

Temperature Selection in the Arizona Tiger Salamander (*Ambystoma mavortium nebulosum*)

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Abstract:

High-elevation ecosystems are experiencing rapid warming and severe impact of global climate change. The Arizona Tiger Salamander (*Ambystoma mavortium nebulosum*) occurs in high elevation ponds in the West Elk Mountains of Colorado and offers a compelling system to test context-dependent responses to climate change. Larval salamanders either metamorphose into terrestrial metamorphs or become gilled, aquatic paedomorphs, thus, the morphs may be differentially affected by climate change. To determine how the different thermal environments experienced by the morphs affects their physiology, we will compare the preferred temperature of metamorphic adult salamanders from high-elevation ponds. A comparison of paedomorphs and metamorphs was initially desired but due to not being able to find any this summer only metamorphs were used. The first objective is to construct and test a water-filled thermal gradient runway. The thermal gradient design will be improved until we can establish a reliable range and distribution of temperatures, we will place a single salamander in the gradient for 3.5 hours and will record horizontal position in the gradient and water temperature next to the animal's middlebody every 5 minutes. We will also compare the horizontal position of salamanders in the chamber without the temperature gradient as a control to test for evidence of active thermoregulation by the animals in temperature gradient trials. We will calculate the mean, and

upper and lower 10% of temperatures and horizontal positions for each individual and utilize an ANOVA test in order to see what effect sex, collection site and mass have on minimum, maximum and average temperature selection.

Keywords: Arizona tiger salamanders, thermal gradient runway, temperature selection, thermoregulation, climate change

Introduction:

Thermoregulation is a critical process in the survival of an organism as it enables the body to return and stay within particular body temperatures and maintain homeostasis. Two main forms of thermoregulation exist, endothermy and ectothermy. Whereas endothermic organisms thermoregulate mostly internally via metabolic processes, ectothermic organisms thermoregulate mostly using external sources in their environment (Libretexts. Homeostasis - Thermoregulation, 2020). Environmental temperature is thus fundamentally important in the ecology of ectotherms (Vitt & Caldwell, 2014). Because ectotherms are reliant on environmental temperature, they are also potentially sensitive to changes in environmental temperatures, thus making them particularly vulnerable to climate change (Paaijmans et al., 2013). Thermoregulatory behaviors allow ectotherms to escape being completely reliant on environmental temperature as long as thermal microhabitats with heterogeneous temperatures are available for them to move between in order to avoid environmental temperatures that are far from their preferred temperatures (Hossack et al., 2009). One taxon that is especially threatened due to climate change is the class Amphibia (Donnelly and Crump 1998), because as ectotherms with permeable skin they have high sensitivity to temperature and precipitation changes (Corn, 2007) which are both being altered by climate change. Temperate ectotherms on average have increased warming tolerances

compared to subtropical species, but temperate zones are predicted to have faster rates of warming (Duarte et al., 2012).

Understanding the thermoregulatory behavior of amphibians is critical to understand how they interact with their environment and how climate change may potentially impact them. Previous research has shown that salamanders have considerable control of their body temperature via behavioral temperature selection (Licht and Brown, 1967). Salamanders thermoregulate using the varying water temperature of the ponds, the shallows of our high elevation sites are warmer during the day and at night the deeper parts are warmer. The Arizona Tiger Salamander (*Ambystoma mavortium nebulosum*), which is the organism of study for this research project, could be vulnerable to climate change due to being restricted to breeding in bodies of water as well as living in and around them. *Ambystoma malvortium nebulosum* occurs within the region around the Rocky Mountain Biological Laboratory at high elevation lakes where fully metamorphosed and paedomorphic adults occur within the ponds. The paedomorphic adults retain gills into adulthood and remain fully aquatic. In contrast metamorphic adults lose their gills and spend a significant time in both the terrestrial and aquatic environments (Whiteman et al., 2011).

High-elevation Arizona Tiger Salamanders offer a compelling system to test context-dependent responses to climate change independent of geographic or phylogenetic variation in a relatively simple montane community (thus limiting confounding effects of species interactions). Because of their polyphenic life history strategies, paedomorphs are primarily subject to environmental changes in the aquatic habitat whereas metamorphs are subject to environmental variation affecting both the aquatic and terrestrial environments. Metamorphs and

paedomorphs are thus likely to be asymmetrically impacted by changes to thermoregulatory opportunities caused by climate change.

