

University of Nevada, Reno

**Patterns of Hydroregulation in Representatives from  
Three Anuran Families: *Anaxyrus*, *Lithobates* and  
*Pseudacris***

A thesis submitted in partial fulfillment of the requirements for the  
degree of Master of Science in Biology

by

Tia R. Pilikian

Dr. C. Richard Tracy/Thesis Advisor

August, 2011

© by Tia Pilikian 2011  
All Rights Reserved



University of Nevada, Reno  
Statewide • Worldwide

THE GRADUATE SCHOOL

We recommend that the thesis  
prepared under our supervision by

**TIA R. PILIKIAN**

entitled

**Patterns Of Hydroregulation In Representatives From Three Anuran  
Families: Anaxyrus, Lithobates, And Pseudacris**

be accepted in partial fulfillment of the  
requirements for the degree of

**MASTER OF SCIENCE**

C. Richard Tracy, Ph.D., Advisor

Lynn Zimmerman, Ph.D., Committee Member

Scott Mensing, Ph.D., Graduate School Representative

Marsha H. Read, Ph. D., Associate Dean, Graduate School

August, 2011

## Abstract

Amphibian homeostatic processes such as thermoregulation and hydroregulation and their effects on physiological parameters have been extensively studied, but the inherent control of hydroregulation as a function of itself remains relatively unknown. This thesis addresses patterns and preferences in hydration levels of three species of anurans with different lifestyles and habits. The aquatic American Bullfrog (*Lithobates catesbeiana*), semi-terrestrial Pacific chorus frog (*Pseudacris sierra*), and terrestrial western toad (*Anaxyrus boreas*) were tested in the laboratory for patterns and preferences in the maintenance of body hydration levels. *L. catesbeiana* and *P. sierra* preferred high hydration levels ranging from 85%-99% and 83%-99% respectively, which are consistent with their propensity to remain near water, even during foraging. *A. boreas* preferred a wide range of hydrations of 62%-99%, suggesting that routine activities away from water including hibernation and active foraging allow western toads to tolerate significantly low hydration levels.

## Acknowledgements

It is my pleasure to express gratitude to the many people who helped make this thesis possible.

First and foremost, I would like to thank my advisor, C. Richard Tracy, who introduced me to the pleasures of my thesis project. Dr. Tracy was a constant source of support in dealing with all facets of this thesis. From multiple talks about the ever-evolving experimental design to the emails refining my thesis defense in the 11<sup>th</sup> hour, I cannot express the amount of gratitude I have for Dr. Tracy. The completion of my degree would not have been possible without his support.

This thesis would also not be possible without the help and encouragement of the additional members of my master's committee, Lynn Zimmerman and Scott Mensing. Thank you both for your guidance and support, and for pushing me to look at the bigger picture of amphibian ecology.

I also owe thanks to my lab mates Fran Sandmeier, Pete Noles, Lee Lemenager, Bridgette Hagerty, Sarah Snyder, and Nichole Maloney for all the guidance they have given me for various help like catching frogs and toads, help with statistics and writing, shaping up my oral thesis defense, and, most important, giving me words of encouragement when they were needed the most.

A big thank you is in order for the Tracy lab animal care manager John Grey for his help in caring for my frogs and toads, and for our office manager Larry Hillerman who always made sure the paperwork for business and grants flowed smoothly.

I also would like to thank the U.S. Fisheries and Wildlife Service for supplying a grant that I was able use to complete my thesis research.

## TABLE OF CONTENTS

Abstract.....	i
Acknowledgements.....	ii
List of Tables.....	v
List of Figures.....	vi
Homeostasis Background Literature Review.....	1
Introduction to Thermoregulation.....	1
Introduction to Hydroregulation.....	4
Methods.....	11
Results.....	16
Discussion.....	20
Literature Cited.....	29
Appendix.....	38

## List of Tables

Table 1. Patterns of hydration preferences from hydroregulation trials for <i>Anaxyrus boreas</i> , <i>Lithobates catesbeiana</i> , and <i>Peudacris sierra</i> . Significant difference in mean hydric state is indicated by * .....	20
---	----

## List of Figures

- Figure 1. Dehydration rate data and lines of best fit for *Anaxyrus*, *Lithobates*, and *Pseudacris* from individual dehydration trials. This illustration represents the dehydration rates as a percent of the lowest dehydration rate for each species.....16
- Figure 2. Hydration (as percent of standard body mass) at times of rehydration for all trials of each species. ◆, *Anaxyrus*; ■, *Lithobates*; ♦, *Pseudacris*. Figure consists of three replicate trials for all individuals .....18
- Figure 3. Percentage of hydoregulation trials that occurred in specified intervals of percent hydration. Three data points occur for each individual.....19

## **Background Literature Review**

Most vertebrates must maintain homeostasis in one or more physiological processes, which can be accomplished using physiological or behavioral processes in response to external stimuli. Homeostasis helps an individual to adjust to, or endure, changes in the environment. Maintaining homeostasis allows individuals to optimize organismal processes such as growth and development, digestion, and physical performance, and ecological processes such as reproductive fitness, foraging, and predator evasion. Mechanisms and maintenance of homeostatic processes are not mutually exclusive, i.e., multiple processes can be controlled simultaneously.

### ***Amphibian thermoregulation as a homeostatic process***

Endothermic animals such as mammals and birds physiologically maintain a high and constant body temperature, which enables individuals to remain active for long periods of time in spite of variable or extreme ambient temperatures. A high, sustained temperature also allows the body to carry out biochemical processes with optimal efficiency. However, endothermy comes at a high energetic cost; active rodents are thought to use up to 90% of consumed energy to thermoregulate (or maintain high internal body temperature,  $T_b$ ) (McNab, 1963). Bennett and Nagy (1977) also found that normal resting metabolic rates of desert fence lizards are only 3-4% that of the total resting rates of rodents or birds of similar body mass. Conversely, body temperatures of ectotherms such as reptiles and amphibians

depend on surrounding environmental temperature to achieve optimal body temperatures.

When energy is not required to maintain a high  $T_b$ , it can be allocated into growth, general maintenance and repair, and reproduction (McNab, 1963). Compared to endotherms, mechanisms of temperature regulation in ectotherms are unique due to significant physiologic differences in maintenance and survival. The inability of ectotherms to maintain an optimal  $T_b$  physiologically presents several restrictions to the animals. First, ectotherms are limited to home ranges and habitats where temperatures are within their physiologically preferred, or survivable, limits as the body temperature of the animals will ultimately assume, or be close to, the operative temperature of the thermal environment (Lillywhite, 1971; Brattstrom, 1963). Second,  $T_b$  determines the extent to which ectotherms can perform physiological activities such as walking, hopping, foraging, and evading predators. Extreme highs and lows in individual  $T_b$  can grossly hinder, or even inhibit, individuals from locomoting. Third, if ectotherms are capable of performing certain activities, temperature can place certain limitations on their physiological performance. Numerous studies have illustrated that extreme high and low temperatures negatively affect activities such as sprint performance and locomotor ability (Tracy et al., 1993; Christian and Tracy, 1981), and growth and digestion (Lillywhite et al., 1973).

However, despite the inability to maintain a constant  $T_b$ , amphibians are not limited by ambient temperature only. Individuals can exhibit behavioral thermoregulation,

the ability to control  $T_b$ , several ways by placing themselves in available microhabitats, and assuming various body postures, as well as limiting activity to certain times of the day. Individuals can increase  $T_b$  by basking on substrate exposed to the sun to absorb solar radiation (Lillywhite 1970; Brattstrom, 1963), and decrease  $T_b$  by seeking refugia from solar radiation in shaded areas, under rocks, and in soil crevasses (Brattstrom, 1979), and limiting activities such as locomotion and digestion to day or night where ambient temperatures are most suitable for different species (Lillywhite et al., 1973; Seymore, 1972; Vallivieso and Tamsitt, 1974).

