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ARTICLE

Special Feature: Pandemic Pivots

Diurnal temperature variation impacts energetics but not reproductive effort across seasons in a temperate dung beetle

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Abstract

Temperature varies on multiple timescales and ectotherms must adjust to these changes to survive. These adjustments may lead to energetic trade-offs between self-maintenance and reproductive investment. However, we know little about how diurnal and seasonal temperature changes impact energy allocation. Here we used a combination of empirical data and modeling of both thermoregulatory behaviors and body temperature to examine potential energetic trade-offs in the dung beetle *Onthophagus taurus*. Beginning in March 2020, universities and laboratories were officially closed due to the COVID-19 pandemic. We thus performed experiments at a private residence near Knoxville, Tennessee, USA, leveraging the heating, ventilation and air conditioning of the home to manipulate temperature and compare beetle responses to stable indoor temperatures versus variable outdoor temperatures. We collected *O. taurus* beetles in the early-, mid-, and late-breeding seasons to examine energetics and reproductive output in relation to diurnal and seasonal temperature fluctuations. We recorded the mass of field fresh beetles before and after a 24-h fast and used the resulting change in mass as a proxy for energetic costs of self-maintenance across seasons. To understand the impacts of diurnal fluctuations on energy allocation, we held beetles either indoors or outdoors for 14-day acclimation trials, fed them cow dung, and recorded mass change and reproductive output. Utilizing biophysical models, we integrated individual-level biophysical characteristics, microhabitat-specific performance, respirometry data, and thermoregulatory behaviors to predict temperature-induced changes to the allocation of energy toward survival and reproduction. During 24 h of outdoor fasting, we found that beetles experiencing reduced temperature variation lost more mass than those experiencing greater temperature variation, and this was not affected by season. By contrast, during the 14-day acclimation trials, we found that beetles experiencing reduced temperature variation (i.e., indoors) gained more mass than those experiencing greater temperature variation (i.e., outdoors). This effect may have been driven by shifts in the metabolism of the beetles during acclimation to increased temperature variation. Despite the negative relationship between temperature variation and energetic reserves, the only significant predictor of reproductive output was mean temperature. Taken together, we find that diurnal temperature fluctuations are important for driving energetics, but not reproductive output.

KEYWORDS
biophysical models, energetics, metabolic rate, Scarabaeinae, seasonality, thermal fluctuations

PANDEMIC PIVOT

After designing an experiment dependent on laboratory incubators that were inaccessible during the pandemic, we improvised by turning the childhood home of lead author and graduate student Fleming into a dung beetle laboratory. Fleming used a spare bedroom as a control temperature treatment for experiments, the garage for processing beetles, and a family freezer for storing cow dung, which was used to feed experimental animals. For the sake of science, the lead author's innocent and supportive family endured a blazing Tennessee summer without lowering the thermostat in a house that smelled like actual cow dung! We appreciate their sacrifice; our pandemic pivot produced an exciting dataset that allows us to examine changes in the energetics and fitness of dung beetles across their breeding season. Because we had no access to laboratory equipment to measure metabolic rate, we turned to a combination of changes in body mass, previously collected data on the thermal sensitivity of metabolic rate, and biophysical modeling to explore the energetic outcomes of increased temperature variation.

INTRODUCTION

Temperature varies on multiple temporal scales that interact to constrain the performance of ectotherms (Dillon et al., 2016; Kefford et al., 2022). Because temperature impacts metabolic rates of ectotherms (Angilletta, 2010; Brown et al., 2004), temperature changes can affect energetics and lead to life history trade-offs between reproduction and somatic maintenance and repair (Andrew et al., 2020; Garland Jr. et al., 2022; Zera & Harshman, 2001). Reproduction is energetically expensive, particularly for females. In thermal environments with higher metabolic demands, lower energetic reserves of adults may lead to reduced investment in the next generation (Crill et al., 1996; Ernsting & Isaaks, 2000; Marty et al., 2022) or abandonment of reproductive attempts (Green et al., 2019; Schou et al., 2021). For example, goldenrod gall flies (*Eurosta solidaginis*) that experience repeated freezing events allocate more energy to cryoprotectant concentrations, which comes at the cost of egg production (Marshall & Sinclair, 2018). Despite such documented temperature-dependent trade-offs, the interactive effects of daily and seasonal temperature fluctuations on energy allocation are largely unknown. Understanding how temperature variation at different timescales and intensities impacts energy allocation is important for predicting organismal responses under changing thermal regimes.

Phenotypic plasticity may modulate the impacts of temperature variation on metabolic rates and thus energetic reserves, although this may vary according to the lifespan of the organism. On the timescales of minutes to hours, rates of physiological processes, such as metabolism, vary by virtue of the acute effects of temperature on molecular reactions. This "passive plasticity" is constrained by biophysical laws beyond the regulatory capacity of the organism (Schulte et al., 2011). In a thermally variable environment, passive plasticity would prompt disproportionate effects of warm and cool temperatures on energetics and performance (Jensen, 1906; Ruel & Ayers, 1999). Spanning days to months, individual ectotherms may alter metabolic rates in response to temperature changes via acclimation, or "active plasticity" (Havird et al., 2020). For instance, an overwintering butterfly, *Erynnis propertius* (Williams et al., 2012) and the dung beetle *Onthophagus taurus* (Carter & Sheldon, 2020) can reduce their thermal sensitivity of metabolism to minimize energy expenditure in thermally variable conditions. The impact that acclimation to diurnal and seasonal temperature changes has on energetic demands may be relative to the lifespan of the organism (Marshall et al., 2021). Multivoltine insects can have multiple overlapping generations within a year that experience different diurnal temperature variations across the breeding season. Seasonal variation in physiological plasticity could impact metabolic rates and the energetic costs of diurnal temperature changes, resulting in variations in trade-offs between survival and reproduction during different times of the breeding season.

