

2013

# Dehydration in Southern Toads (*Anaxyrus terrestris*): Metabolic Costs and Effects of Temperature Selection

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## Recommended Citation

Forster, Claire E., "Dehydration in Southern Toads (*Anaxyrus terrestris*): Metabolic Costs and Effects of Temperature Selection" (2013). *Scripps Senior Theses*. Paper 161.  
[http://scholarship.claremont.edu/scripps\\_theses/161](http://scholarship.claremont.edu/scripps_theses/161)

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Dehydration in Southern Toads (*Anaxyrus terrestris*): Metabolic Costs and Effects of Temperature Selection

A Thesis Presented

by

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To the Keck Science Department

Of Claremont McKenna, Pitzer, and Scripps Colleges

In partial fulfillment of

The degree of Bachelor of Arts

Senior Thesis in Biology

December 10, 2012

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## **Abstract**

Southern toads (*Anaxyrus terrestris*) are ectothermic terrestrial amphibians. As such they are sensitive to thermal and hydric variability. The interactive effect of hydration state and body temperature is not well understood. The aim of this study is to determine if the costs of dehydration vary at different temperatures and if so, whether toads will select different temperatures to minimize these costs. Costs of dehydration were evaluated by measuring metabolic rate at 100% and 85% hydration state, at 30 and 20 °C. Temperature selection of toads at 100% and 85% hydration state was measured using a thermal gradient. Body temperature, but not hydration state, was determined to have a significant effect on metabolic rate. No significant difference in temperature selection was found between toads at 100% and 85% hydration state. Thus, thermoregulatory behavior as influenced by hydration state could not be predicted in *Anaxyrus terrestris*.

## **Introduction**

The physical environment an organism inhabits is integral to its ability to survive and reproduce. This is especially true for ectothermic organisms whose body temperature is determined predominantly by their physical environment as opposed to physiological regulatory processes. Body temperature is important for a number of different biological functions including skeletal and cardiac muscle function, nerve conduction ability, metabolism, digestion, growth, locomotion, and immunity. Endotherms employ high heat production from metabolism and low thermal conductivity to maintain a constant body temperature. These regulatory pathways are not available for anurans and other ectothermic organisms, so the ambient temperature largely determines an ectotherm's body temperature. Behavioral thermoregulation is the predominant way in which these organisms are able to influence their body temperature (Reynolds 1979). Many of the environments anuran species inhabit are thermally variable, and the ability to tolerate a wide variety of temperatures is vital for reproductive success, prey capture success, ion balance, and so on (Snyder and Weathers 1975). Early studies focused on temperatures anuran species select to optimize a given process, such as digestion (Lillywhite *et al.* 1973, O'Connor and Tracy, 1992), growth

(Brattstrom 1979), or immunity (Raffel *et al.* 2006). These studies found that digestion rate and growth rate tended to increase with higher temperatures and that immunity to disease decreased with seasonally cool temperatures. Biological functions however, are influenced by a large number of factors as opposed to a single variable such as body temperature, and more studies have focused on behavioral thermoregulation in conjunction with other environmental factors that also influence these processes (Gatten 1987, Preest *et al.* 1992, Preest and Pough 1989, Preest and Pough 2003).

Another environmental factor that plays an important role in regulating biological functions is hydration state. Many anurans live in environments that are not only thermally variable, but also hydrically variable. Toads have very water permeable skin, and this characteristic allows for toads to lose water at a rate roughly similar to that of an open dish, through evaporative water loss across their skin. As terrestrial amphibians, toads do not always have access to water or moist environments, and so hydration state plays a critical role in the toad's overall well-being and ability to maintain homeostasis. If hydration states below an organism's homeostatic range are reached, fitness level decreases until proper hydration state can be restored, or death occurs (Krakauer 1969). A number of studies have taken a multivariable approach to the assessment of hydration state and body temperature on physiological processes such as metabolic rate (Gatten 1987, Preest *et al.* 1992), feeding, and locomotion (Preest and Pough 1989, Preest and Pough 2003). Metabolic rate was found to be significantly influenced by temperature and to be influenced by dehydration during activity in anurans. Feeding and locomotor performance were also found to be influenced by combinations of body temperature and hydration state. Some studies have focused on behavioral choices influenced by hydric and thermal stress and have found that anurans

species do make behavioral choices to both hydroregulate and thermoregulate (Bundy and Tracy 1977, Tracy *et al.* 1993).

