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Developmental and Immediate Thermal Environments Shape Energetic Trade-Offs, Growth Efficiency, and Metabolic Rate in Divergent Life-History Ecotypes of the Garter Snake *Thamnophis elegans*

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ABSTRACT

Interactions at all levels of ecology are influenced by the rate at which energy is obtained, converted, and allocated. Trade-offs in energy allocation within individuals in turn form the basis for life-history theory. Here we describe tests of the influences of temperature, developmental environment, and genetic background on measures of growth efficiency and resting metabolic rate in an ectothermic vertebrate, the western terrestrial garter snake (*Thamnophis elegans*). After raising captive-born snakes from divergent life-history ecotypes on thermal regimes mimicking natural habitat differences (2 × 2 experimental design of ecotype and thermal environment), we measured oxygen consumption rate at temperatures spanning the activity range of this species. We found ecotypic differences in the reaction norms of snakes across the measured range of temperatures and a temperature-dependent allometric relationship between mass and metabolic rate predicted by the metabolic-level boundaries hypothesis. Additionally, we present evidence of within-individual trade-offs between growth efficiency and resting metabolic rate, as predicted by classic life-history theory. These observations help illuminate the ultimate and proximate factors that underlie variation in these interrelated physiological and life-history traits.

Keywords: metabolic scaling, oxygen consumption rate, developmental plasticity, western terrestrial garter snake.

Introduction

Metabolic rate, as a measure of how quickly individual organisms process energy, is central to interactions at all levels of ecology (Kooijman 2000; Brown et al. 2004; Kearney and White 2012; Iles 2014; Maino et al. 2014). In turn, trade-offs in energetic allocation among growth, reproduction, and maintenance compose the basis for life-history theory (Roff 1992; Stearns 1992). Despite decades of empirical and theoretical work, we still lack comprehensive models of the intrinsic and extrinsic drivers of variation in metabolic rate, including the influences of biophysical constraints, ecological heterogeneity, and natural selection (Burton et al. 2011; Reid et al. 2011; White and Kearney 2013). Even the effects of the three primary determinants of metabolic rate—body size, temperature, and ecological factors—remain unclear and at times debated (Gillooly et al. 2001; Agutter and Wheatley 2004; White et al. 2006). Here, we test the influences of genetic background, physiological plasticity, and temperature on growth and metabolic rate in western terrestrial garter snakes (*Thamnophis elegans*) from natural populations. Our results provide empirical clarity on the complex interactions that drive variation in how organisms process energy in their environment.

Populations of the western terrestrial garter snake (*T. elegans*) near Eagle Lake, California, have provided a natural laboratory for examining the physiological mechanisms and ecological causes of divergent life-history evolution. Here, distinct ecotypes at differing positions along the pace-of-life continuum (Ricklefs and Wikelski 2002) have emerged in replicate populations along the lakeshore and in the surrounding mountain meadows (Bronikowski and Arnold 1999; Bronikowski 2000). The lakeshore populations (hereafter, L-fast) grow fast, are short-lived, reproduce yearly or biennially, and demonstrate greater fecundity per reproductive bout; the meadow populations (hereafter, M-slow) grow slowly, are long-lived, reproduce intermittently, and produce fewer offspring per reproductive bout (see detailed descriptions in Sparkman et al. 2007; Bronikowski and Vleck 2010; Schwartz and Bronikowski 2011). This divergence in life-history strategies, despite significant gene flow between populations (Manier and Arnold 2005, 2006), is putatively driven by differing thermal regimes (Bronikowski 2000), resource availability (Bronikowski and Arnold 1999; Miller et al. 2011), and predation rates (Sparkman et al. 2013). Studies in this system have demonstrated ecotypic differences in immune investment (Sparkman and Palacios 2009; Palacios et al. 2011), endocrine function (Sparkman et al. 2009; Palacios et al. 2012), cellular repair efficiency (Robert and Bronikowski 2010), and stress response pathways (Schwartz and Bronikowski 2012). Adult L-fast snakes have higher resting metabolic rates than M-slow snakes across 15°–32°C (Bronikowski and Vleck 2010), but this ecotypic difference was not present in month-old snakes reared under common laboratory conditions (Robert and Bronikowski 2010),

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which suggests that developmental habitats have permanent effects of metabolic rate. Here, we test the metabolic consequences of variation in early life thermal environment, using a 2 × 2 experimental design of captive-born neonatal garter snakes of both ecotypes reared under thermal conditions designed to mimic field ecological differences. We report measures of the resting metabolic rate of individual snakes at four temperatures spanning the natural activity range of this species, first-year growth, and growth efficiency (defined as increase in size per unit of food consumed; Ivlev 1945; Czarnołęski et al. 2008). Because temperature affects metabolic rate and, concomitantly, energy intake and growth, we are able to test for plasticity and subsequent developmental canalization of metabolic pathways resulting from early life-history experiences.

Many ectotherms exhibit some modulation of the temperature dependency of metabolism (in snakes: Aleksiuk 1971; Seidel and Lindeborg 1973; Davies et al. 1981; reviewed in Glazier 2005, 2010, 2015). Nonavian reptiles in particular demonstrate great variation in thermal physiology across latitudinal clines, which has been interpreted as an adaptation to climate (reviewed in Kingsolver and Huey 1998; Angilletta et al. 2003). In snakes, this is generally manifest as populations from higher latitudes (or otherwise cooler climates) having higher metabolic rates than conspecifics and congeners from lower latitudes across a range of active temperatures (Aleksiuk 1971; Davies and Bennett 1981; Zaidan 2003). Temperature-driven differences among populations in how energy is allocated at different life stages can in turn drive geographic variation in life-history strategies (e.g., Angilletta 2001). Maintenance metabolism, the energy required for an organism to maintain biological steady state in the absence of growth, reproduction, or locomotion, is a significant component of the energy budget of animals. Even in ectothermic reptiles, where this cost is greatly reduced compared with endotherms, maintenance metabolism may account for more than a third of an individual's yearly energy budget (Congdon and Tinkle 1982; Secor and Nagy 1994; Beaupre 1995). Only after the obligatory costs of maintenance metabolism are met can an individual allocate energy to activity, growth, or reproduction (Steyermark and Spotila 2000; Angilletta 2001).

