Life stages differ in plasticity to temperature fluctuations and uniquely contribute to adult phenotype

Amanda W. Carter[1]* and Kimberly S. Sheldon[1]

1. Department of Ecology and Evolutionary Biology, University of Tennessee, Knoxville, TN, USA

acarte82@utk.edu; ksheldon@utk.edu

***CORRESPONDENCE:** A. W. Carter; Department of Ecology and Evolutionary Biology; 569 Dabney Hall, 1416 Circle Dr., Knoxville, TN 37996; acarte82@utk.edu

Abstract:

Adaptive thermal plasticity allows organisms to adjust their physiology to cope with fluctuating environments. However, thermal plasticity is rarely studied in response to thermal variability and is often measured in a single life stage. Plasticity in response to thermal variability likely differs from responses to constant temperatures or acute stress. In addition, life stages likely differ in their plasticity and responses in one stage may be affected by the experiences in a previous stage. Increasing the resolution with which we understand thermal plasticity in response to thermal variation across ontogeny is crucial to understanding how organisms cope with the thermal variation in their environment and to estimating the capacity of plasticity to mitigate costs of rapid environmental change. We wanted to know if life stages differ in their capacity for thermal plasticity under temperature fluctuations. We reared *Onthophagus taurus* dung beetles in either low or high temperature fluctuation treatments and quantified thermal plasticity of metabolism of pupae and adults. We found that adults were thermally plastic and pupae were not. Next, we wanted to know if the plasticity observed in the adult life stage was affected by the thermal conditions during development. We again used low and high temperature fluctuation treatments and reared individuals in one condition through all egg to pupal stages. At eclosion, we switched half of the individuals in each treatment to the opposite fluctuation condition and, later, measured thermal plasticity of metabolism on adults. We found that temperature conditions experienced during the adult stage, but not egg to pupal stages, affects adult thermal plasticity. However, temperature fluctuations during development affect adult body size, suggesting that some aspects of the adult phenotype are decoupled from previous life stages and others are not. Our data demonstrate that life stages mount different responses to temperature variability and uniquely contribute to the adult phenotype. These findings emphasize the need to broadly integrate the life cycle into studies of phenotypic plasticity and physiology; doing so should

enhance our ability to predict organismal responses to rapid global change and inform conservation efforts.

Key words: acclimation, body size, *Coleoptera,* development, dung beetle, metabolism, ontogeny, temperature fluctuation

Introduction:

When adaptive, thermal plasticity allows organisms to adjust their physiology to suit the current conditions and is thought to be especially important in fluctuating environments (Kingsolver and Huey, 1998; Lande, 2014; Woods and Harrison, 2002). Paradoxically, thermal plasticity is rarely measured in response to fluctuating temperatures, and instead, is often measured following exposure to constant temperatures or acute thermal stress (Niehaus et al., 2012). This is problematic because temperature fluctuations can dramatically alter performance (e.g. metabolic rate) (Jensen, 1906; Ruel and Ayres, 1999; Williams et al., 2012) and may trigger plasticity via different suites of mechanisms compared to constant temperatures (Sørensen et al., 2016). Understanding plastic responses to temperature variation is crucial to evaluating how most animals cope with thermal variation in their environment and to deciphering the capacity of plasticity to mitigate costs associated with rapid environmental change.

Thermal plasticity is not likely uniform across an individual's life. Life stages can experience unique selection pressures or exhibit distinct behaviors and physiologies that alter thermal plasticity (Fischer et al., 2014). For example, life stages that live in environments with high temperature variation may experience selection for high thermal plasticity (i.e. Climate Variability Hypothesis) (Colinet et al., 2015; Sheldon et al., 2018; Woods, 2013). On the other hand, life stages with greater mobility may evade selection pressures from fluctuating conditions and exhibit reduced thermal plasticity (i.e. Bogert Effect) (Bogert, 1949; Marais and Chown, 2008; Mitchell et al., 2013). Despite this, the bulk of thermal biology research considers a single life stage at a time, most often the adult stage (Chiu et al., 2015; Kingsolver, 2009; Kingsolver et al., 2011; Radchuk et al., 2013). Knowing if and how life stages vary in thermal plasticity may facilitate significant strides in the accuracy of climate change modeling; estimates not based on the most critical life stages may over- or under- estimate persistence (Chiu et al., 2015; Kingsolver et al., 2011; Levy et al., 2015; Pincebourde and Casas, 2015; Radchuk et al., 2013).

In addition to independent plastic responses in each life stage, temperatures in an earlier life stage may alter the phenotype of a later life stage, including the ability to be plastic. Temperatures early in ontogeny can cause organizational shifts in growth and development trajectories that may permanently impact adult phenotype (i.e. developmental plasticity) (Beldade et al., 2011; Uller, 2008; West-Eberhard, 2003). For example, over 80% of surveyed ectotherms exhibit larger body sizes following cooler developmental temperatures (Atkinson, 1994), which can have outsized effects on adult physiology and fitness (Kingsolver and Huey, 2008; Stillwell and Fox, 2005). In addition to affecting mean trait value, developmental conditions may also modify the capacity for plasticity later in life, an area of research that has received surprisingly little attention (Beaman et al., 2016). For example, development at cooler or warmer temperatures enhances the capacity for thermal acclimation in adult zebra fish (*Danio rerio*) compared to development at an intermediate temperature (Scott and Johnston, 2012). Further complicating life stage-temperature interactions is the diversity of life history patterns among ectotherms. Unlike vertebrate ectotherms where growth and development are incremental, ectotherms with modular life cycles, like insects, may decouple the thermal conditions and physiological responses from one life stage to the next ("life cycle modularity hypothesis" (Potter et al., 2011); "adaptive decoupling hypothesis" (Moran, 1994; Stoks and Cordoba-Aguilar, 2012)) (Gray, 2013; Kingsolver et al., 2011).

Since increasing thermal variation in the environment can increase energetic costs (Ruel and Ayres, 1999; Williams et al., 2012), quantifying shifts in metabolism under increased temperature fluctuation is an especially well-suited measure of plasticity. Metabolism is a multifaceted and dynamic process that can provide insight into overall energy budgets of organisms in fluctuating environments with consequences for organismal fitness and population dynamics (Brown et al., 2004; Chown and

Gaston, 1999; Chown and Nicolson, 2004; Dillon et al., 2010; Lighton, 2018; Norin et al., 2016; Sibly et al., 2012). Whole-organism metabolism can be measured via respirometry, which directly measures CO_2 and/or O_2 consumption. When respirometry is measured across a range of temperatures, this enables simultaneous estimation of metabolic rate and thermal sensitivity of metabolism (Lighton, 2018). Depending on the experimental design, thermal plasticity can also be quantified by comparing shifts in metabolic parameters among genetically similar groups (e.g. clones or full-siblings) exposed to different environments (Seebacher et al., 2015).

Thermal plasticity can be measured as shifts in thermal sensitivity of metabolism or overall metabolic rate when individuals or cohorts are exposed to different environments. Thermal sensitivity of metabolism is the relationship between temperature and metabolic rate (e.g. slope of the function, Q_{10}) and dictates energy expenditure in fluctuating environments (Lake et al., 2013; Ruel and Ayres, 1999; Williams et al., 2012). Seemingly minimal adjustments in the thermal sensitivity of metabolic rate can rapidly compound to alter whole-organism performance (Burton et al., 2011; Metcalfe et al., 1995; Williams et al., 2012). In the larvae of *Erynnis propertius,* for example, individuals exposed to increased thermal variation decreased their thermal sensitivity of metabolism (i.e. reduced the slope of the temperature-metabolism function) to reduce energetic costs (Williams et al., 2012). Metabolic rate, on the other hand, is simply the magnitude of the metabolic response (e.g. intercept) and can be measured with total CO_2 production (Lighton, 2018). Since increases in temperature fluctuations can increase energetic costs, organisms should decrease thermal sensitivity of metabolism and/or decrease overall metabolic rate to conserve energy (Ruel and Ayres, 1999).

