Relationship between Behavioral Thermoregulation and Physiological Function in Larval Stream Salamanders

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ABSTRACT.—Relationships between behavioral regulation, environmental temperatures, and physiological tolerance are critical to conservation policy; however, these relationships may not be consistent among sister taxa. A species geographic range is influenced by various factors including physiological tolerance to temperature change. In this study, we investigated the effects of temperature variation on thermal preference and standard metabolic rates (SMR) of two species of larval salamanders, *Eurycea cirrigera* (Southern Two-Lined Salamander) and *Eurycea wilderae* (Blue Ridge Two-Lined Salamander). These species share similar life histories, but *E. cirrigera* is broadly distributed and *E. wilderae* occupies a smaller range. We measured SMR using volume of oxygen consumption during closed-circuit respirometery trials conducted at 5°C increments between 5°C and 25°C. Standard metabolic rates were influenced by temperature, with a range of temperature-independent SMRs observed at those temperatures above each species laboratory-determined thermal preference. Concordant with their thermal preference (15.70°C) and more-narrow geographic range, *E. wilderae* exhibited a smaller scope of temperatures at which SMR was temperature-independent, relative to *E. cirrigera*, and metabolic rates were depressed at 25°C. Therefore, preferred thermal temperatures corresponded with physiological maxima and environmental temperatures in each species. Our results support the importance of behavioral thermoregulation in maintaining optimal physiological function. Further, these findings indicate that the physiological specialization that occurs in species of narrow geographic ranges may preclude favorable responses to changing environmental temperatures caused by land-cover changes, including loss of riparian forest in the Eastern United States.

Species distributions are related to a number of biotic factors (e.g., predation, competition) and abiotic factors (e.g., precipitation, temperature, soil variables). One critical determinant of a species geographic range is physiological tolerance to temperature change (see review by Cahill et al., 2014). Specifically, metabolic function is one important physiological characteristic affecting an organism's response to fluctuations in environmental temperatures. Coadaptation theory proposes that traits within a species will coevolve via natural selection (Angilletta et al., 2006). In regard to thermal biology and metabolism, coadaptation theory predicts the evolution of traits that optimize metabolic functions to match a species thermal environment and, when given the choice, that species will select temperatures at which metabolic function is optimized (Huey and Bennett, 1987; Angilletta et al., 2006).

One mechanism by which ectotherms, such as amphibians, can respond to changes in environmental temperatures is behavioral thermoregulation (Huey and Tewksbury, 2009). Behavioral adaptations can help an organism maintain body temperatures that optimize the physiological processes associated with survival activities, including resource acquisition and allocation. Species that employ behavioral thermoregulation would be predicted to select temperatures that maximize physiological functions. The total effect of these behaviors on viability and fitness will likely be species-specific. Effects could increase (Hansen et al., 2001; Chamaille-Jammes et al., 2006; Weatherhead et al., 2012) or decrease (e.g., reduced foraging time or increased risk of predation resulting from regulatory behaviors; Sinervo et al., 2010) survival probabilities. Specialization to temperatures could also result in concurrent changes in physiological or behavioral function (or both) that could be

beneficial or detrimental to the organism, and research exploring these factors will help to inform the potential tradeoffs within these systems (Angilletta et al., 2003). Relationships between preferred environmental temperatures and physiological maxima may not always extrapolate, even among sister taxa, and species-specific attributes could prove critical when making conservation policy decisions.

We examined thermal preference and standard metabolic rate (SMR) across a range of ecologically relevant temperatures in two larval stream salamanders, *Eurycea cirrigera* (Southern Two-Lined Salamander) and *Eurycea wilderae* (Blue-Ridge Two-Lined Salamander); we used these two species as a model system for examining the relationship between environmental temperatures and metabolic function. Although these species share similar life histories, such as larval development processes and reproductive behaviors, *E. cirrigera* occupy a more diverse and extensive geographic range than do *E. wilderae* (nine ecoregions occupied for *E. cirrigera* vs. one for *E. wilderae*; Petranka, 1998; Sever, 2005). We hypothesized that metabolic rate would increase as a function of temperature in both organisms; however, in accordance with coadapation hypotheses and differences in experienced environmental temperatures and occupied geographic ranges by these closely-related species, we predicted that *E. wilderae* would experience maximum metabolic rates at lower temperatures than would *E. cirrigera* and show less physiological tolerance to changing temperatures, similar to the pattern observed in other eastern plethodontids (Bernardo and Spotila, 2006).