Ectotherms can use behavioral thermoregulation to maintain their body temperatures and minimize energetic costs and trade-offs between survival and reproduction despite changing environmental conditions. Insects modify their body temperature through basking (Heinrich, 2009), burrowing (Mamantov & Sheldon, 2020), habitat selection (Fey et al., 2019; Huey, 1991; Kearney & Porter, 2009), and shifting temporal activity cycles (Kenagy & Stevenson, 1982). These strategies are used independently or in combination to maintain optimum body temperatures and minimize the energetic costs of thermal stress. For instance, aphids escape hot temperatures by dropping off the leaves of their host plant (Ma & Ma, 2012). Integrating thermal variability into meaningful measures of organismal performance and reproductive success requires an understanding of operative body temperature as a product of the physical characteristics of the individual and its ability to behaviorally thermoregulate (Abram et al., 2017). This integration can be achieved by combining biophysical and microclimate models to estimate the physiological condition of an animal in response to the environment (Briscoe et al., 2022; Kearney & Porter, 2020). Finally, by incorporating empirical respirometry data, and acclimatory responses to thermal variation with the body temperatures calculated by biophysical models, total energy expenditure over the course of an experiment can be estimated.

To understand how seasonal and daily temperature variations impact energy allocation, we examined energetics and reproductive output in the dung beetle *O. taurus*. *Onthophagus taurus* has been repeatedly introduced into the USA for recycling livestock dung from fields (Hoebeke & Beucke, 1997) and is currently established in much of the eastern USA (Floate et al., 2017). At our study site in East Tennessee, *O. taurus* is multivoltine and remains active across a 5-month breeding season. This species is a tunneling dung beetle, meaning individuals dig tunnels beneath dung pats and pack dung into these tunnels to form a brood ball into which they lay a single egg. We can quantify female reproductive effort by counting the size and number of brood balls produced. Given their burrowing behavior, *O. taurus* has access to microhabitat variation across the soil profile, with cooler and less variable temperatures available at greater depths (Kirkpatrick & Sheldon, 2022). This microhabitat variation facilitates plasticity in *O. taurus* reproductive behaviors, such that they bury brood balls deeper in response to warming (Macagno et al., 2018; Mamantov & Sheldon, 2020). Thermal variation also triggers phenotypic plasticity in *O. taurus* including metabolic depression across multiple life stages (Carter & Sheldon, 2020; Fleming et al., 2021). However, we know less about the potential interplay of behavioral and physiological mechanisms to combat thermally stressful conditions and influence energy allocation.

To understand how temperature variation and behavior influence trade-offs between energetic reserves and reproductive output in *O. taurus*, we collected empirical data on energetics and reproductive output from *O. taurus* beetles that experienced low (i.e., indoor) or high (i.e., outdoor) diurnal temperature variation in the early-, mid-, and late-breeding season. We used data loggers to record temperature and gather site-specific microclimate data. We used a combination of these empirical data with biophysical models to predict body temperatures given an individual's behavior, morphology, and available microclimate. We hypothesized that: (1) increased temperature variation is energetically demanding due to elevated metabolic rates associated with fluctuating temperatures. If this is true, we predicted that diurnal and seasonal temperature variations would interact to influence total energetic expenditure and that diurnal temperature variation would lead to greater effects on body mass than seasonal temperature variation. We further hypothesized that: (2) the high energetic demands of increased temperature variation would translate to an energetic trade-off between somatic maintenance and reproductive output. As energetic demands for somatic maintenance increase, we predicted that less energy would be allocated toward reproduction and we would see a reduction in the size and/or number of brood balls that females produce. Because the impacts of temperature variation on insect physiology depend in part on mean temperature (Bozinovic et al., 2011; Fleming et al., 2021), which we could not control for in the outdoor treatments, we used data loggers to record temperatures across all trials. We predicted that mean temperature would have a significant effect on both the somatic maintenance of beetles and their reproductive effort. Our results from the empirical data and biophysical models revealed the relationship between thermal conditions and energy allocation across different timescales, including the extent to which temperature and behavior together can explain reproductive output across the breeding seasons (Appendix S1: Figure S1).

METHODS

General overview and approach

Our goal was to understand how diurnal and seasonal temperature changes impact energy allocation across the 5-month breeding season of the dung beetle *O. taurus* (Scarabaeinae). We first fasted one set of beetles during each season for 24 h and examined changes in body mass to infer energetic costs to self-maintenance under increased diurnal temperature variation. We then held a second set of beetles during each season for 14 days in experimental treatments to examine the effects of increased diurnal temperature variation on energetic demands and reproductive output. Typically, an experimental procedure in the laboratory would place beetles from each season in different incubator treatments (e.g., high vs. low temperature variance). However, given the pandemic, this was not possible. Instead, we used a private residence to establish two treatments: indoors, representing low temperature variance (17.41-23.15°C), and outdoors, representing increased temperature variance (mean of 11.75-29.63°C). We then built a series of biophysical models in NicheMapR that calculated body temperature, activity, and foraging times from both environmental air temperatures and modeled behavioral thermoregulation. Then, using previously collected respirometry data (i.e., Carter & Sheldon, 2020), including responses to thermal variation, we estimated the total energetic cost of each 14-day treatment using the NicheMapR predicted body temperatures. Finally, we examined how body mass and reproductive output varied as a function of this total energetic cost.

Beetle collection

We collected *O. taurus* beetles using baited pit-fall traps on a cattle farm in Clinton, Tennessee (36°09′07.0° N, 84°06′30.3° W) in May, July, and September 2020 which represent the early-, mid-, and late-breeding seasons, respectively (Mamantov & Sheldon, 2020). Each season, beetles were collected over a span of 2–3 consecutive days. After collection, we transported beetles in plastic containers with moist paper towels to a house adjacent to the field where beetle processing and trials took place. We recorded beetle mass using a portable field balance (Ohaus Scout SPK) and placed each individual in 16-cm tall cylindrical containers (0.02 m^3) filled with a moist, autoclaved soil mixture (4:1 parts soil:sand). At each collection period, we randomly assigned female ($n = {\sim}60$) and male ($n = {\sim}60$) *O. taurus* to one of three experimental treatment groups: fasted treatment, indoor treatment, and outdoor treatment. Only major males were selected to control for potential differences in the energy reserves associated with mating strategy and horn ornaments.

Temperature manipulations

During the COVID-19 pandemic, we were unable to access climate-controlled incubators in the laboratory at the University of Tennessee, so we had to find alternative methods for manipulating diurnal temperature variation. We created different temperature variation treatments by comparing beetles held outside at ambient temperatures (hereafter "outdoor treatment") with beetles held inside a home with central heating and air conditioning that could maintain relatively constant temperatures (20 ± 3°C; hereafter "indoor treatment"). Beetles assigned to outdoor temperatures were held on the south side of the house beneath a 4-m high deck to shield the beetles from direct sunlight. A semitransparent umbrella that allowed light to pass through was placed above the beetles to prevent rainwater from flooding the beetle containers. Beetles assigned to controlled temperatures were held indoors on a wire rack in a spare bedroom of the house. The rack was placed against a north-facing window that received no direct sunlight throughout the day. This allowed indoor beetles to experience identical photoperiods as the outdoor beetles without being exposed to direct sunlight that may have caused temperature spikes.