We compared thermal plasticity in response to increased temperature fluctuation in adults and pupae of *Onthophagus taurus* dung beetles. *Onthophagus taurus* is an excellent system for such questions as they are holometabolous insects with a life history that may select for differential thermal

physiology across life stages. Eggs, larvae, and pupae develop underground within a parentally provisioned brood ball (Halffter and Edmonds, 1982). Developing young are relatively buffered from daily temperature extremes compared to adults that fly in open fields in search of dung for foraging and reproduction. First, we wanted to know if life stages vary in their capacity for thermal plasticity, specifically, pupae and adults. We predicted that adults would exhibit greater plasticity than pupae, since they likely encounter greater temperature variation across microhabitats.

We next wanted to know if the conditions experienced during early life stages carry-over to affect adult plasticity. We predicted that adults would exhibit plasticity in response to their current environment, but that the magnitude of the plastic response would be attenuated by developmental conditions. Because developmental temperatures may impact adult phenotype through a myriad of mechanisms, we were also interested in determining if increased thermal variation affected body size, a trait important to fitness in *O. taurus* (Moczek and Emlen, 1999). Since fluctuating environments can be energetically costly, we predicted that beetles reared in the high temperature fluctuation treatment during development would be smaller than those reared in the low fluctuation treatment, regardless of the temperatures experienced as an adult.

Methods:

Do life stages differ in thermal plasticity to increased temperature fluctuation?

We trapped *Onthophagus taurus* beetles in June 2018 in Kings Mountain, North Carolina (n = 115). We brought adults to the lab, housed them in breeding triads (one male with two females) with *ad libitum* access to autoclaved cow dung, and collected and individually reared offspring from resulting F_1 brood balls. At approximately four weeks post adult emergence, we paired a virgin F_1 female with an

unrelated virgin F_1 male creating 31 families. We collected F_2 brood balls every three days. We individually reared F_2 brood balls at either a low fluctuation (24 ± 4 °C) or high fluctuation (24 ± 8 °C) treatment using a split-family design (Fig. 1). Temperature treatments fluctuated in a near-sinusoidal fashion to simulate daily temperature fluctuations in the field. We chose these temperatures because the mean and variance are within the range of temperatures normally experienced by adults and pupae during breeding as verified with temperature logger data (*unpublished data*). Brood balls and adults were housed within individual 2-ounce containers filled with moist soil in incubators. We verified that the soil within the containers did not significantly insulate individuals (Fig. S1), and that the ambient temperatures within incubators reflect realized temperatures of experimental individuals. In total, we used 21 families for analyses since some of the original 31 families did not produce enough brood balls. We conducted metabolic trials on F_2 pupae (3 weeks post-egg laying) and adults (3 weeks post-eclosion) using stop-flow and flow-through respirometry, respectively (see respirometry methods below).

Do temperatures during early life stages carry-over to affect adult responses to thermal fluctuations?

We reared F_2 individuals in a low fluctuation (24 ± 4 °C) or high fluctuation (24 ± 8 °C) treatment through all egg to pupal stages. At eclosion, half of the individuals in each treatment were placed back in their temperature fluctuation treatment, while the other half were switched to the opposite temperature fluctuation treatment. This created four unique fluctuation treatment combinations: low-low, low-high, high-high, and high-low. Approximately four weeks after adult emergence (which mirrors the duration spent in the developmental treatment), we conducted open-flow respirometry trials on the adults (see respirometry methods below). This approach allowed us to examine whether thermal fluctuations during development leave a signal on adult phenotype.

Respirometry methods

We measured CO_2 of each pupa or adult at four sequential trial temperatures:15, 20, 25, and 30 °C. To do this, we used a pump (SS4, Sable Systems International, Las Vegas, NV) to push air free of CO_2 and water vapor ("Zero air"; Airgas, Knoxville, TN) through a metabolic set-up at a rate of 120 mL/min. The zero air was chemically scrubbed of CO_2 and water vapor with Ascarite (Sigma-Aldrich, St. Louis, MO) and Drierite (Xenia, OH) respectively, as an additional precaution. As such, we know any CO_2 measured downstream of the beetle was produced exclusively by the beetle. We measured CO_2 with a combination CO_2 and H_2O analyzer (LI-7000; Li-Cor, Lincoln, NE). For pupal trials, we first weighed each pupa and placed them individually in a 20 mL syringe. We perfused the syringe with zero air, sealed, and placed in an incubator at the lowest of the four trial temperatures (i.e. 15 °C) for 30 mins. After exactly 30 mins, we removed the syringes from the incubator and injected 10 mL of air from the syringe into tubing preceding the combination CO_2 and H_2O analyzer at a rate of 0.5 mL/sec to measure respiration rates. We then repeated the procedure at the next warmest trial temperature until the individual had been trialed at all four temperatures. All individuals received the same increasing temperature series across metabolic trails, as would occur naturally with diurnally increasing temperatures. Though this presents an opportunity for acclimation from previous trial temperatures, any acclimation should be similar across all groups. At the end of the trial, we again recorded mass of pupa.

For measuring respiration in adults, we first weighed an adult and then placed it in a glass chamber connected to tubing in between the flow rate pumps and the combination CO_2 and H_2O analyzer. The chamber was held in the dark to minimize adult activity, as demonstrated in pilot assays. We held the adult in the glass chamber at the lowest trial temperature (15 °C) and passed air continuously over the beetle for 15 min. Data from the last 5 min of this period were used for analyses. We then increased the incubator temperature to the next warmest trial temperature and repeated the respiration measurements. We took baseline CO_2 readings before and after each trial temperature

During baseline readings, CO_2 and H_2O values returned to zero, so any CO_2 measured during a trial reading was from the beetle currently being measured. Baseline readings also allowed us to correct for any drift in the zero air being pushed through the setup and to monitor for contamination or inconsistencies. We also weighed the adult after the metabolic trials.

We used our response variable, CO_2 production (µL/ min), to examine three parameters: metabolic rate, thermal sensitivity of metabolism, and thermal plasticity. Metabolic rate is quantified as overall CO_2 production and gauges total energetic cost. Thermal sensitivity of metabolism is the steepness of the temperature-metabolic rate function and dictates energy expenditure under temperature fluctuations. A steeper function results in greater energetic costs when temperatures fluctuate provided that the area under the curve increases (due to Jensen's inequality). Finally, thermal plasticity is the shift in thermal sensitivity between treatments. For example, a significant difference in thermal sensitivity of metabolism between adults reared in the high and low fluctuation treatments would indicate thermal plasticity. Though all three metrics are inferred from the same dataset, these are individual traits that can respond independently and may often be under unique selective pressures (Brown et al., 2004).

Data analyses:

Do life stages differ in thermal plasticity to increased temperature fluctuation?