Previous research on the preferred temperatures of *A. m. nebulosum* at Mexican Cut Nature Preserve demonstrated that the metamorphs exhibited less precision in their thermoregulation 15° - 29°C while the paedomorphs exhibited a narrow selection in temperature ranging between 21° and 26°C (Heath, 1975). It can be predicted based upon Heath's results that the paedomorphs are narrower in their selection due to a narrower range of aquatic temps. However, air temperature at the site has already increased by 2.5°C over 30 years (Whiteman, personal communication) and preferred temperatures may have shifted as well or may have remained static despite changes in environmental temperature ranges. In addition, (Heath, 1975) had experimental design flaws that we will correct (e.g., estimates of preferred temperature were not independent samples).

Materials and Methods:

The first goal was to construct a thermal gradient runway that could hold water, hold a thermal gradient and produce a repeatable thermal gradient. The thermal gradient runway needed to exhibit temperatures that the animals would experience throughout the active season, roughly 5°C to 30°C, and do so for 3.5 hours, the length of a full trial. The runway had a length of 243.84 centimeters accessible to the salamanders. The heating and cooling aluminum blocks were blocked off to the salamanders. One large 3 meter long gutter was used for the runway and was cooled on one end and heated on the other end using a cold water reservoir and a hot water reservoir.

The cold water reservoir utilized a cooler with salt water and a wort chiller. The target temperature for the cold end of the runway was 5°C. The wort chiller is a coiled series of metal

pipes that water and be pumped through and chilled. The salt water was used in order to decrease the freezing point of the water and thus bring the moving water to an even lower temperature. A water pump pumped the water through the wort chiller which was surrounded with ice packs and ice which brought the water down to roughly 1°C and flowed into the aluminum blocks at one end of the runway in order to chill the water and then pump it back into the reservoir. This cold water reservoir formed a closed loop system and none of the water in the reservoir mixed with water in the runway.

The hot water reservoir heated the opposite end of the reservoir with a target temperature of 27.5°C after realizing that 30°C was too difficult to maintain and preserve the 5°C of the cold end. The hot water reservoir was constructed of an insulated cooler with an immersion heater and pump. The pump pumped the water into aluminum blocks in the runway in order to heat that end and then flowed back into the hot water reservoir thus producing another closed loop reservoir.

Experimental trials consisted of placing the salamanders in the middle position of the runway, roughly 121.92 centimeters from both the coldest and hottest position. The trials lasted 3.5 hours with the first hour not being analyzed in our data analyses and being considered an acclimation period. The animals' middle body temperature was recorded using a thermocouple probe every 5 minutes as well as their horizontal position in the runway, 0 centimeters being the coldest position, 121.92 centimeters being the middle position and 243.84 centimeters being the hottest position. Control trials consisted of the exact method as the experimental trials except no thermal gradient was present, only a flat temperature was present throughout the runway. The control was used in order to determine if the experimental animals were moving throughout the gradient randomly or selecting a temperature via thermoregulation.

The animals used for these trials were collected from high elevation ponds. Specifically, the animals I used were collected from Flat Tire Pond, Lily Pond and Rainbow Lake. These animals were brought back to the animal care room at RMBL and acclimated for 48 hours before being used in experimental or control trials. Appropriate biosecurity protocols were followed: we cleaned all equipment and containers between sites with 10% bleach, rinsed it, and dried it and similar methods were used for equipment and supplies that the animals made contact with during their stay in the animal care room. The runway was also sanitized between uses of salamanders from different collection sites. The animals were then released 6-7 days after being collected at their original point of capture.

For the statistical analysis the maximum, minimum and average temperature recordings for each animal were used (Angilletta 2009). An ANOVA test was utilized in order to see what effect sex, collection site and mass had on minimum, maximum and average temperature selection.