This study of larval salamanders provides particularly useful insight into the relationships between environmental temperatures and physiological tolerances and implications for conservation biology. Larvae are constrained to water during development and, typically, the daily thermal variation in these streams is significantly less than in the terrestrial environment. Stream-dwelling organisms will likely experience increasing water temperatures from anthropogenic climate change (Battin et al., 2007; Nelson and Palmer, 2007), exacerbated by increased insolation caused by removal of riparian forest (Sridhar et al., 2004; Sweeney et al., 2004; Seavy et al., 2009). Further

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information will be needed about the relationship between environmental temperatures and physiological function to best inform species conservation efforts targeted at organisms living in these types of environments.

MATERIALS AND METHODS

Study Species.—The plethodontid genus *Eurycea* includes numerous species that require aquatic environments for reproduction and larval development and use terrestrial habitats as adults (Petranka, 1998). The Southern Two-lined Salamander (*E. cirrigera*) is found throughout the Southeast from the Florida panhandle up to Southern Ohio and is found in a variety of habitats including low-elevation streams, rocky brooks, and river swamps. The Blue Ridge Two-lined Salamander (*E. wilderae*) has a similar life history but occupies a smaller geographic range in the Blue Ridge Mountains (Fig. 1; Petranka et al., 1993; Petranka, 1998; Sever, 2005). *Eurycea wilderae* occupy a variety of microhabitats ranging from high elevation headwater streams into lower valley drainages.

Eurycea cirrigera larvae were collected from a first-order stream in the Cowan's Ford Wildlife Refuge in Mecklenburg County, North Carolina (35.3775°N, 80.9658°W; WGS84) from 20 June to 18 October 2013. *Eurycea wilderae* larvae were collected from the Coweeta Hydrologic Laboratory in Macon County, North Carolina (35.0593°N, 83.4311°W; WGS84) from 12 June to 22 November 2013. We intentionally collected subjects within a narrow body-size range to minimize the confounding relationship between metabolic rate and body mass (mass range: *E. cirrigera* 0.18–0.46 g; *E. wilderae* 0.16–0.29 g). Larvae were
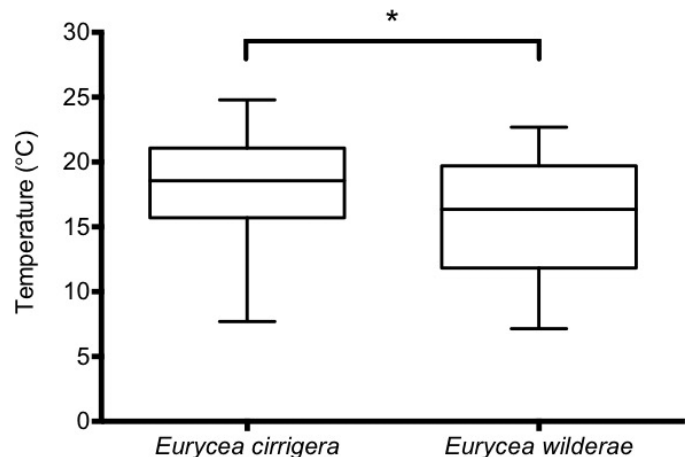
FIG. 2. Thermal preferences of *Eurycea cirrigera* and *Eurycea wilderae*. Mean thermal preference for *E. cirrigera* ($n = 25$) and *E. wilderae* ($n = 26$) over 2 h. Range represents the 2.5% to 97.5% percentiles and box represents 1st and 3rd quartiles.

housed at 4°C in dechlorinated tap water before trials were completed. Subjects were fasted for at least 72 h prior to metabolic rate trials.

Thermal Preference.—We measured thermal preference in an enclosure consisting of a level aluminum rain gutter (150×10 cm) with a sand substrate and 1 cm of dechlorinated tap water. A thermal gradient was created by placing one end of the enclosure on ice and the other on a hot plate set to low. This created a maximum gradient from 1°C to 50°C. All subjects were allowed to acclimate to room temperature for 2 h before placing them in the center of the enclosure, which was also approximately at room temperature. After placement, subjects were allowed to move freely throughout the enclosure so they could immediately begin making decisions based on available thermal cues. We measured the temperature of the individual's location every 15 min for 2 h. Temperatures were measured at the midpoint of the body to minimize disturbance to the behavior of the individual. Each larva was tested separately (*E. cirrigera*, $n = 25$; *E. wilderae*, $n = 26$).