To process metabolic data, we first corrected raw CO_2 data for any drift in baseline readings using a Catmull-Rom spline correction (Catmull and Rom, 1974) and smoothed the data using a Savitsky-Golay filter (Savitzky and Golay, 1964) with an 11-step window. We log +1 transformed all data before

analyses and verified model suitability via visual examination of residual plots. We used a general linear mixed model (Proc Mixed, SAS v 9.4) that included temperature fluctuation treatment, life stage, and metabolic trial temperature and all interactions. We also included beetle mass at the time of the trial as a covariate. We used a repeated statement specifying individual beetle as the subject to account for non-independence among an individual's four CO_2 measurements and specified an unstructured covariance matrix. The model also included the random effects of family and sex. An outlier datum from one adult's 30 °C metabolic temperature trial was removed from final analyses based on a studentized residual greater than an absolute value of 3. Removing the outlier did not change our overall conclusions (i.e. the best fit model remained the same with and without the outlier). The final model included life stage, thermal fluctuation treatment, metabolic trial temperature, and all three-way and all two-way interactions.

To better disentangle the three-way interaction, we also tested for differences in plasticity due to the temperature fluctuation treatments within a single life stage. These two follow-up models utilized a similar approach as above and corrected for multiple comparisons.

We ran a general linear model to calculate effect sizes. The model included temperature fluctuation treatment, life stage, and their interaction. We ran these models at each metabolic trial temperature separately to avoid inflating calculations, since each beetle was trialed multiple times. We determined effect sizes using Type III sums of squares and report partial ω^2 as this measure is less biased than η^2 for two-way models (Yigit and Mendes, 2018). The overall ω^2 of each model (i.e. at each metabolic trial temperature) minimally ranged from 0.68 to 0.79 at the 15 and 30 °C trial temperatures respectively, so we only report partial ω^2 of the fixed and interactive effects at the 30 °C trial temperature.

Finally, to verify that our measures of CO_2 correspond to energy expenditure, we used energy equivalents for CO_2 production to calculate energy expenditure in the situation where adults were catabolizing carbohydrates (21.1 kJ/l) and pupae were catabolizing lipids (27.8 kJ/l) (Fig. S2) (Walsberg and Hoffman, 2005). Our main conclusions did not change, and, thus, we assume CO_2 is a good proxy for energy expenditure.

Do temperatures during early life stages carry-over to affect adult plasticity and phenotype?

To answer this question, each individual was exposed to a developmental condition and an adult condition. We analyzed the data using a two-way approach so that we could parse the relative importance of developmental or adult temperature conditions to adult thermal responses. We used a similar model and selection method as above. The final model included the developmental temperature fluctuation treatment, the adult temperature fluctuation treatment, metabolic trial temperature, all possible two-way interactions, and the three-way interaction. Body mass was included as a covariate.

We ran a general linear model to calculate effect sizes as above. The model included treatment as a main effect (with four levels).

We also tested whether the temperature fluctuation treatments affected adult body size. We used a general linear model that tested for the effect of the developmental temperature treatment, adult temperature treatment, and their interaction on adult body size (mass).

Results:

Do life stages differ in thermal plasticity to increased temperature fluctuation?

Our goal was to determine if thermal plasticity differs between pupae and adults. Thermal plasticity is measured as a significant change in the CO_2 – temperature function (i.e. a shift in thermal sensitivity) between the high and low fluctuation treatments, within a life stage. In the full model, the significant three-way interaction demonstrates that life stages vary in thermal plasticity (Fig. 3) (Table 1; Life stage x thermal fluctuation treatment x metabolic trial temperature interaction: $F_{3, 77} = 5.52$, $p = 0.002$). Follow-up analyses based on two separate mixed effects models for each life stage revealed that adult beetles significantly decreased thermal sensitivity of metabolism under the high fluctuation treatment (Fig. 3 Top panel, $p = 0.02$), whereas pupae showed no change in thermal sensitivity of metabolism between the two temperature fluctuation treatments (Fig. 3 Bottom panel, $p = 0.69$). Thus, we observed plasticity in thermal sensitivity of metabolism in adult beetles, but not pupae.

In addition to examining stage-specific thermal plasticity via *shifts* in thermal sensitivity (above), our data also allowed us to examine thermal sensitivity of metabolism itself (i.e. the steepness of the temperature-metabolic rate function). Life stages differed in thermal sensitivity of metabolism; adults exhibited a steeper slope in the CO_2 production – metabolic trial temperature function than pupae (Table 1, Fig. 3; Life stage x metabolic trial temperature interaction: $F_{3, 77} = 151.16$, $p < 0.0001$). Additionally, thermal sensitivity of metabolism was lower in the high fluctuation treatment than in the low fluctuation treatment (Table 1; $F_{3, 77} = 5.05$, $p = 0.03$); however, this appears to be driven by treatment differences in the metabolism of adults and not pupae.

Finally, our model also allowed us to examine differences in metabolic rate among groups. Adult metabolic rate (CO_2 production) ranged from a low of 0.53 ± 0.03 to a high of 1.68 ± 0.18 µL/ min at trial temperatures of 15 °C and 30 °C, respectively. Pupal metabolic rate was much lower, ranging from a low

311 of 0.12 ± 0.017 to a high of 0.23 ± 0.009 µL/ min at 15 °C and 30 °C, respectively (Fig. 3). Not surprisingly,
312 the covariate of body size affected CO_2 production (Table 1).
313 The general linear model used to calculate effect sizes revealed that life stage has the largest
314 impact on CO_2 production. Effect sizes are presented as partial ω^2 (lower bound, upper bound 90%
315 confidence limits): Life stage 0.79 (0.72, 0.83); Treatment 0.05 (0.004, 0.16); Life stage by treatment 0.05
316 (0.005, 0.16).
317
318 *Do temperatures during early life stages carry-over to affect adult plasticity and phenotype?*
319
320 We wanted to understand whether temperatures during development affect adult responses to
321 temperature fluctuations. We found that temperature fluctuations experienced during development did
322 not affect metabolic rate, thermal sensitivity of metabolism, or thermal plasticity during adulthood
323 (Table 2, Fig. 4 (Top panel)). Instead, the temperature fluctuations experienced during adulthood
324 triggered differential thermal sensitivity of metabolism (adult treatment x metabolic trial temperature:
325 $F_{3,65} = 3.45$, $p = 0.02$), where beetles that experienced the high fluctuation treatment during adulthood
326 (regardless of developmental treatment) exhibited lower thermal sensitivity of metabolism than beetles
327 that experienced the low fluctuation treatment as adults (Fig. 4 Top panel). Metabolic rate was also
328 marginally affected by the adult fluctuation treatment ($F_{1,65} = 4.08$, $p = 0.048$). Specifically, adults that
329 experienced the high temperature fluctuations during adulthood (regardless of developmental
330 conditions), had lower overall metabolic rates than beetles that experienced the low fluctuation
331 treatment during adulthood (Fig. 4 Top panel). The partial ω^2 effect size for the main effect of
332 treatment (i.e. four development x adult temperature treatment combinations) was -0.016 (0.0, 0.08).
333

We examined whether temperature fluctuations affect adult mass. On average, beetles from the low temperature fluctuation treatments weighed 0.095 ± 0.003 g, whereas, beetles from the high temperature fluctuation treatment were 14% smaller, weighing 0.082 ± 0.003 g. Beetles that experienced high temperature fluctuation conditions during development were significantly smaller than those that developed in the low temperature fluctuation treatment (Fig. 4 Bottom panel; Developmental treatment: $F_{1, 65} = 8.16$, $p = 0.006$). Though structural body size is fixed at eclosion, mass can change slightly due to feeding post-eclosion. Thus, we also wanted to determine if the temperature fluctuation treatment experienced during adulthood impacted adult mass. The adult treatment did not affect mass ($F_{1, 65} = 0.98$, $p = 0.33$) nor did the interaction between adult and developmental treatments ($F_{1, 65} = 0.04$ $p = 0.85$).

