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THE ROLE OF TEMPERATURE ON SURVIVAL AND GROWTH OF THE BARTON SPRINGS SALAMANDER (*EURYCEA SOSORUM*)

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***Abstract*.—The Barton Springs Salamander (*Eurycea sosorum*) is listed federally as endangered and is an obligate aquatic salamander with a substantial population occurring in a few spring outflows located in a highly urbanized recreational area near downtown Austin, Texas, USA. The purpose of this study was to gain essential information regarding the response of *E. sosorum* to several thermal manipulations. All salamanders used in this study were produced at the San Marcos Aquatic Resources Center (United States Fish and Wildlife Service) in San Marcos, Texas, USA, as part of a captive breeding program. To examine the effects of thermal stressors, we subjected salamanders to a nominal temperature increase of 0.5° C per day until we observed a loss of righting response (LRR). Additionally, we assessed salamander growth following a 69-d trial in which we reared young *E. sosorum* at five temperature treatments (nominal 15, 18, 21, 24 and 27° C). The cumulative ET50 (Effective Temperature at which half of the salamanders experienced a LRR [mean ± SD]) for the combined replicates observed in *E. sosorum* was 32.6 ± 0.08° C. The optimal temperature for growth of *E. sosorum* based on weight and total length was estimated to be 18.7° C and 18.3° C, respectively. These results will aid in the conservation, management, and ongoing efforts to culture *E. sosorum* in captivity. Further, our results may provide insight to the potential thermal tolerances of other perennibranchiate *Eurycea* salamanders in central Texas thought to be sensitive to thermal fluctuations in their habitats.**

Key Words.—conservation; critical thermal maxima; endangered; loss of righting response; optimal growth; Plethodontidae

INTRODUCTION

The global decline in amphibian populations is an alarming phenomenon, which results in the loss of ecologically significant species and an overall reduction in local biodiversity (Blaustein and Wake 1995). In the early 1990s, public attention in Austin, Texas, USA, was focused on the protection of Barton Springs, a locally revered spring-fed swimming area, and the imperiled Barton Springs Salamander (*Eurycea sosorum*) found within the Barton Springs complex (Petranka 1998). *Eurycea sosorum* was considered to have one of the smallest ranges of any known vertebrate species in North America (Chippindale et al. 1993) with spring habitat located in a rapidly developing area of Austin, Texas. Given dramatically low monthly salamander surveys during the early 1990s (Hansen et al. 1998), citizens, politicians, and developers fervently deliberated proposed conservation measures for *E. sosorum* and its habitat. Ultimately *E. sosorum* was granted endangered status by the US Fish and Wildlife Service (USFWS 1997).

The species occupies a stenothermal habitat with perennial spring flow and substrates consisting of rock, cobble, gravel, and various macrophytes. Given the adaptation of *E. sosorum* to a relatively stable thermal environment, *E. sosorum* is at a minimum partially dependent on consistent water temperatures (USFWS 2005). Temperature is one of the most important abiotic factors affecting amphibians and influences physiological functions, such as metabolic rate, gas exchange, reproduction, development, and growth (Hillman et al. 2009). For stenotherms, maintenance of a narrow range of environmental temperatures is necessary for optimal physiological function. Numerous studies demonstrate the detrimental effects to salamanders caused by temperature extremes (Zweifel 1957; Hutchison 1961; Rohr and Palmer 2012). Annual mean water temperatures in Barton Springs normally range from 21° to 22° C (USFWS 2005). However, intermittent spring flow does occur at Upper Barton Springs (Dries et al. 2013). Despite long-term average temperatures (report period 2004 to 2011) falling within this range, Dries et al. (2013) states that regression analyses from available data indicate a statistically significant increasing trend for water temperatures in both Upper Barton and Parthenia Springs.

