

Experimental Validation of Biophysical Models of Tiger Salamanders (*Ambystoma mavortium nebulosum*)

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Abstract

Arizona tiger salamanders (*Ambystoma mavortium nebulosum*) can thrive in a large variety of environments including high elevation ecosystems where climate change conditions are most drastic. Accurate biophysical models of the water loss and operative temperature for *A. m. nebulosum* can be used as tools to investigate two major environmental constraints faced by amphibians under climate change: exposure to water loss and extreme temperatures. The purpose of this study was to validate plaster biophysical models to use in future Arizona tiger salamander ecophysiology research. Objective 1 was to construct a constant humidity chamber for the biophysical model validation experiments. The chamber maintained a $55 \pm 5\%$ relative humidity at 17 °C with a saturated magnesium chloride solution. Objective 2 was to compare model water loss and temperature to live tiger salamanders in the constant humidity chamber. A model and a live salamander were placed into individual mesh containers in the constant humidity chamber. The water loss and temperature change of models and salamanders were tracked during the 150-minute trials. The loss of water rate proved to be insignificant between the models and salamanders validating this biophysical model. Future field experiments will be able to use these models, but modifications to the models can be used to improve upon their design.

Keywords: tiger salamanders, biophysical models, thermoregulation, climate change, bioindicator species

Introduction

With climate change, the phenology, wintering-ability, reproduction, diet, growth, acclimation capacity, behavior, demographics, interactions, and abiotic conditions of amphibians are all changing in a variety of ways (Muths et al., 2017; Amburgey, 2013; Rollins, 2019; Taylor et al., 2020; Thurman and Garcia, 2017; Rohr et al., 2018). Particularly, average temperatures have increased by 2.5 °C in the past 30 years high up in the Southern Rocky Mountains of Colorado (Whiteman, personal communication). Yet, in these high elevations (3,600 m or higher) Arizona tiger salamanders (*Ambystoma mavortium nebulosum*) still survive. Currently, a subpopulation of tiger salamanders is moving into higher elevations, expanding its breeding range, and restructuring the local biotic communities in unknown ways. (Whiteman, personal communication). This is despite the growing season being only three to four months long and ponds routinely drying up before many salamander larvae can metamorphose in this subalpine habitat (Whiteman, 2012; Wissinger and Whiteman, 1992). Understanding the capability and underlying cause of how tiger salamanders persevere during a period of climatic change is likely to reflect how other amphibian species in high elevation ecosystems might respond as well.

The temperature increase in the southern Rocky Mountains is most likely affecting the biophysical water loss and evaporative water loss (EWL) response in *A. m. nebulosum* as changing climatic conditions are linked to increased thermal and hydric stress (Kearney et al., 2008; Lertzman-Lepofsky et al., 2020). These salamanders are exceptionally sensitive to water loss and internal and external temperature (Angilletta, 2009). Relative humidity (RH) is an important factor for terrestrial amphibians due to their moist and permeable skin which allows for the exchange of water, ions, and gasses in terrestrial habitats (Hillman, 2009). Temperature is equally important in its vast influence upon most physiological systems in amphibians (Hillman, 2009). Now, in response to an increase in external climatic temperatures, amphibians can self-

regulate to lower their internal temperatures by losing water (Hillman, 2009). In most cases the thermal stress is minimal, the water loss is in a small quantity, and the amphibian can quickly recharge water loss in nearby sources of water (Lertzman-Lepofsky et al., 2020). But current climate change predictions estimate that available aquatic habitat in subalpine habitats will decrease and amphibians at these elevations will face longer growing seasons with hotter temperatures (Lertzman-Lepofsky et al., 2020). This is setting up a perfect clash between tiger salamanders losing water to prevent overheating while risking lethal desiccation. This might have major implications in restricting the available range, population levels, and biotic influence for these salamanders and other high elevation amphibians.

