et al., 1999), may be an explanation for the different wing scaling pattern seen in this study.

We found no correlation between body size and wingbeat frequency in *M. rotundata*. For most insects, wing beat frequency varies with body size (Dudley, 2002), with a negative correlation between body size and wingbeat frequency (Tercel et al., 2018; Casey et al., 1985; Darveau et al., 2005. Smaller individuals have a higher wing beat frequency to control navigation through the higher experienced air viscosity (Ellington, 1999; Wang, 2005). In *M. rotundata*, larger bees did not have a lower wing beat frequency, suggesting there is no size compensation based on number of wing beats. There was no correlation between wing beat frequency and body size, as well as between mass-specific flight metabolic rate and body size. This result may be because the number of times an insect beats its wings influences flight metabolic rate, with metabolic rate increasing with number of wing beats (Casey et al., 1985; Darveau et al., 2005; Billardon and Darveau, 2019). Wing beat frequency and wing loading are often positively correlated, insects with a higher wing loading typically have a higher wing beat frequency (Byrne et al., 1988).

In this study, larger bees had higher wing loading and a lower excess power index. Smaller individuals with lower wing loading have the capacity to carry heavier loads per gram of body mass (Marden, 1987) which may decrease cost of flight. Higher wing loading, as we observed in larger *M. rotundata*, indicates that more power is needed to accomplish the same lift as a smaller bee (Marden, 1994). This may be because larger bees in our study have smaller wings than expected based on their mass. Other studies have also observed lower wing loading in smaller individuals (Darveau et al., 2005) and EPI is higher (Seidelmann, 2014). Wing loading, EPI, and wing beat frequency impact how much female bees can carry when building a nest and provisioning offspring. Overall, we observed that smaller bees have more power available to fly beyond hovering flight, and larger bees may be using most of their available flight power to maintain hovering flight.

While being large may have its advantages in some aspects of flight, the advantages to small body size have often been overlooked. Although a larger insect may have increased foraging distance (Greenleaf et al., 2007), smaller individuals may have better performance by other metrics. We found smaller bees had more power available during flight at no apparent metabolic cost. Because there were no differences in mass-specific metabolic rates during flight, it appears that these bees are equally energetically efficient. The smaller bees required less power during flight, and as bees decreased in size their power requirements for lifting loads also decreased. This study demonstrates that smaller bees may not be at a disadvantage in all aspects of flight performance and may even be at an advantage. Other advantages to small size include increased agility and reductions in costs of development, maintenance, heat stress, and reproduction (Chown and Gaston, 2010; Blanckenhorn, 2000). When resources are scarce, female solitary bees provide smaller provisions to offspring, leading to the production of "tiny" female offspring (Kim and Thorp, 2001; Bosch, 2008). General decreases in foraging habitat may lead to bee populations with overall smaller body sizes (Renauld et al., 2016). With concern for pollinator habitat loss, which would decrease resource availability, more research focusing on the potential performance outcomes of smaller body sizes is needed.

5. Conclusion

The goal of our study was to determine how body size affects allometry, metabolic rate, and flight related morphology in a single species, *M. rotundata*. We produced a large range of body sizes by manipulating food quantity to influence adult body size. By using respirometry to measure excurrent CO_2, we measured both resting and flight metabolic rates for a range of bee body sizes. Our results indicate larger bees have higher absolute metabolic rates at rest and during flight. Smaller bees have higher mass-specific metabolic rates at rest, but mass did not impact mass-specific metabolic rate during flight. Differences in nutritional condition led to allometric growth of the body segments (head, thorax, abdomen, IT span) and wings (area and length). As body size increases the thorax and abdomens of bees become disproportionately larger, while their wings become disproportionately smaller compared to mass. These differences in allometry influenced flight-related morphometrics. Larger bees had wings that were smaller than expected based on body size, which may have contributed to these larger bees having less power available during flight based on flight biomechanical formulas. Our study and others contradict the assumption that performance only increases with body size, or bigger is always better. Instead, smaller bees are not at a disadvantage when it comes to flight performance.

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.

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