Experimental trials

Fasted treatment

Due to university closures caused by the pandemic, we were unable to access the laboratory and use respirometry equipment. Thus, we used changes in body mass to infer the energetic costs of self-maintenance for female and male beetles in each season. To examine whether differences among seasons affected changes in body mass independent of diurnal temperature variation, we held female and male beetles individually in containers filled with soil in the outdoor treatment for 24 h without food (experimental design outlined in Appendix S1: Figure S2). We recorded the mass of beetles at the beginning and end of the fasting period. These fresh field beetles allowed us to control for beetle "condition" relative to field temperatures they experienced days prior to the capture date while also limiting the beetles' response time to potential acclimation cues once removed from field conditions.

Indoor and outdoor treatments

To examine the effects of diurnal temperature variation on the energy demands and reproductive trade-offs of *O. taurus*, we conducted 14-day temperature trials in the outdoor and indoor temperature regimes (experimental design outlined in Appendix S1: Figure S3). Given the challenge of parsing the reproductive efforts of female and male beetles in breeding pairs (i.e., male-assisted brood ball provisioning), we focused specifically on the reproductive efforts of females by housing female and male beetles separately in the containers described above. Female *O. taurus* can store sperm for extended periods of time (Hunt & Simmons, 2002) and were assumed to be fertilized upon collection. We recorded the mass of each beetle on day 0 and day 15. Every 3 days, we fed all the beetles *ad libitum* autoclaved cow dung and we searched through the soil of the female containers to remove any brood balls produced. We recorded the mass of each brood ball before opening it to confirm it contained an egg. To record hourly soil temperatures in the treatments, we prepared separate soil containers identical to those housing beetles and placed temperature data loggers (Onset Hobo Pendant Temperature Logger) beneath the soil surface (2 cm) and at the bottom of the container (14 cm). Data from the two loggers were then averaged to represent the hourly soil temperature experienced by burrowing beetles in the outdoor and indoor treatments.

Air, operative, and microclimate temperatures

To record ambient air temperatures at the beetle collection site, we placed a thermocouple data logger (Lascar EL-USB-TC USB) beneath a sunshield (AcuRite Solar Radiation Shield). To record operative temperatures of surface active beetles, we placed another thermocouple data logger in the center of a clay beetle model that was similar in mass to *O. taurus* and exposed to direct sunlight. Both thermocouples recorded temperatures every minute throughout the 14-day trial period each season (Appendix S1: Figure S4). To record microclimate temperatures available to *O. taurus*, which tunnels beneath the soil surface, we buried temperature data loggers (Onset Hobo Pendant Temperature Logger) at 6, 21, or 36 cm below the soil surface at the beetle collection site and recorded temperature every hour. These soil depths are representative of the depths at which *O. taurus* will bury brood balls.

Biophysical models

To interpret the extent to which diurnal and seasonal body temperatures drive changes in beetle mass and reproductive behavior, we built a series of biophysical models that calculated body temperature using the beetles' initial mass, environmental temperatures and modeled behavioral thermoregulation and activity times. Generally, the difference between body temperature and air temperature increases at temperatures further from the species' thermal preference, as beetles burrow to avoid such conditions. We measured food intake rates indirectly by estimating the foraging activity times of beetles using the biophysical models. When activity times were limited, we expected reduced energy intake that may elicit energetic trade-offs. Although it was difficult to estimate food availability in the wild, cow dung was plentiful in the semirural area around Knoxville, Tennessee where we completed our work and thus we expect that our experimental design mimics local conditions. We used mechanistic modeling implemented in NicheMapR (Kearney & Porter, 2020; https://mrke.github.io/), incorporating known ground and below-surface soil temperatures, photoperiod, microclimate data calculated by the model, and information about the study species, to then calculate body temperature and activity times for the treatment duration (Appendix S1: Figure S3). Our model parameters were developed using data from specimens kept in treatment groups held indoors and outdoors, and literature values of the biophysical properties of dung beetles (Appendix S1: Table S1). Details of the model and parameters we used are outlined in Appendix S1: Section S2: Methods.

Metabolism

We used the thermal sensitivity of metabolic rate in response to thermal variation in *O. taurus* from Carter and Sheldon (2020) to model how changes in body temperature in our experimental treatments translated to differences in energy consumption over the course of the experiment, as in Marshall and Sinclair (2012). We first fitted separate nonlinear regressions relating CO_2 production to body temperature for each sex and exposure combination (low amplitude fluctuations vs. high amplitude fluctuations; Appendix S1: Figure S5), assuming that our outdoor treatment was most like the high amplitude treatment and that our indoor treatment was most like the low amplitude treatment from Carter and Sheldon (2020). Then we took the coefficients from this model to calculate CO_2 output for each hour of each experimental treatment for each sex. We then summed these CO_2 outputs for each experimental treatment to calculate the total energetic cost in each experimental group for the time period of the study. Finally, we investigated how this total energetic cost differed among seasons, and whether it was a significant predictor of empirically measured brood ball production and change in body mass.

Statistical analyses of body mass

To examine factors affecting the initial body mass of beetles caught in the field, we used linear models ("lm" function in base R; R version 4.2.2) with the categorical predictors of season and sex and their interaction. To investigate the drivers of change in mass over 24 h of fasting, we built a linear model that included mean and SD in body temperature, then used the "extractAIC" function to test explanatory power (base R; R version 4.2.2). We again used a linear model with the predictors of the mean and SD in body temperature, the season, and sex. We calculated body temperature using NicheMapR, combined it, and weighted it in the same way as the air temperatures. We used the same method to examine the change in body mass of beetles acclimated to 14 days in each treatment.

Statistical analysis of CO_2 production

To examine the potential factors affecting total CO_2 production for beetles acclimated to the 14-day treatments, we ran a linear model a ANOVA as described above (base R; R version 4.2.2). These included mean body temperature, SD in body temperature, season and sex. The model used group averages for all variables, and groups were created by dividing beetles via season, sex, and treatment.

