Effects of Temperature and Thermal Acclimation on Frog Metabolic Performance

Submitted by

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Abstract

The threat of global climate change makes it critical to understand how changing patterns of temperature variability influence ecological processes such as population growth rates and predator-prey interactions. According to the metabolic theory of ecology (MTE), rates of ecological processes are fundamentally limited by organism metabolic rates. Metabolism has in turn been found to scale predictably with temperature according to the Boltzmann-Arrhenius (BA) equation for enzyme kinetics, which can be adapted to account for enzyme deactivation at high temperatures (e.g., Sharpe-Schoolfield model). An important outstanding question is how key metabolic parameters, such as the activation energy for metabolism, are influenced by organismal responses including thermal acclimation. In this study, I investigated how thermal acclimation influences key MTE model parameters for adult spring peeper frogs (*Pseudacris crucifer*), by using the rate of their observable respiratory movements as a proxy for metabolic rate. After holding frogs at one of three "acclimation temperatures" for two weeks, they were transferred to one of eight "performance temperatures." I then recorded frog respiratory rates at several time points following the transfer. Statistical model fitting was used to estimate key MTE model parameters and to determine whether and how thermal acclimation influenced spring peeper thermal performance. I found that the Sharpe-Schoolfield model provided a better description of the thermal performance curve for spring peeper metabolism than the BA equation, and that thermal acclimation did not significantly alter metabolic responses to temperature so long as mass scaling effects were accounted for in the model.

Current Research

Global climate change has had enormous impacts on the living world, causing significant changes in characteristics of animal and plant populations (Root et al., 2003; Parmesan and Yohe, 2003) and influencing species interactions and ecological dynamics (Petchey et al., 1999; Tylianakis et al., 2008; Parmesan, 2006). To develop better predictive models for the ecological consequences of climate change, it is important to understand the thermal biology of individual organisms (Rohr et al., 2013; Dell et al., 2014). However, for most species there are insufficient data available to estimate key model parameters (Gillooly et al., 2001), particularly if we want to increase realism by incorporating adaptive responses to temperature variability, such as thermal acclimation (Rohr et al., 2013). The metabolic theory of ecology (MTE) provides a promising solution to this problem by providing a general mechanistic framework for understanding organism responses to temperature (Brown et al., 2004; Humphries and McCann, 2014).

According to MTE, the metabolic rates of individual organisms can be used to predict ecological processes at all levels of organization, including the rates of interspecific interactions such as predation and parasitism (Brown et al., 2004; Molnár et al., 2017). This assumption is based on the well-supported hypothesis that many characteristics of ecosystems, including carbon cycling (Allen et al., 2005), population growth and carrying capacity (Savage et al., 2004), and even rates of speciation (Allen et al., 2006), are fundamentally limited by organismal metabolic rates. MTE provides quantitative models describing how body size and temperature govern whole-body metabolism. An advantage of MTE-based models is that they provide a more mechanistic approach to modeling thermal performance curves (TPCs), which describe organism performance as a function of temperature (Huey and Kingsolver, 1989; Molnár et al., 2017). Thermal performance curves are typically unimodal, with performance increasing nonlinearly

with temperature up to a temperature of peak performance (T_{pk}), then decreasing to zero above T_{pk}. Metabolic models are based upon the Boltzmann-Arrhenius (BA) relation, which describes the temperature dependence of reaction rates (Gillooly et al., 2001; Brown et al., 2004). The description of TPCs by MTE models allows for predictions of key parameters such as activation energy (E_A) and high temperature of deactivation (T_H). Of particular interest is the Sharpe-Schoolfield (SS) model, a modified version of the BA equation that accounts for enzyme deactivation at very warm temperatures (Sharpe and DeMichele, 1977; Schoolfield et al., 1981). This model is more realistic than the standard BA equation because it captures declines in organism performance at extreme high and low temperatures due to enzyme deactivation (Hochachka and Somero, 1968), and therefore allows more accurate estimation of key parameters (Molnár et al., 2017).

A core assumption of many MTE-based models is that key model parameters remain consistent across taxa and physiological processes (Gillooly et al., 2001; Brown et al., 2004); however, significant variation in activation energy has been found among various taxonomic groups (Dell et al., 2011). Thermal acclimation responses, which are processes by which organisms reversibly adjust their physiologies in response to changing temperatures (Angilletta, 2009), might be one source of this variation, as they often alter the shape of thermal performance curves and metabolic activation energy values (Seebacher et al., 2015). Acclimation responses can include changes in gene expression that result in activation of heat-shock responses, or changes in cell membrane composition (Feder and Hofmann, 1999; Hazel and Williams, 1990). By making key SS model parameters like E_A and T_H into functions of acclimation temperature, thermal acclimation can be modeled and taken into account in analyses of metabolic

performance (Rohr et al., 2013). Incorporating acclimation temperature into the SS model also allows us to study the mechanistic basis of its effects on thermal performance.