I am interested in determining if there are costs associated with dehydration in *Anaxyrus terrestris* (Southern toads), and if so, whether these costs vary with temperature and consequently influence temperature selection. One way to evaluate cost is by measuring an organism's metabolic rate. If, for example, it is more costly metabolically to be dehydrated at a particular body temperature, then you might expect animals to select a different body temperature (one at which metabolic rate is lower) when dehydrated than when fully hydrated.

To evaluate metabolic costs, oxygen consumption rates were measured under four test conditions for each toad. The four test conditions used to evaluate metabolic costs were 100% hydration at 30 and 20°C, and 85% hydration at 30 and 20°C. The same hydration states, 100% hydration and 85% hydration were then used in experiments to determine if toads select for different temperatures at different hydration states. Each toad underwent temperature selection trials at both hydration in a thermal gradient for a period of 1 hour with body temperature measurements taken at 20-minute intervals.

I hypothesized that metabolic rate would increase with increased temperatures due to the tendency for chemical reactions to occur more rapidly at higher temperatures. I also hypothesized that metabolic rate would increase with dehydration due to the physiological stress of being dehydrated. Based on the results of my first experiment, I hypothesized that there would be no difference in temperatures selected for by toads at either 100% hydration or 85% hydration.

## **Methods and Materials**

### *Experimental Animals*

Thirteen adult *Anaxyrus terrestris* of both sexes were used in this experiment. These toads are part of a permanent colony that is kept in the Keck Science Department animal care room (n=13,  $x_{\text{mass}}=15.22\text{g}$ , range=10.30-21.67g). The toads were divided into two groups (n=6 and n=7) for testing purposes, and each toad was tested at four test conditions (100% hydration state at 30 and 20 °C, and 85% hydration state at 30 and 20 °C) for the metabolic rate experiment and two test conditions (100% and 85% hydration state) for the thermal gradient experiment. The order of test conditions was randomly assigned to each toad for both the metabolic and thermal gradient experiments. Because the order was randomly assigned, the number of toads being tested at any of the four conditions varied with each trial date. The toads were given 72 hours rest between both metabolic and thermal trials in order to allow rehydration and recovery from handling and dehydration stress.

### *Preparation for Metabolic Rate and Thermal Gradient Experiments*

Test subjects were not fed the day prior to their trial date to ensure that digestion was not influencing resting metabolic rate. Between 4-5 pm the day prior to the trial date the test subjects were placed in plastic shoeboxes. Each shoebox was lined at the bottom with paper towels and roughly 1 cm of water to allow the toads to hydrate to 100% hydration state. One end of the shoebox was tilted up in order to provide only the choices of a damp region and a wet region.

At 8:30 am the next morning, the bladder of each toad scheduled for dehydration trials was emptied by inserting a glass pipet into the cloaca (Ruibal 1962). The pipet tapped the toad's bladder, emptying it of urine. Some toads spontaneously urinate upon handling, but each test subject's bladder was still emptied via pipet. Each subject was measured to 0.01

g and this weight was assumed to be 100% hydration state. Because the toads were fasted and their bladders emptied, all loss of mass experienced after this is due to evaporative water loss. When a toad reached 85% of its original 100% hydration state weight it was assumed that the toad had reached a hydration state of 85%. To reach 85% hydration state, animals undergoing dehydration trials were put in wire mesh cages (13.5x13.5x13.5 cm, Figure 1) and placed roughly 1 meter in front of a heated fan. The fan was placed on its lowest heat and speed settings. The toads were then weighed every 20 minutes, until the target weight was achieved. Dehydration to the target weight usually required several hours, but varied with the mass and the activity of the toad in question.