Statistics analysis of reproductive output

To examine potential reproductive trade-offs associated with increased temperature variation and energy expenditure, we examined the effects of temperature variation on brood ball number and size. To assess the effects of temperature variation on brood ball number, we constructed negative binomial generalized linear models predicting the number of brood balls produced by a female as a function of season, treatment, parent initial mass, mean air temperature, SD of air temperature, mean body temperature, SD of body temperature, and total CO_2 production. We applied a negative binomial distribution to account for the overdispersion and the high number of zeros present in our dataset (Hartig & Lohse, 2020). We assessed the collinearity of temperature variables using pairwise Pearson's correlation analyses. Mean air temperature and mean body temperature, and the SD of body temperature and CO_2 production, were highly correlated. Thus we chose to remove mean air temperatures and CO_2 production from the model. Our sample sizes restricted model complexity, and we chose to drop the SD of air temperature as body temperature provided a more precise measurement of the temperatures that the beetles experienced. In addition, we dropped treatment from the model as its effects were largely retained in the SD of body temperature given the nature of the experimental design. Thus, the final model included season, parent initial mass, mean body temperature, and SD of body temperature as predictors of the number of brood balls a female produces. To assess whether temperature variability improved the model, we compared the corrected Akaike information criterion (AIC_C) values of the full model with a reduced model excluding the SD of body temperature as a predictor of brood ball number. This model selection approach is useful for testing an *a priori* hypothesis, and therefore model inference to reduce the probability of false-positive errors that commonly occur when comparing many models (Tredennick et al., 2021). We evaluated model fit using the *dharma* package for residual diagnostics (Hartig & Lohse, 2020).

Female beetles did not produce any brood balls in the fall, thus we chose to remove fall beetles from the analysis for brood ball number to improve model performance. We did, however, run another model in which we kept data from the fall beetles, and our results were qualitatively similar (see Appendix S1: Section S1: Methods). Although we bred *O. taurus* in the laboratory in September (Mamantov & Sheldon, 2020), we suspect the fall beetles in the present study may have entered an early reproductive diapause in response to abnormally cool temperatures.

The drivers of brood ball size were similarly assessed. However a linear regression was used to predict the average mass of brood balls produced by each female. We assessed model fit by inspecting the normality of model residuals (Zuur et al., 2010). We assigned the significance level at $\alpha = 0.05$ for all models and calculated confidence intervals around predicted relationships. Data and code are available on OSF (https://osf.io/pgma6/).

RESULTS

Initial body mass

We caught and weighed a total of 180 male and 201 female *O. taurus*. Male beetles were heavier than female beetles in all seasons (Appendix S1: Figure S6, sex: $F_{1,373} = 163.697$, $p < 0.001$; season: $F_{2,375} = 5.084206$, $p = 0.006$; season × sex: $F_{2,375} = 4.148$, $p = 0.017$). Initial mass decreased across the seasons from spring, to summer, to fall (statistics for the entire model are given in Appendix S1: Table S2, and Figure S6).

Fasted beetles

To test whether season affected the metabolic rates independent of daily temperature variation, *O. taurus* beetles were fasted outside for 24 h. For both sexes, the SD in body temperature during fasting was highest in the fall and lowest in the summer (Table 1). The hours of activity predicted by NicheMapR varied by season as a consequence of available daylight and temperature, and were 2 h lower in the summer cmopared to the spring and two hours lower in the fall compared to the summer. The minimum body and air temperatures did not drop below the estimated temperature at which the species would no longer be able to forage (Table 1).

We ran a model to test the effect of initial mass on change in mass for *O. taurus* fasted outside for 24 h. We found that the initial mass had no significant effect on mass loss, justifying removing it from the following model (Appendix S1: Table S3). In the following model, which incorporated mean and SD of body temperature with season and sex (AIC: −1482.646), only mean body temperature significantly predicted change in body mass over 24 h ($F_{1,1265} = 10.652$, $p = 0.001$, full model output given in Appendix S1: Table S4), however the effect of mean body temperature on the change in mass was weak ($\beta = 0.006 \pm 0.06$) relative to SD of body temperature ($\beta = 0.011 \pm 0.16$, full model output give in Appendix S1: Table S5, Figure 1).

Two-week indoor and outdoor acclimation treatments

To test the impacts of acclimation to thermal variation on *O. taurus*, we fed beetles to satiation for 2 weeks during each season. Beetles kept indoors experienced lower fluctuations in body temperature (Table 2), helping to parse out the impact of other seasonal effects from body temperatures. Body temperatures of both male and female *O. taurus* were least variable in summer relative to spring and fall in both the indoor and outdoor treatments (see Table 2). The difference in SD of body temperature between the indoor and outdoor treatments was largest in the fall for both sexes (male and female

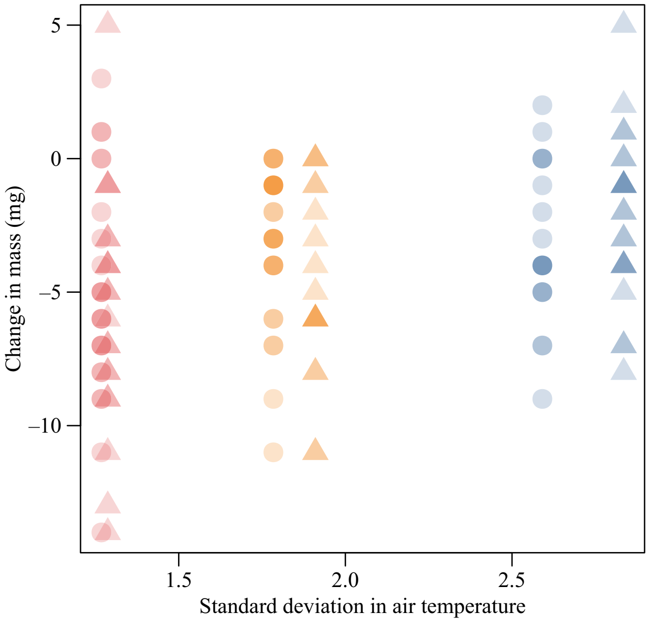

FIGURE 1 Mean daily average of standard deviation in air temperature and change in body mass for beetles that were fasted outside for 24 h. Values have been presented as partially transparent to indicate where multiple *Onthophagus taurus* had the same value for change in mass. Symbols show males (triangles) and females (circles) and colors show data from spring (yellow), summer (red), and fall (blue).