Currently, amphibians are very poorly represented in the literature on activation energy estimates, with sufficient data to estimate key model parameters existing for only two species (Gillooly et al., 2001). Additionally, for amphibians and other taxa, the study of thermal acclimation responses has been neglected, and most prior studies have not measured metabolic performance at enough temperatures to measure key parameters for the SS model (Molnár et al., 2017; Régnière et al., 2012). Therefore, the central objective of this project was to use the SS model to describe thermal performance curves for adult spring peepers (*Pseudacris crucifer*), small frogs that are found throughout the eastern United States and Canada. I estimated key MTE model parameters for spring peepers, using respiration rate as a proxy for metabolic rate and making metabolism a function of thermal acclimation. I hypothesized that the SS model would provide a better description of the spring peeper thermal performance curve than the simpler BA model, due to deactivation of metabolic enzymes at high performance temperatures. I further predicted that the activation energy for metabolism would be lower for cold-acclimated frogs than warm-acclimated frogs (i.e., shallower "slope" for the increasing part of the curve). This prediction is derived from the "beneficial acclimation hypothesis" of thermal biology, which postulates that thermal acclimation responses should lead to increased performance at the acclimation temperature relative to unacclimated animals.

Methodology

Animal Care

This experiment was conducted in 2017 at Oakland University, and used a total of 57 adult spring peeper frogs (*Pseudacris crucifer*). In April 2015 and March 2016, adults were collected from Saginaw Forest in Ann Arbor, MI. Adults were allowed to mate in the lab and were released to the collection location once eggs had been laid. Once the eggs hatched in the lab, tadpoles from all clutches were homogenized. Tadpoles came from at least ten clutches of 2015 spring peepers and at least eight clutches of 2016 spring peepers. Before and after hatching, tadpoles were maintained in aerated artificial spring water (ASW; Cohen, Neimark, and Eveland, 1980) at room temperature (~22°C) and were fed ground fish flakes *ad libitum*. Once most tadpoles reached Gosner stage 25, tadpoles were placed in outdoor mesocosms until metamorphosis (May and June of 2015 and 2016). Mesocosms were 1000-L cattle tanks filled with dechlorinated tap water and seeded with deciduous leaf litter, pond water, and *Daphnia*. Metamorphs were then collected and maintained in the lab until the start of the experiment. Before experiments, spring peeper metamorphs were maintained at room temperature (~22°C) on a 12:12 hour light:dark cycle and were fed *ad libitum* with fruit flies dusted with ZooMed Reptivite amphibian vitamin supplement. Initially, frogs were housed in pairs within 1-L plastic containers filled with unbleached paper towel wetted with ASW. However, it quickly became apparent that there was competition for food between frogs housed together, and frogs were thereafter maintained individually. Bedding was changed weekly and was sprayed with dechlorinated tap water *ad libitum* to maintain appropriate moisture levels before the experiments began.

During the experiment, frogs were housed individually in 300-mL plastic deli cups lined with two sheets of unbleached paper towel and wetted with 15-mL of ASW (Figure 1a). To prevent desiccation, ASW was added to each cup biweekly in amounts determined from preliminary water-loss data, and soiled paper towels were changed weekly. Each frog was fed 20-30 wingless fruit flies biweekly dusted with ZooMed Reptivite amphibian supplement.

Incubators constructed using Styrofoam coolers, heat tape, and adjustable thermostats (Figure 1b; Raffel et al., 2013) were used to control temperature treatments, and were kept in a refrigerated room with an ambient temperature of 8°C. Individual frogs were rotated daily to control for within-incubator temperature variation. Incubator temperature was monitored with both thermometers and HOBO temperature loggers (Onset, Bourne, MA). All animals were maintained on a 12:12 hour light:dark cycle.

Respirometry

At the beginning of the experiment, each animal was randomly assigned to and maintained at one of three "acclimation temperatures" (11°C, 17°C, 23°C). After two weeks of acclimation (on "day 0"), each frog was moved to one of eight "performance temperatures" ranging from 8°C to 29°C in increments of 3°C. After allowing frogs to adjust to their new temperatures for 3-4 hours, a GoPro camera (GoPro Hero 3 and Hero 4 Black, GoPro Inc., San Mateo, CA) was used to record three 10-second videos of each frog's respiratory movements. Each frog was then moved to a second ("final") performance temperature, where it was held for 3-4 hours before taking another set of videos. All of these "day 0" performance measurements were recorded within 7.5 hours of each frog being moved from its acclimation temperature. I kept track of incubator order during this "performance" period, to allow us to test the assumption

that frogs remained metabolically acclimated to their original acclimation temperature throughout this several-hour "performance" period.

Videos of each frog were also recorded 5 days before the temperature shift (day -5) to assess baseline respiration rates at each acclimation temperature. To assess the amount of time required for respiration rate to acclimate to a new temperature, frogs were maintained at their final performance temperatures for several days after the temperature switch, and the video observations were repeated on days 2 and 4.

Video Analysis

All videos were analyzed using Avidemux 2.6, a free video editing program. Original videos were approximately 10 seconds each but were all cropped to 4-second long clips to ensure a consistent sampling period for all frogs. The number of breaths taken per 4 seconds was counted by watching each video frame-by-frame, with the throat moving out and back counting as one breath. Half breaths (0.5) were counted if the throat moved out fully, but not back in fully, or vice versa, and were added to the total breath count.