The components of an individual's energy budget, including maintenance, growth, and reproduction, compete with each other and are thus subject to trade-offs (Gadgil and Bossert 1970). We might expect to observe such trade-offs, however, only when the variance in resource allocation exceeds that of acquisition (van Noordwijk and de Jong 1986; Glazier 1999). Assuming constant acquisition rates, high maintenance metabolism should be associated with reduced energy allocated to other functions (Kooijman 2000), while lowered maintenance metabolic costs should be associated with higher production rates and the attainment of higher growth rates (Danzmann et al. 1987; Killen 2014). In captive-born juveniles, where energy is not yet being allocated to reproduction while acquisition rates are controlled or statistically accounted for, the trade-off between maintenance and growth should be most prominent (Glazier 1999). Laboratory studies controlling for resource availability have found both negative (e.g., Danzmann et al. 1987) and positive (e.g., Hoogenboom et al. 2012) relationships between growth and metabolic rate (reviewed in Glazier 2015). Taken together, these studies indicate that a single optimal metabolic rate may not exist and that fluctuations in resource availability may maintain metabolic polymorphisms within populations (Burton et al. 2011; Reid et al. 2011).

Additionally, we explore ecological and thermal effects on the relationship between metabolic rate and body mass. This relationship is commonly described as the slope of the log-log plot of oxygen consumption rate ($\dot{V}O_2$) on body mass (the scaling exponent b in the power function $R = aM^b$, where R is metabolic rate, M is body mass, and a is a scaling coefficient). A negative allometric relationship ($b < 1$), as is predicted by theory and most commonly found in empirical studies, describes increased energetic efficiency with increasing body size and therefore has direct implications in trade-offs between growth and other energetic demands (Carey et al. 2013). For decades, researchers have debated the universality of the scaling exponent (b), arguing that this value is determined by the physical properties of biological systems and thus is constant across the diversity of life with a value of 2/3 (Rubner 1883) or 3/4 (Kleiber 1932). Empirical studies in snakes have found evidence that extrinsic factors affect metabolic scaling (Buikema and Armitage 1969; Vinegar et al. 1970; Dmi'el 1972; Davies and Bennett 1981), but these studies have been relatively unappreciated. Recently, tests in a wide range of taxa have found support for variation in scaling exponents, leading to a refutation of such universal scaling laws (reviewed in Glazier 2005, 2010).

This experiment tested three primary hypotheses of potential drivers of variation in metabolic rate within a framework of divergent life histories. First, we tested the relative influences of genetic background (maternal ecotype) and environmental conditions (rearing treatment) and their interaction on metabolic rate and growth efficiency, the growth rate accounting for the amount of food consumed. As found previously (Bronikowski 2000), we expect snakes from the L-fast populations to exhibit higher growth rates than M-slow snakes regardless of rearing conditions. A significant effect of rearing treatment on metabolic rate or a significant interaction between ecotype and rearing treatment implies that metabolic rate is developmentally plastic (and possibly adaptive, depending on the direction of the interaction). Second, we tested for evidence of within-individual trade-offs between maintenance metabolism and first-year growth efficiency. While we might expect a positive relationship between growth and metabolic rate in actively growing snakes (Peterson et al. 1999), at the time we measured metabolic rate snakes were fasting and not actively growing. With this in mind, we hypothesize that there will be a trade-off between maintenance metabolism and first-year growth efficiency: those individuals that expend more energy on metabolic rate (after correcting for body size) will have grown less in their first year, accounting for the amount of food consumed. Third, we tested the effects of ecotype and rearing treatment on the metabolic scaling exponent as well as the temperature dependence of the relationship between body mass and metabolic rate. These empirical tests of the complex proximate and ultimate factors determining growth rate,

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metabolic rate, and metabolic scaling in the context of differing life-history strategies provides a solid platform for understanding the adaptive significance and potential of these traits.

Material and Methods

Study Organisms

In June 2010, gravid females were collected from eight populations of *Thamnophis elegans* around Eagle Lake, California—four replicate populations each of the two ecotypes, L-fast ($n = 22$) and M-slow ($n = 22$). These eight populations are representative of the approximately 35 populations in the vicinity (1–25 km) of Eagle Lake. Within this metapopulation, gene flow is such that neighboring populations within each ecotype show evidence of plentiful gene flow, whereas populations of the contrasting ecotypes have significantly diverged at neutral loci (Manier and Arnold 2005; Manier et al. 2007). Gravid females were brought back to the laboratory colony at Iowa State University and individually maintained in 10-gal glass aquariums with ground corn cob substrate and a plastic bowl that served as both water dish and retreat site. They were placed on a thermal gradient for 24 h per day (range: 25°–34°C), kept on a 12 : 12 L : D schedule, and offered 1–2 mice once a week until parturition.

Offspring were born between August 12 and September 19, 2010. Within 24 h of birth, offspring were sexed, weighed, and measured, and they were then housed individually in plastic boxes with paper substrate and a water bowl. Litters were randomly divided, with sex split evenly, into two temperature-treatment groups designed to mimic the differing thermal regimes of the lakeshore (L-fast) and meadow (M-slow) habitats (Bronikowski 2000). Ambient room temperature was 20°C, with the warmer treatment receiving 16 h of supplemental heating per day and the cooler treatment receiving supplemental heating for 8 h per day. This supplemental heating, supplied by under-tank heat tape, provided a gradient of 22°–32°C, allowing the animals to behaviorally thermoregulate. All juveniles were kept on a 12 : 12 L : D schedule and offered a fraction of a frozen/thawed pinky mouse once a week. Individuals that repeatedly consumed all food were offered a greater amount in subsequent feedings. All snakes were weighed (range: 1.70–5.79 g; SD: 0.76 g) and measured (snout-vent length; range: 157–247 mm; SD: 14.3 mm) on November 29, 2010. Snakes were moved to a cold room and hibernated uniformly in the dark at 4°C from January through May 2011. Following hibernation, animals were placed back into their thermal treatment groups and were offered whole pinky mice once per week. We recorded the amount eaten at each feeding. We again weighed (range: 3.27–15.94 g; SD: 2.8 g) and measured (snout-vent length; range: 190–357 mm; SD: 36.3 mm) snakes on November 1, 2011. All experimental animals were treated in accordance with Iowa State University Institutional Animal Care and Use Committee protocol 3-2-5125-J.