Several studies have reported how important thermoregulation is to the performance of an individual as a whole. When placed in a temperature gradient from 3 - 50°C, juvenile Woodhouse's toads selected temperatures between 24-32°C, and when tested for hopping performance at temperatures ranging from 6-36°C optimal hopping performance occurred at 30°C, consistent with preferred body temperatures sought out in a laboratory thermal gradient (O'Connor and Tracy, 1992). Hydrated juvenile American toads also selected temperatures in a laboratory thermal gradient where those temperatures would provide the potential to maximize sprint speed (Tracy et al., 1993). Amphibians have also been shown to select temperatures that maximize growth and digestion; in the laboratory, juvenile western toads sought higher temperatures when satiated, possibly to accelerate digestion, and lower temperatures when starved to reduce energy expenditure (Lillywhite et al., 1973). Toads in that study also preferred temperatures nearly

identical to those where growth and energy efficiency peaked (Lillywhite et al., 1973). Thermoregulation also can act to reduce water loss in amphibians. Unlike birds and mammals that have an increased cutaneous resistance to water loss via scales, thickened skin, feathers, and hair, the integument of amphibians offers little or no resistance to evaporative water loss (EWL). EWL increases with higher temperatures and causes amphibians to dehydrate more rapidly. To reduce the rate of EWL, and subsequent dehydration, amphibians can thermoregulate by selecting lower temperatures along a thermal gradient when hydrically stressed (Malvin et al., 1989; Tracy et al., 1993). Behavioral thermoregulation allows amphibians to choose  $T_b$  that is (1) within a physiologically survivable range, and if possible, (2) near an actual preferred temperature that facilitates optimum performance.

### ***Amphibian hydoregulation as a homeostatic process***

The skin of amphibians is highly vascularized, porous, and has abundant glands to exude mucus onto the skin surface. Significant gas exchange of oxygen and carbon dioxide occurs through the skin via a superficial capillary network, and the skin functions as an ancillary means of respiration. Unlike the water resistant skin of most terrestrial vertebrates, amphibian skin allows for a bidirectional flow of water, making both cutaneous absorption and evaporation possible. Under normal ecological circumstances, amphibians do not ingest water to hydrate, but instead absorb water through the integument (Bentley and Yorio, 1979). The primary location for water absorption through the skin is a patch of vascularized skin on the ventral surface between the hind legs referred to as the pelvic patch or seat patch

(McClanahan and Baldwin, 1968). Skin of the pelvic patch is thinner and more vascular than other areas, and in one study on the desert toad, *Anaxyrus punctatus*, this skin accounted for approximately 10% of the total surface area of an individual (McClanahan and Baldwin, 1968). Using the pelvic patch to absorb water, amphibians can take in water from free water sources such as lakes, rivers, streams, ponds, puddles, and ditches. Water absorption can also occur through wet or moist soil, and by 'scooting' across substrates such as leaves and rocks thus harvesting the water on the surfaces.

For water absorption into the body to occur, the pelvic patch of the individual must make contact with the wet or moist substrate. A Water Absorption Response is elicited, and the individual wipes the pelvic patch on the wet substrate while abducting the hind legs (Stille, 1958). To absorb water from a substrate, the water potential for the anuran must be lower than that of the respective substrate.

Translating water potential to osmotic pressure also shows that for water absorption to occur, the osmolality of body fluid must be higher than that of the wet substrate. When an anuran loses water to its environment, osmoreceptors in the hypothalamus stimulate the neurohypophysis to release the peptide hormone, Arginine Vasotocin (AVT), to enhance water uptake. AVT, whose homologue is Arginine Vasopressin in mammalian vertebrates, is an anti-diuretic hormone (ADH) that when released increases hydraulic permeability and absorption of water in the pelvic patch skin and bladder, and possibly also modifies water potential at the pelvic patch (Tracy et al. 2007; Schloss et al., unpublished). Increasing water

permeability allows absorption of water from a substrate where normally it would be difficult. Once a frog is fully hydrated, its blood osmolality, and its bladder urine concentration decrease as water enters the lymph and bladder. Ruibal (1962) found that the lymph concentration of fully hydrated *Anaxyrus cognatus* is approximately 6-7% of the total lymph/water composition and bladder urine concentration is as low as 1%, with water as the remaining fluid. A fully hydrated bladder can contribute 20-50% of the total body mass of an individual (Main and Bentley, 1964; Ruibal, 1962).

Most anurans lack physiological resistance to evaporative water loss (EWL), and they lose water at rapid rates (Tracy, 1976; Pough et al., 1983; Shoemaker, 1975; Shoemaker and Nagy, 1977; Wygoda, 1984). The rate of water loss for some amphibians can be as high as 10-100 times greater than other non-amphibian tetrapods (Bentley and Yorio, 1978), and most frequently, EWL can occur at rates as high as that of a free-water surface (Schmidt-Nielson, 1969; Bentley, 1971; Tracy, 1976).

As an individual begins to dehydrate, urine production and urination cease. Water stored in the bladder begins to reabsorb into desiccated body tissues. The redirection of bladder water from the bladder causes the urine concentration and subsequently lymph concentration to increase (Ruibal, 1962). If, after release of AVT, the animal is still unable to absorb water (i.e., they are away from a water source or on dry soil), dehydration of tissues will continue.

### ***Factors affecting the rate of dehydration***

A number of factors have been shown to affect the rate of dehydration of amphibians. Body size is a strong predictor of the rate of EWL for individuals. Small anurans have a higher surface area-to-volume ratio, which can account for a higher rate of EWL and lower cutaneous resistance than that in larger anurans (Heatwole et al., 1969; Tracy et al., 2010), and among individuals of the same species, smaller individuals dehydrate faster than do larger conspecifics (Cohen, 1952). This surface-area effect is concordant with literature indicating that body size does not elicit changes in EWL rates per unit surface area (Schmid, 1965; Withers et al., 1982; Wygoda, 1984). Anurans also dehydrate faster at higher temperatures; as temperature increases, evaporation from the skin surface accelerates (Buttemer, 1990; Tracy, 1976). Movement, and activity level, can also increase the rate of EWL insofar as moving individuals expose more surface area to evaporation when legs and head are extended. An increase in activity can raise metabolism, and consequently the body temperature of an individual, accelerating EWL (Cohen, 1952).

Different species can have very different rates of EWL. Studies conducted on dehydration rate of species of different habitats indicate that species with increasing degrees of terrestriality/arboreality can have dehydration rates slower than those who are more aquatic (Warburg, 1965; Wygoda, 1984; Thorson, 1955; Thorson and Svihla, 1943; Tracy et al. 2008; Tracy et al., 2010). The bullfrog, *Lithobates catesbeiana*, has one of the lowest resistances to EWL, and individuals lose water at

the same rate as a free water source (Rey, 1937). Wygoda (1984) found that the dehydration rate was highest for aquatic species, intermediate for terrestrial species, and slowest for arboreal species, which lost water up to 47% slower than non-arboreal species. Contradictory data also indicate that there is no difference in dehydration rate of species of different habitats and degrees of terrestriality (Claussen, 1969; Schmid, 1965).

### ***Ways to reduce EWL***

Similar to achieving a preferred body temperature through behavioral thermoregulation, anurans have developed various adaptations to slow dehydration. By adopting a water-conserving posture, a non-active individual tucks feet close to the body, rests the chin on the substrate, and closes the eyes when necessary; by employing this position, surface area available for cutaneous water loss (CWL) is reduced significantly (Pough et al., 1993). Other ways involve aggregation among conspecifics, selecting cooler and wetter microhabitats, decreasing physical activity, adopting nocturnality, and burrowing into moist substrate (Dole, 1967; Hairston, 1949; Heatwole, 1960, 1962; Hendrickson, 1954; Pough et al., 1983; and Stille, 1952). Some species, which are highly adapted to xeric environments, can prevent water loss by forming cocoons consisting of multiple layers of superficial skin cells and mucus while in their burrows during drought periods. These individuals later emerge during wetter seasons with little to no water loss (Ruibal and Hillman, 1981; Tracy et al., 2007).