Statistical Analyses

All analyses were conducted using R statistical software v.3.0.2 (R Developmental Core Team, 2013). Linear mixed effects models were used for initial tests of evidence for thermal acclimation effects on respiratory performance curves at each time point (function "lmer" in package "lme4"; Bates et al., 2014), including random effects of incubator in each model to ensure acclimation and performance temperature effects were assessed at the correct level of replication (Altman et al., 2016). Linear mixed effects models were based on the central MTE equation, which assumes that mass-specific metabolic rate scales with $mass^{-1/4}$ and with temperature according to the Boltzmann-Arrhenius relationship:

$$B = p_{T0} \cdot M^{-1/4} \cdot e^{\frac{-E_A}{kT}}, \quad (1)$$

where B is mass-specific metabolic rate, p_{T0} is performance at standardization temperature T_0 (19°C), M is mass, e is Euler's number, E_A is activation energy for metabolism, k is Boltzmann's constant (8.617×10^{-5} eV/K), and T is temperature in Kelvin (Brown et al., 2004). Breaths per second (*BPS*) was our measure of mass-specific metabolic rate. Log-transforming the equation above leads to the following:

$$\ln(BPS) = p_{T0} - \frac{1}{4} ln(M) - \frac{E_A}{k}\left(\frac{1}{T}\right), \quad (2)$$

Empirical estimates of p_{T0}, E_A, and the mass exponent can be obtained by fitting data to the following regression model:

$$\ln(BPS) \sim \beta_0 + \beta_1 ln(M) + \beta_2 \left(\frac{1}{T}\right) + \varepsilon, \quad (3)$$

where $\beta_0 \approx p_{T0}$ and $\beta_2 \approx \frac{E_A}{k}$. In this formulation, $ln(M)$ and $1/T$ are treated as independent variables, and the mass exponent is allowed to vary as free parameter β_1. The respiration data was fit to this equation using a nested linear mixed-effects regression model with a random effect of Performance incubator. This allowed for straightforward estimation of $E_A \approx -\beta_2 \times k$.

This model was next compared to an alternative formulation in which the mass scaling coefficient was constrained to equal −1/4, the most commonly accepted scaling coefficient in the primary literature. In this formulation, mass-scaled respiration rate was treated as the dependent variable.

$$\ln\left(BPS \times M^{1/4}\right) \sim \beta_0 + \beta_2 \left(\frac{1}{T}\right) + \varepsilon, \quad (4)$$

I next tested for potential effects of Acclimation temperature (T_{Accl}) by adding them to the linear regression analyses described by equations 3 and 4. Visible curvature was seen in the relationship between $ln(BPS)$ and $1/T$ (Figure 2), possibly due to enzyme deactivation at high

temperatures (as predicted), so a quadratic effect of performance temperature was also included in the model. An interaction between the linear effects of acclimation and performance temperatures was also initially included; however, this was not a significant predictor of respiration and was therefore removed from the final model (Tables 1-2). Type II sums of square errors were used to obtain F-statistics and p-values for each predictor, treating linear terms as marginal to both interaction and quadratic terms. The model describing data from day -5 did not include an effect of acclimation temperature, because that was equivalent to the performance temperature at this time point.

Acclimation temperature did not significantly influence mass-scaled respiration rate (see Results); therefore, data from all time points including data collected prior to the temperature switch were combined to generate a new estimate of E_A using equation 4.

Once acclimation effects had been tested for using the appropriate level of replication via linear mixed effects models, data were fit to a modified SS model using the methods of Molnár et al. (2017). Molnár et al. (2017) provide R code for fitting data to the SS model using the theoretically predicted log-normal error distribution (the regression-based BA model-fitting described above also assumes a log-normal distribution). Unlike the BA model, the SS model accounts for reversible enzyme deactivation at high temperatures, which is a likely source of the curvature in the $ln(BPS)$~$1/T$ relationship noted above. Accounting for enzyme deactivation at high temperatures generates more accurate estimates of E_A when the dataset includes temperatures at or above the peak temperature for performance (T_{pk}; Molnár et al., 2017). Because acclimation temperature did not significantly influence mass-scaled respiration rate, acclimation temperature was not included in the nonlinear models, and all respiration time points were combined. The equation used for the SS model was:

$$p(T) = p_{T0} \cdot M^{-1/4} \cdot e^{-\frac{E_A}{k}\left(\frac{1}{T}-\frac{1}{T_0}\right)} \cdot \left[1 + e^{\frac{E_H}{k}\left(-\frac{1}{T}+\frac{1}{T_H}\right)}\right]^{-1}, \quad (5)$$

where parameters are as described for equation 1 and E_H is the energy required for enzyme deactivation, which occurs at temperature T_H. The model also includes a free parameter for the standard deviation of the log-normal error distribution, s_ε (Molnár et al., 2017). Note that this equation requires temperature to be modelled in Kelvin, but temperature values are presented in degrees Celsius here for clarity. Five parameters were optimized by maximum likelihood model fitting: pT_0, E_A, E_H, T_H, and s_ε. Starting parameter estimates were E_A = 0.3 eV, E_H = 3.5 eV, T_H = 32°C, and pT_0 = 4.5 breaths per second (estimated based on the data) multiplied by the average mass of the frogs used in the experiment.