Metabolic Rate Measurement

Oxygen consumption rate ($\dot{V}O_2$) is widely used as a measure of metabolic rate—in this case, resting metabolic rate—with all the requirements of standard metabolic rate met except that snakes were measured during their normal activity period (Bennett and Dawson 1976; McNab 2002; Careau et al. 2008). We quantified $\dot{V}O_2$ in offspring from both ecotypes (L-fast: $n = 64$, representing 20 litters from four populations; M-slow: $n = 42$, representing 17 litters from three populations) in each of four temperature-treatment groups (20°, 24°, 28°, and 32°C) using closed-system respirometry (Vleck 1987). Snakes were postabsorptive after fasting for 2 wk before the whole-body metabolic rate measurements, to eliminate the metabolic influence of digestion (i.e., specific dynamic action) and ensure that our measures were of maintenance metabolism (Stevenson et al. 1985; Álvarez and Nicieza 2005). At the time of metabolic measurement, we weighed each snake with an electronic balance (range: 3.76–17.30 g; SD: 2.8 g). In the 4 wk before the experiment, snakes did not gain weight (average change in mass from November 1 through December 1, 2011: −0.13 g; range: −2.66 to 1.36 g; SD: 0.71 g), thus reducing the risk of confounding our measures of resting metabolic rate with delayed overhead costs of growth (Rosenfeld et al. 2015). Metabolic chambers consisted of darkened metal cans sealed with modified lids to include a tube with a stopcock on the end. Two can sizes were used in the measurements (245 and 995 mL) for smaller and larger snakes, respectively. Before the start of each trial, 5 mL of water was injected into each can to ensure that water content would remain constant (i.e., saturated) throughout the experiment. Snakes were placed within metabolic chambers in temperature-controlled incubators (Econotemp; Thermo Fisher Scientific, Waltham, MA) 30 min before trials to become accustomed to test conditions (Hare et al. 2004), with stopcocks in open position. Additionally, 50 cm^3 of room air was drawn with a 60-cm^3 syringe and placed within incubators at the trial temperature at the same time. At the start of trials, this volume of air was injected into the cans and mixed by plunging and withdrawing the syringe twice; 30 cm^3 of air was then removed from the can, and the stopcocks were sealed. Air pressure was recorded at the start of each trial to correct calculations to standard temperature and pressure. Each incubator was fit with a thermocouple thermometer, and temperature was measured at time 0, 15 min, 30 min, and every 30 min thereafter to ensure consistency throughout trials. At the end of 2 h, 40 cm^3 of air was removed from each can. We measured the O_2 concentration in initial and final samples using an Ametek N-37M oxygen sensor and an Ametek S-3A/11 oxygen analyzer (Pittsburgh, PA). Water and CO_2 were removed from air samples by injection through Drierite (Hammond Drierite, Xenio, OH) and Ascarite II (Thomas Scientific, Swedesboro, NJ), respectively, before entering the oxygen sensor. Following Vleck (1987), rates of oxygen consumption ($\dot{V}O_2$) were calculated as

$$\dot{V}O_2 = \frac{\text{can volume} \times (\text{initial } O_2 - \text{final } O_2)}{\text{duration} \times (1 - \text{final } O_2)}, \quad (1)$$

where initial O_2 is the initial O_2 concentration and final O_2 is the final O_2 concentration.

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Snakes were randomly grouped into four batches of 26–27 individuals and randomly assigned to a metabolic chamber within each batch. The sequence of test temperatures was then randomized within each block such that individuals experienced a randomized permutation of test temperature order. Snakes showed no signs of decreased activity or sluggishness associated with hibernation in the period before the oxygen consumption rate measurements were made. Each snake was tested once at each of the four temperatures, and measurements were conducted on consecutive days, December 1–5, 2011.

Statistics

Growth Efficiency

First-year growth efficiency was measured as change in snout-vent length (mm) during the first year of growth, accounting for variation in the amount of food consumed. We analyzed growth from November 29, 2010, to November 1, 2011, to ensure that the growth period was uniform for all snakes, because L-fast snakes were born before M-slow snakes. As such, the effects of ecotype and the number of growth days are confounded in an analysis using growth measures from birth. After inspecting data for normality and homogeneity of variances, we used mixed linear models to test for the effects of ecotype, rearing treatment (warm/cool), sex, and interactions thereof on growth. We included the amount of food consumed (g) as a covariate, as well as interactions thereof. To account for variation in growth due to size, we used initial size as a covariate in the analysis. Population nested within ecotype was included in the model, treated as a fixed effect, to account for among-population habitat heterogeneity within ecotypes (Palacios et al. 2013). We also included the random effect of litter nested within population and ecotype, which accounts for among-litter variation within populations (Robert and Bronikowski 2010). The interaction between ecotype and rearing treatment was left in the model, as this interaction is of biological interest; the remaining nonsignificant interaction terms were removed from the model (all $P > 0.35$). The final mixed linear model used for our analysis of first-year growth efficiency was

$$\begin{aligned} Y = \mu &+ \text{initial size} + \text{food consumed} + \text{ecotype} \\ &+ \text{rearing treatment} + \text{sex} + \text{ecotype} \times \text{rearing treatment} \\ &+ \text{population(ecotype)} + \text{litter(population ecotype)} + \varepsilon, \end{aligned} \tag{2}$$

where μ represents the grand mean and ε represents the error term. Denominator degrees of freedom for F-tests were estimated using the Kenward-Roger degrees of freedom approximation, which weights the denominator degrees of freedom according to the variance of the effect (Kenward and Roger 1997).

Oxygen Consumption Rate

We first $\log_{10}$-transformed $\dot{V}O_2$ and body mass to linearize the relationship between these variables. After log transformation, mass measurements were standardized around the midpoint of the log-transformed range to estimate intercepts of the mass-oxygen consumption regression at a biologically meaningful mass as well as to remove statistical covariation between our estimates of slope and intercept (Glazier 2010; Killen et al. 2010). The effect of temperature was treated as a continuous variable and was therefore modeled in kelvins so that differences in temperature between treatments would be correctly proportional, although we present results in degrees Celsius for ease of understanding. To account for a possible curvilinear relationship between metabolism and increasing test temperature, we included a squared test temperature term in the model (Sokal and Rohlf 2011; Iles 2014). After inspecting data for normality and homogeneity of variances, we used repeated-measures mixed linear models to test for the effects of body mass, test temperature, ecotype, rearing treatment (warm/cool), sex, and interactions thereof. As above, population nested within ecotype was included as a fixed effect, litter nested within population and ecotype was included as a random effect, and denominator degrees of freedom for F-tests were estimated using the Kenward-Roger degrees of freedom approximation. The time of day at which the metabolic measurements were made was randomized within batch order for each day; time of day and all interactions with fixed effects were not significant and were removed from the model (all $P > 0.09$). We removed additional nonsignificant interaction terms from the model (all $P > 0.14$) and used the following mixed linear model in our analysis of log ($\dot{V}O_2$):

$$\begin{aligned} Y = \mu &+ \log(\text{mass}) + \text{test temperature} + \log(\text{mass}) \\ &\times \text{temperature} + \text{ecotype} + \text{ecotype} \times \text{temperature} \\ &+ \text{rearing treatment} + \text{sex} + \text{temperature}^2 \\ &+ \text{population(ecotype)} + \text{litter(population ecotype)} \\ &+ \text{individual ID} + \varepsilon, \end{aligned} \tag{3}$$

where μ represents the grand mean and ε represents the error term.