TABLE 1 Body temperature, behavior, and conditions experienced by *Onthophagus taurus* in Knoxville, Tennessee, USA during the 24 h fasting trial.

Season and sex	Air temperature range (°C)	Mean ± SD air temperature (°C)	Mean soil temperature (°C)	Initial mass (mg)	Change in mass (mg)	Body temperature range (°C)	Mean ± SD body temperature	Hours spent foraging (out of 24 h)
Spring								
F	19.50–25.48	22.09 ± 1.78	22.10	67.18 ± 16.68	−3.14	19.89–25.06	22.74 ± 1.58	15
M	19.11–24.77	22.09 ± 1.78	22.10	85.78 ± 9.56	−4.67	19.11–24.51	22.77 ± 1.66	15
Summer								
F	23.89–27.47	25.40 ± 1.27	25.44	65.17 ± 15.63	−5.09	23.96–27.11	25.29 ± 1.09	13
M	23.85–27.50	25.45 ± 1.29	25.50	83.80 ± 16.73	−5.62	23.97–27.32	25.45 ± 1.13	13
Fall								
F	18.06–25.00	20.83 ± 2.59	20.71	51.37 ± 17.06	−3.21	18.1–24.59	21.35 ± 2.67	11
M	19.11–26.84	22.15 ± 2.84	21.50	77.43 ± 12.55	−2.13	19.23–25.46	22.24 ± 2.45	11

Note: Spring treatments were performed at the end of May 2020, summer treatments at the end of July 2020, and fall treatments at the end of September 2020. Females and major males were used. Body temperatures were calculated in NicheMapR.

TABLE 2 The conditions experienced by, and body temperature of, *Onthophagus taurus* in Knoxville, Tennessee, USA during both the indoor and outdoor 14-day treatments.

Season and sex	Treatment	Initial mass (mg)	Change in mass (mg)	Mean ± SD body temperature (°C)	Mean ± SD air temperature (°C)	Body temperature range (°C)	Air temperature range (°C)
Spring							
F	IN	61.53 ± 13.41	3.04	22.66 ± 1.160	21.62 ± 0.27	20.62–24	20.85–22.65
M	IN	81 ± 7.08	0.61	22.70 ± 1.16	21.61 ± 0.26	20.63–24	20.85–22.63
Summer							
F	IN	57.70 ± 12.78	2.65	22.97 ± 0.91	21.97 ± 0.23	21.32–24	21.2–23.15
M	IN	85.14 ± 12.41	−2.68	22.9 ± 0.97	22.00 ± 0.22	21.17–24	21.20–23.148
Fall							
F	IN	53 ± 23.98	4.13	21.54 ± 1.35	20.44 ± 0.72	17.85–24	17.41–22.97
M	IN	80.14 ± 12.79	−4.18	21.62 ± 1.36	20.45 ± 0.71	17.89–24	17.41–22.97
Spring							
F	OUT	70.32 ± 18.18	0.64	22.85 ± 1.61	22.43 ± 1.99	15.77–28.25	14.83–29.63
M	OUT	83.5 ± 6.82	−6.58	22.85 ± 1.6	22.60 ± 1.99	15.65–27.99	14.83–29.63
Summer							
F	OUT	69.28 ± 16.00	2.27	24.36 ± 1.04	24.72 ± 1.59	20.52–27.47	20.26–28.85
M	OUT	73.59 ± 14.78	0.14	24.31 ± 1.05	24.71 ± 1.62	20.48–27.43	20.26–28.85
Fall							
F	OUT	53.95 ± 16.81	0.62	19.15 ± 3.18	18.22 ± 2.58	11.82–24.11	11.75–27.13
M	OUT	80.77 ± 14.74	−8.18	19.20 ± 3.18	18.22 ± 2.53	11.82–24.13	11.75–27.13

Note: Spring treatments were performed at the end of May 2020, summer treatments at the end of July 2020, and fall treatments at the end of September 2020. Only major males were used. Body temperatures were calculated in NicheMapR.

O. taurus, indoor: 1.4°C; female *O. taurus*, outdoor: 3.2°C; see Table 2). The difference in experienced thermal variability between males and females was highest in summer for both the indoor and outdoor treatments (Table 2).

As before, we built a linear model predicting change in body mass over the 14-day period. Here, the term initial body mass was significant and not excluded from the model. We incorporated mean and SD in body temperature with initial body mass, season and sex (AIC: −2453.034). We found that initial body mass ($F_{1,242} = 68.021$, $p < 0.001$) and mean body temperature ($F_{1,242} = 17.18$, $p < 0.001$), significantly predicted mass change over the 14-day period (full model output given in Appendix S1: Table S8). Initial mass had a relatively strong effect ($\beta_{\text{start mass}} = -0.188 \pm 0.03$). However, the effect of mean body temperature on the change in mass was weak ($\beta_{\text{mean body temperature}} = 0.0002 \pm 0.0009$) relative to SD in body temperature ($\beta_{\text{SD body temperature}} = -0.0019 \pm 0.0014$; full model output given in Appendix S1: Table S8).

Opportunities to forage, along with other activities, are important elements of an organism's energy intake and balance. NicheMapR predicts three different types of activity: inactive, basking, and foraging. Foraging occurs when thermal conditions permit; basking occurs when they do not, but when temperatures are warmer above the soil surface than underground; inactivity occurs at night and when it is warmer underground. Therefore, beetles will be inactive for longer in spring and fall as a product of reduced daylight hours. Over the course of the 14-day acclimation experiment, NicheMapR predicted that inactivity would be highest in the fall and lowest in the spring (Table 3).

As above, we found hours of inactivity remain consistent between sexes but vary by season and as a consequence of available daylight (Table 3). For the outdoor treatment, in spring, foraging hours were reduced as a consequence of basking to a slightly greater degree in males than females, no hours were lost in the summer and, in the fall, foraging hours were even more limited.