Next, a BA model was fit to the data by removing the portion of equation 5 that specifies effects of high temperature deactivation, leaving:

$$p(T) = pT_0 \cdot \bar{M} \cdot e^{-\frac{E_A}{k}\left(\frac{1}{T}-\frac{1}{T_0}\right)}. \quad (6)$$

Optimized SS model parameter values were used as the starting parameter estimates for the BA model. The fit of the BA model was compared to that of the SS model by AIC.

Results

Regression analysis

In the linear regression model that allowed the mass-scaling exponent to vary as a free parameter (dependent variable $ln(BPS)$), respiration rate significantly increased with mass at days 0 and 2 with coefficients (estimated mass exponents) of 0.375 and 0.25, respectively (Table 1). These coefficient values were far from the MTE-predicted exponent value of -0.25. However, I also found that the acclimation treatment significantly affected frog body mass with warm-

acclimated frogs having lower mass relative to cold-acclimated frogs (Figure 3), indicating that mass and acclimation temperature were confounded predictor variables in the respiration analysis. When respiration was assumed to scale with mass$^{-1/4}$, acclimation temperature had no significant effect on respiration at any time point (Figure 2, Table 2).

Respiration rate significantly increased with performance temperature at all time points (Figure 2, Tables 1 and 2), and there was a significant negative quadratic effect of performance temperature on respiration at the 0-d time point (Figure 2b, $F_{1, 69} = 8.46$, $P = 0.005$); however, this effect was non-significant at all other time points (Tables 1 and 2). When all time points were combined, E_A for spring peeper respiration was estimated to be 0.26 eV using the linear model for mass$^{-1/4}$ scaled respiration rate.

Nonlinear analyses

The SS model provided a better fit for the spring peeper respiration data than the BA model (AIC = −278.7 versus AIC = −261.1, respectively; Figure 4). Activation energy was 0.27 ± 0.01 eV for the BA model and 0.33 ± 0.05 eV for the SS model (E_A ± SE). The high temperature for enzyme deactivation (T_H ± SE) was estimated as 37.87 ± 2.17°C using the SS model. Estimates for all parameter values including p_{T0}, E_H, and s_ε are provided in Table 3.

Discussion

As expected, respiration rate increased with performance temperature no matter how mass was incorporated into the linear model analysis, and the SS model provided a better description of the dataset than the simpler BA model indicating enzyme deactivation at high temperatures. However, contrary to my predictions, I found no evidence of thermal acclimation effects on mass-scaled respiration rate. This was somewhat surprising, given that several studies

have found effects of thermal acclimation on metabolism of temperate amphibians (Rieck et al.,1960; Dunlap, 1969; Jones, 1972). However, other studies have found no effect of thermal acclimation on respiration (Lukose and Reinhart, 1998) or locomotion (Wilson and Franklin, 2000) in temperate adult frogs. It is possible that acclimation effects might only be apparent at extreme acclimation temperatures not tested in this study (e.g., Kaufmann and Bennett, 1989). It is also possible that acclimation effects would have been detected if respiration rate was measured sooner after the temperature switch, and if frogs were not allowed to acclimate to their performance temperatures for several hours prior to the initial respiration rate recording. However, it seems unlikely that a multicellular organism like a frog would have such rapid acclimation responses as to become fully acclimated within hours of a temperature shift (Raffel et al., 2013).

Most results from linear models were quantitatively similar whether respiration rate was assumed to scale with $mass^{-1/4}$ or the mass-scaling exponent was allowed to vary; however, one important difference was detected. When the mass-scaling exponent was allowed to vary, there was a significant effect of acclimation temperature on respiration rate and the model indicated that larger frogs had significantly faster respiration rates (Table 1). This result was puzzling, because MTE predicts faster mass-specific metabolic rates and shorter process times for smaller animals (Gillooly et al., 2002). In investigating this result, it was found that acclimation temperature had a negative effect on spring peeper mass, leading to a confoundment between the acclimation treatment and frog mass in the first regression model (Figure 3). This could be a result of frogs' inability to replace fat stores to keep up with the high metabolic demand at higher acclimation temperatures, as postulated by the thermal stress hypothesis of Paull et al. (2015). The apparent acclimation effect disappeared when $mass^{-1/4}$ scaled respiration rate was used as the

dependent variable (Figure 2, Table 2). Given the ubiquity of the mass$^{-1/4}$ scaling relationship across many species, it seems unlikely that frog mass actually had a positive effect on respiration rate as implied by the models that allowed the mass scaling estimate to vary. Thus, I concluded that there was no evidence of a thermal acclimation effect on frog respiration rate in this experiment. This finding demonstrates the importance of accounting for mass-scaling relationships, especially when variables of interest (e.g., temperature) might be confounded with mass.