Correlation between Growth Efficiency and Metabolic Rate

To quantify the relationship between growth efficiency and resting metabolic rate, we modeled the residuals of the $\dot{V}O_2$ model (eq. [3]) as function of the growth efficiency residuals (eq. [2]) in a repeated-measures mixed linear model. We used marginal residuals from the repeated-measures $\dot{V}O_2$ model because these represent the distance between data points and overall means, not means within individuals. Additionally, we created simple regression models for each of the four test temperatures and calculated the Pearson correlation coefficients of residuals from the $\dot{V}O_2$ and growth efficiency models at each temperature.

Allometric Scaling Coefficients and Exponents

We estimated scaling exponents and confidence intervals using restricted maximum likelihood from a log-log model of

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oxygen consumption rate on mass at each test temperature using ordinary least squares linear regression, which gives the most accurate parameter estimates across a small range of body sizes (White 2011). Estimates of the intercept of the log-log regression correspond to the log of the scaling coefficient (a), and estimates of the slope of the log-log regression correspond to the scaling exponent (b). We used Proc Mixed and Proc Reg in SAS (ver. 9.4; SAS Institute, Cary, NC) for all statistical analyses, with a level of significance of $\alpha = 0.05$.

Results

Growth Efficiency

In the model of growth efficiency (i.e., change in body length accounting for food consumed), the amount of food consumed, initial size, and rearing treatment were significant factors. Not surprisingly, snakes consuming more food grew more, while larger snakes exhibited higher growth efficiency than smaller snakes. Additionally, snakes receiving the warm rearing treatment had higher growth efficiency than those receiving the cool rearing treatment. Population nested within ecotype was significant in this model, largely driven by low growth efficiency in snakes from one of the M-slow populations (table 1; fig. 1).

Oxygen Consumption Rate

Temperature interactions with both body mass and ecotype as well as the main effects of these three factors were significant in determining $\dot{V}O_2$. Additionally, the squared temperature term was significant, indicating a nonlinear relationship between oxygen consumption rate and temperature (table 2). The significant interaction between test temperature and ecotype indicates differences in the shapes of thermal reaction norms of snakes from each ecotype (fig. 2): L-fast snakes had highest $\dot{V}O_2$ at 24°C, while M-slow snakes had highest $\dot{V}O_2$ at 28°C. A post hoc comparison of least squares means, adjusted for multiple comparisons (Tukey's method), shows that M-slow snakes had lower $\dot{V}O_2$ than L-fast snakes at 20°C ($P = 0.046$). The significant interaction between body mass and test temperature indicates that the allometric relationship of mass to $\dot{V}O_2$ changed across this temperature range (fig. 3). This interaction is analyzed further below ("Allometric Scaling Coefficients and Exponents").

Correlation between Growth Efficiency and Metabolic Rate

Across individuals, there is a significant negative correlation between residuals from the models of growth efficiency and oxygen consumption rate (repeated-measures mixed linear model: $F_{1, 104} = 4.06$, $P = 0.047$). Within each test temperature, Pearson correlation coefficients are all negative, although only the correlation at the highest test temperature (32°C) is marginally significant ($P = 0.054$; table 3; fig. 4). This means that snakes exhibiting lower resting metabolic rates converted a greater proportion of food consumed to an increase in body size over the first year of growth.

Allometric Scaling Coefficients and Exponents

Because neither the interaction between ecotype and mass nor the interaction between rearing treatment and mass was significant in the initial mixed model of oxygen consumption rate, scaling coefficients and exponents were calculated using observations from all individuals. As evidenced by the significant interaction between mass and temperature described above, metabolic rate scaled differently at each temperature (table 4; fig. 5). The scaling coefficient (a) showed a significant increasing trend from 20° to 28°C, which corresponds with our expectations based on the thermal reaction norms (fig. 2). The scaling exponent (b) showed a significant decreasing trend from 20° to 32°C. At the lowest temperature, the relationship between body mass and metabolic rate was positively allometric ($b > 1$), indicating that smaller snakes consumed less oxygen per unit of body mass than larger snakes. As temperature increased, estimates of the scaling exponent decreased, indicating increasing efficiency of larger snakes at higher temperatures.

Discussion

In this experiment, we measured oxygen consumption rate ($\dot{V}O_2$) and growth efficiency of captive-born juvenile garter snakes (*Thamnophis elegans*) from mothers of divergent life-

Table 1: Mixed linear model analysis of *Thamnophis elegans* growth efficiency from November 2010 to November 2011, including food consumed as a covariate

Source of variation	Estimate	df_n, df_d	F	Pr ≤ F	Direction of significant factors
Food consumed	3.04	1, 92	109.03	<.0001**	More consumed > less consumed
Initial size	−.33	1, 65.3	4.62	.04*	Larger > smaller
Ecotype	−6.47	1, 30.4	.65	.43	…
Rearing treatment	14.53	1, 84.1	10.03	.0022**	Warm > cool
Ecotype × rearing treatment	−5.70	1, 78.8	.77	.38	…
Sex	2.31	1, 89	.50	.48	…
Population(ecotype)	…	5, 24.7	3.57	.014*	…

Note. Litter nested within population and ecotype was included as a random effect. Denominator degrees of freedom for F-tests were estimated using the Kenward-Roger degrees of freedom approximation. Significant effects are designated with a single asterisk ($P < 0.05$) or a double asterisk ($P < 0.01$).

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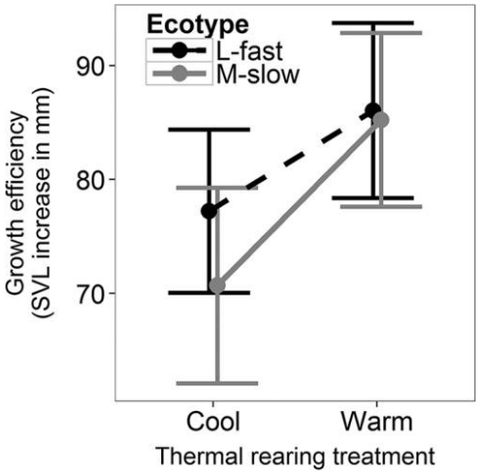

Figure 1. Growth efficiency of *Thamnophis elegans* juveniles, measured by change in snout-vent length (SVL) from November 2010 to November 2011, accounting for the amount of food consumed (see eq. [2]). Data are least squares means from a model also accounting for initial size. Snakes receiving the warm rearing treatment grew more efficiently than snakes receiving the cool treatment for both ecotypes. Error bars represent 95% confidence intervals. L-fast = fast-growing lakeshore ecotype; M-slow = slow-growing meadow ecotype.