Energetic impacts of temperature variation

In all treatments, for both sexes, the calculated total CO_2 output was highest in summer, followed by spring, and

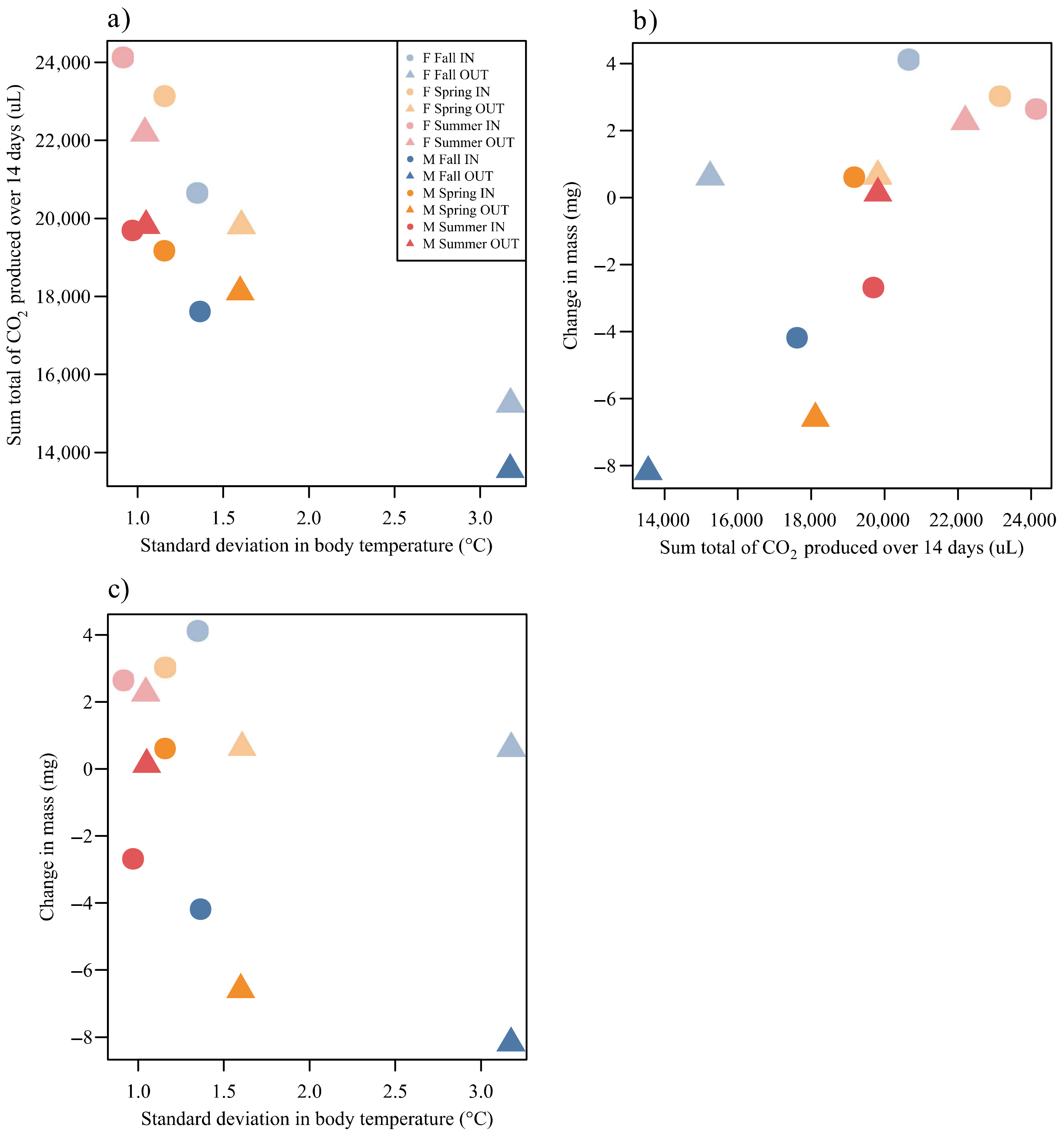

FIGURE 2 (a) The sum total predicted CO_2 production of *Onthophagus taurus* beetles over the 14-day acclimation treatment as a function of the standard deviation in body temperature. (b) The change in mass of *O. taurus* beetles over the 14-day acclimation treatment as a function of the sum total CO_2 produced. (c) The change in mass over the 14-day acclimation treatment as a function of standard deviation in body temperature. Males are represented by opaque symbols and females by partially transparent symbols. All presented values are means for their respective treatment × season × sex, statistics described in the text. Symbols show indoor (circles) and outdoor (triangles) treatments. Colors show data from spring (yellow), summer (red), and fall (blue).

lowest in fall. Similarly, CO_2 output was higher in the indoor versus outdoor treatment for female *O. taurus* for all seasons, and higher in the indoor versus outdoor treatment for males in the spring and the fall (Figure 2). The ANOVA model indicated that total CO_2 output over the course of the 14-day acclimations significantly decreased with increased mean body temperature ($F_{1,6} = 100.269$, $p < 0.001$), SD in body

TABLE 3 The activity of *Onthophagus taurus* in Knoxville, Tennessee, USA during both the indoor and outdoor 2-week treatments.

Season	Treatment	Sex	Inactive (h)	Basking (h)	Foraging (h)
Spring	IN	F	126	0	210
Spring	OUT	F	126	7	203
Spring	IN	M	126	0	210
Spring	OUT	M	126	7.83	202.17
Summer	IN	F	154	0	182
Summer	OUT	F	154	0	182
Summer	IN	M	154	0	182
Summer	OUT	M	154	0	182
Fall	IN	F	182	2	154
Fall	OUT	F	182	24	130
Fall	IN	M	182	2	152
Fall	OUT	M	182	21.14	132.86

Note: Where multiple trials took place in the same season, calculations have been weighted by the number of beetles per day (see *Methods* for details). Only major males were used. Activities were calculated in NicheMapR.

temperature ($F_{1,6} = 24.357$, $p = 0.003$), and sex ($F_{1,6} = 41.048$, $p < 0.001$; full model output given in Appendix S1: Table S10). SD in body temperature had a relatively strong effect ($\beta_{\text{SD body temperature}} = -3.464 \pm 669.2$), as did sex ($\beta_{\text{sex}} = -2822.2 \pm 440.5$; full model output given in Appendix S1: Table S10).

