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# The thermal ecology of the Red-spotted toad, Bufo punctatus, across life history

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# THE THERMAL ECOLOGY OF THE
# RED-SPOTTED TOAD,
# *BUFO PUNCTATUS,*
# ACROSS LIFE
# HISTORY

by

Candice Marie Rausch

Bachelor of Science
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A thesis submitted in partial fulfillment
of the requirements for the

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## ABSTRACT

### The Thermal Ecology of the Red-spotted Toad, *Bufo punctatus,* Across Life History

by

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Compared with other terrestrial vertebrates, amphibians are generally less tolerant of thermal extremes. The Mojave Desert has ambient temperatures outside the proposed thermal tolerance zone of its most abundant amphibian, the Red-spotted toad *(Bufo (Anaxyrus) punctatus).* Few data have been presented regarding the thermal ecology of these animals, including their thermal histories and proposed strategies to avoid temperature extremes. Previous studies suggest *B. punctatus* avoids extreme thermal exposure and adult toads experience body temperature ($T_b$) below a proposed critical thermal maximum ($CT_{max}$) of 35° C, and can maintain $T_b$ at 25° C during the winter. Further, previous studies indicate a $CT_{max}$ for tadpoles of 33° C. A reassessment of the thermal ecology of Red-spotted toads was warranted based on personal observations reported in this thesis.

I recorded environmental and/or $T_b$ of Red-spotted toads across their entire life history: eggs, tadpoles, juveniles and adults. In the field, eggs and tadpoles may encounter water temperatures as high as ~40° C. Juveniles can experience $T_b$ as high as 42.2° C. Adult toads experience $T_b$ as high as 39.1° C. These observations were combined with experimental determination of $CT_{max}$across life history statges: the $CT_{max}$ of tadpoles was variable, but decreased as development progressed; adult toads exhibit a $CT_{max}$ as high as 45.2 ± 1.0° C. I documented thermal preferences ($T_p$) of tadpoles and adults. In contrast to other studies of the effect of ontogeny on preferred temperature, $T_p$ of Red-spotted toads remains fairly constant across development. I documented developmental time from egg deposition to metamorphosis at 18 days in the summer and 47 days in the spring – both periods are less than the previously described 60-day developmental period. Finally, I present preliminary data on apparent basking-like behavior of late stage tadpoles in the field.

Despite seemingly high and presumably stressful environmental temperatures, Red-spotted toads do not generally live on the verge of thermal tolerance. However, later stage tadpoles may encounter potentially lethal temperatures. The collected data document the thermal ecology of Red-spotted toads and contradict previously assumed limits that may not have been experimentally-derived. These data may allow informed decision making in land use and conservation efforts by better defining an important ecological parameter in a species that may be subjected to increased pressures by human activity and climatic change.

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# ACKNOWLEDGEMENTS

This thesis would not be complete without ample gratitude to my advisor, Frank van Breukelen for his guidance, assistance, materials and lab space. A special thank you is extended to the rest of my committee: Dr. Allen Gibbs, Dr. Stanley Hillyard, Dr. Stephen Roberts, and Dr. Peter Starkweather. Additional appreciation is warranted to Dr. Hillyard for use of instrumentation, guidance in the available literature, and undying enthusiasm for desert anurans. Dr. Gibbs, I thank for constructive advice and suggestions. I extend great gratitude to Dr. Roberts and Dr. Starkweather for their tutelage and expertise in the fields of physiology and ecology respectively. I would also like to thank Dr. Starkweather for introducing me to ephemeral pools and the amazing animals that call them home. Thank you to Peipei Pan and Vanja Velickovska for friendship and assistance in the lab. Thank you to Jeff Goldstein for guidance, literature suggestions and friendship. I am grateful to Ilonka Zlatar for her assistance at "the rock" and bringing light and fun into research. Thank you to the many people who joined me in the field and, on occasion, assisted in "frog squeezing".

I would also like to thank two of the best friends the world has to offer, Dr. Megan Litster and Jessica Farrell, for always believing in me and encouraging me to be a better scientist and educator.

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Chris & Katie Happ, thank you for your love and eager ears. Cole and Kallie, thank you for making me smile and feel so loved. And of course, my very best friend, companion, and love of my life, Mitchell Brunscheen, thank you for love that knows no limits, precious time and priceless support.

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# CHAPTER 1

# INTRODUCTION

Despite potentially detrimental extremes in temperature and water availability, North American deserts have been home to various anuran species. Over time, this diversity has been stunted by vast population extinctions and marked declines among individual populations (Bradford et al., 2005). It has been hypothesised that these extinctions have resulted from climatic change and more direct human activity, including regional water diversion and local non-indigenous species introduction (Bradford, 2002; Hayes and Jennings, 1986). As anurans tend to be heavily impacted by environmental temperature, it would be helpful to establish if they are living close to their physiological limits or if they are well within their zone of thermal tolerance.

## 1.1 Background

There is little known of the thermal life history of *Bufo (Anaxyrus) punctatus (*the Red-spotted toad), the most abundant Mojave Desert anuran (Bradford et al., 2005). Personal observations of this species suggest that assumptions of its thermal ecology were in need of reevaluation. The Mojave Desert often experiences air temperatures ($T_{air}$) below freezing in winter and in excess of 45° C in summer (see Figure 1.1). Such seasonal ambient temperature ($T_a$) fluctuations may have significant impacts on the body

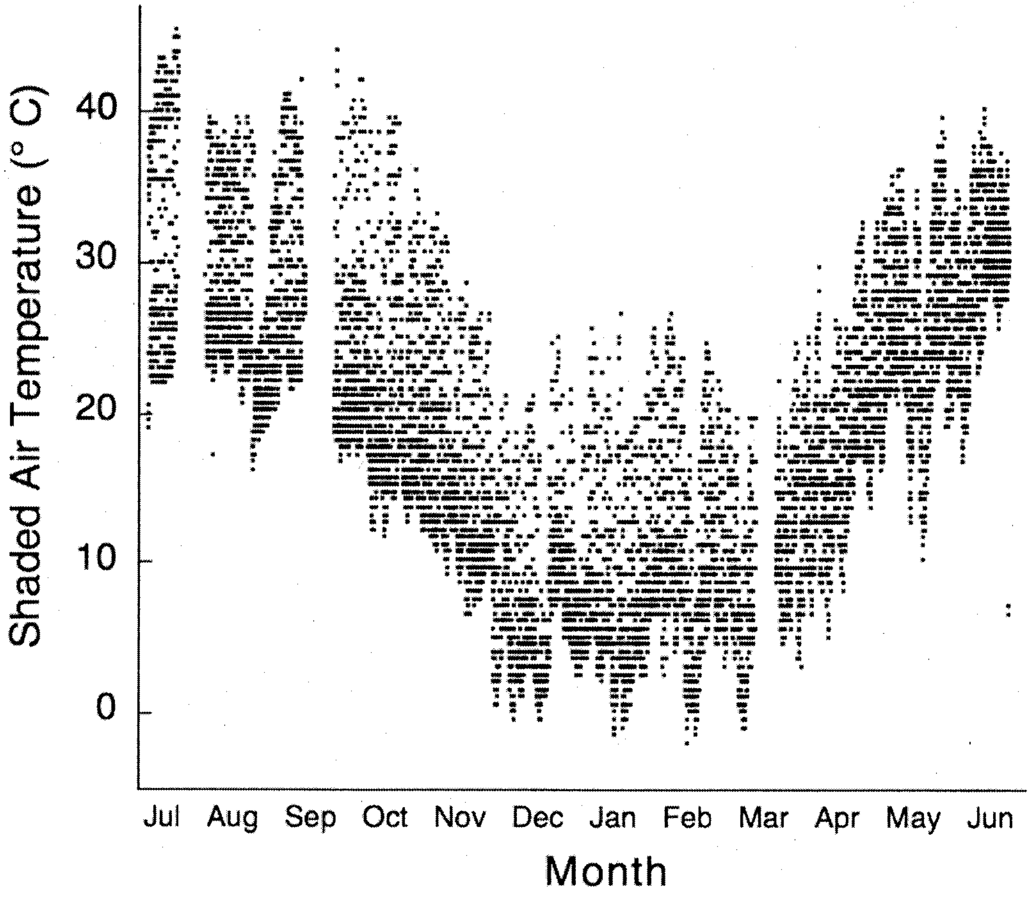

Figure 1.1 Air temperature in Brownstone Basin

Temperature recorded with a small data-logger in a sheltered sandstone crevice where toads have been observed. This location is shaded from solar radiation for the entire year and gives an approximation of air temperature in the study area. Individual points represent temperature (° C) measured hourly over the course of one year.

temperatures ($T_b$) and physiology of ectothermic organisms (Cloudsley-Thompson, 2001). It has been widely assumed that desert anurans do not experience high body temperatures, as most amphibians behaviorally will select thermal microhabitats to regulate body temperature within reasonably narrow limits (Lillywhite, 1970; Seebacher and Alford, 2002). Zweifel (1968) estimated a range of tolerance for developing *B. punctatus* embryos of 16° C – 33° C. However, water temperatures in some local breeding pools frequented by *B. punctatus* exceeds 35° C almost daily in late summer. No temperature data previously have been measured for juvenile Red-spotted toads. McClanahan et al. (1994) propose a critical thermal maximum ($CT_{max}$) of 35° C for adult *B. punctatus* and anecdotally describe an individual Red-spotted toad that experienced a consistent body temperature ($T_b$) of 25° C during the winter months. Moore and Moore (1980) presented $T_b$ data of *B. punctatus* adults in the field. While their observations were limited to only 2-3 summer days, they showed a maximum experienced temperature of 37° C and indicate that the previously proposed $CT_{max}$ of 35° C may be too low.

## 1.2 *Bufo punctatus* – Life History

The Red-spotted toad (*Bufo (Anaxyrus) punctatus*) can be found in much of North America's desert regions, as is illustrated by Figure 1.2 (Korky, 1999; Bradford et al., 2005). *B. punctatus* appears to prefer habitats near springs, persistant and ephemeral pools, and rocky crevices. Ranging in elevation from near sea level to 2,000 m (Stebbins, 1985), *B. punctatus* appears to be more tolerant of habitat change than any other anuran species of the Mojave, having varied little from its historical distribution (Bradford et al., 2005).

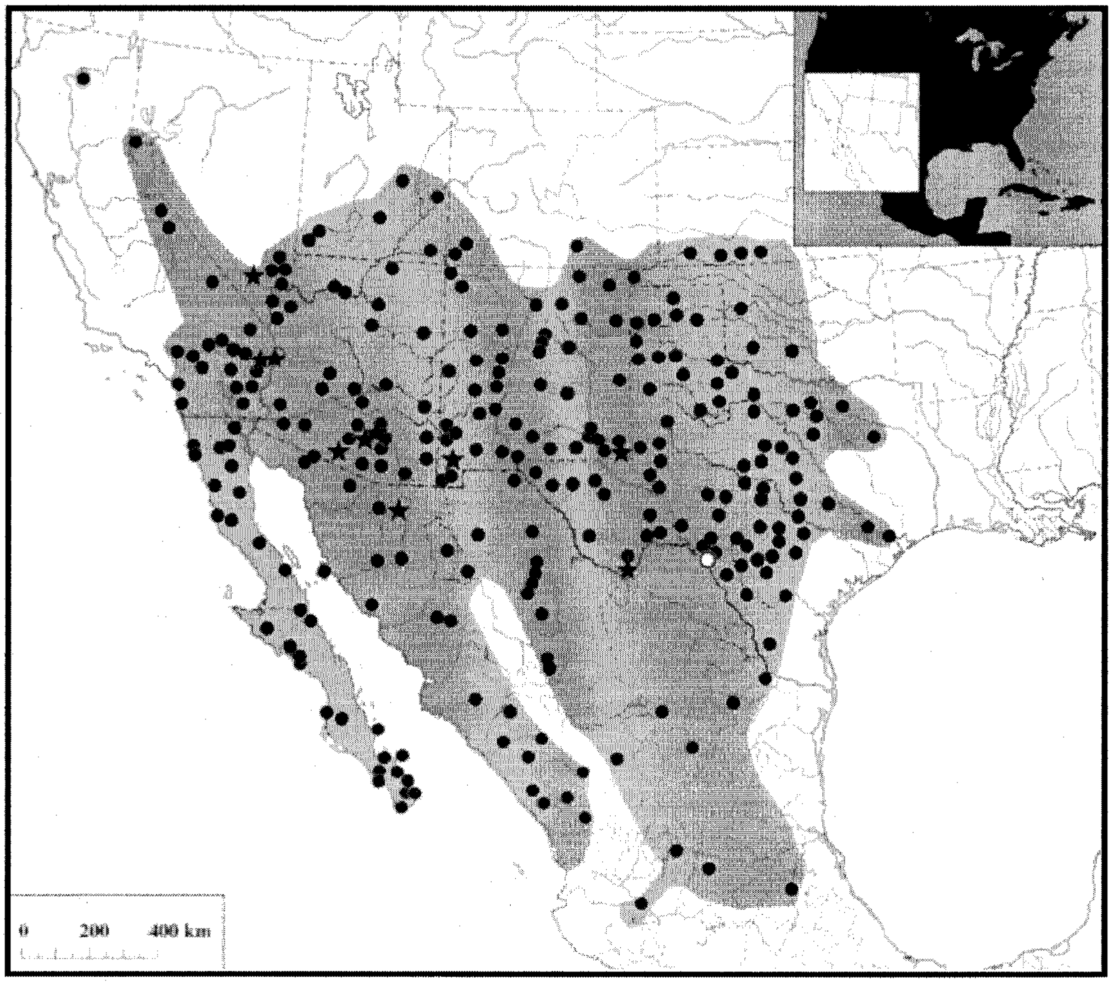

Figure 1.2 Geographic distribution of the Red-spotted toad, *Bufo punctatus*

*(Figure from Korky, 1999)*

*Bufo punctatus* is the most common toad in the Mojave Desert, and its distribution extends into much of the North American deserts/Southwest. The closed circles demonstrate various collection sites, the open circle represents the type locality. Stars indicate locations of known fossil records.

Red-spotted toads typically breed between April and June, although breeding has been noted as late as August and even September (Tevis, 1966; J. Jaeger, personal communication; personal observations). While some data are available for adults in these brief active periods (Moore and Moore, 1980), little is known of the temperatures experienced in other developmental stages and seasons.

## 1.3 Thermal Experience of Anurans in Ephemeral Ponds

Temperature experienced over an anuran's lifetime can be highly variable and life stage dependent. The general thermal intolerance of amphibians is likely a factor of their unique relationship of individual species with multiple distinct ecotypes throughout life history and their ability to behaviourally thermoregulate (Bentley, 1966; reviewed in Alford & Richards, 1999). Mojave Desert anurans start life confined to aquatic environments. Behavioral thermal regulation is limited, as escape from the aquatic environment can only occur after metamorphosis, suggesting increased thermotolerance in larval stages. It has been emphasized that as a result of their elevated evaporation rates and ability to behaviorally thermoregulate adult desert anurans are perhaps buffered from the possibility of experiencing extremely elevated $T_b$ (Tracy, 1976; McClanahan et al., 1994).