To understand the environmental limits of ectothermic and moisture obligated species, it is crucial to identify their thermoregulatory abilities (Taylor et al. 2020). But the specifics of how tiger salamanders self-regulate these factors in their dynamic environments is currently unknown. Experiments to analyze these processes run risks of endangering and subjecting *A. m. nebulosum* to lethal conditions (Alvarado, 1972). An appropriate alternative to evade this ethical dilemma is the use of biophysical models. Biophysical models are tools that might be implemented to accurately predict an organism's physiological response to attributes of interest (e.g., water loss, body temperature; Tracy et al. 2007). To accurately predict water loss and operative temperatures (an estimate of body temperature), biophysical models must lose water at rates similar to an inactive amphibian under the same environmental conditions. Models that do not mimic EWL from amphibian skin will over-estimate operative temperatures and be an ineffective mechanism. Accurate biophysical models are important for amphibian research that can provide significant data about the temperature, water loss, and the relationship of their synergistic interactions while eliminating the risk of exposing live animals to extreme and

dangerous conditions (Peterman et al., 2013; Tracy et al., 2007; Lertzman-Lepofsky et al., 2020; Taylor et al., 2020). The data can then be used for accurate analysis of amphibian life histories, niches, behaviors, and responses to disturbances (Lertzman-Lepofsky et al., 2020; McEntire and Maerz, 2019; Peterman et al., 2013; Tracy et al., 2007). One well established types of biophysical model, successful in mimicking the operative temperature and skin permeability of amphibians, is constructed using plaster of Paris (Peterman et al., 2013; Tracy et al., 2007).

The purpose of my study is to validate the use of biophysical models as surrogates for inactive Arizona tiger salamanders. I will accomplish this by comparing the water loss and temperature of models and live inactive salamanders. Validating these models will provide an invaluable instrument to evaluate the thermoregulatory abilities of tiger salamanders in the high country. It will also provide means to utilize Arizona tiger salamanders as bioindicators of climate variation, model the future range of this species based on climate change models, and track thermal and hydric differences across microcosms in the boreal, subalpine, and alpine zones of the Western United States.

Materials and methods

The first objective was constructing and testing a constant humidity chamber. Three constant humidity chambers, large, medium, and small, were built and tested for their capacity to hold a constant relative humidity for 2.5 hours. These tests involved placing quantities of saturated salt solutions in the different sized containers and recording the RH levels for several hours. The tested saturated salt solutions were magnesium chloride, potassium carbonate, and sodium chloride. The salt solution of magnesium chloride was selected based on its ability to maintain a reasonable $55 \pm 5\%$ RH at 17°C over the two-and-a-half-hour time period and the

ease and safety of using such salt. These trials also revealed that an excessive amount of salt would be needed to maintain the RH in the largest container, so this apparatus was scrapped.

For the model validation test the small and large constant humidity chambers were utilized. The medium container was framed with a rectangular 49 cm x 39 cm x 20 cm plastic storage container. The smaller container used a 44 cm x 30 cm x 20 cm rectangular plastic storage container. A foam gasket and four clamps sealed the lid to prevent air and moisture from escaping both chambers. Within each chamber Truweigh Enigma Digital Mini Scales EN-500-01-SL, three in the medium and two in the small, were attached to the bottom. The scales were calibrated with a 500-gram (g) calibration weight and were warmed up for 30 minutes before use. Upon use of all scales, a rectangular 16 cm x 10 cm x 7 cm plastic container encapsulated a plaster model or live salamander. The plastic container was modified so that there was aluminum mesh screened windows on all 6 sides. Stilts were also attached to the bottom to allow for ventral air exposure and maximize chamber air circulation around the salamander and models. An InTemp CX450 Temperature and Relative Humidity Logger (Onset Computer Corp.) tracked temperature and RH in both constant humidity chambers. An approximately 400 g saturated salt solution of magnesium chloride was utilized to control the humidity within the chamber (Wexler and Hasegawa,1954). A small battery-powered fan was also inserted into the apparatus to provide constant airflow. The apparatus was kept in an animal care room at $16.8 \pm 1^{\circ}\text{C}$ during all validation experiments.