VO₂ Consumption.—We measured SMR as the rate of oxygen consumption (VO_2) using a computer-controlled, closed-circuit respirometer system (RP1LP, Qubit Systems, Inc., Kingston, Ontario, Canada). Each salamander was placed in a sealed plastic respiratory chamber (1.6×10 cm; G113, Qubit Systems, Inc.) with 5 mL of dechlorinated tap water to ensure individuals remained fully hydrated and that cutaneous respiration could be measured (i.e., subjects were fully hydrated but not fully submerged). Lungless salamanders such as *Eurycea* exhibit high levels of cutaneous respiration (Feder, 1983b), thus making aerial measurements a consistent measure of respiration. Previous research using cutaneous respiration indicates correspondence in aerial and aquatic respiration under resting conditions (Bentley, 1975; Feder, 1983a). Metabolic chambers were placed in a temperature-controlled environmental chamber set at five, ecologically relevant temperatures tested in a randomized order (i.e., 20°C, 15°C, 5°C, 10°C, and 25°C). Each trial was separated by at least 48 h, between which subjects were returned to their housing conditions.

Preliminary testing and previous research indicated that lungless salamander metabolic rates stabilize after 3 h (Gifford et al., 2013; Young and Gifford, 2013). We chose to record VO_2 consumption over a 6-h session using the first 3 h to allow for metabolic stabilization and the last 3 h for data collection. The
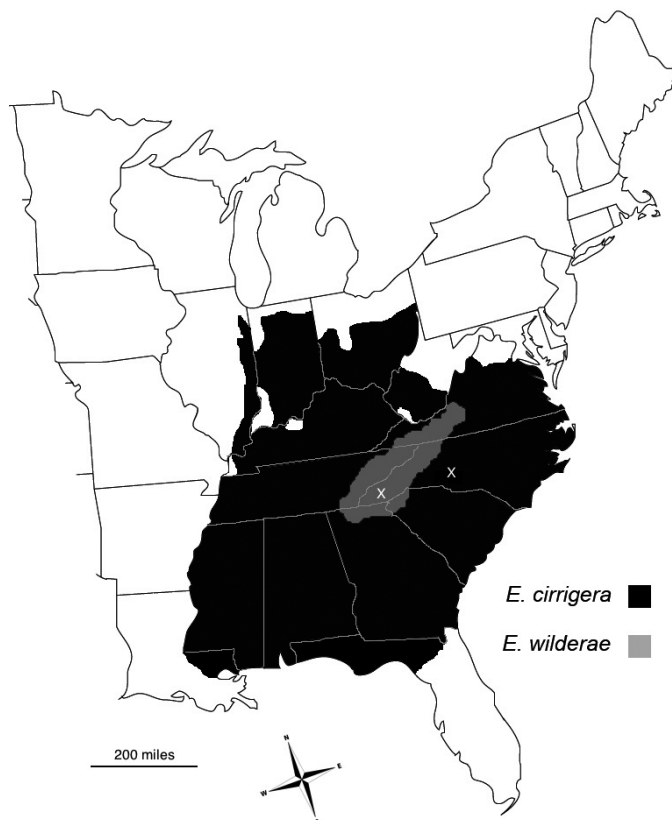

FIG. 1. Geographic range of *Eurycea cirrigera* (black) and *Eurycea wilderae* (gray). Range maps adapted from Lannoo (2005). The white X symbols indicate collection sites for study samples.

rate of VO_2 consumption was estimated from the slope of O_2 concentration vs. time over the last 3 h of each session (mean R^2 = 0.909). During measurement sessions, air-flow was controlled by a mass flow controller (G246, Qubit Systems, Inc.) with flow rates between 150 and 200 mL min^{-1}. The air steam effluent to the chambers was passed through a Drierite column prior to entering the O_2 gas analyzer (S102, Qubit Systems, Inc.). All VO_2 calculations were corrected for standard temperature and pressure in the Qubit software. Each subject was tested individually (E. cirrigera, n = 8; E. wilderae, n = 8).