It has been widely assumed that egg and larval stages, for the most part, are protected from high temperature. The high specific heat capacity of water allows for thermal buffering, especially in larger ponds. Although thermoregulation is limited, tadpoles may select microclimates provided by vegetation, rocks, and variable water depth (Ultsch et al., 1999). Eggs are unable to behaviorally thermoregulate and are dependent on parental site choice of deposition.

Embryonic thermal tolerance is often greater than larval thermal tolerance (Zweifel, 1968; Ultsch et al., 1999). What happens to thermal tolerance after hatch is still quite debatable. In some species, data indicate larval thermal tolerance increases and becomes more restricted as tadpoles approach metamorphosis (Dupré and Petranka, 1985). However, others present data for increased thermal tolerance as early stage tadpoles, followed by a reduction in thermal tolerance in the later stages of metamorphosis (e.g., Cupp, 1980; Noland and Ultsch, 1981; Sherman, 1980; reviewed in Ultsch et al., 1999).

Mojave ephemeral pond systems subject young *B. punctatus* to rapidly evaporating habitats with little vegetation, typically shallow water depths, and thus, likely extreme thermal exposure. Currently, *B. punctatus* is believed to require 60 days to metamorphose after a 72 h embryonic period; evaporating pools in the Mojave seldom provide 63 days of water (Wright and Wright, 1949; Tevis, 1966). Many tadpoles, particularly those indigenous to temporary pools, exhibit phenotypic plasticity in developmental rate as pools experience rapid evaporation (Newman, 1989; Denver, 1997). There is evidence that physiological stress and other correlates of rapidly developing pools can result in a premature activation of the endocrine pathways regulating metamorphosis (Denver, 1997). Additionally, as ponds evaporate, decreases in water volume result in less thermal inertia and wider temperature fluctuations. As temperatures rise and water levels drop, tadpoles develop more quickly and may manage to leave the pond before all the water evaporates. However, if temperatures rise above tadpole thermal tolerance, tadpoles can experience respiratory distress, resulting in a dramatic decrease in survivorship (Ultsch et al., 1999).

## 1.4 Thermal Experience of Juveniles & Adults

Upon completion of metamorphosis, tadpoles emerge from the water as newly metamorphosed juveniles, or toadlets (˜0.2 – 0.5 g). I have witnessed juveniles traversing extremely hot substrates with surface temperatures in excess of 60° C around the ephemeral pond network in Brownstone Basin, NV. The complete thermal experience of juvenile desert anurans has not been well defined. It is known that younger adults are often more heat tolerant than older adults (Mayhew, 1968). In addition, basking behavior has been documented in juvenile *Bufo debilis* and *Bufo spinulosus*, and has been suggested as a means to speed growth by elevating body temperature above ambient temperatures (Seymour, 1972; Lambrinos and Kleier, 2002, respectively).

Due to methodological limitations, most field studies on $T_b$ of free-ranging individuals (using radio-telemetry or thermocouples), while valuable, have been restricted to a few days or include limited numbers of measurements (e.g., Lillywhite, 1970, Seymour, 1972; Moore and Moore, 1980; Carey, 1978; van Gelder et al., 1986; Seebacher and Alford, 2002; Lambrinos and Kleier, 2002). Longer duration studies have been conducted using toads in enclosed constructed habitats in temperate regions (Smits, 1984), but do not address differences among various developmental stages.

As thermal tolerance range is known to be relative to a given animal's $T_a$ range (Snyder and Weathers, 1975), I hypothesize that low water availability and rapidly evaporating ponds may sometimes facilitate more severe thermal exposure of desert anurans than previously assumed, resulting in higher overall thermal tolerance and developmental rate.

## 1.5 Thesis Overview

I define the thermal experience and discuss the thermal ecology of *B. punctatus* across season, as a function of developmental stage (eggs, tadpoles, juveniles and adults), and determine at what life stages Red-spotted toads are likely naturally to experience thermal stress. I document the developmental rate and temperatures experienced by spring and summer clutch tadpoles and evaluate the behavioral response across life history to ecologically relevant thermal extremes. I also address the thermal experience of adults in the winter months in the greatest detail to date using implanted data loggers. The immediate knowledge gained in this work will be critical to our understanding of desert anurans and other desert animals.

## 1.6 Thesis Objectives

### 1.6.1 Objective 1. Thermal experience across development

In order to understand the thermal biology of any organism, we must first have a solid understanding of the thermal experience of the organism in its natural environment. Before this study, few data existed for free-ranging desert anuran body temperature. I present $T_b$ across season and development from eggs to adults.

### 1.6.2 Objective 2. Behavioral response to ecologically relevant thermal extremes

Data on thermal experience are nearly useless without some understanding of their physiological impact. I established $T_p$ and $CT_{max}$ for various stages from tadpole to adult.

### 1.6.3 Objective 3. Thermal biology across developmental stages

The temperatures experienced and thermoregulatory techniques of anurans vary throughout development. This study compares the natural thermal experience and response to thermal extremes of various distinct life stages of the Red-spotted toad.

## 1.7 Achievements

I characterized the thermal history of the Red-spotted toad throughout development (eggs, three tadpole stages, juveniles, and adults) and determined critical temperatures ($CT_{max}$ and $T_p$) throughout development.

I present chemical and thermal profiles for 24-hour periods during tadpole development. I present the thermal experience of eggs and tadpoles as a function of water temperature ($T_{water}$) throughout development, as a function of early or late season breeding, from egg deposition to metamorphosis. I present mid-day summer body temperatures of juvenile Red-spotted toads. This study documents body temperature experienced by four free-ranging adult Red-spotted toads over the course of four seasons of a year. To the best of my knowledge, this study exhibits the most complete record of free-ranging body temperature of any desert anuran.

I document that *B. punctatus* likely is not at the limit of thermal tolerance in terrestrial stages, but rather is most in danger of thermal detriment in late aquatic stages. This study fills in many important voids in the description of the thermal experience of the Red-spotted toad and presents methods that may be appropriate to further investigate the thermal ecology of other animals. Particularly those potentially at risk of population reduction or extinction.

# CHAPTER 2

# THERMAL ENVIRONMENT AND TOLERANCE OF EGGS AND TADPOLES TO ECOLOGICALLY RELEVANT THERMAL EXTREMES

Due to their inability to leave water before metamorphosis, aquatic eggs and tadpoles are less capable of escaping ambient thermal extremes than are terrestrial juveniles and adults. However, their aquatic environments typically do not experience the thermal extremes observed on dry land. Water exhibits increased thermal stability because of its relatively high specific heat and thermal inertia. As ephemeral pools evaporate, decreased volume results in the potential for greater temperature fluctuations.

These ephemeral ponds can experience temperatures frequently in excess of 35° C and periodically exceeding 40° C. I observed thermal environments and developmental rates of tadpoles in a spring pond and rapidly evaporating late summer pond, from time of egg deposition to metamorphosis. Tadpoles were capable of leaving the water and inhabiting land within 47 days in spring and 18 days of egg deposition in summer. To the best of my knowledge, this latter metamorphosis period is the most rapid metamorphosis ever documented for a *Bufo* species. Through the use of water temperature monitoring and laboratory experiments, I show that water temperature in desert pools can exceed the thermal tolerance of some late stage tadpoles. However, typical water temperatures rarely pose a hazard to eggs, embryos or early stage tadpoles.

A basking-like behavior was observed in late stage tadpoles. I document preliminary data on the occurrence of this behavior, which appears to slightly elevate body temperature ($T_b$) as a function of time. Surprisingly, this behavior is commonly exhibited in a life stage that demonstrates decreased thermal tolerance in the laboratory. Further research is necessary to assess possible benefits of this behavior.

## 2.1 Introduction – Eggs & Tadpoles

Eggs and tadpoles are generally subject to the range of habitat temperatures selected by their parents. Tadpoles will often select optimal conditions for development if choices are available (Freidenburg and Skelly, 2004). However, as eggs are immobile, there likely is strong selection for increased and broader thermal tolerance in the embryonic stages (Ultsch et al., 1999). Ephemeral pond environments, like those typically used by *Bufo punctatus,* may experience water temperatures as high as 39.6° C (personal observation). As a result of high temperature and low relative humidity, aquatic stages of *B. punctatus* can be exposed to a rapidly evaporating environment, and subsequent increased population density, decreased food availability, drastic changes in water chemistry, and thermal instability.

Larval development is heavily dependent on environmental temperature. When temperatures rise, developing ectotherms may experience accelerated growth and differentiation rates (Smith-Gill & Berven, 1979; Buchholz and Hayes, 2002). In the field, tadpoles will typically select warmer microhabitats corresponding to the optimal temperature of development (preferred temperature, $T_p$ or PBT; Huey and Stevenson, 1979; Freidenburg & Skelly, 2004). However, further elevation in temperature may expose animals to increased morbidity or mortality. Experimental determinations of preferred temperature and thermal tolerance can be used to better understand an organisms thermal environment. Thermal gradients and varying thermal compartments are used in the laboratory to estimate the preferred environmental temperatures of animals, by allowing individuals to move freely between temperatures and body temperature is monitored. An approximation of the high temperature that will limit an animal's ability to escape danger can be determined in the laboratory and is termed the critical thermal maximum ($CT_{max}$). $CT_{max}$ is typically ~10° C above $T_p$. $T_p$ of many temperate species tadpoles is between 28° C and 32° C (Ultsch et al., 1999). Warm acclimated animals often demonstrate increased thermal tolerance and preference (Brown, 1969; van der Have, 2002).

Concomitant with decreased water availability in an evaporating ephemeral pond, water temperatures ($T_{water}$) may become more variable, and extremes in $T_{water}$ more likely. Ephemeral desert pools, experiencing marked thermal extremes as compared with other aquatic habitats, can exhibit temperatures in excess of 35° C daily. Many bufonids experience a decrease in $CT_{max}$ just before metamorphosis (e.g., Cupp, 1980; Noland and Ultsch, 1981; Sherman, 1980; Ultsch et al., 1999). While rising temperature corresponds to an increase in metabolism and commensurate increase in the rate of metamorphosis, I hypothesize that *B. punctatus* tadpoles experience temperatures that may be lethal or physiologically damaging, particularly as pool volume, and presumably tadpole thermal tolerance, decreases near the end of metamorphosis.

In ephemeral pools, tadpoles that fail to develop before the pool evaporates die. In many bufonids including *B. punctatus*, metamorphosis has been reported to require ˜60 days (Wright and Wright, 1949; Tevis, 1966). It is known that Spadefoot toads, *Scaphiopus couchii,* typically leaves the water ˜60 days after hatch. However, in an evaporating pond, metamorphosis may occur only 8 days after egg deposition (Newman, 1989), and other spadefoot species can develop in about 14 days (Bragg, 1945; Klassen, 1998). Given the environmental conditions of some *B. punctatus* habitats, I hypothesize that metamorphosis may become similarly accelerated and occur more quickly than the previously described 60 days. Indeed, in the Brownstone Basin, near Las Vegas, NV, few periods occur when water is available for 60 days, and yet *B. punctatus* thrives in this area.

## 2.2 Materials & Methods – Eggs & Tadpoles

### 2.2.1 Field Environment of Eggs and Tadpoles

Field research was conducted in the ephemeral pond network of Brownstone Basin near Las Vegas, NV, USA (N 36°10'59.56 W 115°25'51.11, elev. 1487 m). The site is a fairly isolated mixed sandstone and limestone rainwater catchment network of ephemeral ponds, which may be inundated by rainfall and/or snow throughout the year, but which typically dry out and refill during the summer monsoon rainy season.

Thermal environment of eggs and tadpoles was monitored using small programmable I-Button data loggers (Dallas Semiconductor, model DS1922L) submerged in ponds where eggs and tadpoles were observed in Brownstone Basin. For a few representative days during each season, I recorded water chemistry, including pH, dissolved oxygen, conductivity, and temperature, using a YSI (model 556 MPS) water analyzer. To assess thermal variability within a single pond, data loggers were placed at 15 locations in a pond where eggs had been recently deposited. Temperature was also measured at the bottom of three ponds whenever water was present to get an idea of each pond's thermal capacity and thermal extremes.

### 2.2.2 Field Observations of Development in the Field

The rate of development was tracked in spring and summer, from the time of egg deposition until metamorphosis. During the summer, digital photographs were taken of tadpoles almost every day of development. All tadpoles were staged using Gosner's staging (Gosner, 1960). On two occasions, access to the field site was precluded due to flooding in the area. Water temperature was recorded from time of the first rain of the season to the time toadlets left the water.

Temperature data were analyzed for daily mean temperatures and daily range. Tadpoles were measured using ImageJ (www.NIH.gov) image analysis software. Total body length (TB, body including tail) and body length (BL, body excluding tail) were measured in accordance with descriptions of acceptable tadpole measurement (McDiarmid and Altig, 1999). An ANCOVA was used to assess change in developmental rate as compared across developmental stages, defined as pre-leg development (stages 1 – ˜30) and during leg development (stages ˜30 – 46). Further ANCOVAs were used to define the difference in growth rate between TB and BL, to assess changes in growth rate in early and late development.

### 2.2.3 Animal Collection and Care for Laboratory Experiments

Eggs and tadpoles from multiple clutches and ponds were collected in late summer. Eggs and tadpoles were returned to the lab and held in filtered pond water from the collection site (0.22 μm Millipore filters). Tadpoles were fed a suspension of *Spirulina* daily *ad libitum.*

### 2.2.4 Laboratory Determined Thermal Preference of Tadpoles

Tadpoles (n = 5 from each stage) of Gosner stages 23 and 39, were used in laboratory thermal tolerance and thermal preference experiments ($T_p$). To establish $T_p$, I used a Sable Systems, Inc. actively regulated thermal gradient. The gradient is comprised of a 2 cm thick aluminum surface bound by Plexiglas sides and an independently operated Peltier device on either side of a 6 cm x 66 cm long chamber. The Plexiglas walls are lined with 64 LED light gates, spaced approximately every 1 cm, to allow for observation of an animal's location with a computer operated location logging device (see Appendix 1). The gradient was established between ~10° C and ~37° C. Tadpoles were placed, individually, in the center of the gradient and freely allowed to select temperatures within the available range for 30 min. The gradient was base-lined prior to each tadpole trial. The water temperature at every gate was measured manually before and after each trial with a thermocouple. After several trials, the change in temperature was found to be negligible and was only measured at 8 cm increments monitor consistency between trials. The location of each tadpole was logged and integrated over time by LadScan software (Sable Systems, Inc.) and analyzed with water temperature at each location to calculate the average water temperature experienced by each tadpole (preferred body temperature, as defined by Reynolds and Casterlin, 1979). $T_p$ were averaged and SE was determined. Preliminary experiments demonstrated no effect of using either the average experienced temperature or the temperature most frequently experienced, as a result, average experienced temperature was used as an approximation of $T_p$. Linear thermal gradients will typically yield activity profiles demonstrating edge effects as animals will often move to the end of the gradient, turn around, and proceed back, increasing the time spent near the edges of a linear gradient. Although edge effects are often a concern in thermal gradients, they were not evident in any of the trials in the data set (as assessed by changing the gradient range), most likely because of the wide thermal range available and the generally low activity of *B. punctatus* tadpoles.

