



## Influence of temperature and body mass on standard metabolic rate of eastern red-backed salamanders (*Plethodon cinereus*)

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### ABSTRACT

(1) We estimated the standard metabolic rate (SMR) of wild-caught *Plethodon cinereus* across a range of body masses and ecologically relevant temperatures.

(2) We collected 16 salamanders, ranging 0.06–1.11 g and quantified oxygen consumption at 10, 15, 20, 25, and 30 °C with a computer-controlled, closed-circuit, indirect respirometer. The multiple regression model indicated that SMR increased with body mass and temperature ( $F_{2,77}=815.59$ ,  $P < 0.001$ ).

(3) Our allometric relationship explained > 95% of the variation in SMR. The regression equation can be used to investigate questions of applied or basic ecological interest, such as quantifying energetic costs of anthropogenic or natural disturbances on energy budgets.

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### 1. Introduction

Understanding relationships between the thermal environment and energy use of amphibians is critical to evaluating evolutionary, ecological, and behavioral hypotheses (Huey and Stevenson, 1979; Moreno, 1989; Petruzzi et al., 2006; Hossack et al., 2009) and to making inferences about thermal ecological physiology (Rome et al., 1992). Temperature affects virtually all aspects of amphibian physiology, including digestion, vision, locomotion, growth, and metabolism (reviewed by Gatten et al. 1992, Rome et al., 1992). Resting metabolism, a physiological process that can be a large component of the energy budgets of amphibians, is at the intersection of ecology and physiology (Huey and Stevenson, 1979). For ectotherms such as amphibians, an increase in temperature is known to increase metabolic rate (Pough, 1980; Rome et al., 1992). Thus, in the absence of compensatory measures such as behavioral thermoregulation, warming the thermal environment of an amphibian will cause an increase in metabolic rate and overall energy expenditure. In turn, this creates an ecological trade-off that may influence growth or other components of the energy budget that contribute to an individual's fitness (Congdon et al., 1982; Sears, 2005; DuRant et al., 2007).

In light of the changing global environment, more attention has been given to investigating effects of altered thermal regimes on ecological relationships of amphibians (Araujo et al., 2006; Buckley and Jetz, 2006). To best evaluate effects of the thermal

environment on metabolic rate or other physiological processes, predictive models that estimate the physiological rate of interest across temperatures are needed. To date, predictive relationships between temperature and metabolic rate only have been developed for a relatively small number of amphibians (Gatten et al., 1992; Petruzzi et al., 2006). Further, for those available relationships, most experimentation was not conducted at a range of ecologically relevant temperatures, and many fail to incorporate current advances in technology or in quantitative interpretation of data (Huey and Stevenson, 1979; Zaidan and Beaupre, 2003; Hopkins et al., 2004; Beaupre, 2005).

One such methodological issue involves isometric versus allometric scaling of the energetic response. Researchers often divide the energy used or volume of oxygen consumed by body mass to create a standardized mass-specific ratio for many physiological rates (e.g., specific dynamic action, standard metabolic rate). However, standardization assumes an isometric relationship (e.g.,  $y$ -intercept=0, slope=1.0) between body mass and energy output, and this often leads to estimation and statistical errors (Packard and Boardman, 1988; Zaidan and Beaupre, 2003; Beaupre, 2005). To remedy what has been a standard practice, researchers should incorporate mass into a multiple linear regression or as the covariate in an analysis of covariance to account for the allometric influence of body mass on physiological rates (Packard and Boardman, 1988; Zaidan and Beaupre, 2003). Currently for most amphibians, incorporation of body mass into predictive relationships of metabolic rates has not been completed, and even fewer studies have simultaneously considered the influence of temperature and body mass on these allometric relationships (Petruzzi et al., 2006).