Once the majority of the toads scheduled for dehydration had achieved their target weight, toads undergoing trials at 100% hydration state had their bladders emptied in the manner described above, and then were placed individually in metabolic chambers (Figure 1).



**Figure 1.** Dehydration cage (left) and metabolic chamber (right).

*Metabolic Rate Experiment*



The first experiment was performed to determine if there was a difference in metabolic rates for toads at 100% and 85% hydration states at 30 °C and 20 °C. These metabolic trials were performed between June 20, 2012 and July 6, 2012 in the Keck Science Building. The toads, both hydrated and dehydrated, were incubated in metabolic chambers at either 30 or 20 °C for 90 minutes to allow recovery from disturbance and to allow them to acclimate to the test temperature. In order to minimize further water loss while the toads were recovering and acclimating, each metabolic chamber was covered with a wet paper towel that was rewetted as needed to remain damp, and each metabolic chamber had humidified air pumped into it.

Syringes were used to take initial air samples from the metabolic chambers and final air samples 2 hours later. These were run through an Applied Electrochemistry S3A oxygen analyzer to determine oxygen concentration. Air samples were passed through pipets filled with ascarite and drierite to remove CO<sub>2</sub> and H<sub>2</sub>O respectively. Resting metabolic rate was then calculated as oxygen consumption rate, milliliters of oxygen consumed per hour (Vleck 1989, Figure 2, Table 1).

$$O_2 \text{ Concentration } \left( \frac{ml O_2}{hr} \right) = \frac{\left[ \left( \left[ Volume - \left( \frac{Mass}{0.8} \right) \right] - \left[ Volume * \left( \frac{P_w}{P_b} \right) * \left( \frac{RH}{100} \right) \right] \right) * (F_i - F_e) \right]}{(1 - F_e)} * \left( \frac{273.15}{T_k} \right) * \left( \frac{P_b}{760} \right)$$

**Figure 2.** Vleck's equation for oxygen consumption rate.

**Table 1.** Meaning of values in Vleck's equation.

Volume	Metabolic chamber volume (ml)
Mass	Body mass of toad (g)
Time	Time difference between initial and final air samples (hrs)
Fi	Initial oxygen concentration
Fe	Final oxygen concentration
Tk	Temperature (K)
Pb	Barometric pressure (mm Hg)
Pw	Water vapor pressure
RH	Relative humidity

During later trials, Keck Science staff moved the barometer used to determine barometric pressure in the equation above from the second floor of Keck to the basement. This move caused a discrepancy in the barometric pressure measurement used in these trials for the metabolic rate equation. In order to correct this discrepancy, an average of previous barometric pressures was used instead of the measured values.

#### *Thermal Gradient Experiment*

The second experiment was performed to determine if toads at different hydration states would select for different body temperatures in a thermal gradient. Thermal gradient trials were conducted between July 16, 2012 and July 30, 2012 in a constant temperature room in the Keck Science Building. The thermal gradient consisted of eight lanes of insulated track with heating coils underneath the lanes that increased in density at one end to provide a range of temperatures along the track (Figure 3). The thermal gradient was set to provide temperatures across the range of 17 to 36 °C. All toads (n=12) were tested at both 100 and 85% hydration. Toads at 85% hydration were placed in the thermal gradient

immediately after dehydration and toads at 100% hydration were placed in the thermal gradient at roughly the same time.

The same preparation and dehydration process used in the metabolic experiment was used in this experiment. Bench pads were wrapped around the Plexiglas lids of the thermal gradients lanes. The pads were sprayed with water before, and 30 minutes into, the thermal gradient trial to minimize further water loss. The toad's body temperatures were measured initially, and then every 20 minutes afterwards, for an hour using a thermocouple thermometer inserted 1-2 mm into the cloaca. After taking the body temperature measurements the toads were placed in the same position in the thermal gradient that they were removed from. This was done to ensure that any temperature selection was done by the toads and not influenced by the researcher. After the thermal gradient trial was completed the toads were weighed in order to ensure that there was no significant water loss over the course of the experiment.