history ecotypes, reared in controlled conditions to mimic differences in the natural thermal environments. The reaction norms for resting metabolic rate, as measured by oxygen consumption rate ($\dot{V}O_2$), differed in snakes from the two ecotypes across the range of test temperatures, with L-fast snakes having a significantly higher metabolic rate at the lowest temperature. This finding, coupled with a lack of evidence that rearing treatment affected resting metabolic rate, suggests genetic divergence between ecotypes in traits determining resting metabolic rate. For growth efficiency, which is growth rate accounting for food consumption, we found that both initial size and rearing treatment had significant effects on growth efficiency, while there was no difference between the ecotypes. Furthermore, we found that residuals from the models of growth efficiency and oxygen consumption rate were slightly but significantly negatively correlated, demonstrating support for trade-offs or covariation between these important life-history traits. Finally, we found a temperature-dependent allometric relationship between mass and metabolic rate across the temperature range, supporting our emerging understanding of the drivers of variation in this relationship (Glazier 2005, 2010). Together, these results demonstrate the complex interactions among extrinsic and intrinsic forces shaping growth, metabolism, and metabolic scaling.

Snakes from both ecotypes showed greater growth efficiency in warmer conditions, implying that the thermally dependent plasticity of growth rate may be independent of ecotypic background or can be mediated by behavioral thermoregulation. In contrast to Bronikowski (2000), who found evidence of adaptive divergence in growth rates in snakes from different ecotypes for snakes held at constant temperatures, we did not find that ecotype was a significant factor in determining growth efficiency. In the present study, we allowed animals to choose their temperature along a gradient for either 8 or 16 h per day, to more closely resemble natural conditions. In general, food intake, which greatly influences the rate of increasing body size, was largely determined by ecotypic background. However, as we show here, the ability of snakes to efficiently convert food source to body mass was largely determined by thermal rearing environment. Snakes given more opportunity to stay warm both attained larger sizes and grew more efficiently. This is congruent with the findings of Tsai et al. (2009), who used bioenergetic modeling to show that Chinese green tree vipers (*Trimeresurus stejnegeri*) select postprandial thermal environments to maximize both rate of energy gain and efficiency of energy gain.

Table 2: Repeated-measures mixed linear model analysis of log-transformed oxygen consumption rate ($\dot{V}O_2$) in juvenile *Thamnophis elegans*, measured at four test temperatures

Source of variation	Estimate	df_n, df_d	F	Pr ≤ F	Direction of significant factors
Between subjects:					
Log(body mass)	12.29	1, 315	9.44	.0023**	Larger > smaller
Ecotype	3.29	1, 315	7.64	.0060**	L-fast > M-slow
Rearing treatment	−.033	1, 84.5	1.64	.20	…
Ecotype × rearing treatment	−.015	1, 78.7	.07	.79	…
Sex	−.020	1, 87.5	.48	.49	…
Population(ecotype)	…	5, 29.4	.37	.86	…
Within subjects:					
Temperature	.99	1, 314	10.78	.0011**	Higher > cooler
Temperature × log(body mass)	.037	1, 314	7.81	.0055**	…
Temperature × ecotype	.011	1, 314	7.49	.0066**	…
Temperature × temperature	−.0016	1, 314	9.97	.0017**	…

Note. Litter nested within population and ecotype was included as a random effect. Denominator degrees of freedom for F-tests were estimated using the Kenward-Roger degrees of freedom approximation. Significant effects are designated with a double asterisk ($P < 0.01$). L-fast = fast-growing lakeshore ecotype; M-slow = slow-growing meadow ecotype.

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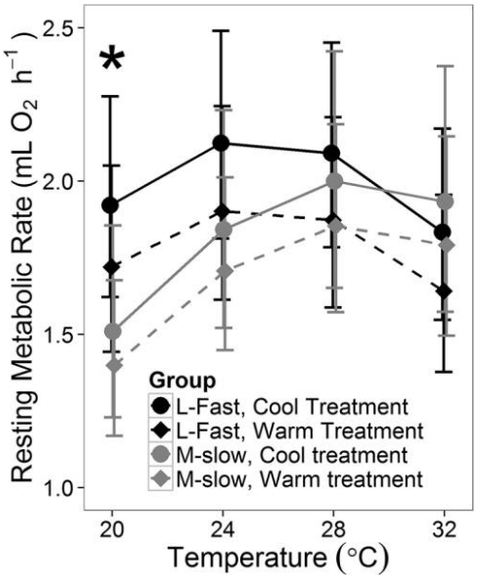

Figure 2. Thermal reaction norms for resting metabolic rate ($\dot{V}O_2$) for *Thamnophis elegans* juveniles by ecotype and rearing treatment group. Higher means correspond to higher mass-specific metabolic rates because calculation of least squares means removes variation associated with body mass (see "Statistics"). A significant ecotype by test temperature interaction demonstrates differences in the shapes of the reaction norms ($P = 0.007$; see table 2). Additionally, least squares mean differences between ecotypes are significant at 20°C ($P = 0.046$), indicated with an asterisk. Error bars represent 95% confidence intervals. L-fast = fast-growing lakeshore ecotype; M-slow = slow-growing meadow ecotype.

In the present study, snakes receiving the warm rearing treatment most likely were able to attain preferred postprandial body temperatures more often and were thus able to optimize both their growth rate and their growth efficiency. This combination of results implies that total growth is influenced by a number of interacting factors, including both thermal environment and genetic background.

Additionally, initial size influenced growth efficiency, with larger snakes growing more efficiently than smaller snakes. This, in combination with evidence that larger snakes also demonstrate increased survival over their first year (Bronikowski 2000), points to clear fitness advantages of being born large. If larger snakes are also metabolically more efficient, as would be expected when $b < 1$, then this is consistent with the principal of allocation (Gadgil and Bossert 1970; Careau et al. 2008) in demonstrating trade-offs between growth and metabolic rate. Indeed, we found evidence of such trade-offs within individuals in our analysis of the relationship between residuals of the growth efficiency model and residuals of the oxygen consumption rate model. The small but statistically significant negative relationship between these residuals indicates that snakes that had higher growth efficiencies had lower resting metabolic rates, providing evidence of a trade-off in energetic allocation between these two important life-history traits. We note that this trade-off is evident in snakes that are in a life-history phase of growth (i.e., juveniles) but that are not actively growing at the time of the metabolic measurements because of food limitation. While snakes are growing, we would expect to see a positive relationship between metabolic rate and growth, as has been demonstrated in field studies of garter snakes (Peterson et al. 1999). Even during short-term fasting, metabolic measurements can be upwardly influenced by delayed overhead costs of growth resulting from tissue synthesis (Rosenfeld et al. 2015). That our animals were on a maintenance ration and did not gain weight during the month before metabolic measurements allows us to test for trade-offs in metabolic rate and first-year growth efficiency without the confounding effect of biosynthesis associated with growth.