Results:

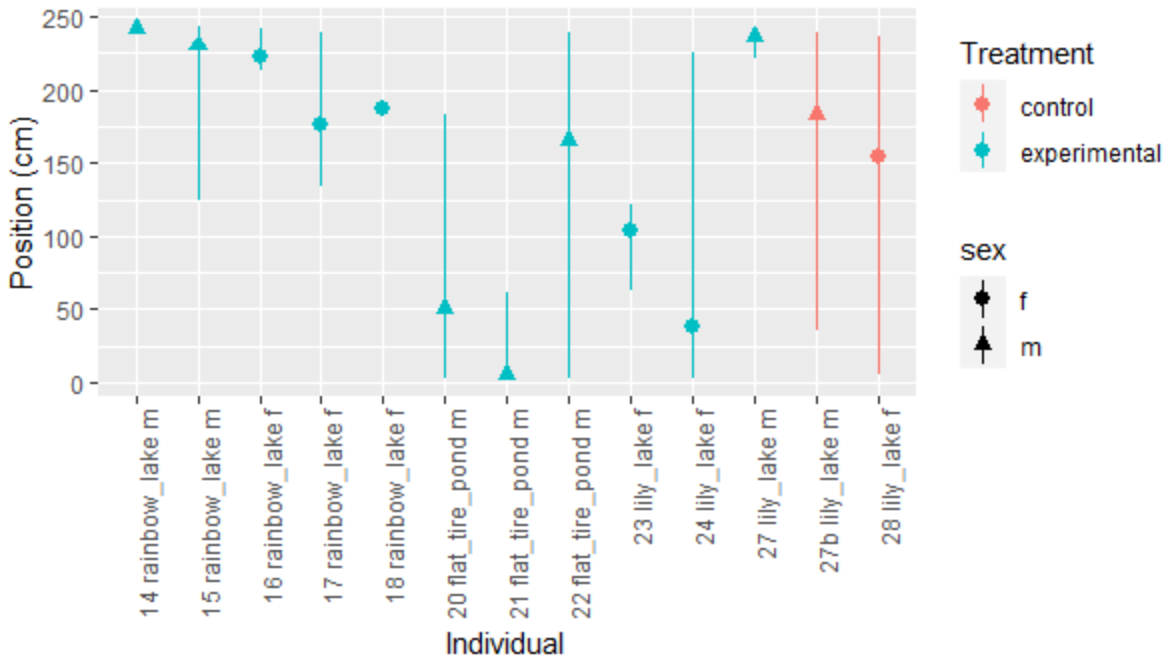
The gradient produced a desirable range of temperatures with stability and replicability between trials. The extreme cold end at position 2.54 centimeters had a range of 8.3°C to 4.6°C with an average of 5.7°C. The position between the middle and the coldest end at 60.96 centimeters had a range of 15.6°C to 12.5°C with an average of 13.9°C. The middle point of the runway at 121.92 centimeters had a range of 19°C to 17.5°C with an average of 18.4°C. The position between the hottest end and the middle end at 182.88 centimeters had a range of 23.1°C to 21.2°C with an average of 22.2°C. The extreme hot end of the runway at position 243.84 centimeters had a range of 30.8°C to 23.8°C with an average of 27.4°C (Table 1).

Table 1. The maximum, minimum and average temperatures of five equally spaced positions within the runway recorded every 5 minutes for 3.5 hours for 5 trials.

Position (centimeters)	Maximum Temperature (celsius)	Minimum Temperature (celsius)	Average Temperature (celsius)
2.54	8.3°C	4.6°C	5.7°C
60.96	15.6°C	12.5°C	13.9°C
121.92	19°C	17.5°C	18.4°C
182.88	23.1°C	21.2°C	22.2°C
243.84	30.8°C	23.8°C	27.4°C

The control trials produced a wide range of positions and an average position roughly in the middle of the gradient, due to having many recordings of being in both extremes of the runway due to the animals moving about freely and not selecting for any temperatures as predicted (Fig 1.) and allows us to interpret that the relatively narrow range of horizontal positions for individuals in the experimental group were actively thermoregulating (Smolinsky and Gvozdk, 2012).

Figure 1.- The maximum (top of bar), minimum (bottom of bar) and average (point on bar) positions during the 3.5 hour trial period of all experimental and control trials.