Discussion

Life stages may experience varying selective pressures from stage-specific environments, and thus, thermal plasticity may differ across an individual's lifetime. As predicted, we found that adults mount plastic responses to increased temperature fluctuation and pupae do not. However, pupae were less thermally sensitive (on average) and, not surprisingly, exhibited lower metabolic rates. Coupled with the broad thermal tolerance of pupae reported elsewhere (Klockmann et al., 2017; Moghadam et al., 2019; Pincebourde and Casas, 2015), our data suggest that while adults may rely on thermal plasticity to cope with thermal variation, pupae may alternatively rely on a broad thermal tolerance and low overall metabolic rate to cope with daily temperature variation. It is unclear if these varying strategies will provide equal protection under future, more variable climates. It is worth noting that, while the amount of exposure to the temperature treatments was similar across developmental and adult stages (i.e. 3 weeks for each), the exposure strictly during the pupal stage was less (~5-7 days) than the exposure during the adult stage since the development also includes egg and larval stages. It is possible that these

differences in stage-specific exposure contribute to differences in thermal plasticity between adults and pupae; however, since we tested mature pupae, our data reflect the maximum acclimation of pupae at these temperatures. Nonetheless, the stage-specific differences herein underscore that the physiology of one life stage should not be used to more generally predict responses across life stages.

The thermal plasticity exhibited by adults is in response to the temperatures experienced during adulthood (i.e. acclimation) rather than during development. Beetles that experienced high temperature fluctuations during adulthood, regardless of developmental conditions, exhibited lower thermal sensitivity of metabolism and lower metabolic rates. This suggests that adult thermal plasticity and metabolism are decoupled from the environment and physiology of egg to pupal stages. This is surprising given the wealth of data linking constant developmental temperatures to variation in adult phenotype in insects (Angilletta, 2009; Atkinson, 1994; Chown and Terblanche, 2006; Gray, 2013). However, it's possible more extreme fluctuations than those used in our study are required to leave a lasting signal on adult phenotype in *O. taurus.*

We found that adult body size decreased with increasing temperature fluctuations during development, demonstrating that adult phenotype is not wholly independent from the thermal environment of previous stages. Increased temperature fluctuations can increase energetic demands (Ruel and Ayres, 1999; Williams et al., 2012). Though we found no evidence of metabolic compensation in early life stages to reduce these energetic demands, our body size data suggests individuals developing in the high fluctuation treatments had fewer energetic resources available for growth and thus, were smaller compared to individuals that developed in the low fluctuation treatment. Since mass was measured three weeks after eclosion, it is possible that differences in the adult stage between treatments, like feeding rates, may contribute to mass differences; however, we did not find any effect

382 of the adult environment on adult mass. Therefore, any systematic differences in feeding in the adult life
383 stage would most likely be triggered by differences in the developmental environment. Our data more
384 broadly suggest that some aspects of adult phenotype may be decoupled from previous life stages while
385 others may not.
386
387 We found that adults exhibited thermal plasticity in response to increased temperature
388 fluctuation by decreasing thermal sensitivity of metabolism. Reducing thermal sensitivity should reduce
389 energetic costs under variable temperatures (due to Jensen's inequality) (Jensen, 1906; Ruel and Ayres,
390 1999), helping beetles conserve energy for other energetically costly activities like searching for dung
391 and mates, reproducing, and mounting immune responses. Previous work in insects has shown
392 decreased thermal sensitivity following acclimation to increased thermal variation (Bozinovic et al.,
393 2013; Williams et al., 2012), suggesting this may be a common plastic response. Our findings
394 demonstrate that even though the capacity to reduce thermal sensitivity may be broadly present across
395 insects, it is not necessarily equivalent across each life stage. More research is needed to discern if the
396 magnitude of adult thermal plasticity can compensate for increased energetic demands under
397 fluctuating environments (Gunderson and Stillman, 2015; Williams et al., 2012).
398
399 Integrating life stage variation in thermal biology has important implications for predicting the
400 impacts of global change (Levy et al., 2015). For example, analyses that accommodate thermal variation
401 suggest that climate change may decrease the fitness of tropical insects and increase the fitness of
402 temperate insects (Deutsch et al., 2008). However, when age-dependent thermal tolerance is included
403 in these models, predictions suggest that temperate species should also experience decreased fitness
404 (Kingsolver et al., 2011). While these examples highlight the necessity of considering age-dependent
405 thermal tolerances, few analyses incorporate the potentially ameliorating effects of thermal plasticity

(Seebacher et al., 2015), and none in a life stage-dependent manner. Existing models that test if plasticity will aid in species persistence come to conflicting conclusions (Gunderson and Stillman, 2015; Seebacher et al., 2015). A demographic model that includes thermal tolerance and thermal plasticity in a life stage-dependent manner (Sinclair et al., 2016), though complex, may help resolve climate change predictions across latitude and taxa.

Acknowledgments

We thank J.M. Fleming and M.A. Mamantov for assistance with field work and laboratory rearing and S. Proffitt and Cruz Dairy Farm for allowing access to property for collecting beetles and cow dung. This research was supported by the US National Science Foundation [Postdoctoral Research Fellowship in Biology grant number 811881 to A.W.C.; Integrative Organismal Systems grant number #1930829 to K.S.S.] The authors have no conflicts of interest to declare.

Data Availability

Data will be made available upon reasonable request.

References:

Angilletta, M. J. (2009). *Thermal Adaptation: A Theoretical and Empirical Synthesis*. OUP Oxford.

Atkinson, D. (1994). Temperature and organism size: a biological law for ectotherms? *Adv Ecol Res* **25**, 1–58.

Beaman, J. E., White, C. R. and Seebacher, F. (2016). Evolution of Plasticity: Mechanistic Link between Development and Reversible Acclimation. *Trends in Ecology & Evolution* **31**, 237–249.

Beldade, P., Mateus, A. R. A. and Keller, R. A. (2011). Evolution and molecular mechanisms of adaptive developmental plasticity. *Molecular Ecology* **20**, 1347–1363.

Bogert, C. M. (1949). THERMOREGULATION IN REPTILES, A FACTOR IN EVOLUTION. *Evolution* **3**, 195–211.

Bozinovic, F., Catalan, T. P., Estay, S. A. and Sabat, P. (2013). Acclimation to daily thermal variability drives the metabolic performance curve. *Evolutionary Ecology Research* **15**, 579–587.

Brown, J. H., Gillooly, J. F., Allen, A. P., Savage, V. M. and West, G. B. (2004). Toward a metabolic theory of ecology. *Ecology* **85**, 1771–1789.

Burton, T., Killen, S. S., Armstrong, J. D. and Metcalfe, N. B. (2011). What causes intraspecific variation in resting metabolic rate and what are its ecological consequences? *Proceedings of the Royal Society B: Biological Sciences* **278**, 3465–3473.

Catmull, E. and Rom, R. (1974). A class of local interpolating splines. In *Computer aided geometric design*, pp. 317–326. Elsevier.

Chiu, M.-C., Kuo, J.-J. and Kuo, M.-H. (2015). Life stage-dependent effects of experimental heat waves on an insect herbivore: Demographic effects of heat waves. *Ecological Entomology* **40**, 175–181.

Chown, S. L. and Gaston, K. J. (1999). Exploring links between physiology and ecology at macro-scales: the role of respiratory metabolism in insects. *Biological Reviews* **74**, 87–120.

Chown, S. L. and Nicolson, S. (2004). *Insect physiological ecology: mechanisms and patterns*. Oxford University Press.

Chown, S. L. and Terblanche, J. S. (2006). Physiological diversity in insects: ecological and evolutionary contexts. *Advances in insect physiology* **33**, 50–152.