Reproductive output

Over the course of the experiment, we measured the reproductive output of 56 females in the spring, 40 females in the summer, and 37 females in the fall. There were a total of 60 brood balls (spring $n = 20$, summer $n = 40$) produced by 24 females. In the spring, the average number of brood balls produced was similar for the indoor (mean 0.393 ± 0.92) and outdoor (0.321 ± 0.72) treatments. In the summer, females produced fewer brood balls on average in the indoor (mean 0.381 ± 0.86) than in the outdoor (1.600 ± 2.26; Appendix S1: Figure S8) treatment.

Our full model for brood ball number did not outperform the reduced model excluding the SD of body temperature ($\Delta\text{AIC}_c = -0.034$), suggesting that temperature variation does not significantly influence the number of brood balls a female beetle produces. The reduced model found mean body temperature to be a significant predictor of brood ball number ($\beta_{\text{mean body temperature}} = 1.0437$, $df = 96$, $p = 0.045$, $R^2 = 0.173$; Appendix S1: Table S12; Figure 3). We found no effect of season (CI = −0.64 to 0.77, $p = 0.833$), or initial mass (CI = −34.74 to 29.11, $p = 0.851$). The models we ran on the full dataset including spring, summer, and fall females confirmed the results above, with mean air temperature as the only predictor for brood ball number (Appendix S1: Tables S8 and S12).

Brood balls produced were significantly larger in the summer (4.310 ± 0.189 g) than in the spring (3.708 ± 0.253 g; Appendix S1: Figure S9). In the spring, there was no significant difference in the brood ball mass between the indoor (3.567 ± 1.280 g) and outdoor (3.879 ± 0.960 g) treatment ($p = 0.23$; Appendix S1: Figure S9). In the summer, females produced significantly larger brood balls in the indoor (5.322 ± 1.588 g) than in the outdoor (4.057 ± 0.946 g) treatment (Appendix S1: Figure S9).

Our full model for brood ball mass did not outperform the reduced model that excluded SD of temperature ($\Delta\text{AIC}_c = 1.005$), suggesting that the temperature variation did not significantly impact brood ball size. Additionally, the reduced model found no significant effect of season ($F_{1,19} = 0.9795$, $p = 0.3347$), treatment ($F_{1,19} = 0.4403$, $p = 0.764$), parent mass ($F_{1,19} = 0.9103$, $p = 0.351$), or mean body temperature ($p = 0.299$) (Appendix S1: Table S13).

DISCUSSION

To understand how daily and seasonal temperature variations interact to affect the energy allocation of *O. taurus*, we exposed beetles to ambient (outdoor) and controlled (indoor) diurnal temperature fluctuations across seasons. We hypothesized that thermal variation was energetically expensive, and that this expense would come at a cost to reproduction. Interestingly, we found evidence to reject both hypotheses. First, the energetic costs of temperature variation can be largely compensated for by the metabolic

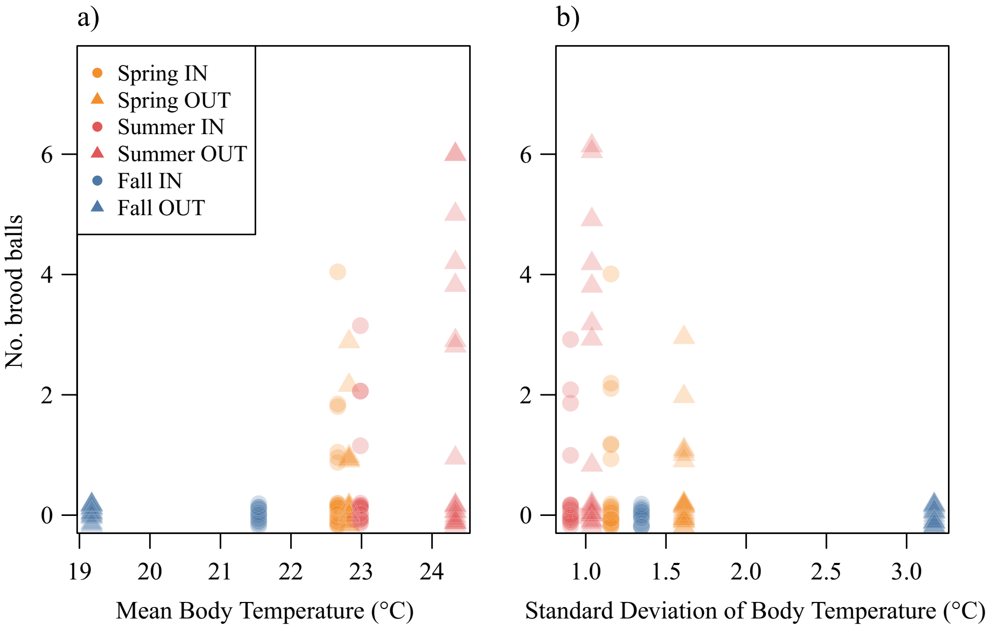

FIGURE 3 (a) Mean body temperature and the number of brood balls individual females produced. (b) Standard deviation of body temperature and the number of brood balls individual females produced. Values have been presented as partially transparent and jittered to indicate where multiple *Onthophagus taurus* females produced the same number of brood balls. All presented values are means for their respective treatment × season, statistics described in the text. Symbols show indoor (circles) and outdoor (triangles) treatments. Colors show data from spring (yellow), summer (red), and fall (blue).

depression that *O. taurus* uses in response to acclimation to thermal variation (Carter & Sheldon, 2020). Second, we found no evidence that energetics drives reproductive effort, instead we found that mean air temperature alone predicted reproductive effort.

Based on the total calculated metabolic cost over the course of the experiment, we found that diurnal temperatures drive the energetics of *O. taurus*, independent of seasonal effects. During periods of fasting, energy metabolism occurs at the expense of body reserves. Therefore, all fasted beetles lost mass over the 24 h period. Mean body temperature significantly impacted the change in body mass of fasted beetles, with beetles losing more mass at warmer temperatures. However, the effects of mean body temperature on the change in body mass were weak relative to the effects of variation in temperature. Although statistically nonsignificant, we found that variation in body temperature negatively correlated with the mass loss of fasted beetles, such that beetles experiencing reduced temperature variance outdoors during the fasting period lost more mass than beetles experiencing high temperature variance outdoors (Figure 1). This suggests that the freshly caught, fasted beetles retained carryover effects such as metabolic depression, from the temperature variation they experienced days prior while in the field. Metabolic depression is likely to be an adaptive response that favors energy conservation by limiting energetic demands of self-maintenance in otherwise stressful thermal conditions (Bennett et al., 1999; Williams et al., 2012). Fasted beetles that experienced high thermal variation preceding collection are likely to possess the energetic benefits of metabolic depression at the beginning of the fasting period. Thus, fasted beetles showed lower energetic costs to self-maintenance in response to high thermal variability as they typically experienced similar conditions in the field prior to collection.