For the ANOVA tests only minimum temperature selection was found to have any significance in relation to sex (Table 3; $p=0.03132$), site (Table 3; $p=0.04086$) sex*mass (Table 3; $p=0.3037$), and mass*site (Table 3; $p=0.04987$). The significance of that sex had on temperature selection was that females did not have any extreme high or low minimum temperatures, average temperatures or maximum temperatures but the ANOVA only stated significance for the minimum temperature selection (Fig. 3). Males on the other hand had the extreme high and low data points for maximum, minimum and average temperatures. Significance that site had on minimum temperature selection was solely from animals from Flat Tire Pond as all of the individuals from that site had the most extreme low minimum temperature selection (Fig. 2). Mass in relation to site had an effect on minimum temperature selection at only Lily Lake where smaller massed individuals had a higher minimum temperature and larger massed animals had a lower minimum temperature. Sex in relation to mass ($p=0.03037$) had a significant impact on

minimum temperature selection (Fig. 3 & Table 3) in that smaller massed females selected for higher minimum temperatures and larger massed females selected for lower minimum high temperatures, this also seems to apply to the average temperature but the ANOVA test stated no significance in this.

Table 2. The ANOVA results for maximum temperature selection with the effects that collection site, mass and sex have on that selection.

	Sum Sq	Df	F Value	PR(>F)
Intercept	2.079	1	0.0474	0.8416
sex	2.770	1	0.0632	0.8178
mass	12.475	1	0.2846	0.6307
site	33.752	2	0.3851	0.7098
sex:mass	3.011	1	0.0687	0.8102
mass:site	28.145	2	0.3211	0.7475
Residuals	131.478	3		

Table 3. The ANOVA results for minimum temperature selection with the effects that collection site, mass and sex have on that selection.

	Sum Sq	Df	F Value	PR(>F)
Intercept	23.732	1	6.2119	0.08830
sex	56.098	1	14.6835	0.03132*
mass	17.390	1	4.5518	0.12260
site	85.158	2	11.1450	0.04086*
sex:mass	57.450	1	15.0376	0.03037*

mass:site	73.134	2	9.5715	0.04987*
Residuals	11.461	3		

Table 4. The ANOVA results for average temperature selection with the effects that collection site, mass and sex have on that selection.

	Sum Sq	Df	F Value	PR(>F)
Intercept	14.185	1	0.3791	0.5817
sex	9.306	1	0.2487	0.6523
mass	6.440	1	0.1721	0.7061
site	41.717	2	0.5574	0.6225
sex:mass	10.074	1	0.2692	0.6397
mass:site	42.88	2	0.5677	0.6179
Residuals	112.262	3		

Figure 2.- Graph of the ANOVA test of the effects collection site, mass and sex have on the maximum, minimum and average temperature selection. Lines show slope in relation to the collection site.