Colinet, H., Sinclair, B. J., Vernon, P. and Renault, D. (2015). Insects in fluctuating thermal environments. *Annual Review of Entomology* **60**, 123–140.

Deutsch, C. A., Tewksbury, J. J., Huey, R. B., Sheldon, K. S., Ghalambor, C. K., Haak, D. C. and Martin, P. R. (2008). Impacts of climate warming on terrestrial ectotherms across latitude. *PNAS* **105**, 6668–6672.

Dillon, M. E., Wang, G. and Huey, R. B. (2010). Global metabolic impacts of recent climate warming. *Nature* **467**, 704.

Fischer, B., van Doorn, G. S., Dieckmann, U. and Taborsky, B. (2014). The Evolution of Age-Dependent Plasticity. *The American Naturalist* **183**, 18.

Gray, E. M. (2013). Thermal acclimation in a complex life cycle: The effects of larval and adult thermal conditions on metabolic rate and heat resistance in Culex pipiens (Diptera: Culicidae). *Journal of Insect Physiology* **59**, 1001–1007.

Gunderson, A. R. and Stillman, J. H. (2015). Plasticity in thermal tolerance has limited potential to buffer ectotherms from global warming. *Proceedings of the Royal Society B: Biological Sciences* **282**, 20150401.

Halffter, G. and Edmonds, W. D. (1982). The nesting behavior of dung beetles (Scarabaeinae). An ecological and evolutive approach. *The nesting behavior of dung beetles (Scarabaeinae). An ecological and evolutive approach.*

Jensen, J. L. W. V. (1906). Sur les fonctions convexes et les inégalités entre les valeurs moyennes. *Acta mathematica* **30**, 175–193.

Kingsolver, J. G. (2009). The Well-Temperatured Biologist: (American Society of Naturalists Presidential Address). *The American Naturalist* **174**, 755–768.

Kingsolver, J. G. and Huey, R. B. (1998). Evolutionary Analyses of Morphological and Physiological Plasticity in Thermally Variable Environments. *Am Zool* **38**, 545–560.

Kingsolver, J. G. and Huey, R. B. (2008). Size, temperature, and fitness: three rules. *Evolutionary Ecology Research* **10**, 251–268.

Kingsolver, J. G., Arthur Woods, H., Buckley, L. B., Potter, K. A., MacLean, H. J. and Higgins, J. K. (2011). Complex Life Cycles and the Responses of Insects to Climate Change. *Integr Comp Biol* **51**, 719–732.

Klockmann, M., Günter, F. and Fischer, K. (2017). Heat resistance throughout ontogeny: body size constrains thermal tolerance. *Global Change Biology* **23**, 686–696.

Lake, S. L., MacMillan, H. A., Williams, C. M. and Sinclair, B. J. (2013). Static and dynamic approaches yield similar estimates of the thermal sensitivity of insect metabolism. *Journal of insect physiology* **59**, 761–766.

Lande, R. (2014). Evolution of phenotypic plasticity and environmental tolerance of a labile quantitative character in a fluctuating environment. *Journal of Evolutionary Biology* **27**, 866–875.

Levy, O., Buckley, L. B., Keitt, T. H., Smith, C. D., Boateng, K. O., Kumar, D. S. and Angilletta Jr, M. J. (2015). Resolving the life cycle alters expected impacts of climate change. *Proceedings of the Royal Society B: Biological Sciences* **282**, 20150837.

Lighton, J. R. (2018). *Measuring metabolic rates: a manual for scientists*. Oxford University Press.

Marais, E. and Chown, S. L. (2008). Beneficial acclimation and the Bogert effect. *Ecology Letters* **11**, 1027–1036.

Metcalfe, N. B., Taylor, A. C. and Thorpe, J. E. (1995). Metabolic rate, social status and life-history strategies in Atlantic salmon. *Animal Behaviour* **49**, 431–436.

Mitchell, K. A., Sinclair, B. J. and Terblanche, J. S. (2013). Ontogenetic variation in cold tolerance plasticity in Drosophila: is the Bogert effect bogus? *Naturwissenschaften* **100**, 281–284.

Moczek and Emlen (1999). Proximate determination of male horn dimorphism in the beetle Onthophagus taurus (Coleoptera: Scarabaeidae). *Journal of Evolutionary Biology* **12**, 27–37.

Moghadam, N. N., Ketola, T., Pertoldi, C., Bahrndorff, S. and Kristensen, T. N. (2019). Heat hardening capacity in Drosophila melanogaster is life stage-specific and juveniles show the highest plasticity. *Biol. Lett.* **15**, 20180628.

Moran, N. A. (1994). Adaptation and constraint in the complex life cycles of animals. *Annual Review of Ecology and Systematics* **25**, 573–600.

Niehaus, A. C., Angilletta, M. J., Sears, M. W., Franklin, C. E. and Wilson, R. S. (2012). Predicting the physiological performance of ectotherms in fluctuating thermal environments. *Journal of Experimental Biology* **215**, 694–701.

Norin, T., Malte, H. and Clark, T. D. (2016). Differential plasticity of metabolic rate phenotypes in a tropical fish facing environmental change. *Funct Ecol* **30**, 369–378.

Pincebourde, S. and Casas, J. (2015). Warming tolerance across insect ontogeny: influence of joint shifts in microclimates and thermal limits. *Ecology* **96**, 986–997.

Potter, K. A., Davidowitz, G. and Woods, H. A. (2011). Cross-stage consequences of egg temperature in the insect Manduca sexta. *Functional Ecology* **25**, 548–556.

Radchuk, V., Turlure, C. and Schtickzelle, N. (2013). Each life stage matters: the importance of assessing the response to climate change over the complete life cycle in butterflies. *Journal of Animal Ecology* **82**, 275–285.

Ruel, J. J. and Ayres, M. P. (1999). Jensen's inequality predicts effects of environmental variation. *Trends in Ecology & Evolution* **14**, 361–366.

Savitzky, A. and Golay, M. J. (1964). Smoothing and differentiation of data by simplified least squares procedures. *Analytical chemistry* **36**, 1627–1639.

Scott, G. R. and Johnston, I. A. (2012). Temperature during embryonic development has persistent effects on thermal acclimation capacity in zebrafish. *Proceedings of the National Academy of Sciences* **109**, 14247–14252.

Seebacher, F., White, C. R. and Franklin, C. E. (2015). Physiological plasticity increases resilience of ectothermic animals to climate change. *Nature Climate Change* **5**, 61–66.

Sheldon, K. S., Huey, R. B., Kaspari, M. and Sanders, N. J. (2018). Fifty Years of Mountain Passes: A Perspective on Dan Janzen's Classic Article. *The American Naturalist* **191**, 553–565.

Sibly, R. M., Brown, J. H. and Kodric-Brown, A. (2012). *Metabolic ecology: a scaling approach*. John Wiley & Sons.

Sinclair, B. J., Marshall, K. E., Sewell, M. A., Levesque, D. L., Willett, C. S., Slotsbo, S., Dong, Y., Harley, C. D. G., Marshall, D. J., Helmuth, B. S., et al. (2016). Can we predict ectotherm responses to climate change using thermal performance curves and body temperatures? *Ecol Lett* **19**, 1372–1385.

Sørensen, J. G., Schou, M. F., Kristensen, T. N. and Loeschcke, V. (2016). Thermal fluctuations affect the transcriptome through mechanisms independent of average temperature. *Scientific reports* **6**, 30975.

Stillwell, R. C. and Fox, C. W. (2005). Complex Patterns of Phenotypic Plasticity: Interactive Effects of Temperature During Rearing and Oviposition. *Ecology* **86**, 924–934.