While we did not systematically vary food availability between treatment groups, snakes ate with different frequencies and therefore consumed different amounts of food. Our analysis, however, statistically accounts for this variation in acquisition and thereby exposes a trade-off between traits driven by variation in allocation (van Noordwijk and de Jong 1986). Evidence of such a trade-off between these traits in a laboratory setting corroborates the majority of such studies reported in the literature (reviewed in Glazier 1999). Snakes, ectotherms with low metabolic and energetic demands, were most likely not resource limited in their ability to allocate sufficient energy to both metabolic rate and growth. Why then does such a trade-off exist? These traits may be regulated, or possibly coregulated, by underlying mechanisms that set boundaries on plasticity when resources are bountiful. Even under favorable ecological conditions, it may not be optimal to increase allocation to these life-history traits simultaneously. For example, Cox and Secor (2007) found evidence of a trade-off between standard metabolic rate and the amount of food energy converted to growth in juvenile Burmese pythons (*Python molurus*), even while maintained on a generous ration, and that this trade-off had a genetic component. In our experiment, we found evidence of such a trade-off between production and metabolic rate as well. That thermal rearing environment, but not ecotype, affects growth efficiency and that ecotype, but not thermal rearing environment, affects resting metabolic rate supports the idea that there is a limit to resource-driven plasticity in shaping metabolic pathways. A context dependency of a trade-off between maintenance metabolism and growth has been found in studies of salmon (*Salmo salar*; Reid et al. 2011) and cotton rats (*Sigmodon hispidus*; Derting 1989) and helps explain polymorphisms in populations where conditions vary temporally and spatially. Given the resource fluctuations found in this garter snake system, both within and between ecotypes (Bronikowski and Arnold 1999; Miller et al. 2011), it is not surprising that we found such high levels of variation in both metabolic rate and growth among individuals.

We see two possible adaptive explanations for the divergence in thermal reactions norms of oxygen consumption rates between ecotypes. The low metabolic rate of M-slow snakes at 20°C may represent a mechanism to conserve energy when temperatures are low and resources are unavailable (Aleksiuk 1971, 1976; Burton et al. 2011). A low metabolism would allow

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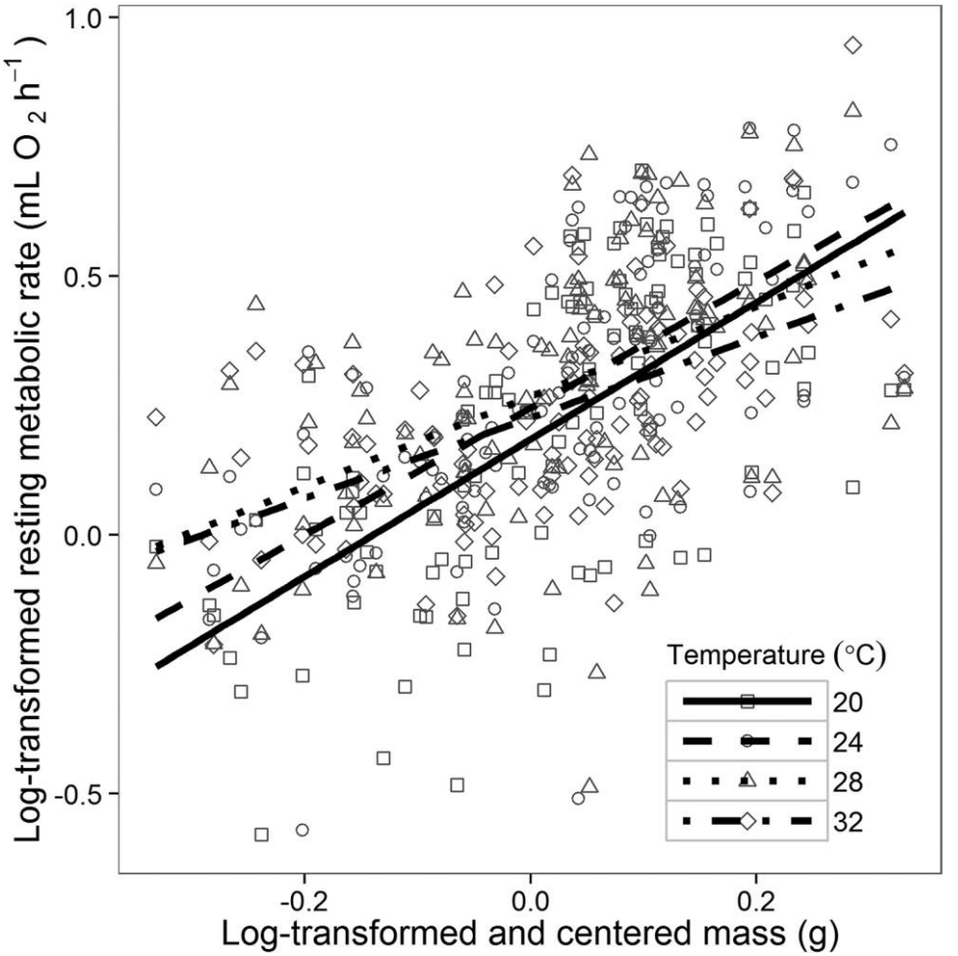

Figure 3. Regression of log-transformed oxygen consumption rate ($\dot{V}O_2$) on log-transformed and centered body mass across the range of test temperatures in juvenile *Thamnophis elegans*. Body mass is centered on 0 so that estimates are made at a biologically relevant mass, the midpoint of the log-transformed range.

these snakes to retreat underground for longer periods of time without depleting energy stores. Alternatively, the higher metabolic rate of L-fast snakes may be a mechanism to maintain a higher aerobic scope as they forage for fish, their primary food source, in the cold lake water (Bronikowski and Arnold 1999). In contrast to adult snakes from these and nearby populations (Stevenson et al. 1985; Bronikowski and Vleck 2010), metabolism in juvenile snakes did not increase monotonically with temperature. Oxygen consumption rate decreased at the highest temperature in all ecotype and rearing treatment groups, with the metabolic rate of L-fast snakes peaking at 24°C and that of M-slow snakes at 28°C. Interestingly, this pattern is similar to the "lag" in oxygen consumption rates across a similar high range of temperatures (28°–34°C) reported by Seidel and Lindeborg (1973) for wild-caught *T. elegans*. Unfortunately, no size range of the tested individuals is reported in that study.