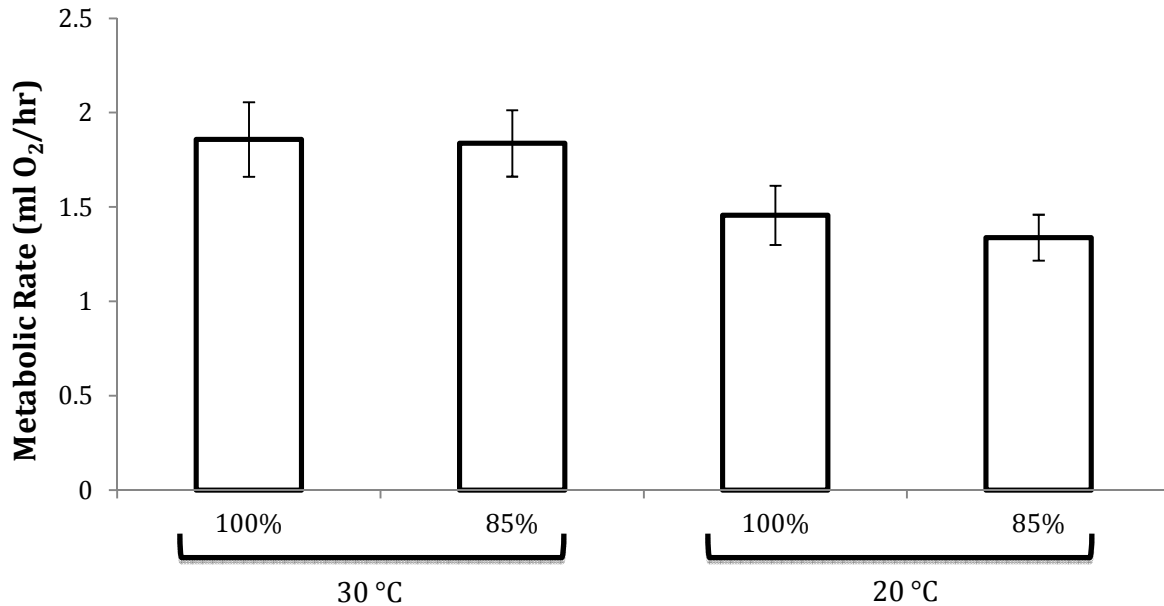
**Figure 3.** Thermal gradient used in experiments consisting of eight lanes, warmest end closest in picture

### *Statistics*

SPSS was used to perform a 2-way ANOVA with metabolic rate as the dependent variable. The two within subject factors were hydration state and body temperature. For the analysis of the thermal gradient data, SPSS was used to perform a repeated measures 2-way ANOVA. The dependent variable was body temperature and the two within subject factors were hydration state and time. For both analyses an alpha level of  $p < 0.05$  was accepted as significant.