Activation energies are often estimated using linear regression models, by log-transforming the response variable and treating the inverse performance temperature (in K) as the independent variable (i.e., equation 3). As expected, this approach yielded the same E_A estimates as nonlinear BA model fitting, which are presented here for direct comparison with the SS model fits. However, the BA model tends to underestimate E_A values, because it does not account for decreased performance at temperatures near or above the organism's T_{pk} (Molnár et al., 2017). Consistent with this expectation, our E_A estimate from the BA model was lower than the estimate from the SS model (Table 3). All estimated spring peeper E_A values fell within the range of published E_A values across many taxa (average 0.65 eV; Dell et al., 2011). Because thermal acclimation did not influence the mass$^{-1/4}$ scaled respiration rate, my prediction that E_A and T_H would increase with acclimation temperature was not supported. In addition to providing a more accurate E_A estimate for performance data than linear and the BA model, the SS model also provides estimates of other parameters of interest, including E_H and T_H. The estimates for E_H and T_H in this study had relatively large standard errors (Table 3), likely because few data were collected above this species' T_{pk} value. Future studies focused on estimating these parameters would need to ensure that additional data are collected at these higher temperatures.

The measure of respiration rate used in this study, buccal respiratory movements, is only one aspect of amphibian respiration, and I had no measure of breath volume or cutaneous respiration rates. A study directly comparing respiratory movements to total oxygen consumption would be beneficial to confirm that respiration rate is indeed a reliable proxy for whole-body metabolism in terrestrial frogs. However, past studies have used many proxies for metabolism, including swimming or sprint speed (e.g., Seebacher et al., 2005), jump distance (e.g., Renaud and Stevens, 1983), photosynthetic rate (e.g., Dwyer et al., 2007), and amphibian calling rate (Canavero et al., 2018); and respiratory movements are likely to correlate with more direct metabolic proxies like breathing volume or oxygen consumption (Hutchinson et al., 1968; Whitford, 1973).

In conclusion, I found no evidence that thermal acclimation responses influenced the (mass$^{-1/4}$ scaled) respiration rate of spring peeper adults. These results highlight the importance of properly mass-scaling metabolic response variables to avoid drawing inaccurate conclusions regarding the effect of the independent variables of interest (e.g., acclimation temperature). Furthermore, these results provided support for using the SS model to describe metabolic data, rather than the BA model, to provide better estimates of E_A. Prior authors have suggested that the outcomes of temperature-dependent species interactions such as predation and parasitism can be predicted by combining the thermal performance curves of interacting organisms (Rohr et al., 2013; Dell et al., 2014). Buccal respiratory movements are an easily attainable, inexpensive, and non-invasive proxy for terrestrial frog metabolism that could allow rapid data collection from many amphibian species for comparisons of metabolic TPCs and predicting temperature-dependent interactions with other species.

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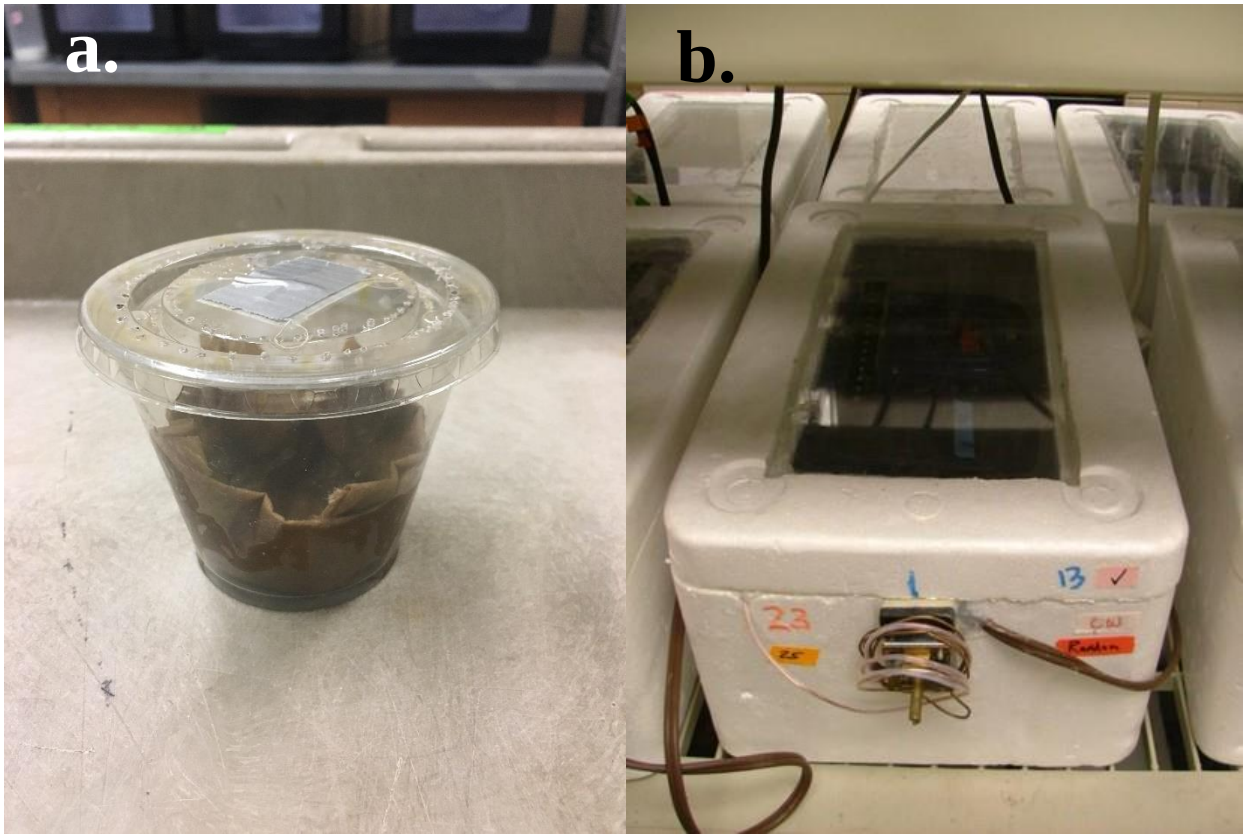