The eastern red-backed salamander (*Plethodon cinereus*) is a widely distributed and abundant salamander species that has

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served as a model for foundational physiological, behavioral, and ecological studies (Spotila, 1972; Burton and Likens, 1975; Jaeger, 1981). However, no predictive relationship has been derived between metabolism and temperature while accounting for body mass in this species. A large component of the energy budget of *P. cinereus* is the standard metabolic rate (SMR), or the metabolic rate of a resting, post-absorptive individual at a specific temperature (Mercer, 2001). To this end, our goal was to develop a predictive relationship of SMR based on temperature and body mass for this widely studied species.

## 2. Methods

### 2.1. Study species

*P. cinereus* is a small species of lungless salamander that makes up considerable biomass in forested ecosystems in eastern North America and may contribute significantly to nutrient cycling through its role as a predator of invertebrates (Burton and Likens, 1975; Wyman, 1998). For this experiment, we hand-captured red-backed salamanders at a forested site on the Jefferson National Forest, Montgomery County, VA and selected individuals that represented a range of snout-vent lengths. We captured eight salamanders on 16 September 2008 and an additional eight individuals on 25 September 2008. Salamanders were transported to the laboratory in re-sealable bags with wet leaves to maintain a fully hydrated state. In the laboratory, salamanders were weighed, measured, and sexed by candling (Gillette and Peterson, 2001). We ensured we had approximately equal numbers of male and female individuals and a wide range of body masses. Salamanders were housed in temperature-controlled conditions in individual plastic containers with a wet paper towel.

### 2.2. Oxygen consumption

Salamanders were fasted for six days at 18 °C prior to the study so individuals were post-absorptive during energetic trials and all acclimated to the same temperature. Salamanders were housed in darkness in the same cabinet where we quantified respiration. For the experiment, we placed a post-absorptive salamander, 5 ml of de-chloraminated tap water, and 0.48 g of KimWipe tissue paper (Kimberly-Clark Corporation, Dallas, TX) in individual 50 ml respiratory chambers in the temperature-controlled cabinet. Providing a moisture source ensured that salamanders maintained a hydrated state for the respirometry trials and estimates of mass.

We estimated energy expenditure across five ecologically relevant temperatures, which corresponded with the range of temperatures observed during a companion study of the thermal environment of free-ranging *P. cinereus* (Homyack, 2009). Oxygen consumption by individual salamanders was quantified with an independent channel on a computer-controlled, closed-circuit, indirect respirometer (MicroOxymax, Columbus Instruments, Columbus, OH) as an indirect measurement of energy use (Hopkins et al., 2004). Eight animals, each in individual respiratory chambers, completed the experiment simultaneously. In addition, one chamber served as a blank control and a second chamber contained a medical battery that consumed a known amount of O<sub>2</sub>/h.

The volume of oxygen consumption ( $\dot{V}O_2$ ) for each salamander was monitored at approximately 70 min intervals for 24 h at each of five temperatures (10, 15, 20, 25, and 30 °C) to quantify indirectly the energy used by each individual at each temperature and to estimate SMR. With the exception of the 30 °C treatment,

we randomized the order of experimental temperatures to which study animals were exposed to avoid potential confounding effects of temperature acclimation. The 30 °C treatment purposely was run last because this temperature was likely to be the most thermally challenging to salamanders. At approximately 1115 h each day, we changed the environmental cabinet to the randomly selected temperature and began a new respirometry trial. There was no other external source of heat, insuring the body temperatures of study animals did not differ from the experimentally controlled temperature. We ran two complete trials, each with eight salamanders.

### 2.3. SMR Estimation

From the respirometry trials, we accumulated approximately 21 estimates of  $\dot{V}O_2$  for each salamander at each temperature. We estimated SMR as the lowest quartile of  $\dot{V}O_2$  measurements (mL/h) for salamanders at each experimental temperature after removing the two first and two last measurements of each trial. Using the lowest quartile value prevented increases in energy output related to salamander movements, diel cycles, or other outliers from influencing estimates of SMR (Hopkins et al., 2004).