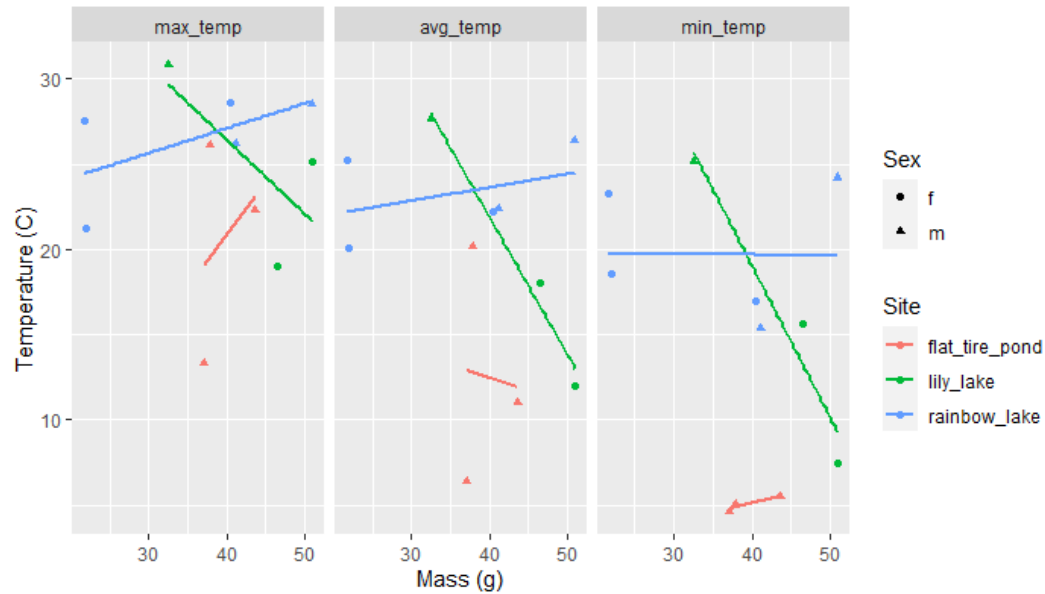
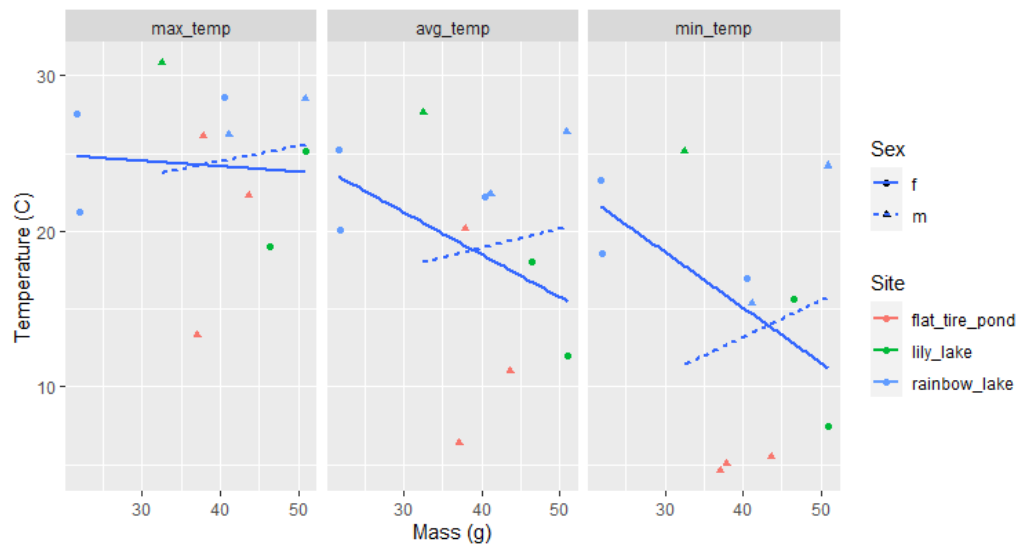


Figure 3.- Graph of the ANOVA test of the effects collection site, mass and sex have on the maximum, minimum and average temperature selection. Lines show slope in relation to sex, dotted lines are male and solid are female.



Discussion:

The most significant of the ANOVA results were those relating to sex. Sex on minimum temperature selection had a significance of $p=0.03132$ and sex in relation to mass on minimum temperature selection had a significance of $p=0.03037$. The significance of sex on minimum temperature selection is most likely due to female salamanders and possibly ectotherms requiring a more conservative range of temperature for internal egg development and production. The males on the other hand did not have conservative temperature selection and had the extreme highs and lows for maximum, minimum and average temperatures. A larger sample size in the future could provide for better statistical analysis and more significance between variables could be found but there is some differential temperature selection between female and male salamanders.

Mass was expected to have an effect on temperature selection but it was only seen in relation to sex having an effect on the minimum temperature selection. In the future a larger sample size would allow the testing of a wider range of sizes and perhaps significance of mass on temperature selection will be found.

One problem that the experiments could have is that only short term temperature selection is being observed and unknown confounding variables could be impacting the trials. 3.5 hours were used for human convenience, so in the future multiple trials for one animal or significantly longer trials should be used.

In conclusion, sex having an effect on temperature selection was unexpected but not surprising. A larger sample size of individuals from each pond and a larger sample size of ponds would allow more data to be analyzed and correlations between mass, sex and collection site/elevation can possibly be found. Multiple thermal gradient runways should be constructed in order to increase the efficiency of data collection since a larger sample size would benefit the

scale of this project immensely since one trial takes 3.5 hours not including setup. In the future a portable/field version of the device can be constructed in order to conduct trials at the Mexican Cut Nature preserve in order to collect data on the salamanders involved in the long term ecological research there.

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