The plaster models were molded out of plaster of Paris (DAP brand) using a 2:1 ratio of plaster:water. The models were air dried for 48 hours and then cured at 60°C for 48-72 hours in a drying oven in the Gothic Research Center. Using a simple PVC mold, the models are a cylinder that is 11 cm x 1 cm. To determine plaster model size we used established surface

area:volume (S:V) relationships from a congener of our study species ($S = 8.34 \times W^{0.684}$; Whitford and Hutchinson, 1967) to calculate surface area (S) for average male (71.41 cm²) and female (79.01 cm²) metamorphs in the population at Mexican Cut Nature Preserve (Whiteman, unpublished data). We then used the equation for S of a cylinder ($S = 2\pi rh + 2\pi r^2$) to determine the appropriate length and diameter of plaster models with an S that falls between the range for average males and female salamanders. A model that is 11 x 1 cm has a S of 75.4 cm². Plaster models also have a built-in cavity that will allow for a cylindrical temperature logger (iButton thermochron; Maxim Integrated) to be inserted on one end of the model. There are many options for future adjustments, such as size, shape, pose, and coloration, that can be made to the different models which might influence their accuracy (Tracy et al., 2007; Lertzman-Lepofsky et al., 2020; Peterman et al., 2013).

Table 1. The average mass, snout-vent length (svl), total length, and surface area of male and female tiger salamanders from the Mexican Cut Nature Preserve (Whiteman, unpublished data)

	Male	Female
Average mass (grams)	23.1	26.8
Average SVL (millimeters)	92.5	92.7
Average Length (millimeters)	172.6	169.9
Average Surface Area (millimeters ²)	71.4	79.0

Live *Ambystoma mavortium nebulosum* were collected from the Kettle Ponds, Rainbow Lake, Rainbow Lake, and Quartz Creek (RMBL) in Gunnison County, Colorado (Fig. 1). Proper biosecurity protocols were followed: we cleaned all equipment and containers between sites with

10% bleach, rinsed it, and dried it. 20 metamorphic adults (N=10 females, N=10 males) were used for the study. Each salamander was housed in an individual container (33 cm x 20 cm x 13 cm) filled with their respective in an animal care room at RMBL. Health checks were made daily and dechlorinated water was changed into each container every 2-3 days. Salamanders were returned to the ponds they were captured in (except one which was taken with permission from Colorado Parks and Wildlife). To ensure the same individuals were not recaptured and retested at later dates, each salamander from sites we visited multiple times (e.g., Kettle Ponds) received a single toe clip before being released.



Figure 1.- A map of a salamander collection site, Kettle Pond 6, nearby streams, and nearby trails.

Once the constant humidity chamber was tested, the models were constructed, and metamorphic salamanders were collected, the validation experiment began. The live salamanders were hydrated and kept in a container of pond water from the salamander's respective pond

before use. Salamanders will be removed from their containers and excess water was blotted off the salamander's skin. The plaster models were dried, weighed to obtain a dry mass, and then soaked in water 24 hours prior to the experiment. Excess water was also blotted off the plaster models before they were placed in the chamber. All plastic containers in the constant humidity chamber were randomly assigned either a plaster model or live salamander, but only one salamander was allowed in the chambers at a time. The constant humidity chamber had a target internal temperature of 17 °C and RH of $55 \pm 5\%$ RH (Reider, unpublished data). A starting weight in grams of each test subject and the internal temperature and humidity of the chamber will be recorded at the beginning of each trial. These measurements were repeated every 10 minutes during the two-and-a-half-hour-long trial. The temperature of each model and live inactive salamander was also recorded at the beginning and end of the trial. Dorsal, ventral, and cloacal temperature measurements for the salamander were conducted with a quick-read thermometer (Risepro Model 8541957450). Once the models and salamander were in place, the fan was turned on, data logger was started, and the saturated magnesium chloride solution was placed in the container, before the lid sealed the air in. For the length of the trial, the lights were turned off in the room and the recorder used a red headlamp to collect measurements. Salamanders were not allowed to lose more than 20% of initial mass to avoid approaching possible lethal limits of water loss (Alvarado, 1972). After each trial, the chamber fan was turned off, data logger was reset, and the saturated magnesium chloride solution had to be dried with transfer pipettes and paper towels and replenished for the next trial.