Figure 1. Plastic containers used to house individual frogs during our experiment (a.) and incubators used to control temperature treatments in our experiment (b.). The 300 mL deli containers were ventilated and contained two pieces of 550-cm^2 unbleached paper towel wetted with 15 mL ASW. Incubators were constructed using Styrofoam coolers, heat tape, and adjustable thermostats (Raffel et al., 2013).

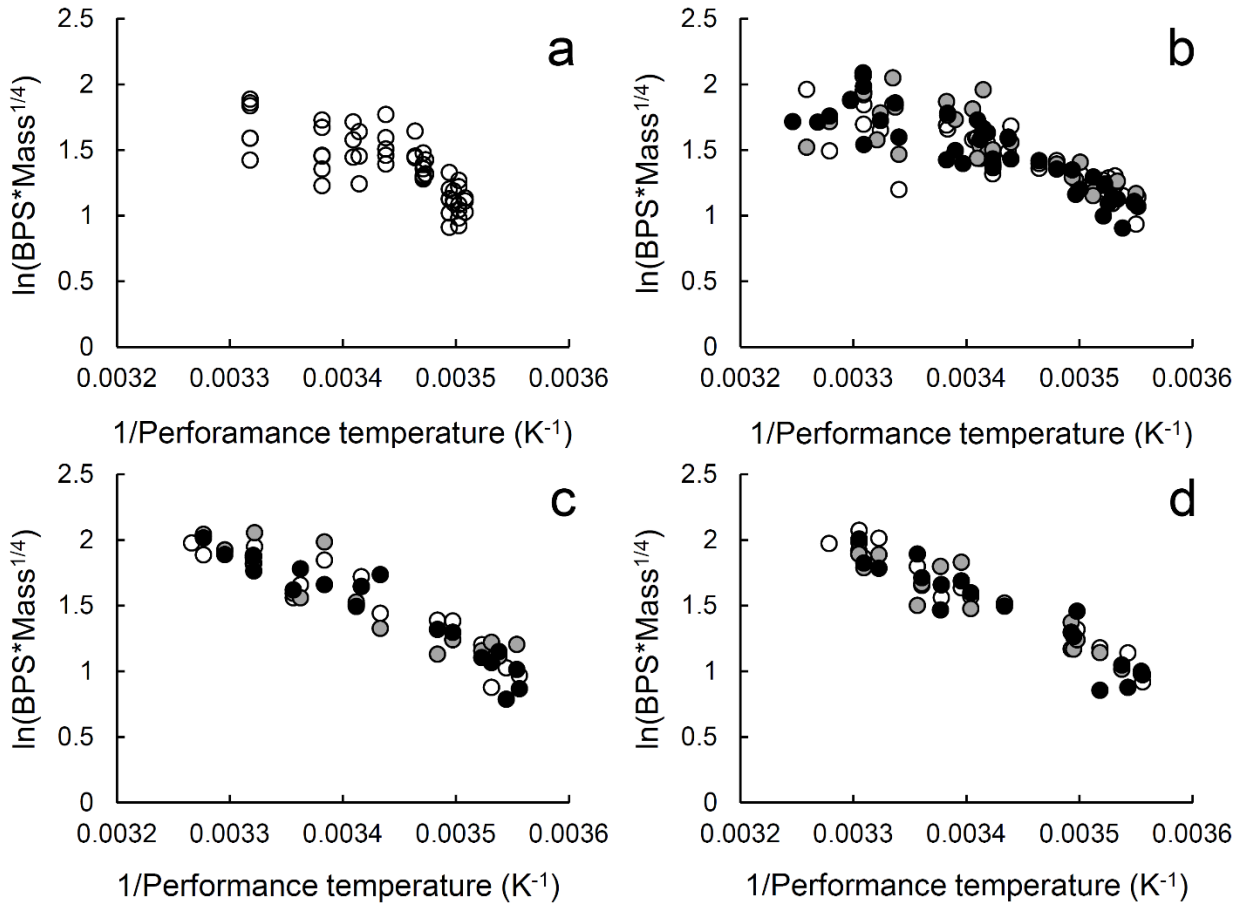

Figure 2. Effect of performance temperature (shown as the inverse of temperature in Kelvin) on mass$^{-1/4}$-scaled respiration rate of spring peepers at (a) -5 days, (b) +0 days, (c) +2 days, and (d) +4 days relative to the temperature shift. In panels (b-d), colored markers represent acclimation temperature treatments: 11°C, open circles; 17°C, gray circles; 23°C, black circles. Because the data in panel (a) were collected before the temperature switch, the acclimation temperature was equivalent to the performance temperature at this time point, and all markers are therefore the same.