### 2.2.5 Laboratory Determined Thermal Tolerance of Tadpoles

Tadpoles (n = 5 of each) from Gosner stages 23 and 35, were exposed to temperatures ranging from 25° C to ˜42° C in a filtered water filled Petri dish. Temperature was increased at approximately 0.5° C/min. Animals were assessed for response to stimulus with soft forceps before every completed degree increment. Body temperature was approximated from water temperature measured with a thermocouple. When animals failed to respond by avoiding the stimulus, they were removed and allowed to recover in cool filtered pond water. All animals recovered in less than 22 minutes and no animals exhibited sustained changes in motility, activity or eating habits one day following the experiment.

### 2.2.6 Preliminary Analysis of Apparent Basking Behavior in Tadpoles in the Field

Tadpoles (typically > Gosner stage 39) had been observed to spend prolonged periods of relative inactivity positioned near the water surface (˜1 – 2 cm below the water surface) on partially submerged rocks in relatively deep murky pools. I asked if this behavior affected their thermal biology. Tadpoles were observed, and time was measured, from the time to first visit a shallow rock surface to the time they left. Tadpoles were caught using a small fish net and held near to the rock surface with the net held taut to avoid thermal effects of handling. I measured tadpole body temperatures by inserting a micro thermocouple into the dorsum. Tadpoles were removed from the pond to avoid resampling and replaced at the conclusion of the experiment.

## 2.3 Results – Eggs & Tadpoles

### 2.3.1 Field Observations

The widest ranges in pH were present in spring. Spring ponds periodically varied nearly 3 pH units on a diel cycle (Figure 2.1). Water temperature in a shallow spring pool (~10 cm in depth), with tadpoles present, exhibited a ~12° C range over the course of 24-hours. Temperature corrected dissolved oxygen demonstrated a similar pattern, as it decreased nearly 100% saturation at night. Temperature corrected conductivity, a proxy for salinity, did not vary appreciably in a 24 hr period. Conductivity increased when pools evaporated and decreased substantially with large volumes of precipitation (Figure 2.2).

The late summer storms typical of the Mojave Desert were often accompanied by massive floods that invoked topographic alteration, faunal displacement, and acute organism exposures to changes in temperature and water chemistry. I was witness to a hail storm in late July. One small pond experienced dramatic changes in water temperature and chemistry as it transitioned towards an icy slurry; temperature was

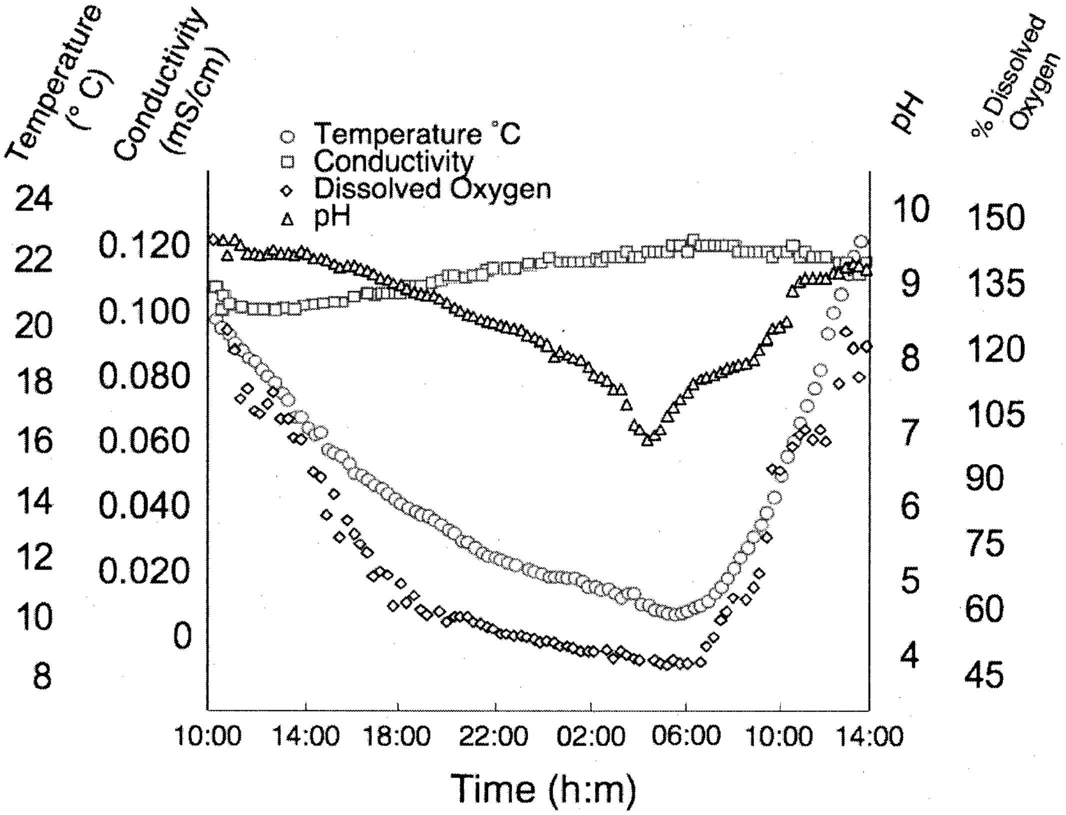

Figure 2.1 The ephemerality of an ephemeral pool in spring

I present water chemistry data, measured with a temperature correcting YSI water analyzer, for a tadpole inhabited pool in spring. Temperature (open circles), salinity or conductivity (open squares), dissolved oxygen (open diamonds) and pH (open triangles) are shown over a 28-hour period. pH can exhibit a nearly 3 pH unit shift in a 24-hour period, becoming lowest in the dark early morning hours. Temperature can change ~12° C. Dissolved oxygen, like pH and temperature, was lowest at night while conductivity changed very little.

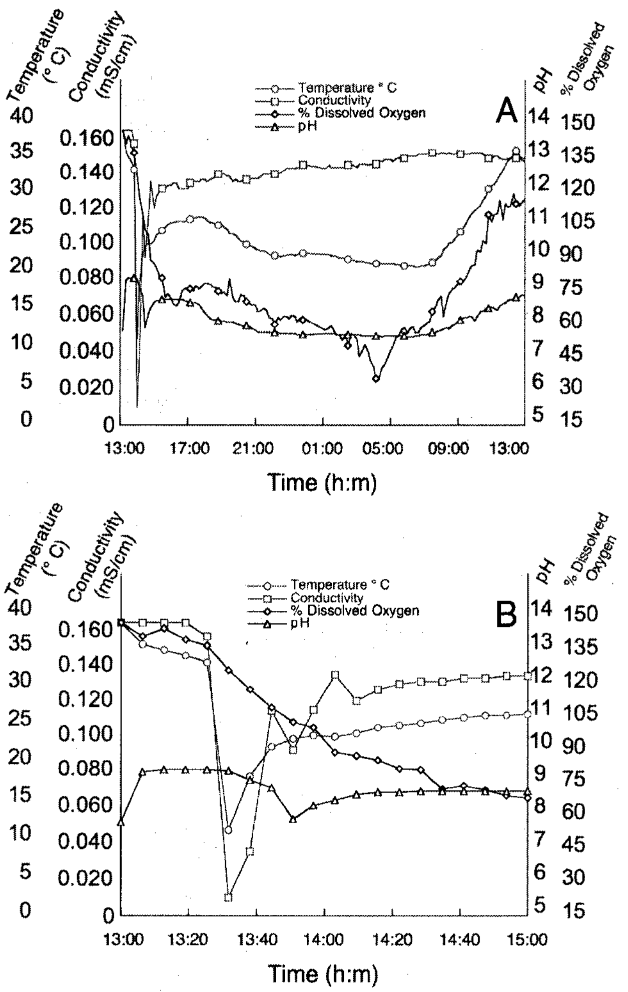

Figure 2.2 Water chemistry of an ephemeral pond during a summer storm

The water chemistry measured in late July during a heavy summer hail storm. (A) 24 – hour period before, during, and after a summer storm. There was a large reduction in temperature and conductivity as a result of the shallow (< 10 cm) pond filling with low ion hail. As a much larger adjacent pond began to overflow, water chemistry rapidly returned to typical summer levels. (B) The onset of the storm (gray box from A) has been expanded to reveal detail. Water temperature of the shallow pond decreased from ~37° C to ~10° C in a matter of minutes, and was restored to 25° C almost as quickly.

reduced ~27° C in a matter of a few minutes (Figure 2.2 B). The rapid influx of low ion content water decreased conductivity to the lowest salinity ever recorded in the pond network, 0.010 mS/cm. As the storm progressed, a much larger pond farther up the catchment network began to overflow, quickly re-establishing more seasonally typical water chemistry (Figure 2.2 B). Tadpoles that survived in the pool did not seem adversely affected by the rapid and violent change in their environment. Summer pool pH will commonly shift ~2 units in a 24 hour period, $T_{water}$ exhibits a ~5° C range in larger ponds and as much as an ~20° C range in small, rapidly evaporating ponds (Figure 2.2). Though changes in measured pH and DO are often an artifact of changing temperature, the YSI water analyzer used in this study corrected for temperature in all measurements.

In addition to temporal variation in the ephemeral pond environment, there is a great deal of spatial variability among ponds and within each pond. With the exception of displacement by the occasional flood, tadpoles and eggs are confined to the pools in which they were originally deposited. Each pool is of different depth and has variable inflow/outflow water relative to other portions of the network. Within a single pool there is variability in depth, vegetation, solute composition and concentration, and the index of solar radiation. To better characterize the thermal microhabitats available to tadpoles at any given time, I measured water temperature in one egg-inhabited pool, in 15 distinct locations (including surface temperature). At various depths and microhabitats within a pond, there is some refuge from thermal extremes. Over a 24-hour period (July 21 – 22, 2006), water temperature fluctuated between 17.61° C and 36.72° C (Figure 2.3). Water temperature varied by water depth and vegetation coverage, forming a gradient of between 0.13° C and 5.58° C, at any given time. The widest thermal gradients occurred

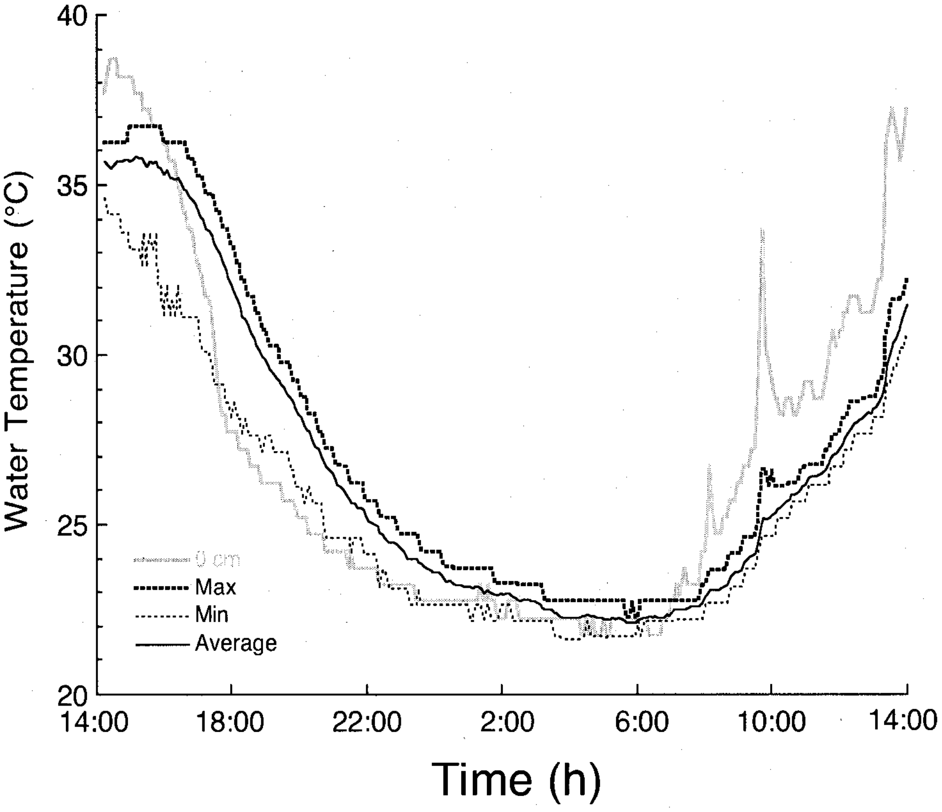

Figure 2.3 Thermal environment available in a single pool over a 24-hour period Maximum (heavy dotted black line), minimum (light dotted black line) and average temperature (light black line) recorded with data-loggers at 14 different locations within a single pond when eggs were present from July 21 – 22, 2006. Surface water temperature measured with a data-logger wet, but not submerged, in the pond (gray line). Maximum range for the 24-hour period was 15.11° C.

with the highest daily temperatures. The warmest times of day presented the widest thermal choices and water temperature reached a high of 36.72° C in some locations (Figure 2.3). In this same pool, there was a 30 minute period when water did not drop below 34° C at any available location (Figure 2.3). It is not abnormal for eggs and tadpoles to be exposed to water temperature of greater than 35° C for extended periods (Figure 2.3 and 2.5).

Spring eggs were deposited in late April and developed in 47 days to metamorphose in mid June 2006. Temperature recorded every 15 min of development is presented in Figure 2.4. Average daily water temperature for spring eggs was 19.63 ± 0.26° C, but daily mean temperature fluctuated across development between 16.21 ± 0.19° C and 22.55 ± 0.15° C. Eggs deposited in late July, 2007, metamorphosed from eggs to toadlets in 18 days (Figure 2.5). The range of daily mean water temperature was variable across development, range = 21.87° C to 27.93° C, but averaged 24.47 ± 0.35° C (mean ± SE). Growth rate was faster in earlier stages (< stage 30; regression analyses, $p < 0.05$). Rate during hind limb differentiation changed significantly: total body with tail (TB; slope = -0.15, $r^2 = 0.78$) and body length without tail (BL; slope = -0.02, $r^2 = 0.69$; $p < 0.05$). There was a significant difference between the slope of TB and BL prior to stage 30 ($p < 0.05$). However, there was no significant difference in growth rate from stage 30 to metamorphosis ($p > 0.05$). As a consequence of heavy storms there was a breach of a natural dam, decreasing the volume of the pond and allowing wider fluctuations in water temperature, as can be seen in Figure 2.5.