Data Analysis.—To examine each species thermal preference, we conducted a repeated-measures ANOVA with species as the between-subjects factor and time as the within-subjects factor. The effect of temperature on SMR as a function of species was analyzed using an ANCOVA with body mass as a covariate (Dorcas et al., 2004; Gifford et al., 2013). Post hoc analyses were conducted using a Bonferonni correction for multiple comparisons. Body mass and SMR were log$_{10}$-transformed prior to SMR analyses to attain linearity. We calculated Q_{10} values over 5°C increments across the entire thermal range tested to examine for differences in thermal sensitivity of metabolic rate. The Q_{10} values indicate the factor by which a physiological process (e.g., metabolic rate) changes as a result of a 10°C increase in temperature, with a value of 1.0 indicating that change is temperature independent (Dorcas et al., 2004; Homyack et al., 2010; Young and Gifford, 2013). For each 5°C increment we report mean Q_{10} values (±1 SE). All statistical tests were conducted in R (R version 2.15.2; R Development Core Team, 2012) or SPSS Statistics (SPSS 20; IBM, 2011); $\alpha = 0.05$.

RESULTS

Thermal Preference.—Summer stream temperatures were collected from May to late-August during previous studies (Cecala et al., 2009; Long, 2011; Cecala, 2012) and ranged from a daily mean minimum and mean maximum of 17.1°C to 25.0°C at Cowan's Ford and 12.9°C to 18.3°C at Coweeta Hydrologic Laboratory. Mean preferred temperatures were 18.0 + 0.83°C for E. cirrigera and 15.7 + 0.87°C for E. wilderae (Fig. 2; $F_{1,609}$ = 29.16, P < 0.001). The higher thermal preference of E. cirrigera did not differ as a function of time (P = 0.915, interaction P = 0.216).

Standard Metabolic Rate.—After controlling for body mass, we observed increases in SMR of E. cirrigera at lower but not higher temperatures (Fig. 3; ANCOVA; log mass: $F_{1,6}$ = 6.194, P = 0.047; temperature: $F_{4,24}$ = 4.012, P = 0.012). Post hoc analyses indicated that SMR increased between 5°C, 10°C, and 15°C (P < 0.006) but not between 15°C, 20°C, and 25°C (P > 0.07). The Q_{10} values supported these findings in that SMR did not increase with temperature in intervals above 15°C (Table 1). Although SMR did not significantly decline above 15°C, we observed mortality at 30°C during pilot testing (data not shown).

The effect of temperature on SMR of E. wilderae was characterized by an inverted U-shape, namely SMR increased at lower temperatures but decreased at higher temperatures after controlling for body mass (Fig. 3; ANCOVA; log mass: $F_{1,6}$ = 0.568, P = 0.563; temperature: $F_{4,24}$ = 3.580, P = 0.020). Post hoc analyses indicated that SMR increased between 5°C, 10°C, and 15°C (P < 0.03) but not between 15°C and 20°C (P = 0.968) and decreased between 20°C and 25°C (P = 0.002). The Q_{10} values supported these findings, with depressed values observed between 20°C and 25°C (Table 1). Comparisons between species at each temperature indicated lower SMR values for E. wilderae at 25°C (t_{14} = 2.092, P = 0.055).
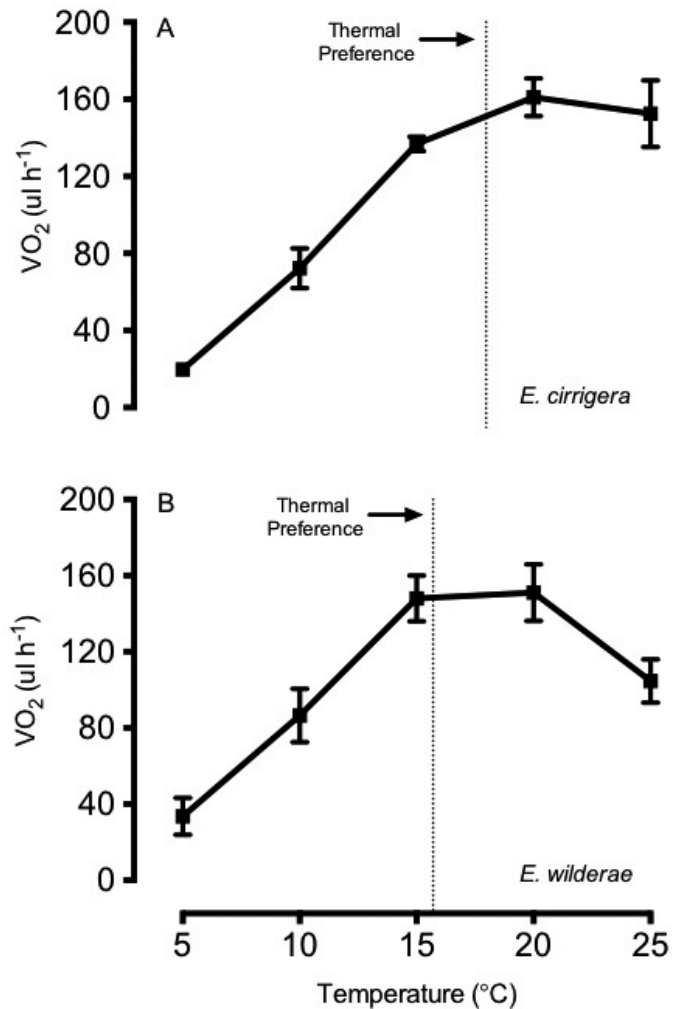