### **Results**

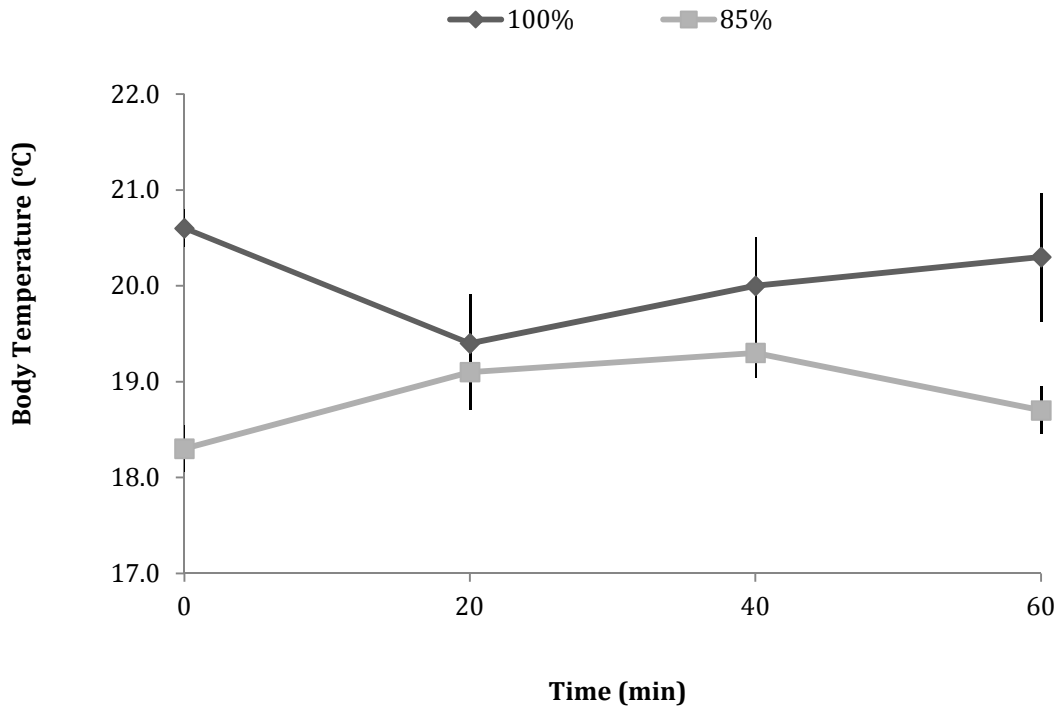
Body temperature had a significant effect on metabolic rate in toads (ANOVA,  $p = 0.010$ ,  $n = 13$ ,  $F_{1,12} = 9.192$ ). Metabolic rate decreased roughly 24% from 30 °C to 20 °C for toads at both 100% and 85% hydration state (Figure 4). In contrast metabolic rate was essentially the same at both 100% and 85% hydration states for toads at 30 °C. Test subjects also showed relatively little difference between 100% and 85% hydration state at 20 °C (Figure 4). The effect of hydration state was found to not be significant (ANOVA,  $p = 0.446$ ,  $n = 13$ ,  $F_{1,12} = 0.620$ ). The interactive effect between hydration state and body temp was also found to be not significant (ANOVA,  $p = 0.332$ ,  $n = 13$ ,  $F_{1,12} = 1.024$ ).



**Figure 4.** Mean metabolic rate and standard error at four test conditions (100% hydration at 30 °C and 20 °C, and 85% hydration at 30 °C and 20 °C) for *Anaxyrus terrestris* (n=13).

The effect of hydration state on temperature selection was found to be significant (ANOVA,  $p=0.014$ ,  $n=12$ ,  $F_{1,11}=8.568$ ). The interactive effect of time and hydration state was not found to be significant (ANOVA,  $p=0.077$ ,  $n=12$ ,  $F_{3,33}=3.192$ ) and the effect of time was also not found to be significant (ANOVA,  $p=0.763$ ,  $n=12$ ,  $F_{3,33}=0.390$ ). In the thermal gradient experiment, toads at 100% hydration state selected temperatures that were a mean of  $1.24 (\pm 0.046 \text{ SE})$  °C higher than 85% hydrated toads at each time period. The measurements for initial body temperatures in toads at 100% and 85% were found to be significantly different (T-test,  $p<0.001$ ,  $n=12$ ,  $t_{1,11}=6.6037$ ). The difference between hydrated and dehydrated toads in initial body temperature is most likely due to the cooling effect of evaporative water loss in the dehydrated toads (Figure 5). At 20 and 40 minutes, body temperatures in toads at 100% and 85% were found to be not significantly different (T-test, 20 minutes,  $p=0.689$ ,  $n=12$ ,  $t_{1,11}=0.41$ , 40 minutes,  $p=0.159$ ,  $n=12$ ,  $t_{1,11}=1.51$ ). It was only at 60 minutes that a greater divergence in temperature selection is seen, with toads at 100%

hydration state selecting higher temperatures than at 85% hydration state. This difference was determined to be marginally significant (T-test,  $p=0.0709$ ,  $n=12$ ,  $t_{1,11}=1.9992$ ).