I compared early and late clutch developmental temperature and time to metamorphosis (Figure 2.6). When water temperature and time were integrated $(\int (\Sigma T_w /$

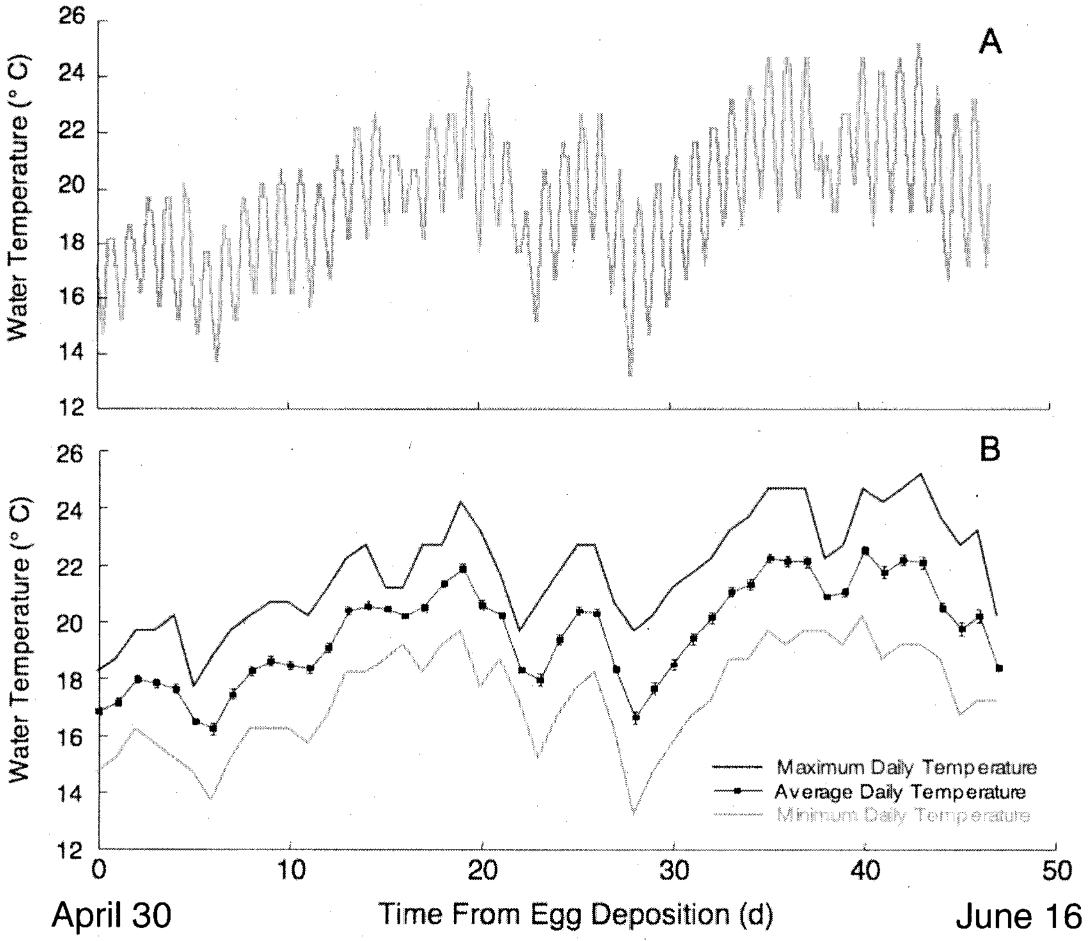

Figure 2.4 Water temperatures during the developmental period of Red-spotted toads in a pond in spring

Eggs were deposited on April 30th, the first toadlets left the water on June 16th of 2006. I present the (A) water temperature during this 47 - day period of development. (B) Maximum, average, and minimum, daily temperatures are displayed for clarity.

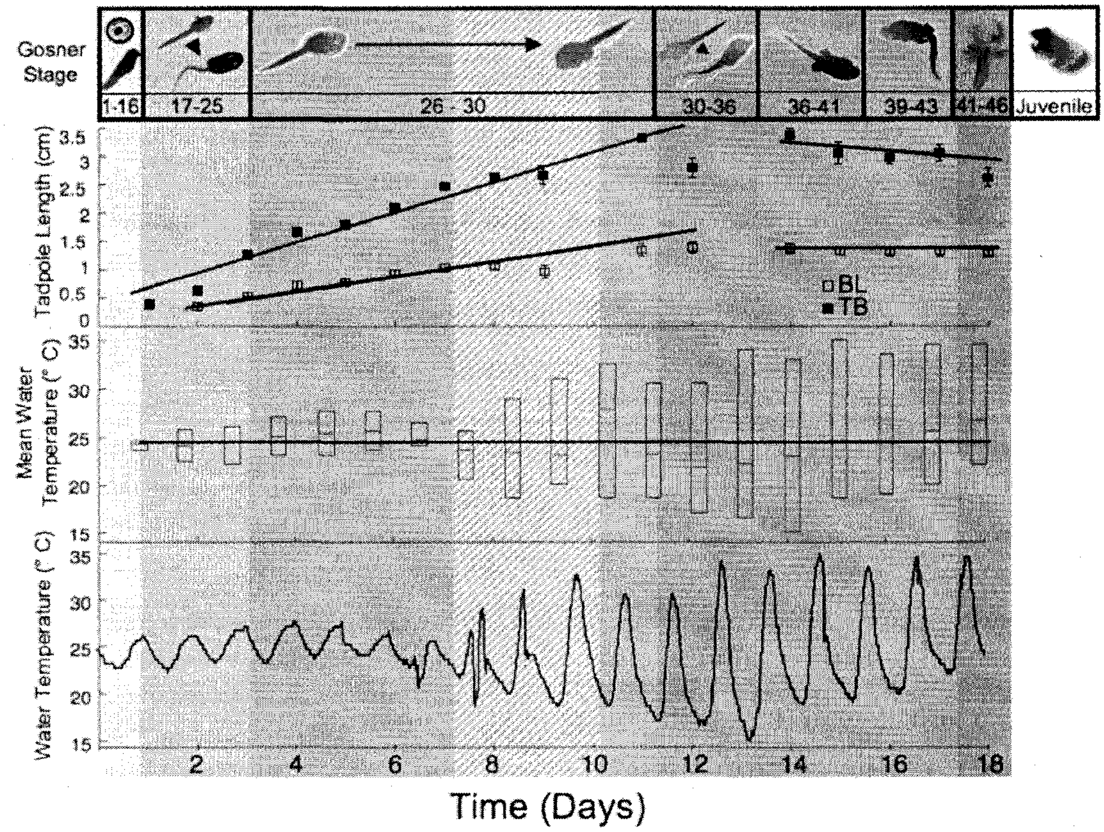

Figure 2.5 Development of *Bufo punctatus* tadpoles in an evaporating pond

I present late summer clutch development from egg deposition in late July to metamorphosis in early August. From bottom: $T_{water}$ (° C) across development; thermal variation in terms of daily mean and range (mean daily $T_{water}$ averaged ~25° C); total body length (TB, closed squares) and body length excluding tail (BL, open squares) are expressed in the top panel (mean ± SE). Solid gray bars correspond to major developmental phases (from left to right: developing embryo, hatchling, larva with leg buds, larva with developed hindlimbs, larva with developed forelimbs). Diagonal white stripes represent rainstorms. Developmental stage is represented in the top bar with corresponding Gosner stage.

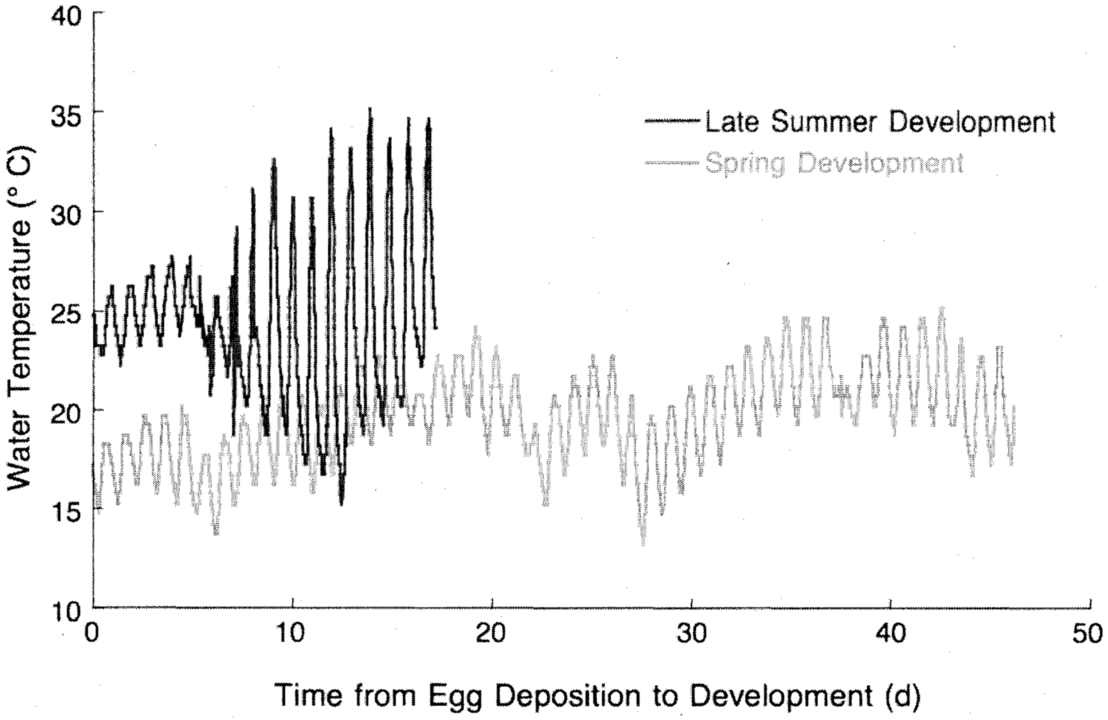

Figure 2.6 Summer and spring developmental temperatures and time to develop

On the same time and temperature scale, summer (black) and spring (gray) developmental temperatures are displayed. Temperature plots stop at the time of metamorphosis (summer = 18 days, spring = 47 days).

$\Sigma$time)) and spring and summer clutches were compared, I found a ratio between of summer to spring of 38.3%. When only time to develop was compared between the two seasons, there was a 33.5% decrease from spring to summer. The similarity of these ratios suggests the difference in developmental rate is primarily due to developmental temperature. However, when minimal temperature of development, 16° C, was accounted for (Brattstrom, 1966), spring tadpoles developed at 18.65% of the rate of summer tadpoles.

Late stage tadpoles (post stage 35) were observed to be resting on the shallow surfaces of partially submerged rocks in the ponds. $T_{water}$ was generally lower than the temperature of tadpoles when they first visited the rock surface. $T_{rock\ surface}$ was higher than any tadpole temperature measured. Tadpole $T_b$ increased with time spent on the rock surface (Figure 2.7; $n = 11$, $r^2 = 0.67$).

### 2.3.2 Laboratory Observations

Tadpoles do not appear to prefer different temperatures across metamorphosis (Figure 2.2). In the laboratory, tadpoles preferred similar temperatures across development, $T_p$ range = 24.02 ± 0.37° C (stage 26; mean ± SE) to 25.85 ± 0.39° C (stage 35), with a range of less than 2° C, there was no significant difference between stages ($p > 0.005$). These temperatures are comparable to the average daily temperatures of many late summer ponds. However, $CT_{max}$ exhibited marked variation across development. Thermal tolerance was not testable by behavioural assessments in eggs. However, late summer eggs were capable of surviving up to 40° C thermal shocks, but not 45° C shocks of up to 2 h (see Future Work and Figure 5.3.1). Hatchling tadpoles (stage 23) demonstrated tolerance to 41.6 ± 0.3° C (mean ± SE). Thermal tolerance was reduced in stage 35 tadpoles (early in hind limb development) to 36.6 ± 0.5° C. Stage 39 tadpoles (developed hind limbs) did not survive even one hour at 35° C in the laboratory.

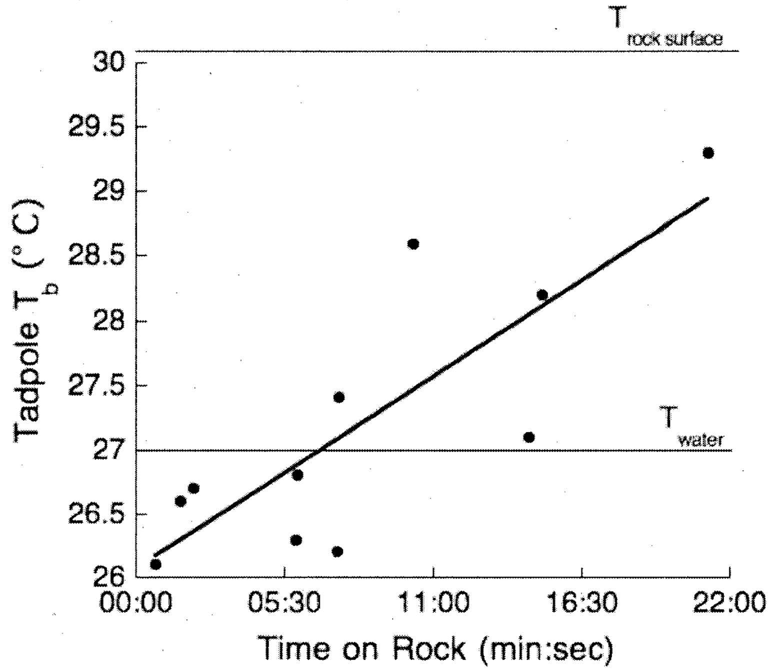

Figure 2.7 Tadpole $T_b$ while presenting basking-like behavior

I observed tadpoles approximately 1 – 2 cm below the surface of the water, on a partially submerged rock surface. Temperature of the submerged rock surface (30.1° C, $T_{\text{rock surface}}$) and the temperature of the water column at the depth of the tadpoles are shown (27.0° C, $T_{\text{water}}$). The time spent on the rock, by each tadpole, is shown graphed with $T_b$. As tadpoles left the rock, they were captured and held near the rock with a small net as their $T_b$ was measured with a micro thermocouple. Diagonal line represents a line of best fit to the data ($n = 11$, $r^2 = 0.67$).

## 2.4 Discussion – Tadpoles & Eggs

*B. punctatus* eggs are laid between late spring and late summer. In the face of environmental variability, *B. punctatus* young appear to 'bloom where they are planted.' Even with water temperatures and chemistry that exhibit marked variation between seasons and across the course of a single day, many *B. punctatus* manage to survive to metamorphosis. Wide fluctuations in pH and DO characteristic of ephemeral ponds may have stressful effects independent of temperature. DO during the day, often greater than 100%, may allow ephemeral tadpoles the advantage of higher body temperature without limitations of $O_2$ availability (Noland and Ultsch, 1981). This period corresponds to the time of highest tadpole activity and metabolic demand. pH, temperature, and DO all decrease rapidly at night. Drastic changes in chemistry, specifically pH, can have huge physiological implications. Additionally, lower pH and DO reduce efficiency of gas exchange as low pH reduces the ability of aquatic organisms to eliminate $CO_2$ (Cloudsley-Thompson, 1970) and low DO requires increased gill ventilation for comparable $O_2$ extraction (Burggren and West, 1982).

In early spring clutches, developmental $T_{water}$ averages ~20° C, while late summer water temperatures average ~25° C and can experience much wider diel oscillations as volume decreases. Surprisingly, even at a developmental temperature averaging ~20° C, tadpoles developed in 47 days, rather than the previously suggested 60 days (Wright and Wright, 1949; Tevis, 1966). Tadpoles averaging ~5° C warmer experience more than a 3 fold increase in developmental rate (Figure 2.6). The ratio of temperature integrated to time, juxtaposed to the ratio of time alone (in comparing spring and summer clutches) suggests this difference in developmental rate to be primarily due to increase temperature rather than phenoplasticity of the endocrine system regulating metamorphosis. However, when minimal temperature of development, 16° C, was accounted for (Brattstrom, 1966), spring tadpoles developed at 18.65% of the rate of summer tadpoles. This variation does support the hypothesis of increased phenotypic plasticity as a result of endocrine upregulation during times of stress and a precocious induction of metamorphosis hormones should not be overlooked (Denver, 1997).