To validate the models, a linear regression analysis was utilized. The analysis regressed water loss in grams over time (Tracy et al., 2007) and used generalized linear models to compare the coefficients of both models and salamanders. The water loss $\% \text{ hr}^{-1}$ on surface area using a

dry mass value of a tiger salamander was also regressed and compared to the water loss % hr⁻¹ on surface area of the models (Alvarado, 1972; Peterman et al., 2013). The % water loss for the models was calculated as:

$$(\text{Initial wet mass} - \text{end wet mass}) / (\text{Initial wet mass} - \text{dry mass}) / \text{time}$$

The dry mass value to determine the salamander % water loss was based on a dehydration study which found that water composes 83% of a tiger salamanders total body mass. (Alvarado, 1972). The following equation was used for this calculation:

$$(\text{Initial wet mass} - \text{end wet mass}) / (\text{Initial wet mass} * .83) / \text{time}$$

A t-test was also conducted to establish if the end temperatures of the models and salamander's dorsal temperature was significantly different or not.

Results

In validating the constant humidity chamber the saturated salt solutions produced a higher constant humidity than was predicted by previous studies. It was expected the magnesium chloride, potassium carbonate, and sodium chloride solutions would produce humidity's of approximately 33%, 43%, and 75% respectively (Wexler and Hasegawa, 1954). The magnesium chloride produced an RH of $55 \pm 5\%$ RH over 7 trials. The potassium carbonate's RH stabilized at around 64% during 2 trials. The sodium chloride had an RH of $80 \pm 8\%$ during 3 trials. These RH's could be held for longer periods in the small and medium chambers as compared to the large chamber.

The rates of water loss for both models and salamanders had a significant linear relationship. The salamander's lost water at a rate of $-0.012 + 0.318$ grams per ten-minute

interval ($p=0.009$), while the model's loss rate was at $-0.007 + 0.232$ grams per ten-minute interval (Fig. 2; $p=0.038$). The coefficients of these regressions were found to be statistically insignificant ($p=0.115$). This correlated to an average percent body loss per hour of $4.92 \pm 1.49\%/h$ for the salamanders. The models lost an average of $6.91 \pm 2.08\%/h$ body mass per hour (Fig. 3). The average end temperature of the models and salamander's dorsal temperature was 14.16°C and 14.14°C respectively and was not statistically significant (Table 2; $p=0.955$). These generalized linear trends, means, and statistically insignificant results were used to justify these models as appropriate surrogates for the EWL response of Arizona tiger salamanders.