### ***Consequences of severe dehydration***

If dehydration continues to a critical level, blood viscosity and hematocrit increase as blood volume is reduced, which will increase cardiac workload. Aerobic capacity is compromised and, paired with increased hyperosmolality of body fluids, tissues become anoxic resulting in systemic failure of organs and muscles (Churchill and Storey, 1995; Hillman, 1980, 1987; Hillman et al., 1987). The individual becomes increasingly lethargic and eventually reaches the critical activity point (CAP), the level of dehydration where the animal loses its righting reflex, or the ability to right itself if flipped on its back (Ray, 1958). The ultimate cause of death is theorized to be a cardiovascular system failure (Hillman, 1980).

### ***Hydration and Performance***

Dehydration can also have negative implications for physical performance. Several studies have shown that dehydration can cause significant declines in jumping distance in *Lithobates*, *Anaxyrus*, *Eleutherodactylus*, and *Acris* (Beauchat et al., 1984; Moore and Gatten, 1989; Rogowitz et al., 1999; Walvoord, 2003). Xeric species seem to be more tolerant, and experience less hindrance of performance than aquatic species (Heatwole and Newby, 1972; Hillman, 1987).

## Introduction to Thesis

A fully functioning frog typically must balance control of multiple physiological, behavioral, and ecological properties and processes such as foraging, mating, predator evasion, thermoregulation, and hydroregulation. Often one property or process must be loosely controlled in order to free the organism to regulate another process with greater precision (Bundy and Tracy, 1977). For example, on warm dry nights, mate calling or foraging in coquís frogs may be decreased or halted as a means to reduce dehydration through evaporation (Pough et al., 1983).

Hydroregulation also can be sacrificed as a means to avoid lethally high body temperatures. For example, in hot thermal environments, Australian tree frogs can reduce cutaneous resistance to water loss resulting in increased evaporation as a means to cool the frog (Tracy et al., 2008). Thus, all regulatable processes likely are not simultaneously regulated, and some properties and processes may be held at acceptable levels while others are regulated to near-optimal levels.

Because most frogs can lose water by evaporation at very high rates, those individuals must return to a source of water as a means to maintain their water balance. However, little is known about the precision with which frogs can control their hydration. Precise regulation, i.e., maintaining a narrow range of hydric states, could allow individuals to optimize various performance measures, but that regulation likely requires the frog to be vigilant about its proximity to water sources. On the other hand, loose hydroregulation could allow individuals to venture far from water sources, and those individuals may more precisely regulate processes

other than body hydration. Data exist on the relationship between hydration preferences and performance, and patterns of dehydration tolerance among species, but there is a dearth of literature on voluntary control of hydration in frogs. My objective was to assess patterns, and preferences, of water balancing behavior in three species of frogs with different ecological habits as a start of a survey of variations in water economy among frog species.

## **Methods**

### ***Study Species***

Three species differing dramatically in ecological habit were subjects of this research. The western toad *Anaxyrus boreas* is a terrestrial anuran whose geographic range spans from western and southern California (excluding the Mojave Desert) to western Colorado, and as far north as southern Alaska. Western toads are generally active between January and October, depending on the local climate. During breeding season toads are found near ponds, lakes, reservoirs, springs, marshes, and streams, but in other seasons, toads become more terrestrial and can be found away from water sources (Ruthven and Gaige, 1915; Wright and Wright, 1949).

The American bullfrog, *Lithobates catesbeiana*, is almost exclusively aquatic and inhabits warm, open, permanent bodies of water. The bullfrog is native to eastern and southern United States, but dispersal by humans has resulted in this species' wide range over most of the United States, as well as other continents. Bullfrogs are

largely found in, or around, water. Bullfrogs inhabiting climates that are mild, wet, and warm year round often don't hibernate, but those found in colder climates will remain dormant in soft, moist mud at the bottom of ponds during winter months.

The Pacific chorus frog *Pseudacris sierra* (and its close relatives) is a small arboreal frog found in most areas of California excluding deserts, and parts of Nevada, Oregon, Idaho, Montana, and southern British Columbia. Chorus frogs can use temporary or permanent water sources for breeding and general activity and retreat to higher ground to hibernate and estivate during non-breeding seasons and droughts respectively (Calhoun and Jameson, 1970). While much of the chorus frogs' activity occurs on the ground, individuals are able to climb onto branches of shrubs and low trees. Dehydration in these small frogs can be slowed by nocturnality, water conserving postures and aggregating (Nussbaum et al., 1983; Goin, 1978).

Sixteen adult male western toads were captured by hand from Rancho San Rafael Park in Reno, NV on 15 April 2008, and 14 juvenile bullfrogs were also collected from a large pond in Rancho San Rafael Park in August 2009. Six male chorus frogs were collected from the same location (IACUC #00108).

### ***Habituation***

Toads and frogs were grouped by similar size and habituated in glass aquaria (31cm x 25 cm x 50.5cm). *Anaxyrus* were grouped four per aquarium, *Lithobates* three per

aquarium, and *Pseudacris* three per aquarium. Habituation occurred for six weeks to familiarize the animals with people, laboratory temperature, and feeding and cleaning schedules. Free water on the aquarium floor was available at all times for rehydration. During the last three weeks of habituation, the aquarium water on the floor of the aquarium was removed and red dishes (12cm x 12cm x 5cm for *Anaxyrus* and *Lithobates*, and 12cm x 12cm x 2cm for *Pseudacris*) filled with water were placed in the center of each aquarium. Animals still had free access to water, but now they were required to jump in the dish to rehydrate. When all animals learned to use the dish to rehydrate, testing for each species began.

### ***Testing***

Laboratory data collection for each species consisted of two components: (1) measurement of individual dehydration rates in the test arena, and (2) assessing hydroregulation behavior. Each individual was allowed to dehydrate in its aquarium once, and then underwent three hydroregulation behavior tests (one toad was tested only twice as it died before the final replicate). To abate defecation during an experiment, food was withheld for 72 hours prior to testing. Laboratory temperature for all tests ranged from 25-27°C. All tests began at 12:00 pm.

### ***Dehydration Rate***

The night before assessing dehydration rate, frogs were partly submerged overnight in approximately 2 cm of water to ensure 100% hydration. Just prior to testing, to prevent re-absorption of bladder water, the bladder was manually voided by inserting a smooth plastic cannula into the cloaca to drain all fluid. Slight pressure

was also applied on the abdomen to ensure that all bladder water was expelled. Each individual was weighed and the standard mass (mass of a fully hydrated animal with an empty bladder, SM) was recorded. Each individual was placed in a 625cm x 430cm x 160cm plastic testing arena with a plastic mesh lid to prevent the animal from escaping. A red water-filled plastic rehydrating dish (same as in rehydration learning) was placed in the center of the arena, but a wire mesh cover was placed over the water dish to prevent the animal from coming into contact with the water.

The behavior of all animals was logged using a Logitech quick cam Pro 9000 video surveillance camera positioned above the testing arenas. Data were retrieved using Evocam version 3.6.4 Universal video software. When the test started, video at 0.2 frames/s was recorded for the test duration to monitor individual activity.

Preliminary testing indicated that it takes 8-9 hours for *A. boreas* and *L. catesbeiana* and four hours for *P. sierra* to dehydrate to approximately 75%-80% of SM. Tests ran uninterrupted for these respective times. The goal of dehydrating was not to reach the critical activity point, but to reach a level of dehydration that induced fatigue, decreased physical activity, and encouraged the adoption of water conserving postures. At the termination of the test, video was stopped and body mass of the individual was recorded. Animals were returned to their cages to rehydrate and to eat. Using the time duration of the test, initial standard mass, and body mass at test termination, linear dehydration rates were calculated for each individual. The video of each animal's behavior allowed us to assess the amount of

activity time (as a percentage of the total test time) for each individual during the test. Multiple regression analysis was then used to determine the extent to which amount of activity had an effect on the rate of dehydration.