Information concerning the response of *E. sosorum* to thermal changes is lacking. To develop and implement effective conservation measures, including improved culture techniques and a review of the current maximum temperature criteria for Barton Creek (Texas

Commission on Environmental Quality 2014), an understanding of the environmental requirements of an organism is necessary. For the following study we determined a relationship between a slow increase in temperature (nominal increase of 0.5° C per day) on the loss of righting response (LRR) reflex, and we determined the effect of temperature on growth of *E. sosorum*. This information may be incorporated into the recovery plan (USFWS 2005) for this federally listed species and may benefit habitat managers as they try and estimate the effects of changing environmental conditions on *E. sosorum* (Turner 2004) and other central Texas stream-dwelling salamanders.

MATERIALS AND METHODS

We conducted the following experiments at the San Marcos Aquatic Resources Center (SMARC, US Fish and Wildlife Service facility), in San Marcos, Texas, USA, using captive bred *E. sosorum* produced as part of a captive breeding program at the SMARC (USFWS 2005). We filled multiple 1,135 L temperature-controlled, fiberglass tank systems approximately half full with well water from the Edwards Aquifer to serve as thermally stable water bath reservoirs. We placed salamanders into one of three 75 L experimental tanks, which we submerged approximately two-thirds into each water bath reservoir during trials. We situated polyvinyl chloride (PVC) pipe halves (5 cm diameter; thermal maxima experiment) or rocks and macrophytes (optimal growth experiment) in each experimental tank to provide shelter for the salamanders. Additionally, we provided a submersible pump in each experimental tank for water recirculation.

***Thermal maxima experiment*.—**We placed 15 *E. sosorum* into each of three experimental tanks and allowed the salamanders 24 h to acclimate prior to treatment. Initial water temperature was 21 ± 1° C (mean ± SD), which is similar to their natural spring habitat and captive assurance colony holding systems at the SMARC. We then raised the temperature a nominal 0.5° C/d (actual mean temperature increase = 0.46° C/d, r^2 = 0.997) until all salamanders exhibited a LRR. The LRR is characterized by a loss of equilibrium so that when sinking to the bottom after a period of swimming, the salamander may rest upon its back for a time before righting itself (Zweifel 1957). The gradual increase in temperature should have provided the animals an opportunity to physiologically acclimate to the increasing temperature (Lowe and Vance 1955; Spotila 1972), and served to determine maximum tolerable temperature. When the temperatures approached the suspected upper thermal limits of the salamander, based on decreased feeding, hyperactivity, and thermal maxima values for salamanders obtained from literature (Zweifel 1957; Sealander and West 1969; Spotila 1972; Berkhouse and Fries 1995; Lutterschmidt and Hutchison 1997), we began monitoring the salamanders for several minutes every hour until the temperature stopped increasing, and then every 3 h after that. At the first indication a salamander was experiencing a LRR, we removed, weighed (to the nearest g), and measured to the nearest millimeter the snout to vent length (SVL, the distance from the tip of the snout to the posterior margin of the cloaca) and total length (TL, the distance from the tip of the snout to the tip of the tail) of the individual. Immediately following measurements, we placed each salamander in a separate experimental tank maintained at the same experimental temperature, and allowed them to cool gradually (approximately 1° C/h) to facilitate their recovery. Mean salamander weight in the thermal maxima experiment was 1.2 ± 0.40 g (mean ± SD), and mean SVL and TL were 37 ± 3.5 mm and 73 ± 9.4 mm, respectively.

We measured temperature, pH (8.0 ± 0.31), and dissolved oxygen saturation (86 ± 10.0%) daily during the trial using a Hydrotech MS5 meter (Hydrotech ZS Consulting, Round Rock, Texas, USA). We measured total ammonia-nitrogen (0.3 ± 0.31 mg/L) up to three times per week throughout the trial by direct Nesslerization (American Public Health Association 1989). We fed the salamanders *ad libitum* a diet of commercially produced live Blackworms, *Lumbriculus variegatus* (California Blackworm Co., Fresno, California, USA), and Brine Shrimp, *Artemia salina* (Mariculture Technologies International Inc., Oak Hill, Florida, USA). We maintained the light cycle under natural photoperiod conditions via overhead skylights and uncovered windows.