Such a leveling or decrease in oxygen consumption rate could be an adaptive mechanism to conserve energy at higher temperatures (Glazier 2015) or a hint at some limit to the thermal dependence of metabolic rate. For example, the oxygen and capacity limitation of thermal tolerance hypothesis (Pörtner 2002; Pörtner and Knust 2007) predicts that thermal limits in ectotherms are set as a result of a mismatch between oxygen demand and supply at high temperatures. This hypothesis has not found support in terrestrial ectotherms (Stevens

Table 3: Pearson correlation coefficients of growth efficiency residuals and $\dot{V}O_2$ residuals measured at four test temperatures in juvenile *Thamnophis elegans*

Test temperature	Pearson correlation coefficient	Pr ≠ 0
20°C	−.13	.17
24°C	−.12	.24
28°C	−.12	.21
32°C	−.19	.054

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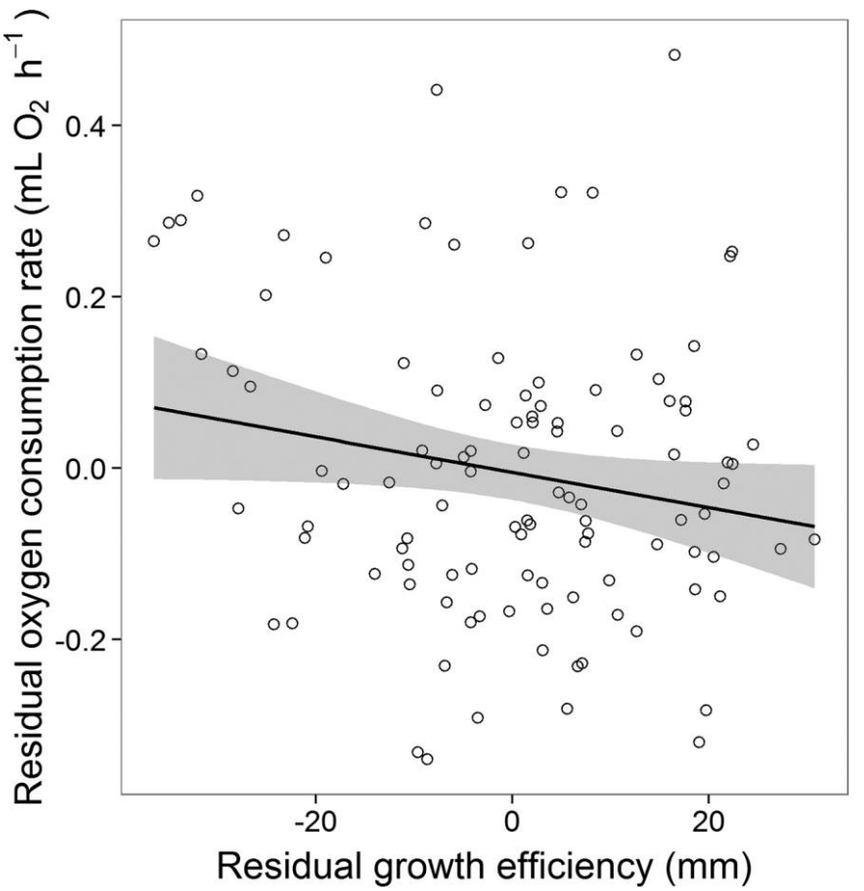

Figure 4. Regression of oxygen consumption rate residuals ($\dot{V}O_2$; see eq. [3]) and growth efficiency residuals (change in snout-vent length; see eq. [2]) for juvenile *Thamnophis elegans*. Data shown are from measures of oxygen consumption rate at 32°C, where the negative correlation was most significant (Pearson correlation coefficient $= -0.19$, $P = 0.054$). The repeated-measures mixed linear model demonstrates the significance of the negative correlation when data from all test temperatures are analyzed ($F_{1, 104} = 4.06$, $P = 0.047$). The shaded area represents the 95% confidence envelope for the regression line.

et al. 2010; Overgaard et al. 2012; Fobian et al. 2014), but these tests have not included juveniles, which may experience limitations of oxygen delivery at early stages of development. For example, Steyermark and Spotila (2000) report such a leveling off of oxygen consumption rate at high temperatures in juvenile snapping turtles (*Chelydra serpentina*). That juvenile garter snakes from Eagle Lake populations exhibited this pattern while adults did not could be due to ontogenetic changes in physiology, such as more effective oxygen delivery and increase in blood oxygen capacity (Pough 1977) or shifts in the relative mass of organs with high oxygen demand (Oikawa and Itazawa 2003; Landgraf et al. 2006). We are planning future

Table 4: Allometric equation parameter estimates relating standard metabolic rate to body mass in juvenile *Thamnophis elegans*

Test temperature	Scaling coefficient (a)	Scaling exponent (b)	R^2
20°C	1.53 (1.38–1.70)	1.33 (1.02–1.63)	.416**
24°C	1.76 (1.61–1.93)	1.23 (.95–1.50)	.426**
28°C	1.85 (1.68–2.05)	.87 (.57–1.17)	.242**
32°C	1.69 (1.56–1.82)	.78 (.55–1.01)	.299**

Note. The scaling coefficient (a) is a measure of metabolic level at the range midpoint of log-transformed sample mass. The scaling exponent (b) describes the relationship between body mass and metabolic rate at the indicated test temperature. Parentheses indicate 95% confidence intervals for estimates. All correlations were negative and highly significant ($P < 0.001$), indicated with a double asterisk.

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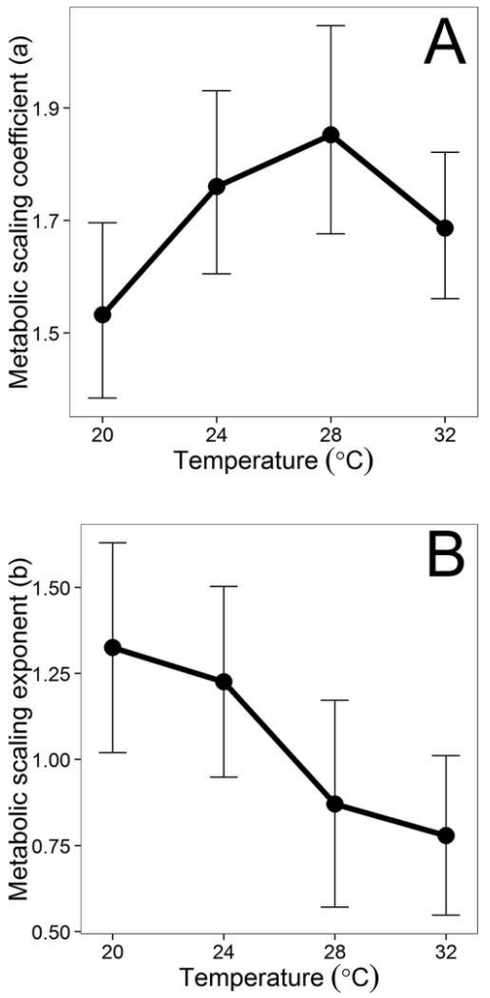

Figure 5. *A*, Estimates of metabolic scaling coefficients (*a*) across temperature range in juvenile *Thamnophis elegans*. *B*, Estimates of scaling exponents (*b*) across temperature range. Error bars represent 95% confidence intervals. Scaling estimates did not differ between ecotypes or treatments, so data are from all individuals ($n = 106$).

experiments to test the capacity of oxygen delivery systems at thermal extremes and the subsequent consequences for metabolic rate.