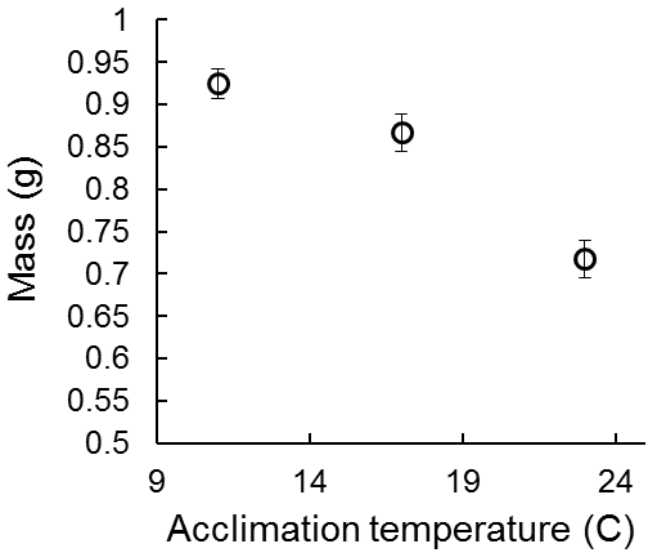

Figure 3. Effects of acclimation temperature on spring peeper body mass, measured at the end of the acclimation period. Error bars are ± SE.

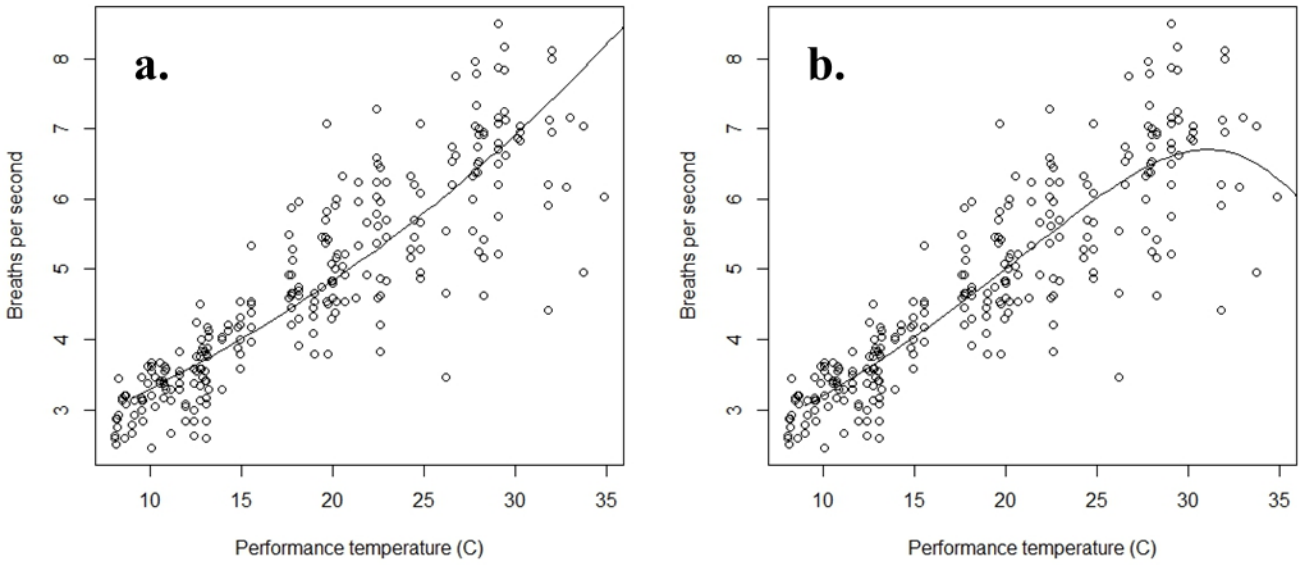

Figure 4. Nonlinear model fit for (a.) Boltzmann-Arrhenius and (b.) Sharpe-Schoolfield models describing the effect of performance temperature on spring peeper respiration rate.

Table 1. Regression statistics from linear mixed-effects models describing the effects of acclimation and performance temperature on spring peeper respiration rate, when the mass-scaling exponent was allowed to vary (response variable was ln[BPS]). Acclimation temperature was not included in -5 day models, because it was equivalent to performance temperature at that time point. The interaction between acclimation and performance temperatures was tested for at all post-temperature switch time points but was not a significant predictor of respiration rate and was therefore not included in the final models.