FIG. 3. Effect of temperature on standard metabolic rate in *Eurycea cirrigera* (A) and *Eurycea wilderae* (B). Values are presented as mean ± SE for eight subjects for each species. Vertical dotted lines represented mean thermal preference for each species (Fig. 2).

DISCUSSION

As predicted, SMRs of E. cirrigera and E. wilderae were influenced by temperature after controlling for mass, with a range of temperature-independent SMRs observed at those temperatures above each species thermal preference. Furthermore, physiological tolerance to temperature change was observed at the highest temperature tested in E. cirrigera but not in E. wilderae. As indicated by Q_{10} values, E. wilderae exhibited a smaller scope of temperatures at which SMR was temperature-independent, potentially as a consequence of their lower thermal preferences, smaller geographic range, and the cooler temperatures experienced in the natural environment. These findings suggest that physiological function may be correlated with the larger geographic range of E. cirrigera. Our findings relating preferred thermal temperatures, physiological maxima, and environmental temperatures can help inform future research of factors that influence species range limits as well as conservation policy regarding the effects of rapid and robust environmental temperature fluctuations on aquatic organisms.

Several hypotheses could explain how specific species might respond to fluctuations in temperature and the relationship between physiological maxima and environmental climate.

TABLE 1. Mean Q_{10} (SEM) for oxygen consumption calculated at 5°C increments for *Eurycea cirrigera* and *Eurycea wilderae*.

Interval (°C)	*Eurycea cirrigera*	*Eurycea wilderae*
5–10	2.30 (0.44)	1.80 (0.24)
10–15	1.36 (0.08)	1.60 (0.34)
15–20	1.08 (0.04)	1.02 (0.07)
20–25	0.98 (0.08)	0.85 (0.06)

First, this response could be geographically variable and dependent on extrinsic more than intrinsic characteristics. All the subjects in our study were collected from single populations of each species. As a result, the observed patterns could be phenotypic plasticity carried over from the home population sites (i.e., uncontrolled residual phenotypes; Urban et al., 2014). Natural selection pressures in these populations could have differed based on thermal and hydrologic regimes specific to each site. For example, the availability of food and other habitat resources are critically important to determining growth rates, and differences in these factors between population sites could have led to geographically specific responses.

Second, species could respond to fluctuations in temperatures with behavioral adaptations. Behavioral adaptations allow organisms to maintain body temperatures that might optimize physiological processes (e.g., metabolism) associated with survival. Numerous studies have revealed the importance of environmental temperatures and humidity in determining salamander activity patterns, with many species foraging longest at high humidity and cool temperatures (Spotila, 1972; Feder, 1983b). Preferred temperatures corresponded with environmental temperatures and physiological maxima in each species. Therefore, these species engaged in behaviors that optimized the physiological processes crucial for survival and growth, supporting the importance of behavioral thermoregulation in maintaining optimal physiological function. We measured only SMR, precluding information about metabolic rates during active periods and costs related to food processing (i.e., specific dynamic action). Although species may be able to digest food more rapidly at elevated temperatures, opportunities to forage may be reduced if surface activity becomes limited by elevated temperature. Behavioral adaptations such as changes in the time and topography of foraging could influence a species response to changing climates, and these adaptations may be critically influenced by associated metabolic costs.