### ***Hydroregulation Behavior***

Three replicate tests were performed on each individual to document species-specific behaviors, and to quantify individual differences. Each individual animal was treated to the same preliminary protocol before each hydroregulation experiment: animals fasted for 72 h, then submerged in approximately 2 cm water the night before the experiment to ensure 100% hydration at the time of the test. A plastic catheter was used to empty the bladder, and slight depression on the abdomen voided any residual water in the bladder. At the beginning of the experiment, standard body mass was recorded, and the animal was placed in the testing arena with the red dish of water positioned in the center of the arena. In these tests, the red dish was uncovered so that the animals had access to the water to rehydrate at will. Surveillance cameras positioned above the arena (recording at 0.2 frames/s) allowed natural behavior without distracting noise or movement within the room. The test was terminated when an individual jumped into the rehydrating dish and remained for at least one minute. Upon test termination, the animal was placed back in its cage for rehydration and opportunity to feed. After all *A. boreas* individuals had been tested three times, testing began on *L. catesbeiana*, and subsequently on *P. sierra*. Analysis of the video allowed the determination of the time elapsed until an individual moved into the red dish to rehydrate. Hydration

state at the time of rehydration of each individual was calculated using the individual's own dehydration rate, and activity level.

## Results

### *Dehydration Rate*

Individual dehydration rates ranged from 0.72 - 1.96 g/h (for *A. boreas*), 0.73 - 1.29 g/h (for *L. catesbiana*), and 0.2 - 0.39 g/h (for *P. sierra*) (Fig. 1). Activity levels (expressed as a percent of time active) were variable among and within species, and

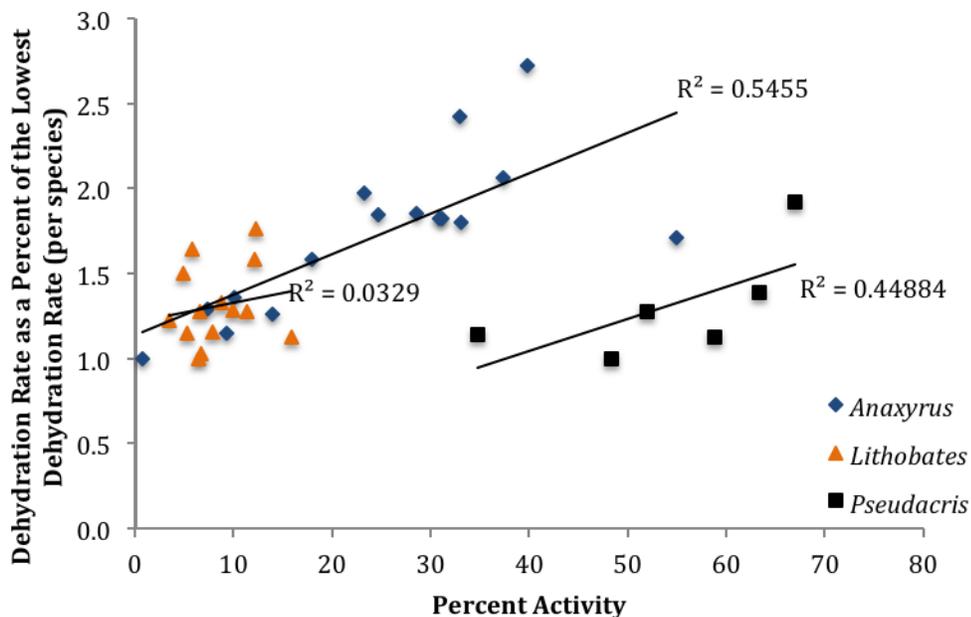


Figure. 1. Dehydration rate data and lines of best fit for *Anaxyrus*, *Lithobates*, and *Pseudacris* from individual dehydration trials. This illustration represents the dehydration rates as a percent of the lowest dehydration rate for each species.

also varied among individuals: 0.7 -54.9% (*A. boreas*), 3.5 -15.9% (*L. catesbeiana*), and 34.7 - 61% (*P. sierra*). Activity did not have a significant effect on the individual dehydration rates for *Lithobates* and *Pseudacris*, which had limited ranges of activity. However, *Anaxyrus* had a very broad range of activity among individuals, and activity significantly increased the rate of dehydration;  $R^2 = 0.53$ ,  $F(1,15) = 16.0$ ,  $p < 0.001$ .

### ***Hydroregulation Trials***

Hydration level at the time at which individuals went to the water source to rehydrate was different among individuals and among species. Thus, *L. catesbeianas'* hydric state at the point at which they went to the water source to rehydrate ranged from 99.7 - 85.2% of standard body mass. *P. sierra* ranged from 99.8 – 83.0%. *A. boreas* ranged from 99.8%-62.5%, a range more than twice as large as both *Lithobates* and *Pseudacris* (Fig. 2).

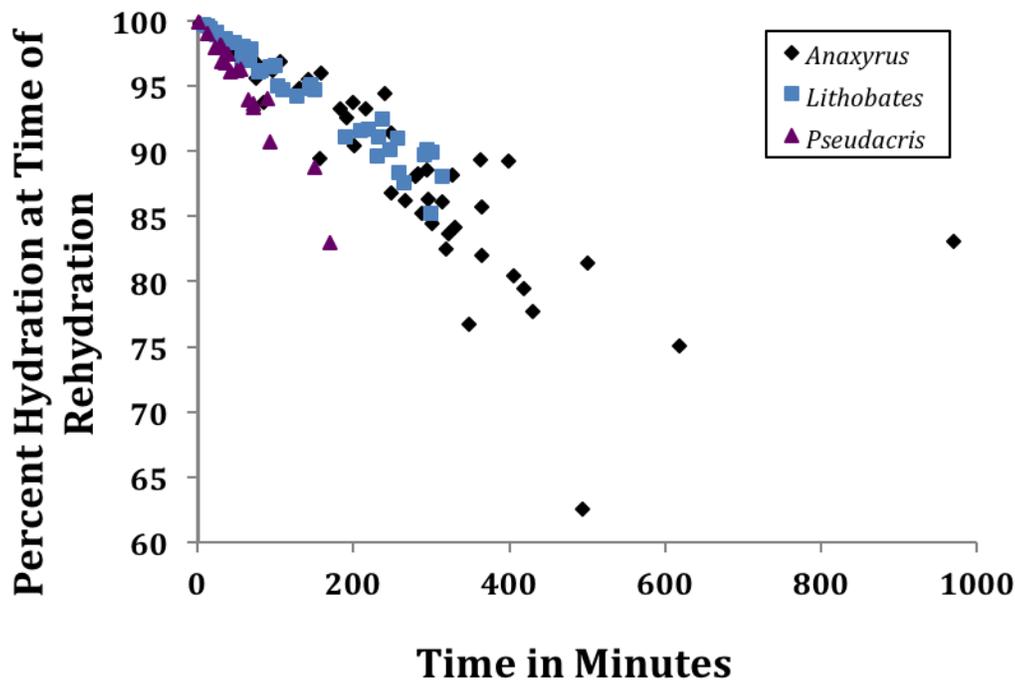


Figure 2. Hydration (as percent of standard body mass) at times of rehydration for all trials of each species.  $\blacklozenge$ , *Anaxyrus*;  $\blacksquare$ , *Lithobates*;  $\blacktriangle$ , *Pseudacris*. Figure consists of three replicate trials for all individuals.

Differences in mean hydration level at the time of voluntary rehydration varied among the three species,  $F(2, 107) = 7.17, p < 0.05$ . Post hoc analysis using the Tukey Kramer HSD test indicated that mean hydration levels of *Anaxyrus* were significantly different than both *Lithobates* and *Pseudacris* ( $p \leq 0.001$ ). Mean hydration levels for *Lithobates* did not differ significantly from *Pseudacris*. Individuals of *Anaxyrus* went to water to rehydrate when their hydration level dropped to only 90% of standard body mass less than half of the time (46%), whereas individuals from both *Lithobates* and *Pseudacris* whose hydration levels dropped to only 90% moved to

water to rehydrate in 90% of the trials (Fig. 2, 3; Table 1). In fact, of those individuals who moved to the water bowl to rehydrate while between 100 and 90% of standard body mass, over half of the returns to water for *Lithobates* and *Pseudacris* actually occurred when the frogs were at or above a 95% hydration level, where only 27% of the replicate trials for *Anaxyrus* were at or above 95% hydration level.