Time point	Predictor	Coefficient	df	F	P
-5 days	Block	-4.58×10^{-2}	1, 41	0.93	0.340
	Mass	2.34×10^{-1}	1, 41	1.78	0.190
	1/Performance temp.	-3.91×10^{3}	1, 41	51.04	< 0.001
	$(1/\text{Performance temp.})^2$	-2.23×10^{7}	1, 41	7.98	0.007
0 days	Block	4.67×10^{-2}	1, 68	3.57	0.063
	Mass	3.75×10^{-1}	1, 68	17.25	< 0.001
	1/Performance temp.	-2.68×10^{3}	1, 68	278.83	< 0.001
	$(1/\text{Performance temp.})^2$	-7.48×10^{6}	1, 68	12.83	0.001
	Acclimation temp.	1.27×10^{-2}	1, 7	16.29	0.005
	Acc. temp. × (1/Perf. temp.)	6.92×10^{0}	1, 94	0.07	0.795
2 days	Block	4.39×10^{-2}	1, 16	1.68	0.214
	Mass	2.54×10^{-1}	1, 31	4.44	0.043
	1/Performance temp.	-3.58×10^{3}	1, 16	425.04	< 0.001
	$(1/\text{Performance temp.})^2$	-4.66×10^{6}	1, 16	3.74	0.071
	Acclimation temp.	1.08×10^{-2}	1, 7	7.49	0.029
	Acc. Temp. × (1/Perf. temp.)	2.44×10^{1}	1, 39	0.58	0.450
4 days	Block	4.29×10^{-2}	1, 16	2.35	0.145
	Mass	9.00×10^{-2}	1, 30	0.58	0.454
	1/Performance temp.	-3.74×10^{3}	1, 16	607.64	< 0.001
	$(1/\text{Performance temp.})^2$	-3.21×10^{6}	1, 16	1.94	0.183
	Acclimation temp.	5.06×10^{-3}	1, 7	1.84	0.217
	Acc. Temp. × (1/Perf. temp.)	3.21×10^{1}	1, 38	0.94	0.339

Table 2. Regression statistics from linear mixed-effects models describing the effects of acclimation and performance temperature on the mass$^{-1/4}$-scaled respiration rate of spring peepers. Acclimation temperature was not included in -5 day models, because it was equivalent to performance temperature at that time point. The interaction between acclimation and performance temperatures was tested for at all post-temperature switch time points but was not a significant predictor of mass$^{-1/4}$-scaled respiration rate and was therefore not included in the final models.

Time point	Predictor	Coefficient	df	F	P
-5 days	Block	-2.42×10^{-2}	1, 42	0.23	0.639
	1/Performance temp.	-3.20×10^{3}	1, 42	33.14	< 0.001
	(1/Performance temp.)^2	-1.90×10^{7}	1, 42	3.85	0.057
0 days	Block	1.00×10^{-1}	1, 69	13.08	0.001
	1/Performance temp.	-2.62×10^{3}	1, 69	220.07	< 0.001
	(1/Performance temp.)^2	-6.68×10^{6}	1, 69	8.46	0.005
	Acclimation temp.	-1.10×10^{-3}	1, 7	0.12	0.740
	Acc. temp. × (1/Perf. temp.)	-3.32×10^{1}	1, 95	1.25	0.266
2 days	Block	7.32×10^{-2}	1, 16	3.70	0.072
	1/Performance temp.	-3.59×10^{3}	1, 16	324.52	< 0.001
	(1/Performance temp.)^2	-4.47×10^{6}	1, 16	2.61	0.126
	Acclimation temp.	-4.54×10^{-4}	1, 7	0.01	0.910
	Acc. temp. × (1/Perf. temp.)	-1.71×10^{1}	1, 40	0.25	0.620
4 days	Block	6.51×10^{-2}	1, 16	5.10	0.038
	1/Performance temp.	-3.75×10^{3}	1, 16	538.24	< 0.001
	(1/Performance temp.)^2	-2.62×10^{6}	1, 16	1.13	0.304
	Acclimation temp.	-2.37×10^{-3}	1, 7	0.68	0.438
	Acc. temp. × (1/Perf. temp.)	-4.52×10^{0}	1, 39	0.02	0.893

Table 3. Parameter estimates (value ± SE) for Boltzmann-Arrhenius (BA) and Sharpe-Schoolfield (SS) models describing the effects of performance temperature on spring peeper respiration rate. Units of measurement are breaths per second for p_{T0} and s_ε, eV for E_A and E_H, and °C for T_H.

Parameter	Nonlinear model type	Estimate
p_{T0}	BA	4.46 ± 0.04
	SS	4.66 ± 0.25
E_A	BA	0.27 ± 0.01
	SS	0.33 ± 0.05
T_H	BA	NA
	SS	37.87 ± 2.17
E_H	BA	NA
	SS	1.79 ± 1.33
s_ε	BA	0.15 ± 0.01
	SS	0.14 ± 0.01