Tadpoles have the ability to select among available thermal microhabitats in their parent-selected ponds to regulate body temperature (Ultsch et al., 1999). This ability allows for upregulation of metabolism and developmental rate to the limit of lethal temperatures. Of the ponds monitored in the Brownstone Basin rainwater catchment, even those exhibiting the most variable microhabitats, demonstrate relatively narrow thermal gradients (Figure 2.3). Extreme temperatures typical of small evaporating ponds are often inescapable in late summer.

$CT_{max}$ demonstrated significant variation across development. Thermal tolerance decreased drastically during the late stages of metamorphosis, as is consistent with the literature (Cupp, 1980; Noland and Ultsch, 1981; Sherman, 1980; Ultsch et al., 1999). Irrespective of the substantial variation in thermal extremes of ephemeral desert ponds verses temperate persistent ponds, $CT_{max}$ remains fairly constant across species, between ˜36° C - ˜43° C over the course of development (Ultsch et al., 1999, Noland and Ultsch, 1981; Sherman, 1980).

Evaporating pools exhibit temperatures below the $CT_{max}$ of most tadpole stages. However, water temperatures in small evaporating pools occasionally surpassed the $CT_{max}$ of late stage tadpoles during late summer. Increased differentiation in late stage tadpoles during limb development is synonymous with increased rate of metabolism and protein synthesis, increasing demands on oxygen (Ultsch et al., 1999). Increased metabolic demand, due to high temperature and accelerated differentiation, coupled with low oxygen availability, as a result of low $PO_2$ at high temperature, have the potential to force late stage tadpoles into respiratory distress (Ultsch et al., 1999). It is possible that the high DO of ephemeral pools during the warmest part of the day essentially rescues late stage summer clutch *B. punctatus* from respiratory distress as a result of increased oxygen availability, but it is likely that other physiological and behavioral methods exist that act to reduce this danger (e.g., Wassersung and Seibert, 1975).

Evaporation and radiation are largely unavailable to tadpoles as thermoregulatory mechanisms. However, tadpoles are subject to conductive and convective heat exchange making them generally the same temperature as the surrounding water (poikliothermic). Tadpoles appear to behaviorally increase developmental temperature using social aggregation (Brattstrom, 1962). There is some evidence for tadpole basking in the present study (Figure 2.6). Tadpole temperature after short periods of basking-like behavior was often lower than the temperature of the water column, suggesting tadpoles had been in cooler, possibly deeper waters earlier. $T_b$ increased slightly with time on the rock. However, $T_b$ was typically only slightly higher than the temperature of the water column (27.0° C), and never exceeded rock surface temperature (30.1° C). Higher preference for this behavior or increased site fidelity was associated with higher $T_b$. There seemed to be a trend towards larger, later stage tadpoles exhibiting this behavior. It is possible that these animals are attempting to increase body temperature to speed development, as their larger body size may have resulted from lower developmental temperatures (Smith-Gill and Berven, 1979). While it is likely that the behavior is associated with increasing body temperature, it is equally possible that the intent is unrelated to body temperature, e.g., to select locations with increased oxygen availability, reduce metabolism by resting, or access a preferred food source. More research is necessary to address the true reason for and effects of this behavior.

# CHAPTER 3

# THERMAL ENVIRONMENT OF JUVENILE RED-SPOTTED TOADS

To the best of my knowledge, there are no data regarding the thermal experience of newly metamorphosed juvenile Red-spotted toads (*Bufo (Anaxyrus) punctatus*). In other species, juveniles toads experience higher body temperatures than in any other life stage. Having observed juvenile *B. punctatus* on substrates that exceed 60° C, I hypothesized that juveniles experience more extreme temperatures than any other life stage. I observed newly metamorphosed toads in late summer and recorded time exposed to the sun and body temperature ($T_b$). There is marked individual variation in the experienced temperatures. The highest $T_b$ recorded in the field was 42.2° C. As expected, $T_b$ of juvenile *B. punctatus* generally increases with time spent in the sun. Juvenile $T_b$ was often higher when traversing dry substrates as compared with moist substrates. Body temperature was more closely associated with air temperature ($r^2 = 0.57$) than substrate temperature ($r^2 = 0.23$). Juvenile Red-spotted toads exhibit a preferred temperature ($T_p$) of 24.2 ± 0.7° C on dry substrate and 24.5 ± 0.7° C in an aquatic gradient. These data suggest juvenile Red-spotted toads experience higher temperatures than in any other life stage.

## 3.1 Introduction – Juveniles

The thermal experience of juvenile desert anurans has not been well defined. Younger toads are more often heat tolerant than older adults (Mayhew, 1968). I observed active juvenile Red-spotted toads throughout the day even when conditions seemed conducive to rapid water and heat stress. Newly metamorphosed toads are often exposed to full sun on hot summer days and will commonly cross substrates in excess of 60° C. Although no data have been presented for the thermal environment of juvenile Red-spotted toads, there have been a few studies in other species evaluating the effect of basking behavior on juvenile body temperature (e.g., Seymour, 1972; Lambrinos and Kleier, 2002).

Seymour (1972) reports body temperature ($T_b$) and activity of wild juvenile *Bufo debilis* along a moist riverlet bank in New Mexico. Seymour found $T_b$ in basking juveniles was as high as 35° C (mean = ~30.9° C). However, juveniles traversing a 45° C substrate experienced mean $T_b$ of ~25.8° C. Water availability was critical for successful basking behavior. Juveniles kept in an open container in full sun reached temperatures of 43° C and died within 40 – 50 minutes. In my ephemeral pond system, water availability is unreliable. I asked what the body temperatures of juvenile Red-spotted toads were in this system. I relate these data to the preferred temperature and the amount of time spent exposed to sunlight.

## 3.2 Materials & Methods – Juveniles

### 3.2.1 Body Temperature in the Field

The handling of small poikliothermic organisms has the potential to substantially affect $T_b$ (Seymour, 1972). To reduce the effect of handling, a device was fashioned from a wire coat hanger formed into a loop (~10 cm in diameter) with a handle. I stretched a small portion of pantyhose over the loop to produce a gentle “toad swatter.” I observed toads as they left their shaded refuges. I recorded the time each juvenile toad spent in the sun and followed at a sufficient distance not to impact the movement of the toadlet. As toadlets neared shaded regions on the periphery of the dry pond, I restrained them and measured body temperature with a dorsally inserted micro thermocouple through the pantyhose "swatter". I measured the time spent by toads in the sun. Following measurement, toads were placed in a shaded plastic box to avoid re-sampling.

### 3.2.2 Thermal Preference

Thermal preference ($T_p$) in an aquatic system was determined in an aquatic system as described in Section 2.2.6. Thermal preference also was assessed using moist paper towels as a substrate.

## 3.3 Results – Juveniles

### 3.3.1 Field Observations

Average high $T_b$ measured in the field was 33.7 ± 0.76° C (n = 22, mean ± SE). The highest $T_b$ recorded for any juvenile was 42.2° C. $T_{air}$ for this toad was 43.6° C (measured ~1 cm above the ground), while the dry sand was 51.5° C (Figure 3.1). It is interesting to note that this body temperature was found after ~5 minutes of exposure. I observed toads traversing warmer substrates, ˜60° C, and/or for longer periods of time, however the micro thermocouple was not available. As would be expected with the effect of evaporative heat loss, surface temperatures and $T_b$ were lower when associated with moist substrates. Toadlets generally exhibited lower body temperatures on moist substrates as compared to dry substrates (Figure 3.1). $T_b$ exhibited a closer association with $T_{air}$ than with $T_{surface}$ (Figure 3.2; $r^2 = 0.57$ and $r^2 = 0.23$, respectively). Juvenile $T_b$ increased slightly with time spent in the sun (Figure 3.3, $r^2 = 0.66$).

### 3.3.2 Lab Observations

Juveniles preferred 24.81 ± 0.10° C (n = 5, mean ± SE) on dry substrates and 24.50 ± 0.69° C in aquatic media. Unfortunately, critical thermal maxima ($CT_{max}$) could not be obtained as juveniles failed to consistently right at any temperature.

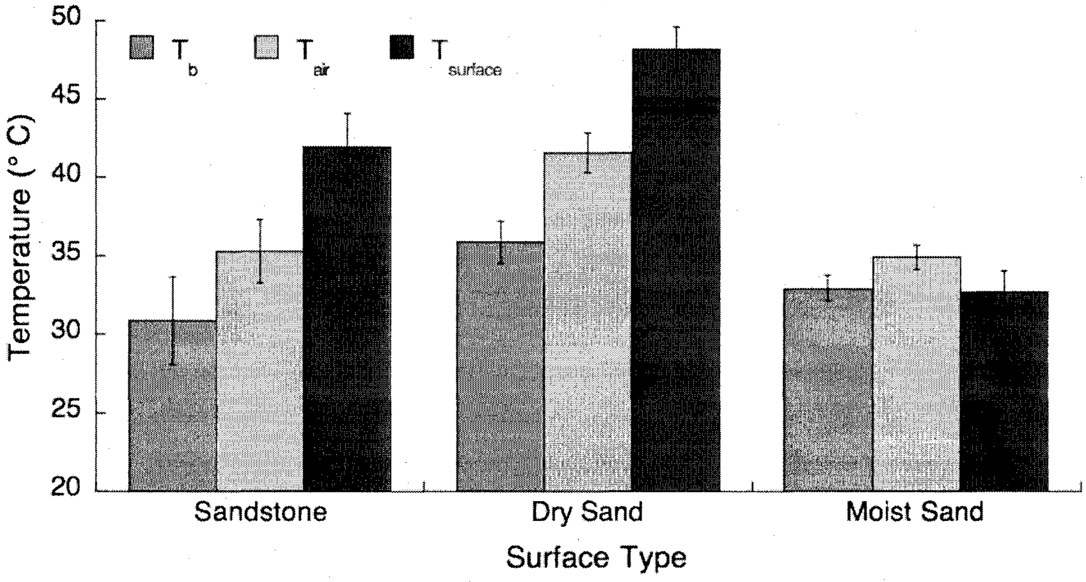

Figure 3.1 Juvenile body temperature, air temperature and surface temperature during mid-day activity

I measured temperature of juveniles ($T_b$, medium gray) found on sandstone (n = 3), dry sand (n = 8), and moist sand (n = 11). For each measurement, air temperature (~1 cm from the ground surface, as an approximation of toad ambient temperature) was also measured ($T_{air}$, light gray), and ground surface temperature at the location of each toad is presented in dark gray ($T_{surface}$).

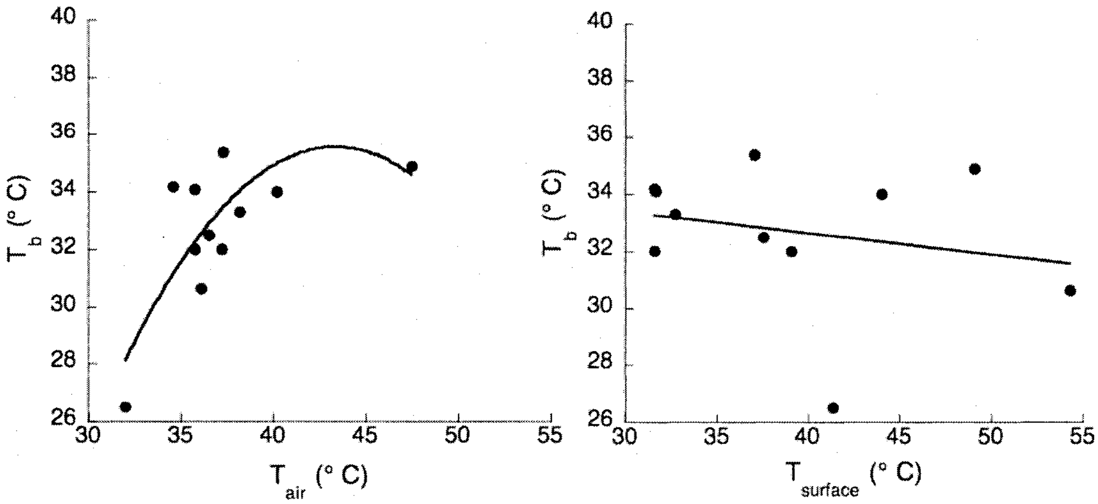

Figure 3.2 Juvenile body temperature plotted against ambient temperature and surface temperature

Body temperature of dry toadlets was measured using a micro thermocouple and (A) plotted against ambient air temperature ($T_{air}$), measured at approximately 1 cm above the substrate. The data have been fit to a polynomial line of best fit ($r^2 = 0.57$). (B) Toadlet body temperature is plotted against surface temperature ($T_{surface}$), and fit to a line of best fit ($r^2 = 0.23$).

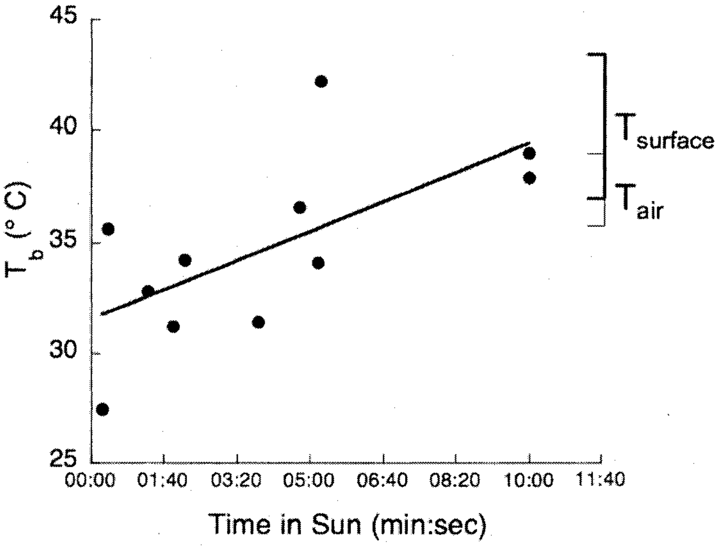

Figure 3.3 Juvenile body temperatures after time in the sun

I measured body temperature ($T_b$) of juveniles, with a micro thermocouple. Toads were followed from the time emerged from a shaded refuge. Time in the sun was recorded and body temperature measured. As a reference, I indicate average $T_{air}$ and $T_{surface}$ with brackets (mean ± SE). The data were fit to a line of best fit ($r^2 = 0.66$).

## 3.4 Discussion – Juveniles

Thermal accounts of juvenile anuran body temperature focus heavily on body temperature while basking (Seymour, 1972; Lambrinos and Kleier, 2002). I observed and measured $T_b$ newly metamorphosed (juveniles or toadlets), *B. punctatus* during late summer in their natural habitat. None of the animals observed appeared to bask.