We found that both metabolic level (*a* from the allometric equation of metabolic rate) and scaling exponent (*b*) are temperature dependent within our range of test temperatures. This is consistent with the metabolic-level boundaries hypothesis (MLBH; Glazier 2005, 2010), which acknowledges variation in this scaling exponent and adopts a focus on physical attributes that set boundary conditions on scaling exponent values. The MLBH predicts a negative relationship between the scaling exponent (*b*) and the scaling coefficient (*a*) in inactive ectotherms at cool temperatures (see fig. 6 for details). Empirical studies testing the MLBH hypothesis with ectotherms across a range of temperatures have demonstrated the ecological dependence and adaptive significance of the relationship between mass and metabolic rate (Killen et al. 2010; Vaca and White 2010; Ohlberger et al. 2012; Carey et al. 2013), but not with universal support (Gifford et al. 2013). Our estimates of scaling exponents at 20°C ($b = 1.33$) and 24°C ($b = 1.23$) were significantly higher than those of both adult and month-old snakes from these populations ($b = 0.59$ [Bronikowski and Vleck 2010]; $b = 0.38$–0.58 [Robert and Bronikowski 2010]). At 28°C ($b = 0.87$) and 32°C ($b = 0.78$), our estimates are not statistically different from the estimates from these previous studies or from the average for squamate reptiles ($b = 0.80$; Andrews and Pough 1985). The scaling exponent estimate at 20°C is significantly greater than 1. This is surprising because it indicates that smaller snakes are more metabolically efficient than larger snakes at cool temperatures. Such high scaling exponents ($b > 1$) have been reported in rapidly growing juvenile ectotherms (reviewed in Glazier 2015) and may be attributable to ontogenetic shifts in the relative mass of organs with differing oxygen demands or a size dependency of underlying mechanisms determining metabolic rate, such as T4 thyroxine or mitochondrial density (Steyermark et al. 2005). The scaling exponent estimates did not differ between ecotypes or rearing treatment groups, implying that variation in the allometric scaling of metabolic rate did not drive the observed divergences in growth rate or metabolic rate.

In ectotherms, colder temperatures are generally associated with lower whole-organism metabolism, activity level, and maintenance costs. In *T. elegans*, 20°C (the lowest temperature at which we measured metabolic rate) represents a threshold between active and inactive snakes, with crawling speed, swimming speed, tongue-flicking rate, and digestive rate all decreasing rapidly below this temperature (Stevenson et al. 1985). Within

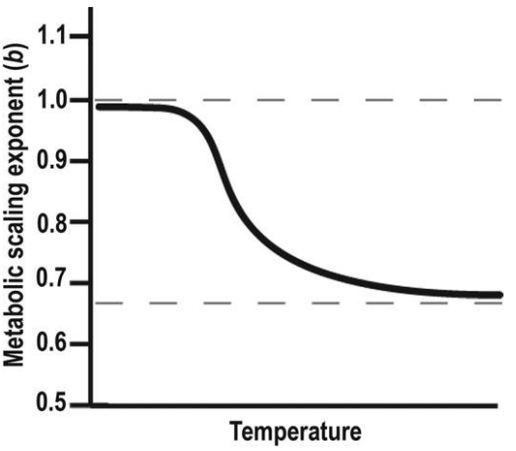

Figure 6. Schematic relationship between metabolic level (*a*, the back-transformed intercept of the log-log plot) and metabolic scaling exponent (*b*, the slope of the log-log plot), as predicted by the metabolic-level boundaries hypothesis (Glazier 2005, 2010). While inactive at cool temperatures, ectotherms will have a low metabolic level (*a*) and a metabolic rate that scales approximately isometrically ($b \approx 1$), limited by the energetic requirements to sustain body tissues. At higher temperatures, metabolic level will increase (increasing *a*) while the metabolic scaling exponent will decrease, now bounded by fluxes of resources and heat across surfaces ($b \approx 2/3$).

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the activity range of this species, the scaling exponent decreased with increasing temperature but was never significantly less than 1, even at the highest temperature measured (table 4). At the two highest temperatures, this estimate also did not differ significantly from 2/3, as predicted by the MLBH (Glazier 2005). We may expect the value of *b* to be greater than 2/3 if body shape allometry—and therefore surface area to volume ratio—changes across ontogeny (Glazier 2014; Hirst et al. 2014). However, previous work with garter snakes showed no evidence that body shape is affected by increased growth rates associated with higher temperatures (Arnold and Peterson 1989). In future experiments, we will test whether the trend of decreasing *b* values continues at higher temperatures and the scaling exponent becomes less than 1, meaning that larger snakes have reduced maintenance metabolic demands. Such increased efficiency of larger snakes could potentially facilitate divergences in growth rates and thus potential life-history differences (Carey et al. 2013). Furthermore, we will test the dependency of metabolic scaling on activity and energetic demands, as found in other snake systems (e.g., Taylor and Davies 1981; Beaupre 1993).

Understanding the relative contributions of developmental plasticity and local adaptation in producing divergence of traits between populations is a central question in an era of rapid environmental changes (Carroll et al. 2014). The thermal dependence of growth efficiency, energy allocation, and metabolic rate in ectotherms has immense implications not only for the fitness of individual organisms but also for population dynamics (Burton et al. 2011; Ohlberger et al. 2012; Carey et al. 2013), yet mechanistic models of the physiological impacts of temperature are still needed (Angilletta 2009; Somero 2011; da Silva et al. 2013). The empirical data provided here offer evidence that important aspects of the phenotype—in this case, metabolic rate and growth—have complex interdependent relationships with both developmental and immediate environments. By making explicit the mechanisms linking physiology with life history, we can understand better how variation in physiological traits affects higher levels of biological organization.

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