Stoks, R. and Cordoba-Aguilar, A. (2012). Evolutionary ecology of Odonata: a complex life cycle perspective. *Annual review of entomology* **57**, 249–265.

Uller, T. (2008). Developmental plasticity and the evolution of parental effects. *Trends in ecology & evolution* **23**, 432–438.

Walsberg, G. E. and Hoffman, T. C. (2005). Direct calorimetry reveals large errors in respirometric estimates of energy expenditure. *Journal of Experimental Biology* **208**, 1035–1043.

West-Eberhard, M. J. (2003). *Developmental plasticity and evolution*. Oxford University Press.

Williams, C. M., Marshall, K. E., MacMillan, H. A., Dzurisin, J. D. K., Hellmann, J. J. and Sinclair, B. J. (2012). Thermal Variability Increases the Impact of Autumnal Warming and Drives Metabolic Depression in an Overwintering Butterfly. *PLOS ONE* **7**, e34470.

Woods, H. A. (2013). Ontogenetic changes in the body temperature of an insect herbivore. *Funct Ecol* **27**, 1322–1331.

Woods, H. A. and Harrison, J. F. (2002). INTERPRETING REJECTIONS OF THE BENEFICIAL ACCLIMATION HYPOTHESIS: WHEN IS PHYSIOLOGICAL PLASTICITY ADAPTIVE? *Evolution* **56**, 1863–1866.

Yigit, S. and Mendes, M. (2018). Which effect size measure is appropriate for one-way and two-way ANOVA models? A Monte Carlo simulation study. *Revstat Statistical Journal* **16**, 295–313.

Table 1. Do life stages differ in thermal plasticity to increased temperature fluctuation?

Effect	*df num, den*	*F-value*	*p-value*
Life Stage X Temperature fluctuation treatment X Metabolic trial temperature	3, 77	5.52	0.002
Life Stage X Metabolic trial temperature	3, 77	151.68	<0.0001
Temperature fluctuation treatment X Metabolic trial temperature	3, 77	5.05	0.003
Life Stage X Temperature fluctuation treatment	1, 77	4.84	0.031
Life Stage	1, 77	368.92	<0.0001
Temperature fluctuation treatment	1, 77	5.16	0.026
Metabolic trial temperature	3, 77	235.95	<0.0001
Body size (g)	1, 77	20.74	<0.0001

Note: Response variable is CO_2 production.

Table 2. Do temperature fluctuations during early life stages carry-over to affect adult plasticity and phenotype?

Effect	*df num, den*	*F-value*	*p-value*
Developmental treatment X Adult treatment X Metabolic trial temperature	3, 65	0.14	0.936
Adult treatment X Metabolic trial temperature	3, 65	3.45	0.022
Developmental treatment X Metabolic trial temperature	3, 65	1.31	0.278
Developmental treatment X Adult treatment	1, 65	0.18	0.674
Adult treatment	1, 65	4.08	0.048
Developmental treatment	1, 65	0.91	0.343
Metabolic trial temperature	3, 65	207.54	<0.0001
Body size (g)	1, 65	25.78	<0.0001

Note: Response variable is CO2 production.

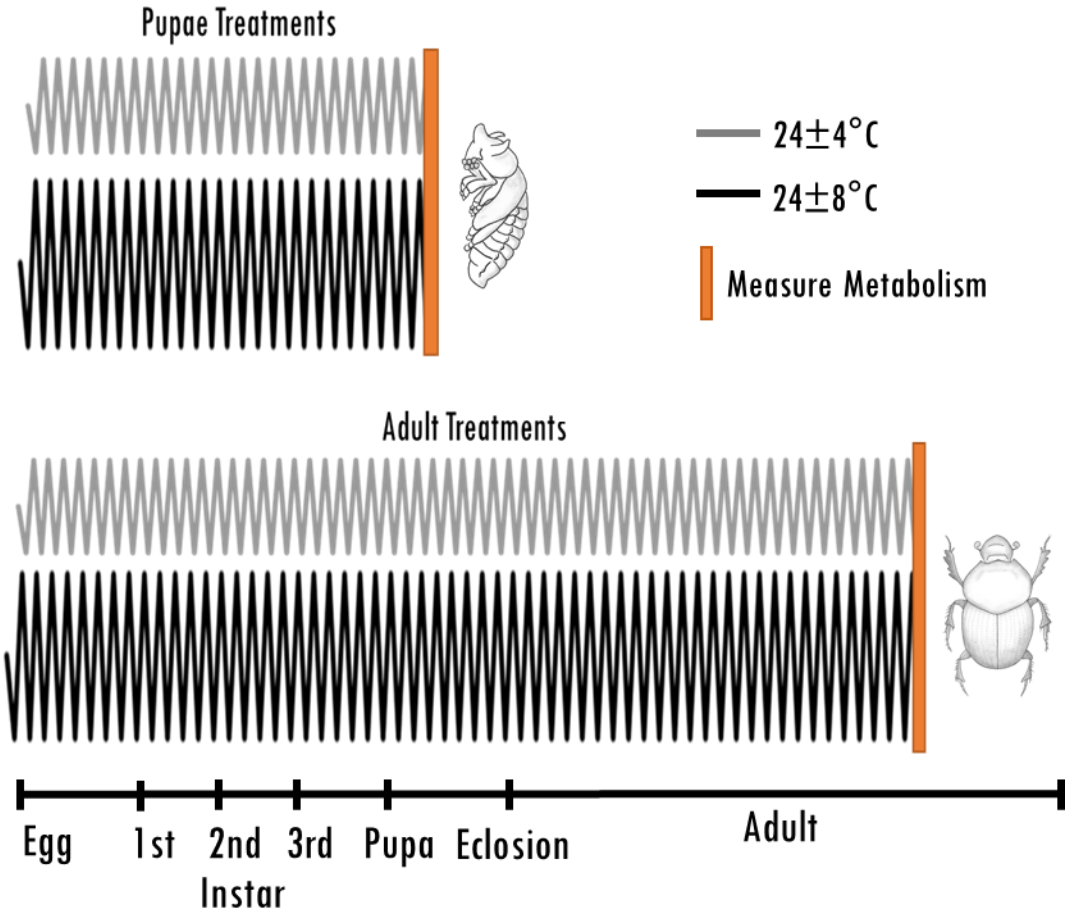


Figure 1. Do life stages differ in thermal plasticity to increased temperature fluctuation? Using a full-sibling design, the F_2 generation of *O. taurus* brood balls were placed into an incubator running either a low or high temperature fluctuation condition during the egg stage. At either the pupal or adult stage, we used respirometry to test for life stage differences in thermal plasticity, thermal sensitivity of metabolism, and metabolic rate.