Juvenile anurans will bask in order to increase body temperature and speed development (Lillywhite, 1970). From my observations, *Bufo punctatus* does not appear to bask. Rather, juvenile Red-spotted toads may be occasionally found traversing extremely hot surfaces (>60° C) in the heat of the day. Previous studies of other species and my data suggest juveniles experience higher temperatures than in other life stages (Mayhew, 1968). Evaporative water loss may serve to cool toads (Tracy, 1976). When water is available, use of evaporative water loss may confer a selective advantage (Seymour, 1972). However, in dry environments, evaporative water loss could result in death. Basking behavior may not be continuously available to *B. punctatus* in ephemeral environments, as water availability is unreliable. However, further data are necessary to confirm this hypothesis.

The data presented here likely underestimate the thermal experiences of juvenile toads. Substrate temperatures in previous years were as high as 65° C, and frequently exceeded 60° C, while knots of newly metamorphosed toads were observed on these substrates (unpublished data). Future efforts will be aimed at collecting $T_b$ data when similar conditions are presented.

# CHAPTER 4

# THERMAL ENVIRONMENT OF ADULT FREE-RANGING TOADS

I measured body temperature ($T_b$) in free-ranging adult *Bufo punctatus* across all four seasons of a year using implanted data loggers. There is marked individual variation in the temperatures experienced by these toads. As expected, toads generally escape extreme seasonal and diel temperature fluctuations. However, these data demonstrate a much wider $T_b$ range than was previously assumed. Although often for short periods, Red-spotted toads do experience $T_b$ as low as 3.1° C and as high as 39.1° C. All animals experienced periods of prolonged thermal stability in cooler months and wider diel oscillations in warmer months. As previously described for many amphibians, Red-spotted toad thermal history is likely a function of site choice; the exploitation of different refuges results in diverse thermal experiences. My field data indicate that *B. punctatus* must exhibit a higher critical thermal maxima ($CT_{max}$) than originally presumed. $CT_{max}$ was experimentally determined to be 45.2 ± 1.02° C (mean ± SE; n = 5). Adult Red-spotted toads (n = 5) had a preferred temperature ($T_p$) that closely matched their average temperature experienced in active seasons, 26.4 ± 0.4° C for dry substrate and an aquatic $T_p$ of 25.4 ± 0.5° C.

## 4.1 Introduction – Adults

While some data are available for a brief period in mid-summer (e.g. Moore and Moore, 1980), the thermal ecology of adult Red-spotted toads during the rest of the year has not been well described. Although no data are shown, McClanahan et al. (1994) refer to a single Red-spotted toad that maintained a body temperature ($T_b$) of 25° C between September and December, and further suggest a maximal thermal limit of 35° C for *B. punctatus*. Based on personal observations, the work of Moore and Moore (1980) and ambient temperature ($T_a$) monitoring of a typical habitat in Southern Nevada, I hypothesize *B. punctatus* to experience more extreme and variable temperatures than previously suggested.

By exploiting small programmable data loggers, I was able to track $T_b$ hourly in free-ranging adult toads for nearly an entire year, including the winter period. These observations were correlated with the determination of thermal preference ($T_p$) and critical thermal maxima ($CT_{max}$) in an effort to characterize more fully the thermal ecology of the Red-spotted toad in nature.

## 4.2 Materials & Methods – Adults

### 4.2.1 Monitoring of Adult Body Temperature in the Field

I studied the Red-spotted toad in the same ephemeral pond system as Chapters 2 and 3 (Brownstone Basin near Las Vegas, NV, U. S. A.; N 36°10'59.56 W 115°25'51.11, elev. 1487 m). Adult toads, *B. punctatus* (n = 15), were collected during the summer. The toad collection and release area is adjacent to a gravel arroyo and is found within a mixed sandstone and limestone rainwater catchment network of ephemeral ponds, which may be inundated by rainfall throughout the year, but which typically dry out and refill during the summer.

Only larger adult animals were collected to ensure adequate size to accommodate data loggers (July 2005). I anaesthetized toads in 3% ethyl-m-aminobenzoate (MS-222; Sigma Chemical Company) dissolved in a 50 mM bicarbonate solution, pH 7.2. A small abdominal incision was made and a ~3 g, ~1.5 cm diameter paraffin-coated I-Button data logger (Dallas Semiconductor model DS1922L), preset to log temperature every hour, was inserted into the abdominal cavity. The incision was sutured and treated with antimicrobial ointment. Animals were rinsed in fresh water until alert, allowed to recover with access to fresh water, and released in the locations from which they were originally collected. I allowed animals to recover for several hours and observed them to be fully ambulatory prior to the time of release. I recovered data loggers from four toads. All four toads were in good body condition and exhibited normal activity level as evidenced by mating behavior. I collected one toad after approximately 2 years in the wild (August 2007); the others were collected approximately one year after release (June and July 2006).

### 4.2.2 Monitoring of Environmental Temperature

As a reference, I logged air temperature and humidity by placing an I-Button data logger (model DS1923) in a shallow rock crevice (~10 cm depth) where toads had been observed. Pond water temperatures were recorded when water was present using submerged I-Buttons (DS1922L) in three adjacent ponds in the toad collection habitat.

### 4.2.3 Data Analysis

Upon retrieving data loggers, data were analyzed for patterns in thermal fluctuation, daily means were calculated along with mean seasonal daily mean with standard error for all toads, air and pond water (Table 1).

Seasonal categories were based on the solstice calendar: fall from September 22 to December 20, winter from December 21 to March 20, spring from March 21 to June 20, and summer from June 21 to September 21.

### 4.2.4 Characterization of Available Thermal Microhabitats

In order to characterize the available thermal microhabitats on a typical summer day, I placed data loggers in a variety of microhabitats that may be used by the toads. Data loggers that may have been exposed to direct sunlight, I encrusted them with pebbles to minimize the effect of direct solar radiation.

### 4.2.5 Critical Thermal Maxima in the Laboratory

I defined $CT_{max}$ of adult toads collected in late summer ($n = 5$) using righting response. $T_a$ was increased, starting from 25° C, at ~0.5° C/min, in a narrow 1 L beaker (with ~5 cm of moist sand) submerged to 1 cm below the brim in an 8 L water bath. I assessed animals for righting response before every completed degree increment. When animals failed to right, I removed them and allowed them to recover on a cool moist paper towel. All animals recovered in less than 10 min and no animals showed changes in motility, activity or eating habits one day following the experiment. I measured body temperature with a cloacal thermocouple. I also measured substrate and air temperature during each trial. $T_b$ varied < 2° C from substrate temperature.

### 4.2.6 Thermal Preference in the Laboratory

I determined thermal preference (n = 5) using a dual Peltier thermal gradient, as in tadpoles and juveniles (Sections 2.2.6, 3.2.3 and Appendix I), in both dry and aquatic gradients. Toads (n = 5) were allowed to select thermal preference in 5 cm of water, between 12.7° C and 36.5° C. The same toads were allowed to select preferred temperature on a moist surface gradient, surface temperature ranged from 11° C – 52° C. Toad position was monitored for one hour by computer logged light gates indicating the location of the toad within the gradient (Appendix I). Recordings were only made when I observed toads to be calm and not intent on escape.

## 4.3 Results – Adults

Body temperatures in red-spotted toads were highly variable throughout the year and ranged from a low of 3.1° C to as high as 39.1° C (Figure 4.1 A - D). In general, diel change in $T_b$ during what are apparently active periods (i.e., April to October) oscillated in parallel to $T_a$ (Figure 4.1 E). I logged $T_a$ in a rock crevice as a reference. While these data likely are more representative of conditions experienced by the toads, the collected $T_a$ data underestimate the extremes in available temperature microhabitats (e.g. see Figure 4.4). Nevertheless, $T_b$ for all four toads rarely exceeded $T_a$ during the warm months and $T_b$ demonstrated less diel variability than $T_a$ (Figure 4.2).

I present temperature data for what I consider typical 3-day intervals for each season (Figure 4.3). These data demonstrate relatively less tracking of $T_b$ to diel $T_a$ changes during fall and winter (Figure 4.3 A - B) than during spring and summer (Figure 4.3 C - D). Figure 4.2 supports this observation.

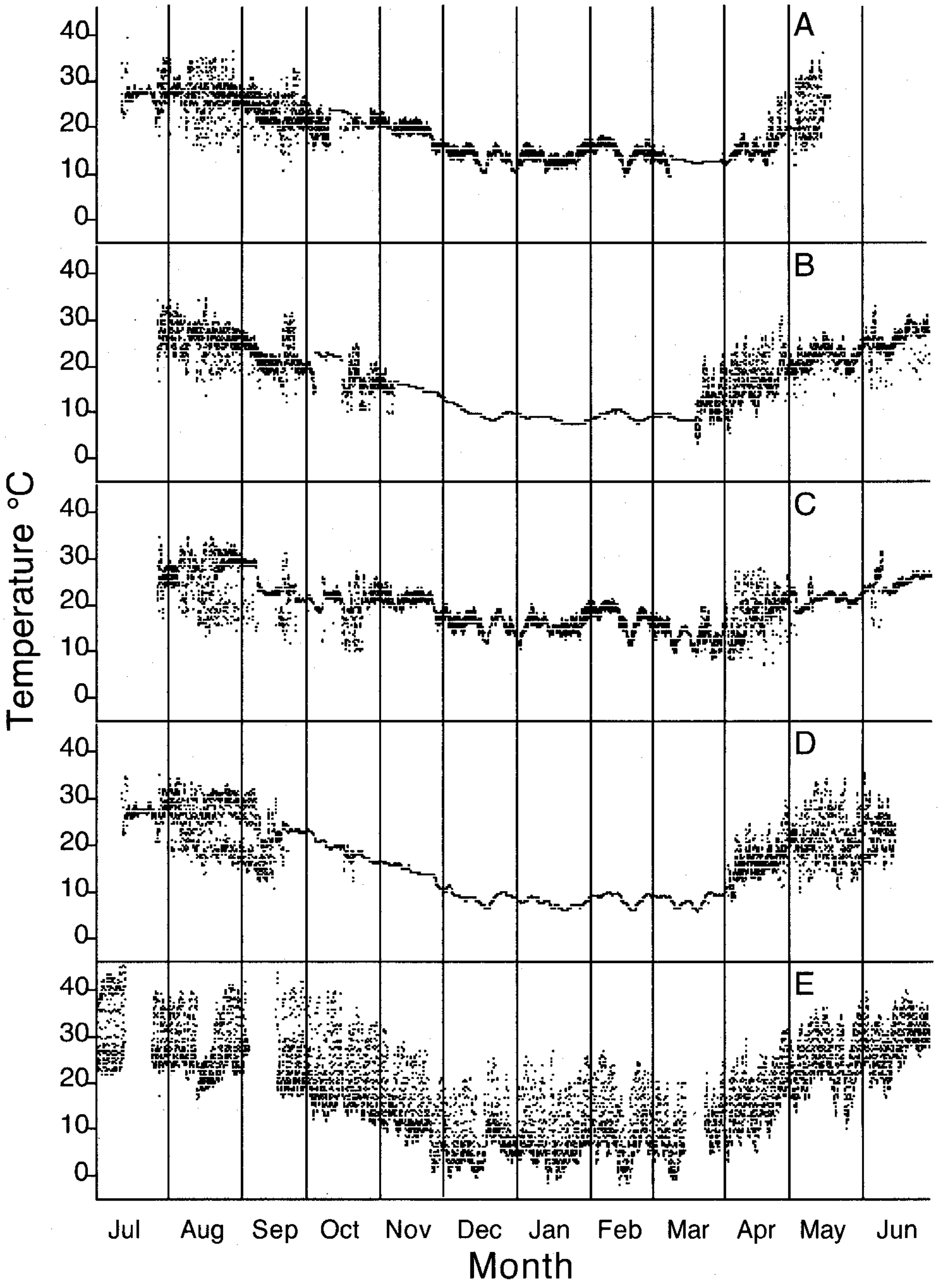

Figure 4.1 Annual patterns of $T_b$ in free-ranging adults

I present $T_b$ of four individual adult Red-spotted toads, *Bufo punctatus*, from time of release until time of recapture (A - D) for each. Ambient temperature ($T_a$) of a shallow rock crevice (E) is included as a reference of environmental fluctuation. Data represent hourly temperature recordings.

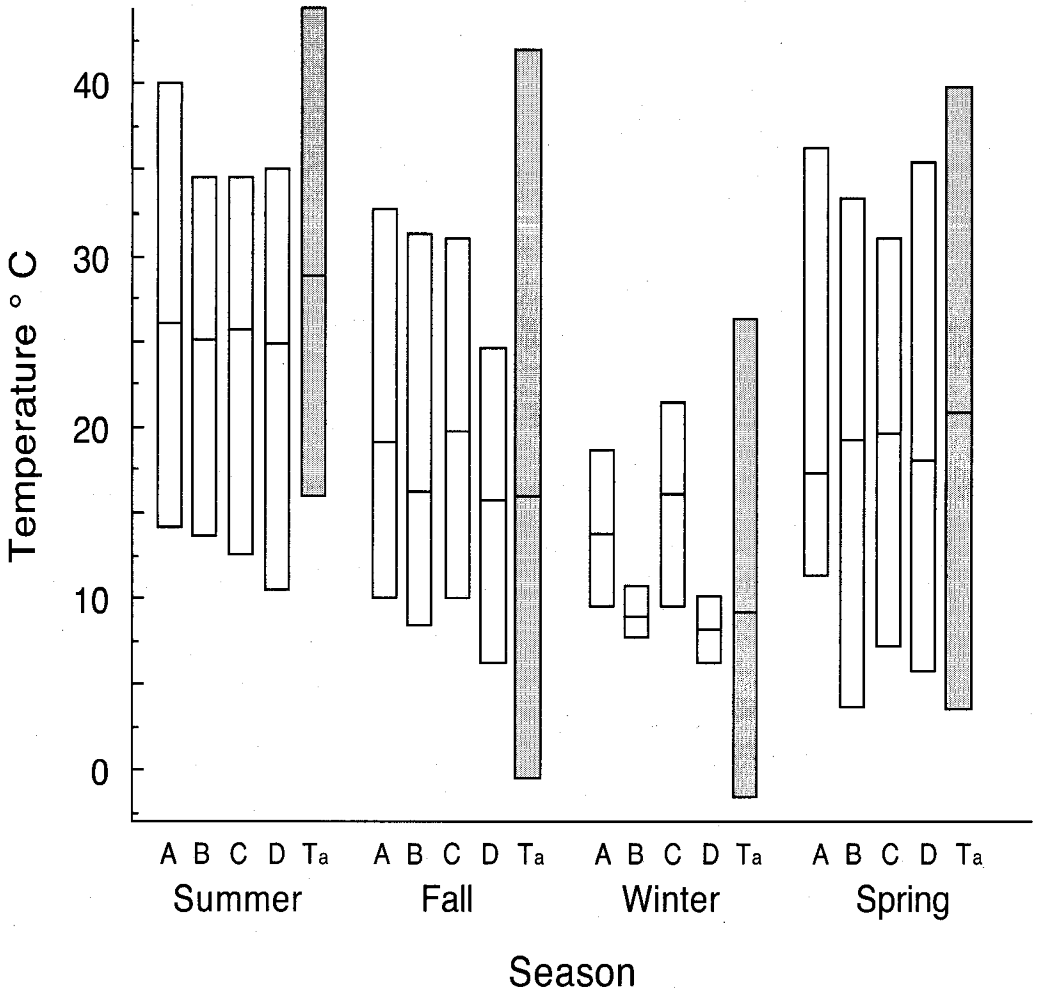

Figure 4.2 Daily mean temperature and range by season

Mean daily $T_b$ of each toad (white bars, A-D) and ambient air in a shallow crevice (grey bars, $T_a$) by season. Bars represent range in temperature, the crosscutting lines represent seasonal means in daily temperature.