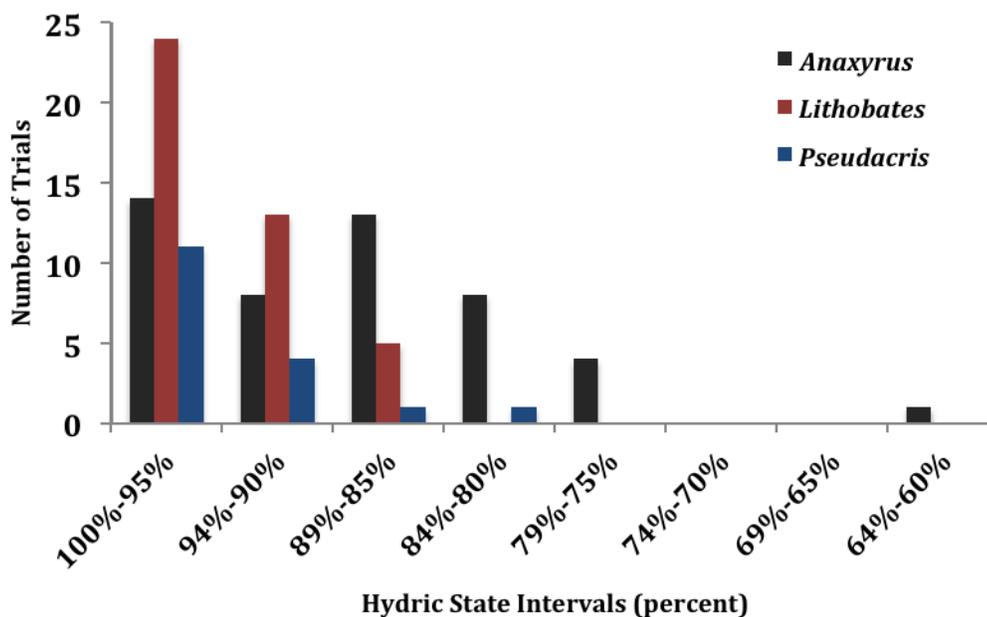


Figure 3. Frequency of hydroregulation trials that ended in specified intervals of percent hydration. Three data points occur for each individual.

In addition to species-specific patterns, individuals within species also showed certain hydration preferences. Twenty-five percent of *Anaxyrus* individuals, 50% of *Lithobates* individuals, and 67% of *Pseudacris* individuals in all three trials rehydrated with hydric states in the 90th percentile, and 18% of *Anaxyrus* individuals rehydrated in the 80th percentile in all trials. Many animals even

displayed strict hydration ranges; 25% of *Anaxyrus*, 14% of *Lithobates*, and 50% of *Pseudacris* rehydrated in all trials with hydration levels within 5% of each other.

Table 1. Patterns of hydration preferences from hydroregulation trials for *Anaxyrus boreas*, *Lithobates catesbeiana*, and *Pseudacris sierra*. \* Indicates significance using Friedman's Test.

	Hydric State Range at Rehydration	Hydric State Span	Average Hydric State	Percent Occurrence of Rehydrating >90%	Percent Occurrence of Rehydration >80%
<i>A. Boreas</i> (n=50)	99.8%-62.5%	37.3%	89.0%*	48%	90%
<i>L. catesbeiana</i> (n=42)	99.7%-85.2%	14.5%	94.5%	83%	100%
<i>P. sierra</i> (n=18)	99.8%-83%	16.9%	95.0%	89%	100%

## Discussion

### *Patterns of Hydration Preferences*

Hydroregulation, has been shown to be important in maintaining homeostasis in amphibians. Analogous to thermoregulation, the ability to control hydration within optimal, or acceptable, levels is necessary for individuals to carry out daily functions efficiently.

Both differences and similarities were discovered in re-hydration “set point” among the three species. The bullfrog *L. catesbeiana* exhibited a set point range (range of hydration levels within which the animal does not behaviorally rehydrate) that

could be somewhat predicted based upon its known close association with water (Currie and Bellis, 1969). During dehydration, *Lithobates* individuals, over 80% of the time, chose to rehydrate before desiccating by as much as 10%. The frogs also appeared never to dehydrate to levels below 85% of standard body mass. This indicates that individuals of this species choose to remain hydrated well above levels of dehydration that would threaten physiologically negative consequences. Aquatic ranids have been found to dehydrate faster, and suffer higher aerobic capacity and physical endurance problems than some terrestrial species (Dumas 1966; Moore and Gatten Jr., 1989; Warburg, 1965); that bullfrogs prefer a somewhat high, and narrow, hydration range fits agreeably with observations that anurans remain close to water sources, and likely remain well hydrated (Currie and Bellis, 1969). While some individuals might travel distances of over a half mile away from ponds (Willis et al., 1956), some studies have found that individuals are found either on or near the shore, or in the water of the same pond of their original capture location (Currie and Bellis, 1969; Ingram and Raney, 1943; Raney, 1940). Additionally, bullfrogs are known sit-and-wait predators; individuals will sit and wait for prey to approach, at which point they will zot and eat the prey (). Employment of the sit-and-wait foraging strategy in or near ponds ensures contact of the ventral patch with moist or wet substrate, and ultimately results in continuous hydration.

Under normal circumstances, *L. catesbeiana* individuals may place more importance on remaining well hydrated than on other activities that might put them at risk of dehydration.

The laboratory hydration preferences of the Pacific chorus frog, *P. sierra*, were somewhat similar to those of *L. catesbeiana*, insofar as in most trials frogs moved to water to rehydrate while at levels of at least 90% hydrated, thus, maintaining a high, and narrow range of hydration. It is interesting that this species maintains high hydration levels similar to the aquatic bullfrog, given that the chorus frog is known to be a more terrestrial species that occasionally frequents water sources. While bullfrogs are rarely found far from water, chorus frogs have been found in locations up to one half mile away from any water source (Storer, 1925). It is possible, however, that *Pseudacris* can be terrestrial and still maintain high hydration for several reasons. First, Claussen (1973) suggested that *Pseudacris* is mostly found in a 'squatting' position where the ventral surface of the body is directly over a moist substrate, and its legs are tightly held near the sides. *Pseudacris* are also known to sit and wait on shrubs and algae masses atop water sources and attack when prey approach (Brattstrom and Warren, 1955). Adoption of squatting and ambush allows more contact of the ventral patch to any substrate from which it could draw moisture. This position also decreases the surface area of exposed skin in danger of dehydration from ambient air. Claussen also discovered that *Pseudacris sierra* could absorb water rapidly from a moist or wet substrate, which if away from a water

source, would allow individuals to replenish water supply quickly from moist surfaces and not dehydrate to suboptimal or threatening hydration levels.

Additionally, *P. sierra* are small anurans, typically not as large as 4 cm in length, thus they have a high surface area-to-volume ratio, and rapid dehydration rates (Heatwole et al., 1969; Tracy, 1976). These characteristics may increase awareness of staying well hydrated. Finally, studies conducted by Claussen found that when *Pseudacris* was given a choice of dry sand or water, chorus frogs chose water, and when faced with wet sand or dry sand, they chose wet sand. Individuals are likely aware of their vulnerability to desiccation, and they may rehydrate whenever possible, resulting in a tightly regulated hydration level that safeguards them against reaching desiccating situations that can be difficult to reverse.