Finally, physiological acclimation to new climatic regimes could contribute to species survival in a changing climate. Our findings suggest that organisms occupying smaller geographic ranges may be physiologically constrained and less likely to physiologically adapt. Indeed, a recent meta-analysis of factors determining species range and warm-edge limits found that abiotic factors, and in particular physiological tolerances to temperature, play a critical role in determining range limits (Cahill et al., 2014). Although these findings would suggest that *E. cirrigera* could occupy high elevation sites currently occupied by *E. wilderae*, other factors, including interspecific competition (Gifford and Kozak, 2012), likely are important in determining distribution. The effects of changing temperatures on behavioral processes such as competition will be important in future research.

The relationship between SMR and temperature for both species in our study supports the coadaptation hypothesis, namely that thermal preference matches biological or physio-

logical (or both) processes such as metabolic function (Dorcas et al., 1997; Angilletta et al., 2006; Dorcas and Peterson, 2007; Gaby et al., 2011). These findings indicate an important role for behavioral thermoregulation and provide a specific physiological process related to the maintenance of these optimal temperatures. We suggest that optimization of metabolic function to the thermal environment enhances biological functions necessary for salamander growth and survival, including predation and digestion. These findings are in accord with production models which predict that more-productive lifestyles (i.e., those that include increased growth and reproduction) require higher metabolic maintenance costs (Careau et al., 2008; Careau and Garland, 2012). In organisms with a biphasic life cycle (e.g., aquatic larval stage and terrestrial adult stage), body size at metamorphosis plays a key role in adult fitness (Werner, 1986) and is moderated by environmental factors including temperature (Alvarez and Nicieza, 2002; Walsh et al., 2008). Larger body size at metamorphosis can predict subsequent viability and fitness in amphibians (Smith, 1987; Berven, 1990; Altwegg and Reyer, 2003; Cabrera-Guzman et al., 2013). Therefore, larvae would be predicted to select temperatures that maximize growth rates to survive to metamorphose at the largest sizes. Concordant with this prediction, in our study the range of temperatures at which thermal independence was observed for metabolic function also matched laboratory-determined thermal preferences. Our findings suggest that species, particularly during larval development, may become metabolically adapted to their thermal environment to maximize growth and survival processes.

Several limitations of our study are noteworthy. First, aerial rather than dissolved gas measurements were recorded during respirometery trials. Although the study species occupies the aquatic environment during larval development, *Eurycea* larvae exhibit high levels of cutaneous respiration. Further, previous research indicates close correspondence in aerial and aquatic measurements under resting conditions (Bentley 1975; Feder, 1983a). Second, all individuals from each species were collected from a single population. Selection pressures in these specific populations may have differed based on hydrologic regimes specific to each site. For example, the availability of food and other habitat resources are critically important in determining growth rates, and differences in these factors between population sites could have led to site-specific adaptations. Marked variability in SMR may occur within separate populations, although previous research has demonstrated consistency in SMR within ectothermic species as a function of population site (e.g., Niewiarowski and Waldschmidt, 1992). Third, we did not measure other important factors related to range size. We recommend this as an important future research direction to determine how species will ultimately respond to changing thermal environments. Systematically exploring these potential covariates of species distribution (e.g., species predation/ competition, soil composition; Cahill et al., 2014) will help to clarify these relationships. Taken together, studies on more species and on more populations of each species will provide a more-robust description of the relationship between behaviorally determined preferred thermal temperatures, physiological maxima, and environmental temperatures.

Despite these limitations, our study poses potentially important considerations related to conservation efforts for species living in narrow geographic ranges. *Eurycea cirrigera* showed a greater thermal performance breadth for SMR, suggesting that greater geographic range occupation may be related to

metabolic physiology and tolerance capabilities. A correlational analysis of range size and SMR of *Desmognathus* spp. indicated that narrower range sizes at cooler temperatures predicted lower SMRs (Bernardo et al., 2007). Similarly, Hossack et al. (2013) found a reduced probability of death and greater acclimation to warm temperatures in *Ascaphus* tadpoles from populations exposed to greater stream temperature variance. Our study provides further evidence to support these findings and suggests that larger geographic ranges may be associated with greater thermal performance breadths under which temperature-independent SMRs are observed. Future research into these functional links between the thermal environment, physiological processes, and species survival will be imperative to informing species conservation decisions and predictions of species-specific responses to changing environmental temperatures.

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