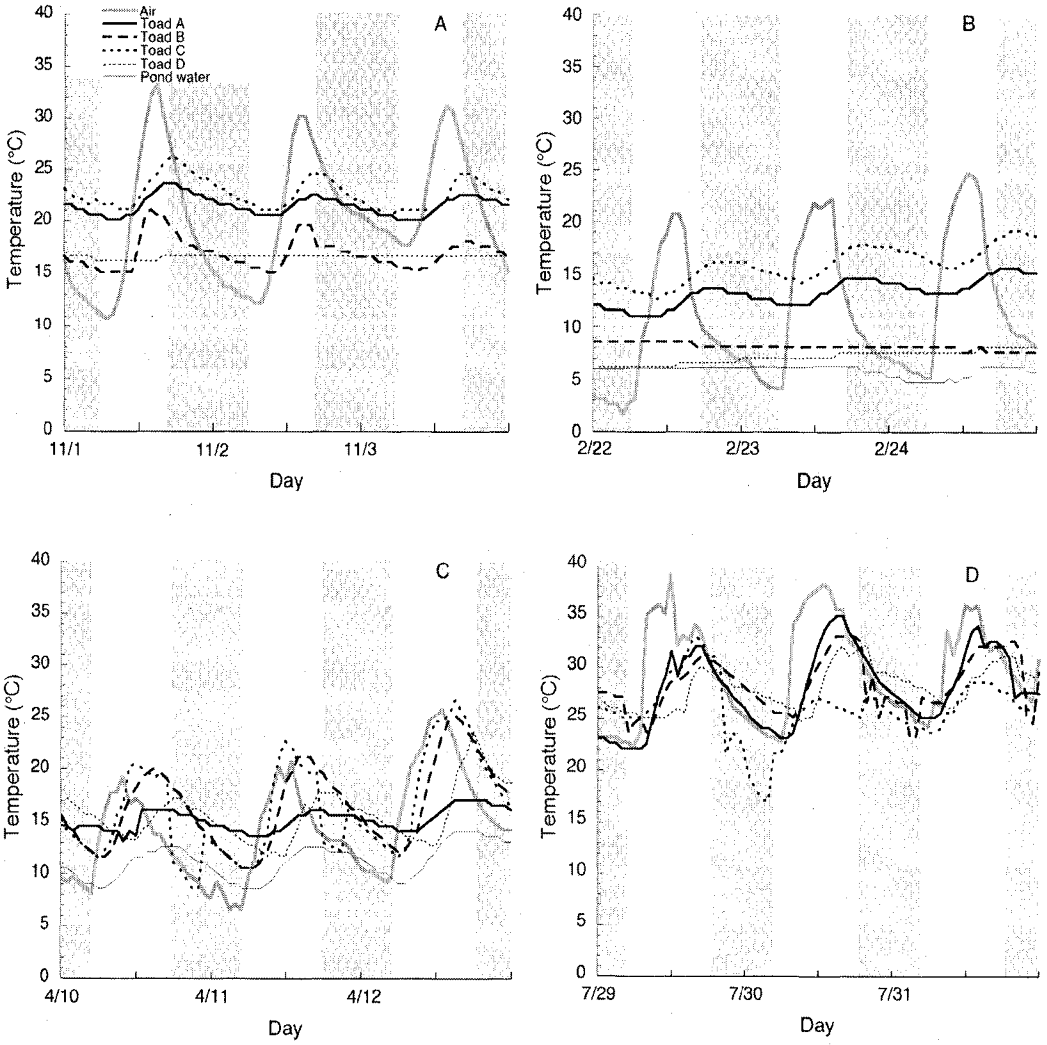

Figure 4.3 $T_b$ for all four toads, $T_a$, and water temperature (if available) for typical representative 3-day sequences

(A) fall, November 1 - 3; (B) winter, February 22 - 24; (C) spring, April 10 - 12; and (D) summer July 28 - 30. Data represent temperatures obtained hourly from midnight the first day to midnight of the third day in the sequence. The vertical shaded bars represent the periods between sunset and sunrise.

Two toads (B and D) demonstrated little to no diel variation in $T_b$ throughout the winter (Figure 4.1 A, D). During the same period, the other two toads had greater (although still damped) diel oscillations (Figure 4.1 B, C). I presume these differences represent site choices for over-wintering. When toad B apparently exited its thermally stable site, its $T_b$ immediately plunged to the lowest value recorded (3.1° C; Figure 4.1 B). Unfortunately, ambient temperature data are not available for this period since the data logger was washed away in a heavy storm.

On a typical summer day, toads had choices of microhabitats that ranged from below 20° C to ~60° C (Figure 4.4). Yet, the highest $T_b$ recorded was 39.1° C. Although exposure to this very high $T_b$ was brief (< 1h), this same toad experienced multiple several-hour periods above 35° C (Figure 1 A, Table 4.1).

My field data indicated that *B. punctatus* must exhibit a higher $CT_{max}$ than previously presumed (e.g. McClanahan et al., 1994). This is further demonstrated by laboratory observations that $CT_{max}$ was 45.2 ± 1.02° C (mean ± SE; n = 5). Red-spotted toads (n = 5) had a preferred temperature ($T_p$) of 26.4 ± 0.4° C for dry substrate and an aquatic $T_p$ of 25.4 ± 0.5° C.

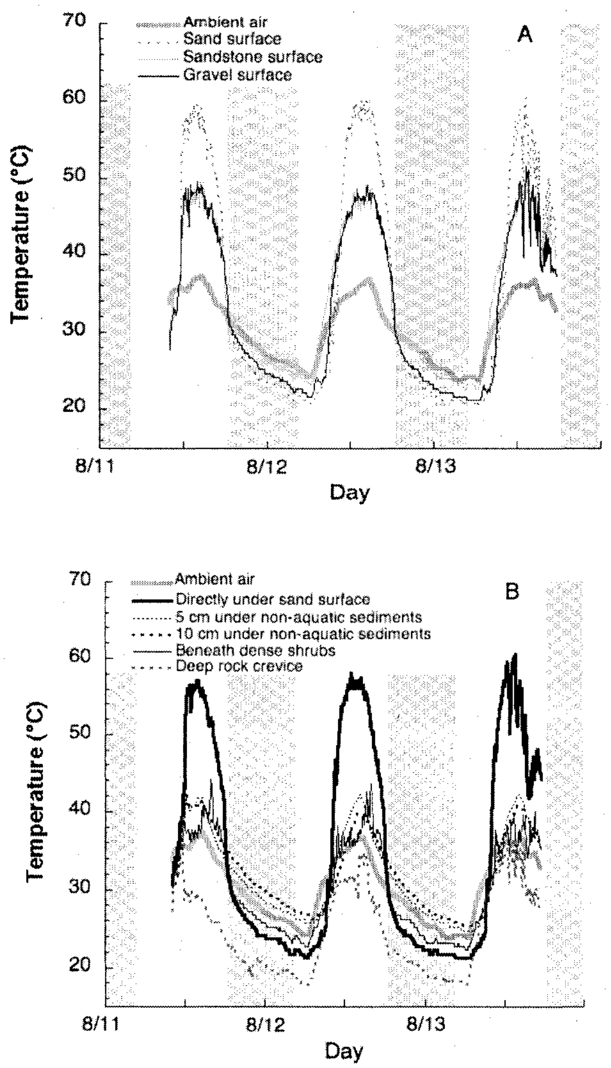

Figure 4.4 Temperature of various microhabitats for three typical summer days

I logged (A) surface temperatures of sand (dotted line), sandstone (thin grey line), gravel (thin black line), and ambient air temperature of a shallow rock crevice for reference (thick grey line). (B) Temperatures of possible toad refuges including: directly under the sand surface (thick black line); under 5 cm of dry sediment adjacent to pond areas (thin dotted line); under 10 cm of dry sediment adjacent to pond areas (thick dashed line); beneath dense shrubs (thin black line); and a deep sandstone rock crevice (approximately 30 cm; thick dotted grey line).

Table 4.1 – SUMMARY DATA FOR SEASONAL CHANGES IN TEMPERATURE
Error terms indicate standard error of the mean.

| Temperature | Season | Air (Shade) | Pond Water* | Toad A** | Toad B*** | Toad C*** | Toad D**** |
|---|---|---|---|---|---|---|---|
| Mean | Summer | 29.2 ± 0.16 | 23.6 ± 0.16 | 26.1 ± 0.09 | 25.2 ± 0.09 | 25.7 ± 0.10 | 24.8 ± 0.12 |
| | Fall | 15.9 ± 0.19 | 9.4 ± 0.13 | 19.2 ± 0.08 | 16.2 ± 0.10 | 19.7 ± 0.07 | 15.7 ± 0.11 |
| | Winter | 9.2 ± 0.13 | 5.5 ± 0.04 | 13.8 ± 0.04 | 8.9 ± 0.02 | 16.1 ± 0.05 | 8.2 ± 0.02 |
| | Spring | 20.9 ± 0.17 | 16.5 ± 0.11 | 17.2 ± 0.14 | 19.3 ± 0.12 | 19.5 ± 0.10 | 18.1 ± 0.14 |
| Maximum | Summer | 45.5 | 35.7 | 39.1 | 34.6 | 34.6 | 35.2 |
| | Fall | 42.2 | 21.5 | 32.6 | 31.6 | 31.1 | 24.7 |
| | Winter | 26.7 | 10.2 | 18.1 | 10.6 | 21.6 | 10.2 |
| | Spring | 39.7 | 27.7 | 36.1 | 33.1 | 31.6 | 35.7 |
| Minimum | Summer | 16.2 | 15.0 | 14.1 | 13.6 | 12.6 | 10.7 |
| | Fall | -0.4 | 3.0 | 10.1 | 8.1 | 10.1 | 6.7 |
| | Winter | -1.9 | 2.1 | 9.6 | 7.6 | 9.6 | 6.2 |
| | Spring | 3.1 | 5.1 | 11.6 | 3.1 | 7.1 | 5.7 |

Temperatures recorded in Brownstone Basin, NV
*When water present
**Toad internal temperature recorded between July 12, 2005 and May 22, 2006.
***Toad internal temperature recorded between July 27, 2005 and July 3, 2006.
****Toad internal temperature recorded between July 12, 2005 and June 16, 2006.

## 4.4 Discussion – Adults

I am unaware of any previous description of $T_b$ for free-ranging anurans that spans all four seasons. Although important contributions were made in the elucidation of thermal ecology of anurans by many previous workers (e.g. Carey, 1978; Lillywhite, 1970; Moore and Moore, 1980; O'Connor and Tracy, 1992; Seebacher and Alford, 2002; Seymour, 1972; Sinsch, 1989; Smits, 1984; van Gelder et al., 1986), these authors were limited by experimental constraints to short periods of monitoring and/or artificial environments. I employed implanted data loggers and documented $T_b$ in free-ranging *B. punctatus* over the course of the entire year.

Despite $T_a$ in accessible crevices that may be as low as –1.9° C or as high as 45.5° C or (Table 4.1, Figure 4.1 E), surface temperatures that may be greater than 60° C (Figure 4.4) and as much as a 40° C thermal gradient available to them (Figure 4.4), Red-spotted toads appear to escape most temperature extremes. Of note, no toad in this study experienced freezing temperatures, although I have measured microhabitat temperature as low as –12° C in the area (data not shown). The coldest measured $T_b$ was 3.1° C. Further, toads may be warmer than the average crevice temperature for the entire winter (Figure 4.2). In summer, all toads are cooler than the average daily temperature (Figure 4.2) and despite a wider range of microhabitat temperatures, toads experience relatively moderate $T_b$ (Table 4.1). In contrast to the work of Carey (1978) on boreal toads in montane regions, where summer $T_b$ fluctuated as much as 20-30° C in a diel oscillation, toads in the present study experienced much more damped diel oscillations (average diel $T_b$ oscillations for all toads = 5.04 ± 0.15° C, maximum recorded diel $T_b$ oscillation was

21° C). Summer active toads avoided extreme heat. These data, and those of Moore and Moore (1980) indicate that Red-spotted toads experience $T_b$ above 35° C (Table 4.1).

Adult toads experience damped diel oscillations in $T_b$ as compared to $T_a$, presumably through thermal microhabitat selection (Huey, 1991). Adult toads are rarely active during the midday in warmer months (personal observations); rather these toads are active at night when radiative heat gain is minimized (Tracy, 1976).

Although data loggers were recovered from four toads, I am unaware of the fate of the remaining implanted toads. Toads may have avoided recapture, or have been lost due to relocation, predation, or mortality. Although it is tempting to speculate that some animals experienced fatal temperatures, our experimentally derived $CT_{max}$ of ~45° C would suggest very few opportunities for toads to experience lethal temperatures in the wild.

In accordance with expectations, adult *B. punctatus* do not appear to experience broad temperature fluctuations in an extreme environment like the Mojave Desert. However, in the laboratory, I found these toads to be capable of tolerating much higher and lower temperatures than those reported previously. Although toads apparently withstand extreme temperatures for short periods, it is likely that these toads exploit behavioral selection of thermally moderate microhabitats to avoid the most extreme of desert temperatures.

# CHAPTER 5

# CONCLUSION & FUTURE DIRECTIONS

## 5.1 Summary

I documented ecologically relevant body temperature ($T_b$) for all stages of the Red-spotted toad. I found temperatures that exceeded previously suggested limits. Furthermore, I found Red-spotted toads to rarely experience temperatures that approach the thermal limits determined in these studies. However, later stage tadpoles are more sensitive to environmental temperatures and may occasionally experience lethal temperatures in nature.

Contrary to the literature, these data suggest a slightly higher thermal tolerance of early stage tadpoles as compared to eggs, but consistent with the literature, generally lower thermal tolerance as larval development progresses (e.g., see table 5.1; Cupp, 1980; Ultsch et al., 1999; Noland and Ultsch, 1981; Sherman, 1980). The severity and extent of reduced thermal tolerance during the late stages of metamorphosis was not expected, particularly as water becomes warmest just before the completion of metamorphosis.

As expected, juvenile *B. punctatus* do experience the highest temperatures of any life stage and will voluntarily spend ~10 minutes on extremely hot dry substrates, in excess of 60° C. The data in Chapter 3 may underestimate ecologically relevant $T_b$ in juveniles, as surface temperatures were not nearly as hot as in previous years.