**Figure 5.** Body temperature of *Anaxyrus terrestris* in a thermal gradient over the period of 1 hour at two hydration states (mean $\pm$  SE,  $n=12$ ).

Further analysis was performed to determine to what extent differences in initial body temperature between the two hydration states determined the significance of hydration state in the statistical tests. When initial body temperature data were removed for both 100% and 85% hydration state the effect of hydration state, time, and the interactive effect of hydration state and time were found to not be significant (ANOVA, hydration state  $F_{1,11}=2.755$ ,  $p=0.125$ , time  $F_{2,22}=0.608$ ,  $p=0.553$ , HS\*time  $F_{2,22}=1.550$ ,  $p=0.235$ ).

## Discussion

Regulation of both body temperature and hydration state are key for an animal's ability to overcome stress caused by environmental variability. In an experiment that focused

on how temperature and hydration state affected a number of organismal processes such as feeding, locomotion, and metabolic rate in *Anaxyrus americanus*, body temperature played a more important role in metabolic rate than hydration state (Preest and Pough 2003). In a similar study in *Hyla arenicolor* body temperature also played a more significant role in determining metabolic rate than dehydration (Preest *et al.* 1992).

Another factor that influences metabolic rate is the activity level of the frogs. There was a greater difference in metabolic rate at different hydration states in active frogs than in resting frogs (Preest *et al.* 1992). In the data I collected there was no significant difference in metabolic rate between hydration states at either of the given body temperature (Figure 4). This implied that there was perhaps no metabolic cost associated with dehydration to 85% of standard mass in resting toads I hypothesized that there would be no difference in temperature selection for toads at either 100% or 85% hydration state based on these findings. In the thermal gradient experiment, a significant effect of hydration state was initially found, but upon further analyses was shown to be solely dependent on the difference in initial body temperature. After initial body temperature was removed hydration state was not found to have a significant effect.

Temperature selection in the thermal gradient is dependent on the toads moving up and down the lanes. This activity level, though not strenuous, could be enough to create a larger difference in metabolic rate between hydrated and dehydrated animals than indicated by my first experiment. Metabolic rate increases in order to sustain activity. When under the stress of dehydration as well as increased activity, metabolic rate is increased to a greater degree. Dehydration has been found to moderately inhibit ATP production by aerobic means in Anuran species, *Rana pipens* and *Anaxyrus americanus* (Gatten 1987). If the dehydrated

toads in my experiment had aerobic ATP production inhibited, they would be forced to rely more heavily on anaerobic ATP production, which is significantly less efficient. In order to decrease the need for ATP, the metabolic rate for dehydrated organisms would need to slow, which can be seen in Preest's *et al.* (1992) work in active metabolism as well as Gatten (1987).

Thermoregulation and hydroregulation are intimately connected in terrestrial amphibians. They experience such high rates of evaporative water loss that it can be difficult for organisms to select for higher temperatures that are optimal for biological function such as locomotion, growth, and digestion without suffering the cost of increased water loss. Therefore, these organisms must always be including both factors in the behavioral decisions given their current environment.

A number of studies have looked at the interactions between thermoregulation and hydroregulation. One experiment (O'Conner and Tracy 1992) found that toads on dry substrate choose lower temperatures for minimizing water loss as opposed to higher temperatures for optimizing other biological processes. They also found that toads have a 20-minute exploratory period in which they familiarize themselves with a thermal gradient. This trend was also found in a similar experiment in which *Anaxyrus americanus* preferentially thermoregulate to achieve cooler temperatures in order to reduce evaporative water loss in environments where water was not readily available (Tracy *et al.* 1993). In work looking at behavioral responses to stressful thermal and hydric environments, the underlying goal in hydroregulation and thermoregulation is survivorship over optimization (Bundy and Tracy 1977).