***Optimal growth experiment*.**—We stocked six to seven salamanders (TL = 15–40 mm) into each of fifteen 75 L experimental tanks. We partially submerged each experimental tank in one of five temperature-controlled fiberglass tank systems, which served as a thermally stable water bath reservoir (three experimental tanks per water bath reservoir). We then allowed the salamanders 24 h to acclimate at a water temperature of 21° C. We adjusted the temperature in each of the five water bath reservoirs to a nominal 15, 18, 21, 24 and 27° C at a rate of ± 1° C/d (Sadler 1979). This rate of temperature change allowed the salamanders to acclimate to the treatment temperatures. When each water bath reservoir reached the assigned temperature, we removed the salamanders, weighed (g) and measured (mm TL) them, and returned each to their respective experimental tank. Mean weight and TL of salamanders at the start of the trial were 0.1 ± 0.06 g (mean ± SD) and 31 ± 4.6 mm, respectively.

While in the experimental tanks, we observed the salamanders at least twice per day and offered them an alternating diet of Brine Shrimp (*Artemia salina*) larvae (INVE Aquaculture Inc., Salt Lake City, Utah, USA), zooplankton (pond raised at the SMARC), and Blackworms, *ad libitum*. We monitored temperature (temperature within a mean of 0.2 ± 0.21° C of nominal settings across temperature treatments), dissolved oxygen saturation (92 ± 7.3% across temperature treatments), and pH (8.3 ± 0.11 across temperature treatments) in each experimental tank daily using a Hach HQ40d meter (Hach Co., Loveland, Colorado, USA). We tracked total ammonia-nitrogen (0.8 ± 0.59 mg/L across temperature treatments) in each experimental tank twice per week by direct Nesslerization. If total ammonia-nitrogen levels exceeded 0.5 mg/L in experimental tanks, we performed a 40% water change using fresh Edwards Aquifer well water that had been maintained at the same experimental temperature as the respective experimental tank. After 69 d at their respective temperature treatments, we removed the salamanders from their experimental tanks and weighed and measured each salamander. We then returned the salamanders to their respective experimental tanks and allowed them to acclimate to their natural habitat and holding temperature of 21° C using the method of a ± 1° C change in temperature per day (Sadler 1979) as previously described.

We applied regression analysis to analyze the data from both experiments, with a significance level set at a $P \leq 0.05$. We used statistical software R (R Core Team 2014) to determine probability values. Data was tested and met the assumptions of normality, independence, and homoscedasticity necessary for regression analysis. In the thermal maxima experiment, we applied linear regression to each replicate (n = 3) and to all replicates combined (Fig. 1). We then calculated the effective temperature at which half of the salamanders displayed a LRR (ET50) for each replicate, as well as the combined replicates, along with the 95% confidence interval. We used the linear equations generated by each regression to calculate ET50 values by assuming a 50% value for the dependent variable (% LRR) and solving for the independent variable value (Temperature). Additionally, to compare our LRR values to those of other plethodontid species, we calculated the mean LRR for each replicate and for the combined replicates. For the optimal growth experiment, we used the mean of all logged temperature readings (70 temperature readings per experimental tank) obtained during the trial and mean salamander growth (% increase in weight and TL of salamanders in experimental tank) in each experimental tank in the polynomial regression analyses. This resulted in three replicates per temperature treatment. We calculated the optimal relationship between either length or weight and temperature from the quadratic equations of each regression (Fig. 2A and 2B) by calculating the estimated vertex of the curve using the following equation;

$$\hat{x} = -\frac{b}{2a}$$

where $\hat{x}$ is the estimated peak of the curve for temperature (° C), a represents the coefficient of the quadratic term, and b represents the coefficient of the linear term.