Hydration levels for the western toad *A. boreas* were remarkably more variable than *Lithobates* or *Pseudacris*. Preferred hydration ranged from near 100% (fully hydrated) to as low as 62%. In general, toads at hydration levels between 85 and 100% hydrated exhibited approximately equal tendency to seek water to rehydrate. And, while *Lithobates* and *Pseudacris* never dehydrated to levels below 85% and 82% respectively, individual toads allowed themselves to dehydrate to as low as 60-70% of standard body mass. Thus, western toads appear to tolerate dehydration much more than do the frogs in our study. It is unknown at what hydration levels toads function in the field, but *A. boreas* are known to spend a significant period of time away from water sources. Bartelt and Peterson (1994) reported that western toads would move away from breeding ponds for several summer months, relying

entirely on moist soils and morning dew for rehydration. At the end of the breeding season, these toads seek hibernacula for the winter, and may travel as much as several kilometers to find a suitable hibernaculum (Bartelt et al., 2004; Campbell, 1970). *Anaxyrus* dehydration tolerance might also be explained from their foraging technique; at night, western toads leave water sources and actively forage for prey for extended periods of time (Hailman, 1984). This act of 'getting up and moving around' inhibits ventral patch contact with moist or wet substrate, and, coupled with traveling extended distances from water sources, likely results in some degree of dehydration that animals must tolerate to survive. The broad range of hydrations tolerated by toads in our study, coupled with their well established independence from water sources indicates that *A. boreas* may find dehydrations of 20% or 30%, or even nearly 40% of standard body mass to be tolerable for normal function, if only for a short period of time.

### ***Maintenance of Hydration Ranges***

There has been some controversy about the underlying anuran strategies of controlling processes like thermoregulation and hydroregulation (Tracy et al. 2010). The majority of frogs in our study sought out a water source even when they were well hydrated- hydrated enough that physical performance was likely not compromised. However, the toads allowed themselves to become dehydrated potentially to physiologically compromising levels before returning to water.

Dehydration, to some degree, inevitably occurs when individuals leave a water source to feed, evade predators, hibernate, etc. Examining hydration preferences

from this study revealed that individuals tolerate varying ranges of hydrations. The lowest hydration level tolerated can be taken to be a lower 'set point' of the regulatory system that represents a level of dehydration an individual is not willing to exceed under normal circumstances. Laboratory studies of anuran locomotor performance tests show a decline in performance with increasing dehydration (Beuchat et al., 1984; Claussen, 1974; Moore and Gatten Jr., 1989; Rogowitz et al., 1999). Thus, instead of narrowly regulating hydration at the physiologically most favorable level, individuals instead appear to maintain a range of *acceptable* hydration levels allowing them to function adequately (Bundy and Tracy, 1977). Maintaining a range of acceptable hydrations instead of maintaining the physiologically 'best' hydration enables animals to perform other functions such as foraging and thermoregulating.

Acceptable ranges of hydrations for *Anaxyrus*, who displayed a broad range with a somewhat extreme set point (62%), differed from both *Lithobates* and *Pseudacris*, whose set points were 85% and 82% respectively. Studies by Heatwole and Newby (1972) and Hillman (1987) found that significant dehydration does not adversely affect physical performance in xeric species such as *A. boreas* as much it does in aquatic species. So, it is possible that loosely regulated ranges of terrestrial *Anaxyrus* are no more physically compromising than tightly regulated ranges of *Lithobates* and *Pseudacris*.

### ***A balancing act of hydroregulation and thermoregulation***

Here, we attempted to parse out the importance of hydroregulation from other behaviorally regulated processes to determine, to what degree pattern in hydroregulation exists, and to provide ecologically derived interpretations of pattern. In the field, however, anurans typically must regulate several processes simultaneously for comprehensive function. Given anurans' high rates of evaporative water loss, and the link between water loss and temperature, it seems that hydroregulation and thermoregulation may be the two most crucial processes to regulate. From previous reports, anurans have been shown to maintain certain body temperatures and hydration levels at which they function best, and when frogs deviate from optimal temperatures or hydrations, they tend to have reduced physiological performance. Thus, regulating body water and temperature is a balancing act between the multiple processes, where anurans will seek to stay within an acceptable range of temperatures and hydrations. At certain times, the importance of one process may be subordinated to maintain the other process within acceptable limits. For example, when ambient temperatures increase to levels that threaten dehydration via evaporation, anurans will seek moist or wet substrates or shelters under shrubs or rocks, and in burrows where water loss is slowed (Bundy and Tracy, 1977). When anurans undergo hydric stress, they may seek lower body temperatures to reduce water loss (Tracy et al., 1993). The results of this study offer a solid and fundamental glimpse into the relative importance of

hydroregulation in representatives from terrestrial, aquatic, and semi-arboreal backgrounds.

### **Conclusion**

Anurans, whose skin provides little or no barrier to evaporative water loss, face unique obstacles in normal daily activities. The degree to which individuals dehydrate, and later begin to sacrifice optimal performance, depends on multiple factors such as species type, body size, activity level, and habitat. When performing tasks including, but not limited to hydroregulation, thermoregulation, breeding, foraging, seeking shelter, and evading predators, anurans must prioritize and execute these activities based upon their relative importance to sustain the individual. This study sought to provide a deeper understanding of the importance of hydroregulation in three species with differing sizes, habitats, and life history traits. We have discovered that our aquatic representative, *Lithobates catesbeiana*, maintains a high and narrow range of hydric states, and appears to place high importance on staying well hydrated. *Lithobates'* high hydration preference is concordant with an aquatic habitat where water is usually plentiful and highly exploited. Employment of a sit-and-wait foraging strategy near water also enables bullfrogs to stay substantially hydrated when feeding. Similarly, the chorus frog *Pseudacris sierra* also maintains a high and narrow hydration range. Although chorus frogs are known to be more terrestrial, they have a small, compact body posture, have the ability to absorb water rapidly, and may slow evaporative water loss behaviorally. Like *Lithobates*, *Pseudacris* are also use a sit-and wait foraging

strategy, which, if executed near water will slow, inhibit, or even reverse water loss. Consistently well-hydrated chorus frogs may rarely pass up a chance to rehydrate, if water should become available. For both *Lithobates* and *Pseudacris*, avoiding high levels of dehydration likely safeguards individuals from compromising physical performance. Different from *Lithobates* and *Pseudacris*, the terrestrial western toad *Anaxyrus boreas* displayed a loosely regulated range of hydrations, sometimes dropping to physiologically compromising levels. *Anaxyrus* often ventures away from water sources (which ultimately results in water loss) during activities such as hibernation and nocturnal foraging. That western toads have developed both physiologic and behavioral adaptations for retarding water loss is likely the reason for frequent abandonment of tight water regulation. Terrestrial toads have shown less physiologic impairment from dehydration than more aquatic species, thus may place less importance on water economy.

The concept of hydroregulation and its importance as a major homeostatic processes is, deservedly so, gaining more recent exposure. Further research of the role of behavioral water economy as a function by itself as well as its interactions with other behaviors will ultimately deepen our understanding of anuran physiological ecology and the degree of significance it has on a wide breadth of species.

### Literature Cited

- Bartelt, P. E., and Peterson, C.R. 1994. Riparian habitat utilization by western toads (*Anaxyrus boreas*) and spotted frogs (*Rana pretiosa*) on the Targhee National Forest. USDA Forest Service Contract #INT-93780-CCSA Final Report.
- Bartelt, P.E., Peterson, C.R., and Klaver, R.W. 2004. Sexual differences in the post-breeding movements and habitats selected by western toads (*Anaxyrus boreas*) in southeastern Idaho. *Herpetologica*. 60: 455-467.
- Bennett, A.F., and Nagy, K.A. 1977. Energy expenditure in free-ranging lizards. *Ecology*. 58: 697-700.
- Bentley, P.J., and Yorio, T. 1979. Evaporative water loss in anuran amphibia: A comparative study. *Comp. Biochem. Physiol.* 62: 1005-1009.
- Bentley, P.J., and Yorio, T. 1979. Do frogs drinks? *J. Exp. Biol.* 79: 41-46.
- Beuchat, C.A., Pough, F.H., and Stewart, M.M. 1984. Response to simultaneous dehydration and thermal stress in three species of Puerto Rican frogs. *J. Comp. Physiol.* 154B: 579-585.
- Bentley, P.J. 1971. Endocrines and osmoregulation: A comparative account of the regulation of water and salt in vertebrates. Springer, Berlin.
- Brattstrom, B.H., and Warren, J.W. 1955. Behavior of the Pacific Treefrog, *Hyla regilla*. *Copeia*. 1955: 181-191.
- Brattstrom, B.H. 1963. A preliminary review of the thermal requirements of amphibians. *Ecology*. 44: 238-255.