In my experiment, dry sand was used as the substrate lining the thermal gradient. As a precaution toads were weighed before and after trials to ensure that no further dehydration was occurring. The area where absorption of water is highest is the pelvic patch, located on the lower trunk and medial hind leg area. This area is what is in contact with the substrate in the thermal gradient. The substrate itself was dry so the toads had no signal indicating that they would be able to maintain their current hydration state. Therefore, the toads would be most concerned with regulation of their hydration state. This makes it difficult to divest thermoregulatory behavior from hydroregulatory behavior. If for example, the toads were hydroregulating and not thermoregulating, both hydrated and dehydrated toads would select for lower temperatures to minimize evaporative water loss.

Studies have shown that anurans do actively select for specific temperatures in both dry and aquatic environments. For example *Rana catesbeiana* can achieve near constant body temperatures through behavioral temperature selection (Lillywhite 1971). The data for the thermal gradient experiment demonstrated that overall, dehydrated toads selected lower temperatures than hydrated toads (Figure 5). However, once initial body temperature was removed from the analysis no significant effect of time or hydration state was found. Due to the fact that no significant effect of hydration state was determined in metabolic rate, I predicted that toads would not select different temperatures at different hydration states. The secondary analysis of my data supports this prediction. If the effect of hydration state was still determined to be significant after the removal of initial body temperature in the analysis I would be able to more confidently infer that the toads were selecting for different temperatures at different hydration states. Testing for a longer time period may show a more significant difference, but there are some issues with testing for longer periods of time.

During the course of an hour, even with a dry substrate, the toads didn't experience more than a decrease of 1-2% of their body mass. This was in part due to the wet lids placed on the thermal gradient lanes in order to reduce evaporative water loss. For longer periods of time it may be hard to maintain hydration levels. One way this could be remedied is by placing the toads on a moist substrate in the thermal gradient. This solution presents its own set of problems however. It would be difficult to control the amount of water the toads were absorbing, and therefore difficult to maintain a set hydration state. Evaporative water loss would also occur in the substrate, especially at the more heated end of the thermal gradient.

By selecting for lower body temperatures, toads will decrease evaporative water loss, and also decrease their metabolic rate. Both of these effects help to increase a toad's chance of survive during prolonged exposure to stressful thermal environments. *Anaxyrus terrestris* are nocturnal, burrowing during the day unless humidity is high (Elliot et al., 2009, p.138). Some hypotheses can be postulated for thermoregulatory behavior in *Anaxyrus terrestris* based on research with other anuran species, but temperature selection cannot accurately be predicted in *Anaxyrus terrestris* in response to dehydration based on my data.

Although my data do not indicate that dehydration to 85% affects metabolism or temperature selection greatly, other studies have shown the importance of hydration state and body temperature in anuran species (Bundy and Tracy 1977, O'Conner and Tracy 1992, Tracy *et al.* 1993). Climate change, urban sprawl, and invasive species pose threats in their ability to alter the native habitat of species (Krakauer 1968). Because of factors like these it is important to understand behavioral patterns important for survivorship. Greater understanding of temperature selection in *Anaxyrus terrestris* can help to better predict where this species may move if its previous habitat is changed in such a way to become inhabitable.

Further research could be done to determine at what hydration state toads begin to select for different temperatures. Further research could also be done to determine if temperature selection varies at different hydration states in a moist substrate. If the issue of evaporative water loss could be effectively solved, a repetition of this experiment with a longer time period used in the thermal selection trials could be useful in helping to determine if toads at 85% hydration state will select different temperatures than do fully hydrated toads.

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