Results

Thermal maxima experiment.—We observed the first instance of LRR in *E. sosorum* at 31.7° C and recorded a maximum temperature of 33.5° C for a LRR. We noted episodes of LRR by the preliminary loss of posterior limb function, consistent with Hutchison (1961), followed by moments of disoriented swimming. The ET50 for the three replicates ranged from 32.5 ± 0.15° C (95% confidence interval of 32.42–32.58° C) to 32.9 ± 0.07° C (95% confidence interval of 32.87–32.93° C). For all replicates combined, the ET50 for the LRR in *E. sosorum* was 32.6 ± 0.08° C (95% confidence interval of 32.58–32.62° C; LRR = -1761.9 + 55.485Temperature; $r^2 = 0.98$; $F_{1,44} = 2083$; $P < 0.001$; Fig. 1). Additionally, the mean LRR for *E. sosorum* was 32.7 ± 0.51° C (95% confidence interval of 32.44–32.96° C) for all replicates combined with a range from 32.5 ± 0.45° C (95% confidence interval of 32.27–32.73° C) to 33.0 ± 0.44° C (95% confidence interval of 32.78–33.22° C).

Optimal growth experiment.—Three *E. sosorum* died during the growth experiments (97% survival). Initial weights and initial TL were not significantly different

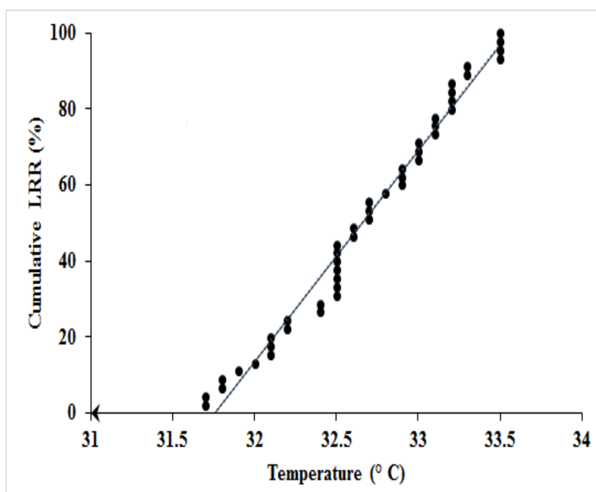

Figure 1. Cumulative loss of righting response (%), for combined replicates of Barton Springs Salamander (*Eurycea sosorum*) in the course of daily temperature increases of 0.45° C (initial temperature = 20.8° C).

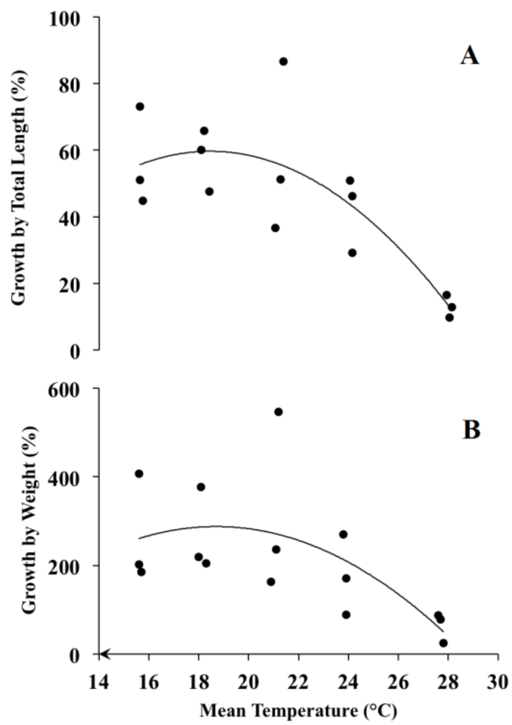

FIGURE 2. Relationship between mean temperature (° C) and growth by percentage total length (A) and percentage weight (B) of Barton Springs Salamander (*Eurycea sosorum*) after a 69 d exposure to nominal temperatures of 15, 18, 21, 24 and 27° C. Growth by TL (A) was equivalent at all tested temperatures, except 27° C, where a significant decline ($P = 0.005$) in growth occurred**.**