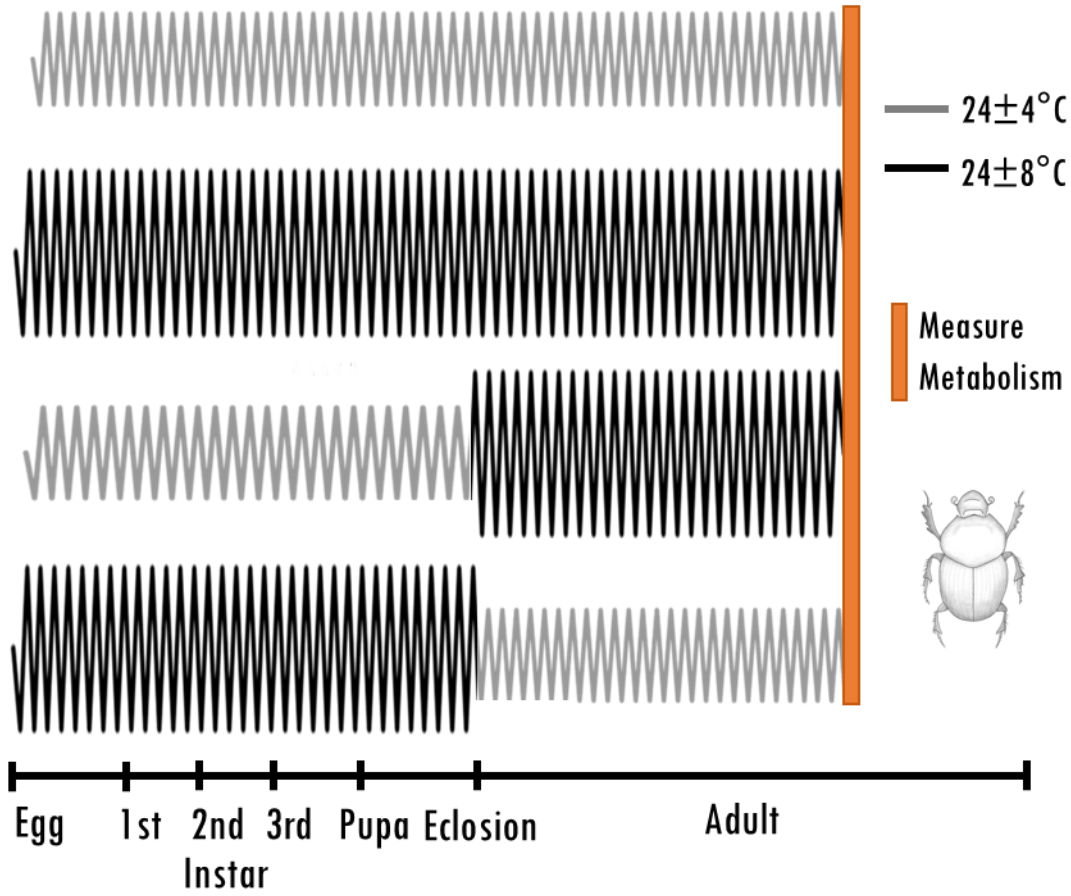
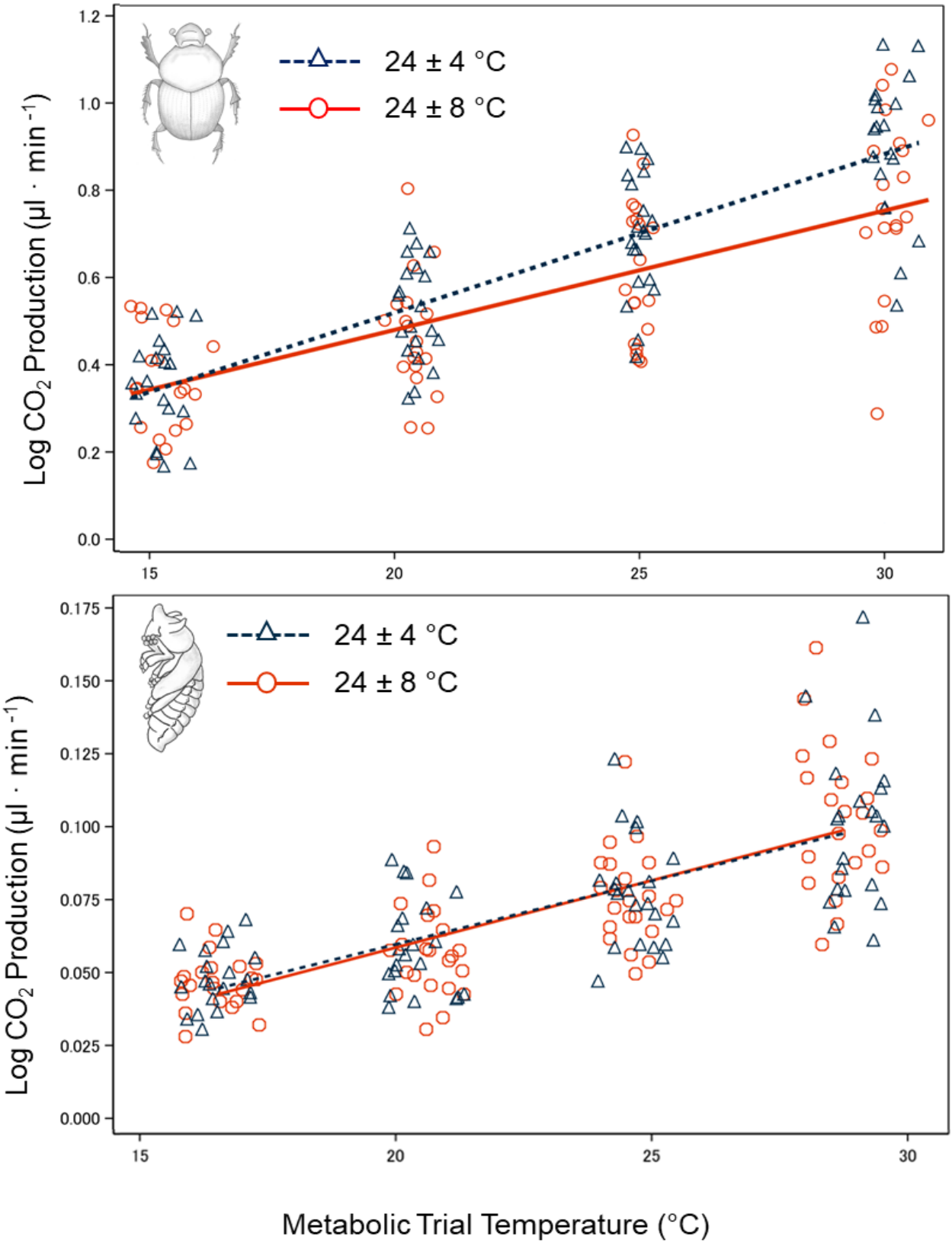
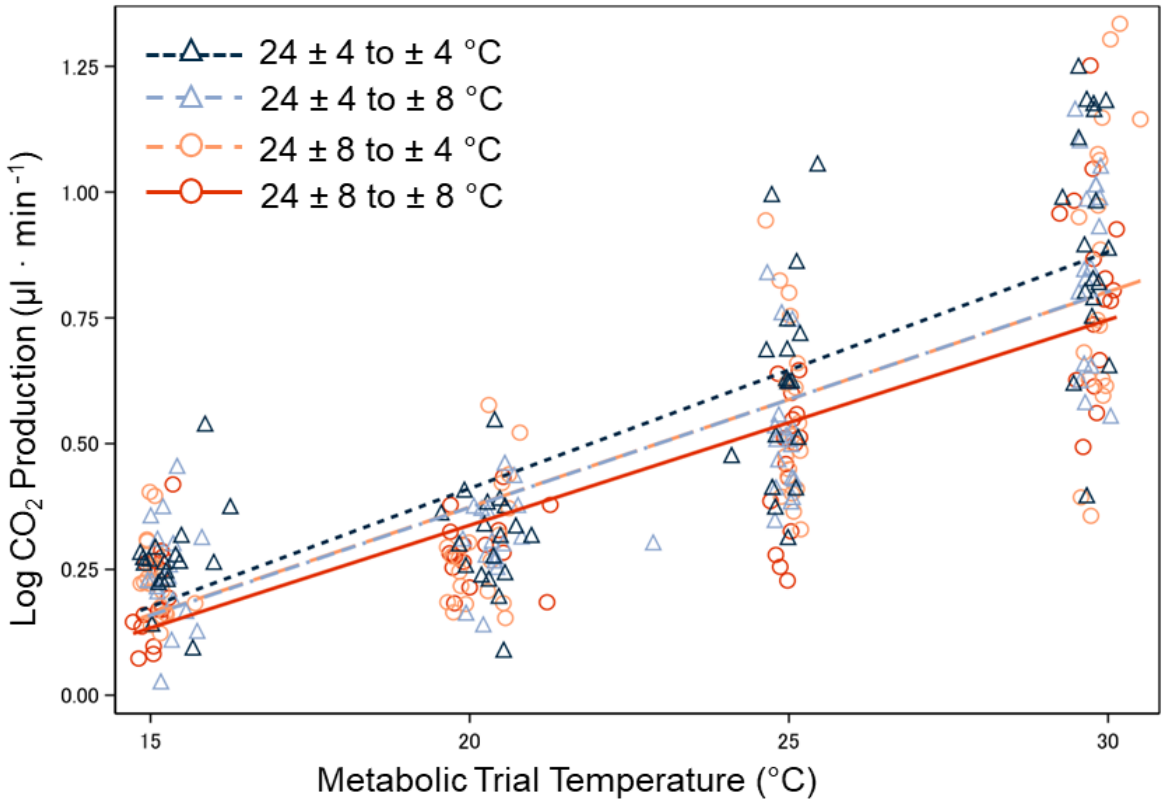