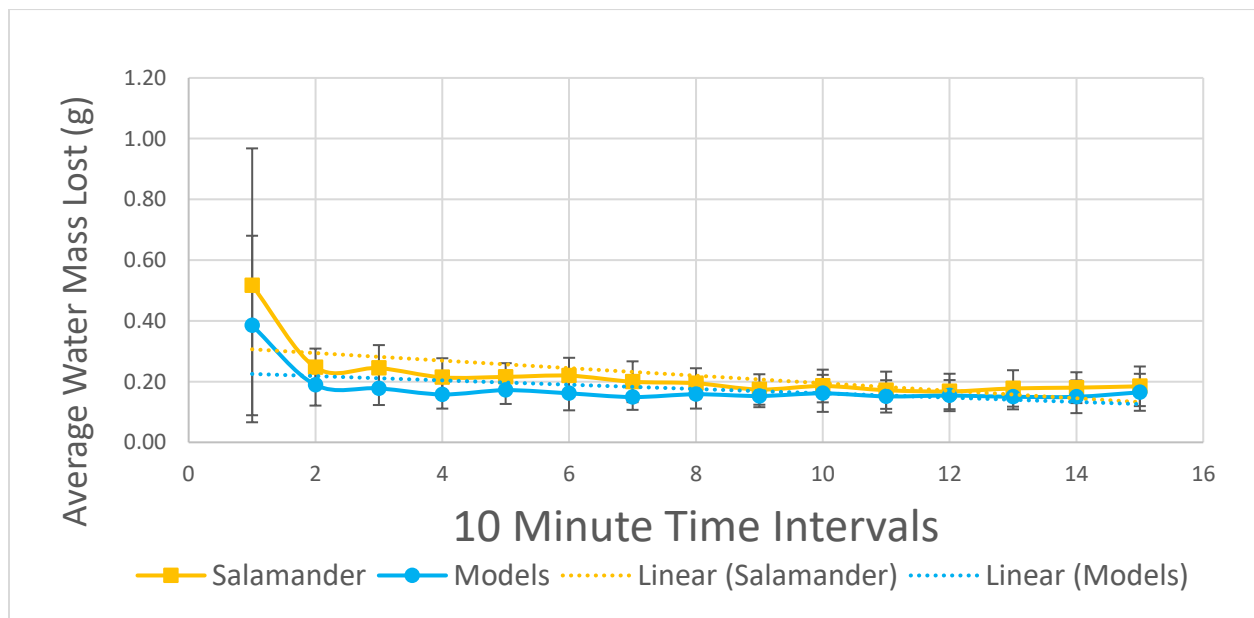


Figure 2.- Graph of the water loss of the models (blue circles) and Arizona tiger salamanders (yellow squares) every ten minutes for two-and-a-half-hours ($n=20$). The significant linear regressions of the salamanders and models are shown as well ($Y = -0.012x + 0.318$, $P = 0.009$, $\alpha = 0.05$; $Y = -0.007x + 0.232$, $P = 0.038$, $\alpha = 0.05$)