To estimate the functional relationship between SMR and temperature while accounting for body mass, we used a multiple linear regression (PROC REG, SAS 9.2; SAS Institute, Cary, NC) that incorporated body mass and temperature as independent variables (Zaidan and Beaupre, 2003). Both body mass and SMR were log<sub>e</sub>-transformed prior to regression analysis to attain linearity. We examined regression residuals for normality and homogeneity and found no significant deviations from the parametric assumptions. We considered statistical significance at  $\alpha=0.05$ . We also calculated Q<sub>10</sub>-values, the rate at which SMR increased with a 10 °C increase in temperature, for each salamander at each 10-degree interval. We report mean Q<sub>10</sub>-values for each 10-degree increment.

## 3. Results

Mass of salamanders ranged from 0.06–1.11 g ( $\bar{X}=0.63$ , SE=0.08) and we included six adult male, five adult female, and five juvenile salamanders (unable to be sexed with candling) in our study. Across the range of body mass and temperatures examined, SMR of *P. cinereus* ranged from 0.003–0.266 mL O<sub>2</sub>/h. SMR increased significantly ( $F_{2,77}=815.59$ ,  $P<0.001$ ) with both body mass and temperature for red-backed salamanders (Table 1, Fig. 1). The predictive multiple regression equation was:

$$\text{Log}_e(\text{SMR}) = 0.102(\text{temperature}) + 0.681(\text{Log}_e(\text{mass})) - 4.849$$

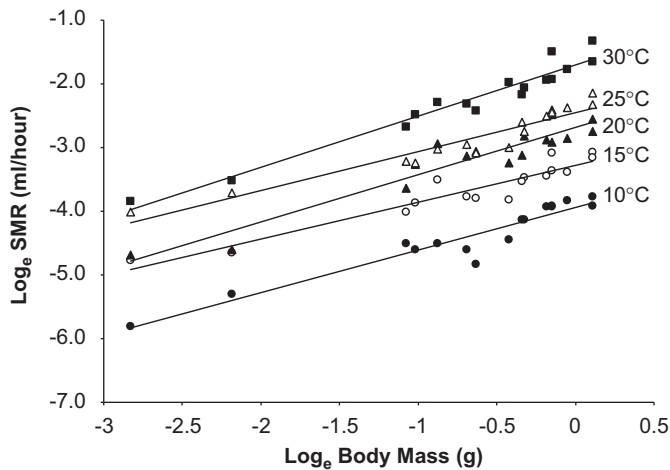
Most of the variability in SMR was accounted for with temperature and body mass ( $R^2=0.955$ ). The relationship between temperature and residual SMR after accounting for body mass was approximately linear (Fig. 2), providing additional support that the linear regression was an appropriate fit to the data.

**Table 1**

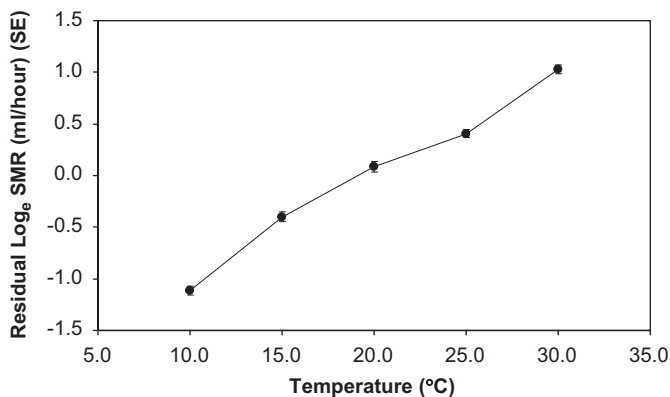
Multiple linear regression of the effects of temperature and body mass on the standard metabolic rate (SMR) of eastern red-backed salamanders (*Plethodon cinereus*). Model  $P<0.001$  and  $R^2=0.955$ . SMR was estimated for 16 salamanders at 10, 15, 20, 25, and 30 °C with a closed-circuit, indirect respirometer.