Figure 2. Do temperatures during early life stages carry-over to affect adult plasticity and phenotype? We reared the F_2 generation of *O. taurus* individuals from the egg stage through the pupal stage at either a low or high temperature fluctuation condition. At eclosion, half of the individuals in each treatment were switched to the opposite temperature fluctuation condition and remained there for an equivalent duration as development (approximately four weeks). Then, we used respirometry to test for treatment differences in thermal plasticity, thermal sensitivity of metabolism, and metabolic rate of adults. At this time, we also measured adult body mass to determine if temperature fluctuations during development affect this trait.


24 ± 4 °C
24 ± 8 °C
Log CO_2 Production (µl · min^{-1})
1.2
1.0
0.8
0.6
0.4
0.2
0.0
15
20
25
30
24 ± 4 °C
24 ± 8 °C
Log CO_2 Production (µl · min^{-1})
0.175
0.150
0.125
0.100
0.075
0.050
0.025
0.000
15
20
25
30
Metabolic Trial Temperature (°C)

29

Figure 3. Life stages differ in thermal plasticity as demonstrated by a significant shift in thermal sensitivity of metabolism between temperature fluctuation treatments in adults, but not pupae. The top panel shows adult data and the bottom panel shows pupal data. The low temperature fluctuation treatment is depicted in navy with a dashed line and navytriangles. The high temperature fluctuation treatment is depicted in red with a solid line and red circles. Each point represents the CO_2 production of an individual at that temperature (see methods for details on adult and pupal respirometry) and are jittered along the x-axis to better display overlapping points.


24 ± 4 to ± 4 °C
24 ± 4 to ± 8 °C
24 ± 8 to ± 4 °C
24 ± 8 to ± 8 °C
Log CO_2 Production (µl · min^{-1})
1.25
1.00
0.75
0.50
0.25
0.00
15
20
25
30
Metabolic Trial Temperature (°C)

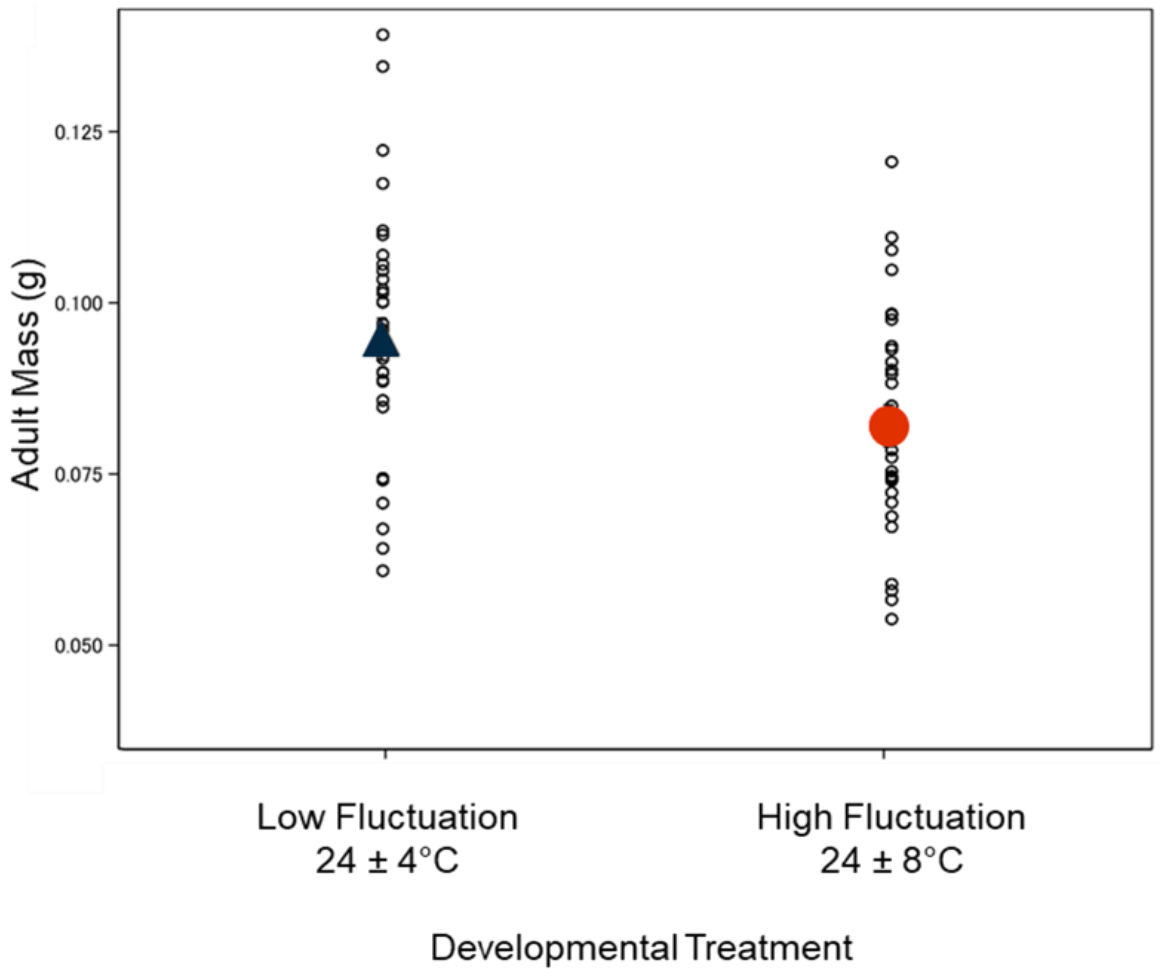

Adult Mass (g)
0.125
0.100
0.075
0.050
Low Fluctuation
24 ± 4°C
High Fluctuation
24 ± 8°C
Developmental Treatment

Figure 4. Temperatures during early life stages carry-over to affect adult body size but not thermal plasticity. The top and bottom panels show adult thermal plasticity and body size, respectively, in relation to different temperature treatments experienced during development. For thermal plasticity (top panel), the temperature fluctuation treatments included high-high (red circles and solid red line), low-low (navy triangles and dotted navy line, high-low (orange circles and orange dashed line), low-high treatment (light blue triangles and dashed light blue line). The bottom panel shows adult body mass (least square mean ± 1 S. E.) as a function of the developmental temperature condition.