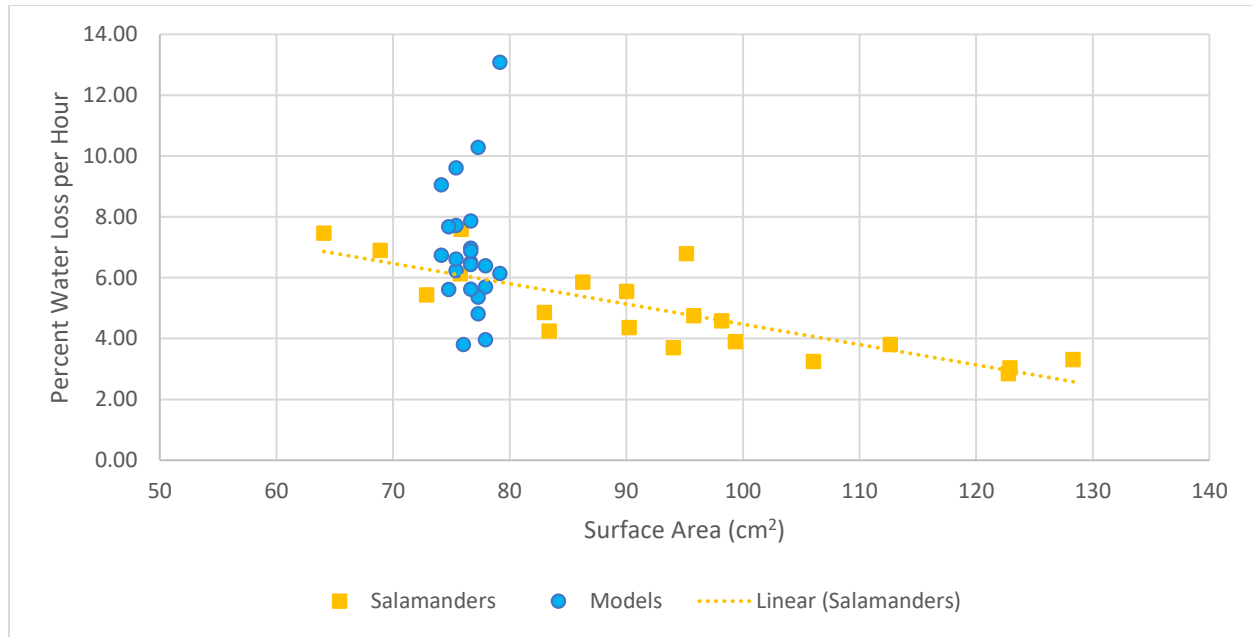


Figure 3.- Graph depicting the water loss percentage of the models (blue circles) and salamanders (yellow squares) per hour (%/hr) over surface area (cm²; n=20). There is also a linear regression line for the salamanders ($Y = -0.006x + 11.13$, $P = 0.00001$, $\alpha = 0.05$).

Table 2. The average operative start and end temperatures in Celsius for the models and salamander's dorsal measurement.

	Models	Salamander's Dorsal
Average Operative Start Temperature (°C)	18.06	17.6
Average Operative End Temperature (°C)	14.16	14.14

Discussion

Under conditions of $55 \pm 5\%$ RH and 17°C for 150 minutes there is support that these models are accurate biophysical models of *Ambystoma mavortium nebulosum*. The validation of these biophysical models allows for the advancement of the understanding of the physiological water loss and operative temperature in *A. m. nebulosum*. These tools might be used in an array of future field experiments. The model can be used along climatic and elevational gradients to track how major these conditions affect the thermal and hydric stress of these amphibians. These

studies can also occur in microcosms and track what features of a site tiger salamanders rely upon. Mapping out these niches and fundamental conditions can also be used to extrapolate the future range of this species using future climate change prediction models. These studies will be able to define the relationship between the physiological EWL of Arizona tiger salamanders and the habitats they currently, and might one day, occupy.

Another important impact of this tool is it enables methodical use of this species as bioindicators. The data collected from using these biophysical models of a boreal, sub-alpine, and alpine amphibian species can track the biotic impacts of dynamic conditions on local thermal and hydric environments (Spotila, 1972; Taylor et al., 2020). How these models react to these changing stressors will render valuable data as climate change alters the regimes of these factors (Kearney, 2008). This can then be extended to the physiological responses of other amphibians occupying similar habitats and niches across the world. Understanding these changes will allow for future management considerations and guide studies concerning the future of high-elevation amphibians.

These plaster models have great potential as investigative research tools in the future and can be improved and modified for future utilization. In their manufacturing, it is important to eliminate air bubbles as these imperfections can erroneously increase the surface area and skew data. Proper and thorough shaking of the wet plaster before it sets should offset this flaw. In the field, the average weight and surface area of the local tiger salamanders should be considered for proper deployment of different sizes of models. As Arizona tiger salamanders which populate lower elevations in the Gunnison Valley tend to have a greater surface area, models deployed in these elevations should have a correspondingly larger surface area as well. Field deployment might also need to consider the effects of coloration of *A. m. nebulosum* on water loss and

operative temperature change. Dyeing and painting the plaster models would provide an appropriate solution when exposing these models to direct or even partial sunlight in the field (Tracy et al., 2007). Behavioral responses such as water conservation postures can also be considered in designing future plaster models (Peterman et al., 2013).

Plaster models make a great surrogate for Arizona tiger salamanders' natural loss of water and operative temperature as biophysical models. Their durability and reusability, adaptability to different field experiments, and aversion from harming live animals establish them as an efficient gizmo for any field biologist. The validation of these biophysical models ensured they can be implemented into the field as a practical and effective metric in a variety of studies. Application of these tools will provide data concerning the physiological response of high elevation amphibians, their life histories, and how climate change continues to alter everyone of these aspects across the world.

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