Variable <sup>a</sup>	df	Parameter	SE	t Value	P value
Intercept	1	−4.849	0.069	−69.95	<0.001
Temperature	1	0.102	0.003	32.39	<0.001
Log <sub>e</sub> body mass	1	0.681	0.028	24.12	<0.001

<sup>a</sup> Body mass and SMR were log<sub>e</sub>-transformed prior to analysis.



**Fig. 1.** Effects of body mass and experimentally manipulated ambient temperatures on the standard metabolic rate (SMR) of *Plethodon cinereus*. Sixteen individuals were measured under each of the five temperatures, 10 °C (●), 15 °C (○), 20 °C (▲), 25 °C (△), and 30 °C (■). Each temperature was significantly related to oxygen consumption ( $P < 0.001$ ,  $R^2 = 0.90$ – $0.95$ ).

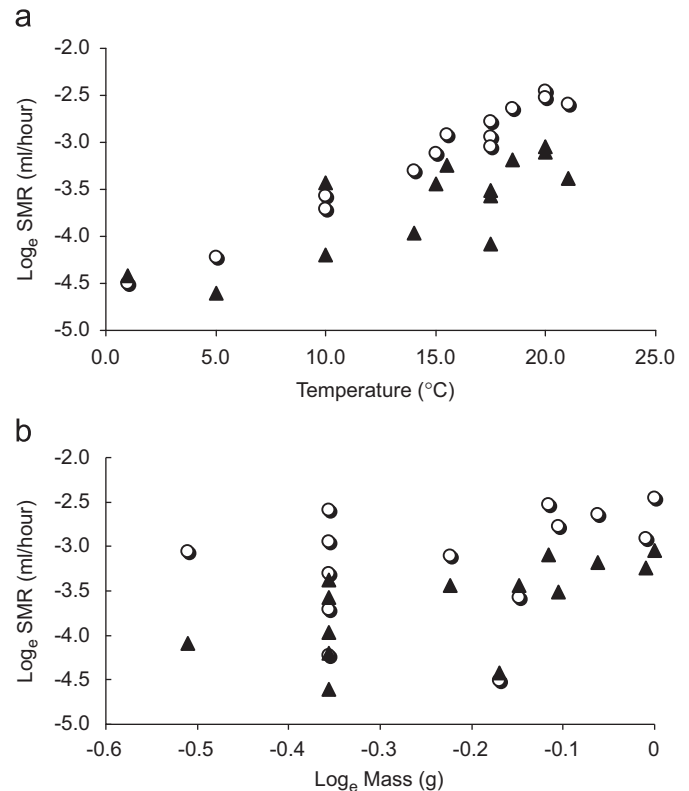


**Fig. 2.** Mean (SE) residual  $\log_e$  (standard metabolic rate, SMR) of *Plethodon cinereus* after adjusting for body mass across five experimentally manipulated temperatures, 10–30 °C. We obtained residual values by regressing  $\log_e$  SMR against  $\log_e$  body mass and quantified means and standard errors of individuals for each temperature.

At temperatures  $\leq 20$  °C, respiration of salamanders tended to peak in the early evening (approximately 1930–2330 h), but this pattern was not evident at 25 or 30 °C. However, our quantitative methods to calculate SMR as the lowest quartile value prevented activity bouts associated with diel cycles from influencing our estimates of basal metabolism. Mean (SE)  $Q_{10}$ -values for SMR were 3.45 (0.25) for 10–20 °C, 2.26 (0.08) for 15–25 °C, and 2.62 (0.15) for 20–30 °C.