among treatments ($F_{4,90} = 1.33$, $P = 0.265$ and $F_{4,90} = 0.905$, $P = 0.464$, respectively). Temperature had a significant effect on the percentage increase in both weight (Weight = -705.35 + 106.28Temperature - 2.84Temperature^2; $r^2 = 0.40$; $F_{2,12} = 3.97$; $P = 0.047$; Fig. 2) and TL (TL = -119.47 + 19.54Temperature - 0.53Temperature^2; $r^2 = 0.66$; $F_{2,12} = 11.48$; $P = 0.018$; Fig. 2) of *E. sosorum*. We estimated the optimal temperature for growth based on increase in weight of *E. sosorum* to be 18.7° C resulting in a 287.3% increase in weight (95% confidence interval of 201.54–373.29%). We estimated the optimal temperature for growth based on increase in TL of *E. sosorum* to be 18.3° C resulting in a 59.7% increase in TL (95% confidence interval of 49.63–69.82%). Further, the 27° C temperature treatment was found to be significantly different for percentage increase in TL ($t = -3.53$; df = 4; $P = 0.005$), suggesting an optimal temperature range of 15–24° C for the tested temperatures. We observed the least overall increases in both weight and TL in the 27° C treatment (actual mean temperature = 27.7° C), which is typically outside the normal thermal range currently encountered in *E. sosorum* habitat.

DISCUSSION

Thermal maxima experiment.—Our results were consistent with numerous thermal tolerance studies of plethodontids (Sealander and West 1969; Spotila 1972; Berkhouse and Fries 1995; Lutterschmidt and Hutchinson 1997), which indicate episodes of LRR at similar temperatures for the Rich Mountain Salamander, *Plethodon ouachitae* (30.6° C), Southern Ravine Salamander, *Plethodon richmondi* (31.3° C), San Marcos Salamander, *Eurycea nana* (35.8° C for adults and 34.3° C for juveniles), and Allegheny Mountain Dusky Salamander, *Desmognathus ochrophaeus* (29.2° C). However, many past studies used heating rates of 0.5 to 1.0° C/min. These rapid heating rates are often justified as necessary to account for the rapid rate at which most reptiles absorb or lose heat (Cowles and Bogert 1944). We chose a reduced heating rate of 0.5° C/d for the current study to allow the animals to physiologically acclimate to changing temperatures, perhaps giving a better estimate of their upper thermal limits. Moreover, these are more likely to approximate conditions in the field in the absence of spring flow or during reduced spring flows.

Optimal growth experiment.—Our optimal growth temperature estimate is slightly below the lower end of the temperature range typically occurring in *E. sosorum* surface habitat (21 to 22° C). However, as none of the treatments in the range of 15–24° C were significantly different, the surface habitat temperatures known for *E. sosorum* are within the range of temperatures determined for optimal growth in TL. Our results are consistent with those from other studies, which indicate that within the temperature range normally encountered by an organism, a decrease in rearing temperatures causes a majority of ectotherms to attain larger sizes at a given developmental stage (Atkinson 1994). Additionally, reduced growth occurs with increased rearing temperatures in ectotherms (Atkinson 1995). However, this reduced growth generally results when an ectotherm is outside of its optimal temperature range for performance (Huey and Kingsolver 1989). Temperature essentially controls the metabolic rate and metabolic scope of such organisms. Metabolic scope is described as the difference between maximum sustained metabolic rate and the standard metabolic rate (SMR) for poikilotherms (Randall et al. 1997). At SMR for poikilotherms, metabolic oxygen demand generally increases exponentially with temperature. However, the physiological capacity to supply this oxygen increases sigmoidally with increasing temperatures (Neill and Bryan 1991). Thus, the temperature of maximum metabolic scope is the optimal temperature for an organism. As temperatures increase beyond the optimal temperature of an organism, metabolic scope begins to decrease, subsequently affecting the physiology and growth of an organism. Our results support an optimal temperature range of 15–24° C for optimal growth of *Eurycea sosorum*, which is much broader than the thermal tolerance often speculated (USFWS 2005; Benjamin Pierce and Ashley Wall, unpubl. report).