- Brattstrom, B. H. 1979. Amphibian temperature regulation studies in the field and laboratory. *Am. Zool.* 19: 345-356.
- Bundy, D., and Tracy, C.R. 1977. Behavioral response of American Toads (*Anaxyrus americanus*) to stressful thermal and hydric environments. *Herpetologica.* 33: 455-458.
- Buttemer, W.A. 1990. Effect of temperature on evaporative water loss of the Australian tree frogs *Litoria caerulea* and *Litoria choris*. *Physio. Zool.* 63: 1043-1057.
- Campbell, J. B. 1970. Hibernacula of a population of *Anaxyrus boreas* in the Colorado Front Range. *Herpetologica.* 26: 278-282.
- Calhoun, R.E., and Jameson, D.L. 1970. Canonical correlation between variation in weather and variation in size in the Pacific tree frog, *Hyla regilla*, in Southern California. *Copeia.* 1970: 124-134.
- Christian, K.A., and Tracy, C.R. 1981. The effect of the thermal environment on the ability of Galapagos Land Iguanas to avoid predation during dispersal. *Oecologia.* 49: 218-223.
- Churchill, T. A., and Storey, K.B. 1995. Metabolic effects of dehydration on an aquatic frog, *Rana pipiens*. *J. Exp. Biol.* 198: 147-154.
- Claussen, D.L. 1969. Studies on water loss and rehydration in anurans. *Physiol. Zool.* 42: 1-14.

Claussen, D.L. 1973. The water relations of the tailed-frog *Ascaphus truei*, and the Pacific tree frog, *Hyla regilla*. *Comp. Biochem. Physiol.* 44A; 155-171.

Claussen, D. L. 1974. Water balance and jumping ability in anuran amphibians. *Am. Zool.* 14:1257.

Currie, W., and Bellis, E.D. 1969. Home range and movements of the bullfrog *Rana catesbeiana* shaw, in an Ontario pond. *Copeia.* 1969; 688-692.

Cohen, N.W. 1952. Comparative rates of dehydration and hydration in some California Salamanders. *Ecol. Soc. Amer.* 33: 463-479.

Dole, J.W. 1967. The role of substrate moisture and dew in the water economy of leopard frogs, *Rana pipiens*. *Copeia.* 1967:141-149.

Dumas, P.C. 1966. Studies of the *Rana* species complex in the Pacific Northwest. *Copeia.* 1966: 60-74.

Goin, C.J., Goin, O.B., and Zug, G.R. 1978. Introduction to Herpetology. W.H. Freeman, San Francisco.

Hailman, J.P. 1984. Bimodal nocturnal activity of the Western Toad (*Bufo boreas*) in relation to ambient illumination. *Copeia.* 1984: 283-290.

Hairston, N.G. 1949. The local distribution and ecology of Plethodontid salamanders of the Southern Appalachians. *Ecol. Monogr.* 19: 47-73.

Heatwole, H. 1960. Burrowing ability and behavioral responses to desiccation of the salamander *Plethodon cinereus*. *Ecology.* 41: 661-668.

- Heatwole, H. 1962. Environmental factors influencing local distribution and activity of the salamander *Plethodon cinereus*. *Ecology*. 43: 460-472.
- Heatwole, H., Torres, F., Blasini de Austin, S., and Heatwole, A. 1969. Studies on anuran water balance-dynamics of evaporative water loss by the coqui, *Eleutherodactylus portoricensis*. *Comp. Biochem. Physiol.* 28: 24-269.
- Heatwole, H., and Newby, R.C. 1972. Interaction of internal rhythm and loss of body water in influencing activity levels of amphibians. *Herpetologica*. 28:156-162.
- Hendrickson, J. R. 1954. Ecology and systematics of salamanders of the genus *Batrachoseps*. *Univ. Calif. Publ. Zool.* 54: 1-46.
- Hillman, S.S. 1980. Physiological correlates of differential dehydration tolerance in anuran amphibians. *Copeia*. 1980:125-129.
- Hillman, S.S. 1987. Dehydrational effects on cardiovascular and metabolic capacity in two amphibians. *Physiol. Zool.* 60:608-613.
- Hillman, S.S., Zygmunt, A., and Baustian, M. 1987. Transcapillary fluid forces during dehydration in two amphibians. *Physiol. Zool.* 60: 339-345.
- Ingram, W.M. and Raney, E.C. 1943. Additional studies on the movement of tagged bullfrogs, *Rana catesbeiana* Shaw. *Am. Midland Nat.* 29: 239-241.
- Kirk, R.L., and Hogben, L. 1946. Studies on temperature regulation II. Amphibia and Reptiles. *J. Exp. Biol.* 22: 213-220.

- Lillywhite, H.B. 1970. Behavioral temperature regulation in the bullfrog *Rana catesbeiana*. *Copeia*. 1970: 158-168.
- Lillywhite H.B. 1971. Temperature selection by the bullfrog, *Rana catesbeiana*. *Comp. Biochem. Physiol.* 40A: 213-227.
- Lillywhite, H.B., Licht, P., and Chelgren, P. 1973. The role of behavioral thermoregulation in the growth energetics of the toad, *Anaxyrus boreas*. *Ecology* 54: 375-383.
- Main, A.R., and Bentley, P.J. 1964. Water relations of Australian burrowing frogs and tree frogs. *Ecology*. 45: 379-382.
- Malvin, G. M., Wood, S.C., and Riedel, C. 1989. Behavioral hypothermia in dehydrated toads. *FASEB Journal* 3: A234.
- McClanahan Jr, L., and Baldwin, R. 1968. Rate of water uptake through the integument of the desert toad *Anaxyrus punctatus*. *Comp. Biochem. Physiol.* 28: 381-389.
- McNab, B.K. 1963. Bioenergetics and the determination of home range size. *Am. Nat.* 97: 133-139.
- McNab, B.K. 1963. A model of the energy budget of a wild mouse. *Ecology*. 44:521-532.
- Moore, F.R., and Gatten Jr, R.E. 1989. Locomotor performance of hydrated, dehydrated, and osmotically stressed anuran amphibians. *Herpetologica*. 45:101-110.

- Mullally, D.P. 1952. Habits and minimum temperatures of the toad *Anaxyrus boreas halophilus*. *Copeia*. 1952: 274-276.
- Nussbaum, R.A., Brodie, E.D. Jr., and Storm, R.M. 1983. Amphibians and reptiles of the Pacific Northwest. Univ. Idaho Press, Moscow, Idaho.
- O'Connor, M.P., and Tracy, C.R. 1992. Thermoregulation by Juvenile Toads of *Bufo woodhousei* in the Field and in the Laboratory. *Copeia*. 1992: 865-876.
- Pough, H., Taigen, T.L., Stewart, M.M., and Brussard, P.F. 1983. Behavioral modification of evaporative water loss of a Puerto Rican frog. *Ecology*. 64: 244-252.
- Ray, C. 1958. Vital limits and rates of desiccation in salamanders. *Ecology*. 39: 75-83.
- Raney, E.C. 1940. Summer movements of the bullfrog, *Rana catesbeiana* Shaw, as determined by the jaw-tag method. *Am. Midland Nat.* 23: 733-745.
- Rey, P. 1937. Recherches experimentales sur l'economie de l'eau chez les Batraciens. Paris, Dissertation.
- Rogowitz, G.L., Cortes-Rivera, M., and Nieves-Puigdoller, K. 1999. Water loss, cutaneous resistance and effects of dehydration on locomotion of *Eleutherodactylus* frogs. *J. Comp. Physiol.* 169: 179-186.
- Ruibal, R. 1962. The adaptive value of bladder water in the toad, *Anaxyrus cognatus*. *Physio. Zool.* 35: 218-223.