#### 4. Discussion

Based on our regression, SMR of *P. cinereus* was significantly and positively related to body mass and temperature (Table 1), an observation expected for ectotherms and seen across amphibian taxa (Gatten et al., 1992). Unfortunately, we cannot qualitatively compare our mass-exponent (0.681) or temperature exponent (0.102) to prior research on plethodontid salamanders because earlier studies did not incorporate body mass into regression equations. This further highlights the importance of consistently incorporating mass into analyses of relationships between



**Fig. 3.** Standard metabolic rate (SMR) of *Plethodon cinereus* at (a) a range of temperatures while accounting for body mass, and (b) by mass while accounting for temperature. These include estimates of SMR reported by Gatten et al. (1992) (▲) and predicted by the equation developed here (○).

temperature and metabolism in future work for synthesis and comparison (Beaupre, 2005). Reported SMR values for individual *P. cinereus* as reviewed by Gatten et al. (1992) were, however, highly positively correlated with predicted values from our regression ( $r = 0.864$ ,  $P < 0.001$ ) (Fig. 3). For reasons that are unclear, predicted SMR generally was somewhat greater than individual values from the literature, particularly at the higher temperatures (Fig. 3a) and masses examined (Fig. 3b). Although we can only speculate on causes of these patterns, methodological (e.g., respirometry equipment, differences in acclimation regimes), statistical (e.g., our predicted estimates resulted from a wider range of temperatures and body masses than point estimates) or biological factors (e.g., geographic or population-level variation in SMR) may have contributed.

Our calculations of  $Q_{10}$ -values for *P. cinereus* indicated that incrementally increasing the temperature led to up to 3.5-fold increases in SMR, which were generally at the high end of values reported for or calculated from other studies of plethodontid salamanders (Vernberg, 1952; Merchant, 1970; Fitzpatrick et al., 1972; Fitzpatrick, 1973; Maguire, 1981; Stefanski et al., 1989; Petruzzi et al., 2006), but comparable to other ectotherms, including turtles (Litzgus and Hopkins, 2003), snakes (McCue and Lillywhite, 2002; Dorcas et al., 2004), and ambystomatid salamanders (Whitford and Hutchinson, 1967). Differences in salamander species, methods to estimate SMR, or seasonal variation (Vernberg, 1952) may have contributed to observed differences in  $Q_{10}$ -values among studies. Interestingly, the lowest  $Q_{10}$ -value (for 15–25 °C) in our study roughly corresponded with the preferred range of temperatures reported for *P. cinereus* (16.0–21.0 °C) (Spotila, 1972; Feder and Pough, 1975). This suggests that salamanders may prefer a range of body temperatures where small deviations in temperature will have a minimal metabolic cost, but that are

maximally efficient for physiological or behavioral processes. However, should body temperatures exceed this preferred range, either from anthropogenic or natural causes, salamanders will incur a greater metabolic cost (higher  $Q_{10}$ -value).

The estimation of effects of a wide range of body mass and temperature on SMR is integral to understanding how an organism allocates energy and the fitness consequences of changes to an individual's energy budget (Sears, 2005). This study provides the foundational equation to model the bioenergetics of *P. cinereus* under various environmental conditions (e.g., temperature regimes). For example, temperature regimes to which salamanders are exposed vary with season, time of day, habitat type, and may be altered with disturbances to their habitats (Rothermel and Luhring, 2005; Homyack, 2009). Thus, with appropriate temperature data, our equation can be used to estimate the energetic costs of a wide variety of natural and anthropogenic factors. For example, Homyack (2009) recently used this allometric relationship to compare the energy needed by *P. cinereus* in unharvested forests to that in habitats where temperatures increased after recent logging.

Additionally, researchers could expand upon past work to compare differences in metabolic costs of color polymorphic *P. cinereus* (Moreno, 1989; Petrucci et al., 2006), or evaluate evolutionary relationships among salamanders (Bobka et al., 1981; Licht and Lowcock, 1991). Future work should broaden our knowledge of physiological relationships to include additional energetic parameters, such as specific dynamic action and costs of dehydration or egg production, for more species of plethodontid salamanders at a relevant range of temperatures while incorporating effects of body mass. This information is critical to understanding energy budgets, ecological trade-offs, and ultimately effects on fitness from natural or anthropogenic factors.

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