Given the ongoing efforts to culture *E. sosorum* in captivity (Chamberlain and O'Donnell 2003; USFWS 2005), our results provide basic thermal information for the culture of *E. sosorum* for captive assurance colonies. Currently, the City of Austin and the US Fish and Wildlife Service maintain captive assurance colonies of *E. sosorum*. Given the inherent difficulties of maintaining a very narrow temperature range in aquaculture (e.g. 21–22° C for *E. sosorum*), these data demonstrate a broader temperature range (15–24° C) for which *E. sosorum* experiences appreciable growth in an aquaculture setting. Thus, captive rearing temperatures that would fall within this range should not negatively impact growth of *E. sosorum*. However, the long term effects of these temperatures on *E. sosorum* have not been investigated.

Further, our results provide vital thermal tolerance information for *E. sosorum*, which may be of value to habitat managers as they try to estimate the impacts of changing habitat temperatures and conditions on *E. sosorum* (Turner 2004). For example, temperatures exceeding 26° C have been documented in Old Mill Spring (Dries et al. 2013), a known spring habitat for *E. sosorum* located within the Barton Springs complex in Austin, Texas. As Turner (2004) reasonably concludes, if spring flows within Barton Springs decrease significantly, water temperatures in Barton Springs pool as a whole will subsequently increase. Historical records indicate decreased spring flows coinciding with episodes of drought in Barton Springs, including the infamous drought of the 1950s, which led to the lowest measured monthly average spring discharge levels ever recorded at Barton Springs of 0.31 m^3/s (11 ft^3/s; Smith et al. 2013). Additionally, the drought of the 1950s led to the complete cessation of spring flow at Comal Springs, the largest spring system in Texas, located in Landa Park, New Braunfels, Texas (Smith and Hunt 2010). Given the probability of such events occurring again in the region and the vulnerability of the Edwards Aquifer to global warming trends (Loaiciga et al. 2000), proactive conservation measures may be warranted. Such measures may include the confinement of wild salamanders into temporary holding facilities during thermal extremes and a review of the current Texas surface water quality standards maximum temperature criteria within Barton Creek (Texas Commission on Environmental Quality 2014).

Fourteen recognized spring and cave-dwelling salamander species in the genus *Eurycea* inhabit the Edwards Plateau region of central Texas (Chippindale et al. 2000). Of those, three are federally listed as endangered and four are federally listed as threatened (USFWS: Environmental Conservation Online System. Endangered species search: Texas. Available from http://ecos.fws.gov/tess_public/reports/species-listed-by-state-report?state=TX&status=listed [Accessed 18 September 2015]). Temperatures up to and exceeding 30° C have been documented (Bowles et al. 2006) in a known habitat of the Jollyville Plateau Salamander, *Eurycea tonkawae*, a federally listed threatened (USFWS 2013) species found along the Jollyville segment of the Edwards Plateau. However, Bowles et al. (2006) did not indicate that this site was occupied by *E. tonkawae* at the time of data collection. Based on our data, we suggest that this temperature approaches the limits of the species thermal maxima, but is clearly beyond the temperatures supporting optimal growth. Further, Bowles et al. (2006) observed that Jollyville Plateau Salamanders are more likely to occur near spring outflows, which are areas of constant water temperature, and determined a negative relationship between salamander density and the standard deviation in water temperature across sites. This apparent dependence on thermal consistency has also been documented in the federally listed threatened Georgetown Salamander, *Eurycea naufrugia* (USFWS 2014), whereby habitat temperatures within 25 m of a known spring habitat for this species remained within the range of 20.2 to 21.0° C for an entire year (Pierce et al. 2010).

Considering the pressures on wild populations of *E. sosorum*, including the presence of predators and competition, salamanders may exhibit less tolerance to thermal extremes in the wild because of these added stressors than did the captive organisms used in this study. Further, the temperatures tested in our study have not been formally tested on the eggs or larvae of *E. sosorum* and would likely have similar, if not exaggerated effects on these life cycle stages. The US Fish and Wildlife Service recommends that to help protect aquatic federally listed species in Texas from adverse effects, temperatures in waters with these species should be maintained at levels that will not affect any life cycle stage of these species (White et al. 2006). With the potential for continued increases in habitat temperatures for *E. sosorum*, as well as other central Texas *Eurycea*, an understanding of the thermal requirements for all life history stages will be necessary for the continued conservation of these species.

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