- Ruibal, R., and Hillman, S.S. 1981. Cocoon structure and function in the burrowing hylid frog *Pterohyla fodiens*. *J. Herp.* 15: 403-408.
- Ruthven, A.G., and Gaige, H.T. 1915. The breeding habits of *Prostherapis subpunctatus*. *Cope. Occ. Pap. Mus. Zool. Univ. Michigan.* 10: 1-7.
- Schloss, J. P., Sparkman, A. M., Hamann, C.P., Schloss, M.J., Hamann, D., and Tracy, C.R. 2007. Vasotocin reverses water flow across amphibian integument. Unpublished Manuscript.
- Schmid, W.D. 1965. Some aspects of the water economies in nine species of amphibians. *Ecology.* 46: 261-269.
- Schmidt-Nielson, K. 1969. The neglected interface: The biology of water as a liquid-gas system. *Q. Rev. Biophys.* 2: 283-304.
- Shoemaker, V.H. 1975. Adaptations to aridity in amphibians and reptiles. Pages 143-154 in F.J. VERNBERG, ed. *Physiological adaptation to the environment.* University of New Mexico Press, Albuquerque.
- Shoemaker, V.H., and Nagy, K.A. 1977. Osmoregulation in amphibians and reptiles. *Ann. Rev. Physiol.* 39: 449-471.
- Stille, W.T. 1952. The nocturnal amphibian fauna of the Southern Lake Michigan beach. *Ecology.* 33: 149-162.
- Stille, W.T. 1958. The water absorption response of an anuran. *Copeia.* (No.3) 217-218.

Storer, T.I. 1925. A synopsis of the amphibia of California. Univ. Calif. Publ. Zool. 27; 1-342.

Thorson, T., and Svihla, A. 1943. Correlation of the habitats of amphibians with their ability to survive the loss of body water. *Ecology*. 24: 374-381.

Thorson, T.B. 1955. The relationship of water economy to terrestriality in amphibians. *Ecology*. 36: 100-116.

Tracy, C.R. 1976. A model of the dynamic exchanges of water and energy between a terrestrial amphibian and its environment. *Ecol. Monogr.* 46: 293-326.

Tracy, C.R., Christian, K.A., O'Connor M.P., and Tracy, C.R. 1993. Behavioral thermoregulation by *Anaxyrus americanus*: The importance of the hydric environment. *Herpetologica*. 49: 375-382.

Tracy, C.R., Reynolds, S. J., McArthur, L., Tracy, C.R., and Christian, K.A. 2007. Ecology and aestivation in a cocoon-forming frog, *Cyclorana australis* (Hylidae). *Copeia*. 2007: 901-912.

Tracy, C.R., Christian, K.A., Betts, G., and Tracy, C.R. 2008. Body temperature and resistance to evaporative water loss in tropical Australian frogs. *Comp. Biochem. Physiol.* 150: 102-108.

Tracy, C.R., Christian, K.A., and Tracy, C.R. 2010. Not just small, wet, and cold: Effects of body size and skin resistance on thermoregulation and arboreality of frogs. *Ecology*. 91: 1477-1484.

- Valdivieso, D., and Tamsitt, J.R. 1974. Thermal relations of the neotropical frog *Hyla labialis* (Anura: Hylidae). *Life. Sci. Occ. Pap. Royal Ontario Mus.* 26: 1-10.
- Walvoord, M. E. 2003. Cricket frogs maintain body hydration and temperature near levels allowing maximum jump performance. *Physiol. Biochem. Zool.* 76: 825-835.
- Warburg, M.R. 1965. Studies on the water economies of some Australian frogs. *Aus. J. Zool.* 13: 317-330.
- Willis, Y.L., Moyle, D.L., and Baskett, T.S. 1956. Emergence, breeding, hibernation, movements and transformation of the bullfrog *Rana catesbeiana*, in Missouri. *Copeia.* 1956; 30-41.
- Withers, P.C., Hillman, S.S., and Drewes, R.C. 1982. Water loss and nitrogen excretion in sharp-nosed reed frogs (*Hyperolius nasutus*-Anura, *Hyperolidae*). *J. Exp. Bio.* 97:335-343.
- Wright, A.H., and Wright, A.A. 1949. Handbook of frogs and toads. *Comstock Publishing Co., Ithaca: xii +640pp.*
- Wygoda, M.L. 1984. Low cutaneous evaporative water loss in arboreal frogs. *Physio. Zool.* 57: 329-337.

APPENDIX A. Results from the individual dehydration experiment for *Anaxyrus boreas*, *Lithobates catesbeiana*, and *Pseudacris sierra*. The table for *A. boreas* includes results for percent activity because activity was shown to significantly affect dehydration rate.

Table 1. *Lithobates catesbeiana*

<b>Frog ID</b>	<b>Standard Mass (g)</b>	<b>Time Lapse (min)</b>	<b>Stop Mass (g)</b>	<b>Dehydration Rate (g/hr)</b>
A1.A	38.5	543	31.1	0.82
A1.B	40	548	29	1.2
A1.C	35.5	539	28.7	0.75
A2.A	46.3	539	38.2	0.89
A2.B	47.1	536	38.8	0.93
A2.C	44.3	536	35.9	0.94
A3.A	45.3	513	34.2	1.3
A3.B	31.9	513	24.6	0.85
A3.C	42.5	508	35.4	0.84
A4.B	42.1	502	34.2	0.94
A4.C	31	502	24.8	0.73
A5.A	32.1	523	23.6	0.97

<b>Frog ID</b>	<b>Standard Mass (g)</b>	<b>Time Lapse (min)</b>	<b>Stop Mass (g)</b>	<b>Dehydration Rate (g/hr)</b>
A5.B	49.7	523	39.5	1.16
A5.C	40.6	487	31.7	1.1

Table 2. *Anaxyrus boreas*

<b>Toad ID</b>	<b>Standard Mass (g)</b>	<b>Time Lapse (min)</b>	<b>Stop Mass (g)</b>	<b>Dehydration Rate (g/hr)</b>	<b>Percent Activity</b>
T1	59.3	540	47.4	1.32	30.9
T2	51	540	42.7	0.93	42.7
T4	86.2	540	73.4	1.42	23.2
T5	75.2	540	63.2	1.33	24.7
T6	80.4	540	70.1	1.14	17.9
T7	86.3	540	79.8	0.72	0.7
T9	72.9	505	62.5	1.23	54.9
T10	79.8	540	68	1.31	31.1
T11	53.1	505	41.9	1.33	28.6
T12	67	498	59.5	0.91	13.9
T14	60	498	51.9	0.98	10.1
T15	69.7	490	63	0.83	9.3
T16	66.4	490	55.9	1.29	33.1
T17	84.3	545	70.9	1.48	37.4

<b>Toad ID</b>	<b>Standard Mass (g)</b>	<b>Time Lapse (min)</b>	<b>Stop Mass (g)</b>	<b>Dehydration Rate (g/hr)</b>	<b>Percent Activity</b>
T18	103	534	87.5	1.74	32.9
T19	121.4	534	104	1.96	39.8

Table 3. *Pseudacris sierra*

<b>Frog ID</b>	<b>Standard Mass (g)</b>	<b>Time Lapse (min)</b>	<b>Stop Mass (g)</b>	<b>Dehydration Rate (g/hr)</b>
F1	4.97	240	3.86	0.28
F2	4.29	240	3.27	0.25
F3	5.11	240	4.2	0.23
F4	7.63	240	6.09	0.38
F5	4.32	240	3.42	0.22
F6	5.01	240	4.21	0.2