In contrast, our results for the 14-day acclimation trials suggest metabolic depression could not fully compensate for energetic demands. During the 14-day acclimation trials, beetles were fed *ad libitum* and, thus, beetles could lose or gain mass depending on their food intake and energy expenditure. Similar to the 24-h fasted beetles, we found that the effects of mean body temperatures on change in body mass of the 14-day acclimated beetles were weak relative to the effects of variation in body temperature. However, in contrast with the 24 h fasted beetles, we found that 14-day acclimated beetles that experienced reduced variation in body temperature gained more mass than beetles experiencing high variation in body temperature. This suggests that the beetles experiencing higher variation in body temperature had higher metabolic rates. Yet, our models predicted that beetles exposed to high temperature variation over the 14-day acclimation trial would produce less total CO_2 than the indoor beetles, suggesting that beetles experiencing higher temperature variation had lower metabolic rates. Taken together, our results of outdoor beetles having lower CO_2 production and yet less mass gain compared with the indoor beetles suggests that any metabolic compensation of the outdoor beetles was not adequate to maintain energy balance over the longer 14-day time period, or potentially that the energetic costs of the additional thermoregulation required in the outdoor treatment partially outweighed the savings of metabolic compensation. Importantly, *O. taurus* has been shown to reduce its metabolic sensitivity in response to temperature variation (Carter & Sheldon, 2020), and our results emphasize the importance of this phenotypic plasticity in compensating for the added energetic load imposed by thermal variation.

We found no energetic trade-off between somatic maintenance and reproductive output. The number of brood balls an individual female produced varied in response to mean air temperature, with females producing more brood balls when conditions were warmer. These findings are in line with theory and empirical evidence suggesting that hotter is better for most performance metrics of ectotherms (Angilletta et al., 2010; Kingslover & Huey, 2008). For small insects that have limited thermal inertia, the challenge becomes maintaining enough heat to maximize performance. Insects can leverage a high metabolic rate during warmer temperatures to increase feeding rates and energy intake that fuel reproduction (Deutsch et al., 2018). Therefore, from a productivity standpoint, the adaptive pressure to respond to thermal variation lessens. We found that reproductive performance increased when the metabolic rates of females were among the highest in the experiment. We expect that if trial temperatures were warmer, exceeding the thermal optimum of the species, we may begin to see greater physiological stress that would alter reproductive performance.

We were surprised to find that fall beetles in both indoor and outdoor treatments did not produce any brood balls throughout the 14-day acclimation trials. While our current design cannot deduce the mechanism causing *O. taurus* females to forgo reproduction, we can make some speculations given the known life history of the species and our biophysical models. Reproductive diapause is common in temperate insects, and *O. taurus* more specifically (Beckers et al., 2015), and can be triggered by a suite of environmental cues including reduced food availability, short photoperiods, and low temperatures (Saulich & Musolin, 2012; Wilches et al., 2016). For dung beetles living in cattle pastures, food availability remains consistent throughout the year and is likely to not be a limited resource. Moreover, the fall beetles were collected at the same time of year as our previous studies in which *O. taurus* continued breeding later into the season when the photoperiod was undoubtedly shorter (e.g., Mamantov & Sheldon, 2020). Therefore we found it unlikely that food availability or photoperiod would trigger diapause so early in the season. More likely is that the low mean temperatures limited the activity times females had available to reproduce. Given that we found a strong relationship between warm temperatures and high reproductive output, and that mean temperature during the fall trials was substantially lower than any temperatures where females reproduced in the spring and summer, there is reason to believe that low mean temperature was the limiting factor preventing females from producing brood balls. However there was no way of knowing if females resumed reproduction later in the breeding season on days when temperatures were warmer, or if the cold snap cued reproductive diapause that would persist into the winter.

There were limitations to our study, partly due to constraints from the pandemic. We had minimal control over the magnitude of temperature variation, we did not measure feeding consumption rates to estimate energy intake, and we only had data for a single breeding season. To better examine the physiological and behavioral response of dung beetles to temperature variation at different timescales, future research should expose beetles from across the breeding season to a full factorial of mean temperatures and temperature fluctuations. The temperatures used in our study fell well within the thermal limits of temperate dung beetles (Sheldon & Tewksbury, 2014) and represent thermal regimes relevant to *O. taurus* currently living in East Tennessee. However, temperature variation is expected to increase as a result of climate change (Vasseur et al., 2014) and *O. taurus* may respond differently to temperatures that are near their critical thermal limits (Colinet et al., 2015). While the results of this study reveal the thermal sensitivity of the physiological and reproductive performance of *O. taurus* in generally permissive temperatures, research examining more extreme temperature means and fluctuations across multiple breeding seasons is necessary for further unpacking the interaction of temperature variation at multiple timescales and predicting responses to future climate conditions.

CONCLUSION

We found that thermal variation drives the energetics of *O. taurus*, however measurable energetics do not drive reproductive output. Our approach to combining empirical data on beetle energetics and reproduction with biophysical models allowed us to highlight the dynamic relationship between phenotypic plasticity and behavioral thermoregulation. The findings of this study are important for predicting responses to future climate change by underlying the significant impact of daily temperature variation on the energetic demands and allocation of ectotherms relative to seasonal temperature changes.

AUTHOR CONTRIBUTIONS

Conceptualization: J. Morgan Fleming, Kimberly S. Sheldon, and Katie E. Marshall. Methodology: J. Morgan Fleming, Kimberly S. Sheldon, Katie E. Marshall, and Alexander J. Coverley. Investigation: J. Morgan Fleming, and Alexander J. Coverley. Formal analysis: J. Morgan Fleming, Katie E. Marshall, and Alexander J. Coverley. Visualization: J. Morgan Fleming, and Alexander J. Coverley. Writing original draft: J. Morgan Fleming and Alexander J. Coverley. Writing, editing: Kimberly S. Sheldon and Katie E. Marshall.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT

Data and code (Fleming et al., 2023) are available from Open Science Framework at https://doi.org/10.17605/OSF.IO/PGMA6.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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