Table 5.1 – SUMMARY DATA FOR DAYS SURVIVED BY ALL GROUPS AFTER HEAT SHOCK. Error terms indicate standard error of the mean.

| | Temp. | | Average Days Survived by Heat Shock Group | | | | |
|---|---|---|---|---|---|---|---|
| Stage | Reared | Control | 35° C, 1h | 35° C, 2h | 40° C, 1h | 40° C, 2h | 45 |
| Eggs (7/26) | 15 | 7.54 ± 1.84 | 11 ± 2.30 | 6.83 ± 1.43 | 5.88 ± 1.65 | 4.58 ± 1.55 | 0 |
| | 25 | 3.5 ± 1.41 | 8.46 ± 2.63 | 4± 1.92 | 2.46 ± 1.33 | 2.02 ± 3.42 | 0 |
| Eggs (8/05) | 25 | 3.04 ± 0.04 | 8.46 ± 2.34 | 28.17 ± 3.17 | 9.71 ± 2.85 | 6.83 ± 1.98 | 0 |
| Eggs (8/09) | 15 | 28.29 ±4.02* | 11.25 ±2.68* | | 29.92 ± 4.66* | | 0 |
| | 25 | 3.33 ± 0.66 | 10.08 ± 1.70 | | 16.04 ± 3.68 | | 0 |
| Tadpoles Hatchlings | 15 | 33.86 ± 4.45 | 39.86 ±8.40 | | 9.57 ±4.91 | | 0 |
| | 25 | 32.29 ± 5.83 | 31.86 ± 5.14 | | 38.71 ± 7.95 | | 0 |
| Tadpoles Stage 35 | 15 | 24.88 ± 4.83 | | 2.5 ± 1.31 | | | 0 |
| | 25 | 26.5 ± 5.95 | | 10.71 ± 6.78 | 0 | 0 | 0 |
| Tadpoles Stage 39 | | 0 | 0 | 0 | 0 | 0 | 0 |

* Eggs did not develop

Adult Red-spotted toads do not appear to be subjected to the extremes I originally expected of their thermal environments. However, like juveniles, adults do experience temperatures higher than most tadpoles. High $T_b$ experienced in nature, was several degrees higher than their previously assumed $CT_{max}$. I found their $CT_{max}$ to be much higher (~6° C) than any temperature they experienced in the field and even sufficient to allow, admittedly brief, excursions on the hottest desert days.

*B. punctatus* tadpoles do not persist in these pools during winter. The fate of juvenile toads is largely unknown during the colder months. Adult toads over-winter in a habitat that provides severe cold ($T_a$ below 0° C). However even at prolonged low $T_a$ in an available crevice, internal body temperatures of the toads never approached freezing, suggesting selection of thermally buffered crevices or burrows. It is notable that all individuals managed to stay cooler than $T_a$ in the heat of the day, even in the cool winter months (though very cold periods showed exception), suggesting a behavioral tendency to stay in reduced temperatures and the use of evaporative cooling. Selection of cooler microhabitats may be a physiological advantage on many fronts. Decreases in $T_b$ afford substantial declines in water loss, some toads will go into a behavioral hypothermia in times of low water availability (Malvin and Wood, 1991). It is likely that desert toads like *B. punctatus* exploit a similar behavior to avoid desiccation. Alternatively, decreased temperature as a function of water loss may subject toads to more rapid desiccation.

Preferred temperature did not change significantly across development (Figure 5.1, $p > 0.05$), as would be expected (Dupré and Petranka, 1985). Low thermal variability within warm pools during the heat of the day may suggest the preference for a moderate

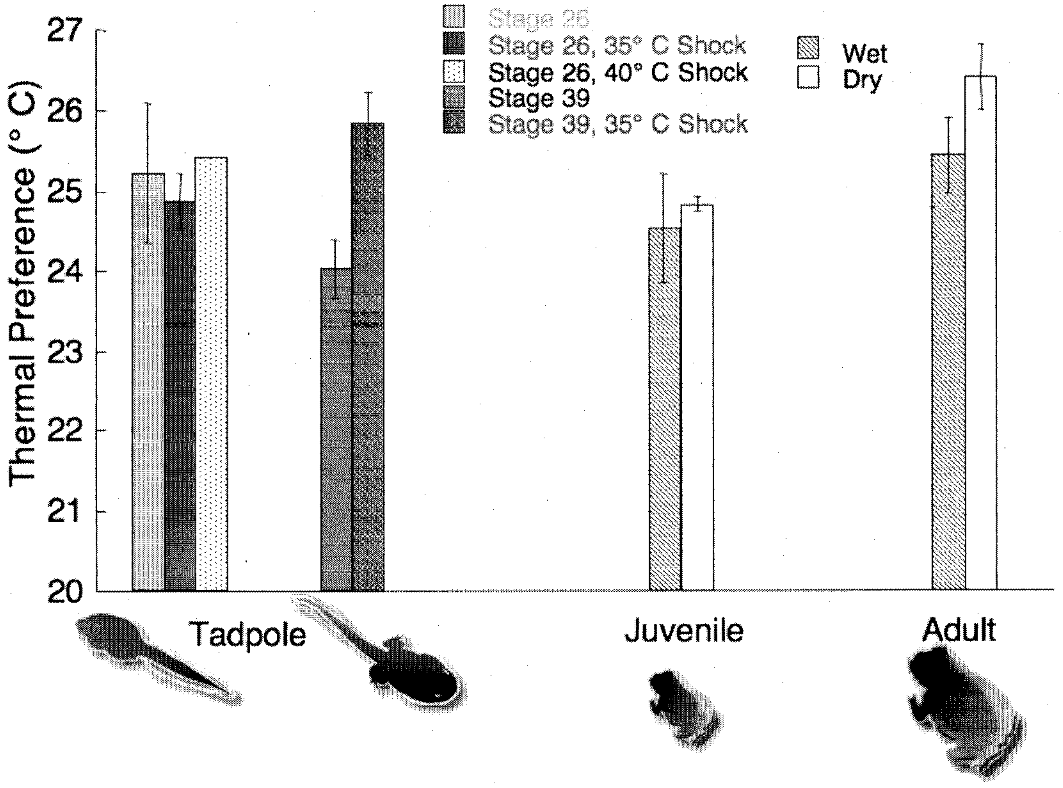

Figure 5.1 $T_p$ of Red-spotted toads across development

Thermal preferences of tadpoles staged to Gosner stage 26, reared at 15° C, tadpoles of stage 35, reared at 25°, and juveniles and adults in both wet and dry gradients. With the exception of stage 39 heat shocked compared with controls, $p < 0.05$, there was no change between treatments or developmental stage ($p > 0.05$). When assessed as a group, stage 39 tadpoles were not significantly different from any other developmental stage ($p > 0.05$). There was no difference between wet and dry treatment juveniles and adults ($p > 0.05$).

temperature (~25° C), may be advantageous across development, particularly as late stage tadpoles become more thermally intolerant. Adults may select moderate temperatures to reduce fuel consumption and water loss.

In contrast to thermal preference, $CT_{max}$ showed substantial variability across development (Figure 5.2). As opposed to other species, these data suggest adult *B. punctatus* to be more thermally tolerant than larvae (Cupp, 1980; Floyd, 1983; Sherman, 1980). In light of the comparatively higher temperature of the adult thermal environment this finding is somewhat intuitive as thermal $CT_{max}$ is often correlated with environmental temperatures (Snyder and Weathers, 1975). Unfortunately, a reliable test of thermal maxima was not successful in juveniles. However, the highest field $T_b$ recorded, 42.2° C, suggests the possibility of a $CT_{max}$ near that of adults.

Late stage tadpoles undergo differentiation events resulting in higher metabolic demand (Smith-Gill & Berven, 1979). The partial pressure of oxygen in water decreases as temperature increases, this tendency, coupled with increased metabolic demand of rapid differentiation, likely exposes late stage tadpoles to respiratory distress (Ultsch et al., 1999). Late stage tadpoles are often observed swimming ventral-side-up at the air/water interface and resting on rocks just below the water surface (Figure 2.7). It is possible that these behaviors may be utilized to increase body temperature or acquire food, respectively. However, one or both of these behaviors may play a role in increased thermal tolerance by increasing oxygen up-take. Oxygen is more available at, and just below, the water surface. The basking-like behavior I observed was accompanied by very little activity and close proximity to the water surface, these factors together may

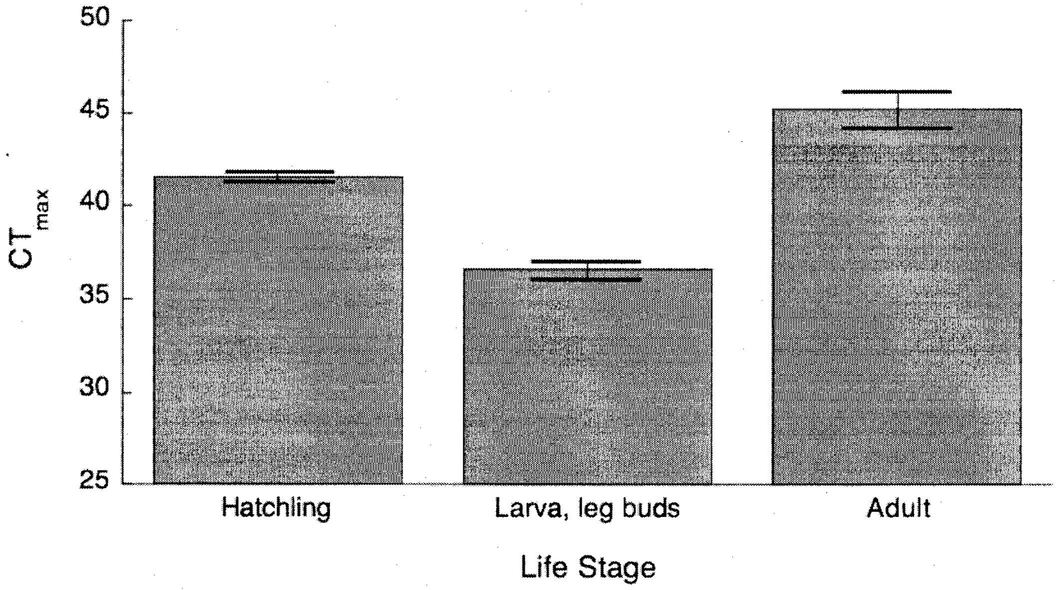

Figure 5.2 Thermal tolerance across life history

I present thermal tolerance across development: hatchling, larva with hind leg buds, and adults. Values were derived from laboratory heat shock experiments of response to stimulus (Hatchling, stage 23; Larva, leg buds, 35) and righting response (Adults) and are presented with SE (n = 5).

help reduce metabolic rate and increase $O_2$ availability. Although surface-skimming tadpoles previously appeared to be eating algae from the water surface, it is possible that they are exhibiting atmospheric air gulping (described in fish; Ultsch, 1989). Some tadpoles, including *Bufo woodhousii*, exhibit this and slightly modified behaviors. Woodhouse tadpoles do not take in atmospheric oxygen directly as bufonids generally lack developed lungs, but take advantage of increased dissolved oxygen and aeration at the pond surface in hypoxic waters (Wassersug and Seibert, 1975). This behavior has not been previously associated with *B. puncatus*, but could potentially reduce the metabolic demand of gill function and increase oxygen availability sufficient to reduce oxygen deprivation, thereby increasing thermal tolerance and survivability in hot ephemeral ponds. More research is necessary to support this hypothesis (see Future Work, Section 5.3.1).

## 5.2 Evaluation

Due to the abnormally dry spring and summer season in the year tadpoles were closely analyzed (2007), there was only sufficient rainfall to fill the ponds in the late summer, resulting in a few small clutches in late July and early August. In past years, eggs were deposited much earlier in the year (e.g., late April, the previous year, Figure 2.). In years when late spring eggs were deposited, there were also clutches as late as July or August. This multi-voltine tendency could present diverse thermal physiology between clutches that was unavailable for study in the year of the presented research.

Previous years also provided much larger clutch sizes, as a result, newly metamorphosed toads numbered in the thousands and were observed traversing substrates where surface temperatures may exceed 60° C. No surface temperatures recorded during the juvenile study approached such extremes. For this reason, I feel the juvenile data to be underestimated and in need of additional assessment. Additionally, the thermal tolerance of juveniles was not obtained as attempts to use righting response were unreliable as an indication of physiological thermal limit. Other assays could not be implemented, as the animals collected were likely to have become lab acclimated and field animals were quickly becoming less heat adapted as a function of early fall.

## 5.3 Future Work

### 5.3.1 Behavioral Methods of Reduced Respiratory Distress and Increased Thermal Stability in Late Stage Thermally Sensitive Tadpoles

Late stage tadpoles appear to be on the verge of their lethal thermal limits. However, development appears to continue through metamorphosis, even in high temperature pools. I hypothesize that late stage tadpoles must reduce the impact of high temperature by some behavioral means. Two distinct behaviors emerged in this study and seem heavily correlated with late stage, but seemingly not early stage, tadpoles. Both surface-skimming and basking-like behaviors appear to be demonstrated more frequently in late stage tadpoles, particularly in the heat of the day and in deeper murky pools. Future work is necessary to better quantify and qualify the frequency and occurrence of this behavior across development, season, oxygen availability, radiation levels, and water temperature. Other possible outcomes of this behavior and possibly surface skimming are increased or preferred food acquisition, increased solar radiation, or increased oxygen availability.

### 5.3.2 Heat Shock Response

Increased body temperature often results in denaturation of critical proteins. These modifications result in loss or alteration of function, eliciting an up-regulation of various protective mechanisms (Hochachka & Somero, 1984). In an effort to reduce the detrimental effects of thermal stress, cells often induce expression of proteins that function to maintain native protein structure (Hubbard and Sandler, 1991). These heat-shock proteins (Hsps), are members of a larger group of molecular chaperones that are essential in protein trafficking, folding, intracellular localization, assembly, secretion, regulation, and degradation in both stressed and content cells (Feder & Hoffmann, 1999; Hubbard and Sander, 1991). These highly conserved chaperones, and the genes that encode them (hsps), are nearly universal among all organisms and can be used as indications of stress.

Using Hsp70 as a marker for physiologic thermal stress, we can establish if toads are naturally enduring physiological stress and if this stress response changes across development. The comparison of Hsp70 profiles across developmental stages and season will allow a better understanding of thermal tolerance in Red-spotted toads, and may yield insight into thermal tolerance and stress of other desert organisms.

In order to assess the physiological consequences of ecologically relevant temperature exposures, I exposed eggs and tadpoles to temperatures of 35° C, 40° C, and 45° C, for one or two hours, and reared a subset to assess developmental time and possible abnormalities. A subset of eggs and tadpoles from each test group were snap frozen and stored at -80° C for later analysis of Hsp70 expression.

My preliminary data reveal that heat shock may facilitate development (Table 5.1). However, some eggs failed to develop and further work is required before definitive conclusions can be drawn. No egg or tadpole survived the 45° C thermal shock process. Eggs and tadpoles were reared at 15° C and 25° C to approximate cooler spring and warmer summer conditions. All embryos reared at 15° C, either did not hatch or died before metamorphosis. Due to sporadic and/or retarded hatching, survival (but not hatching) was determined for eggs reared at 15° C. Three different groups of eggs, from three late summer clutches were shocked and reared (designated Eggs 1; Eggs 2; and Eggs 3 (only shocked for one hour; Figure 5.3.1). Similar trends in survival persisted throughout trial groups and are presented in Table 2.1. A profile of one egg group is presented in Figure 5.3.2 (Eggs 1).

Surprisingly, control embryos typically died within only a few days of egg deposition. This event presented a problem in assessing the effect of a heat shock in respect to a non-treatment control. However, this striking die-off event suggests a developmental advantage for late summer *B. punctatus* experiencing a thermal shock early in

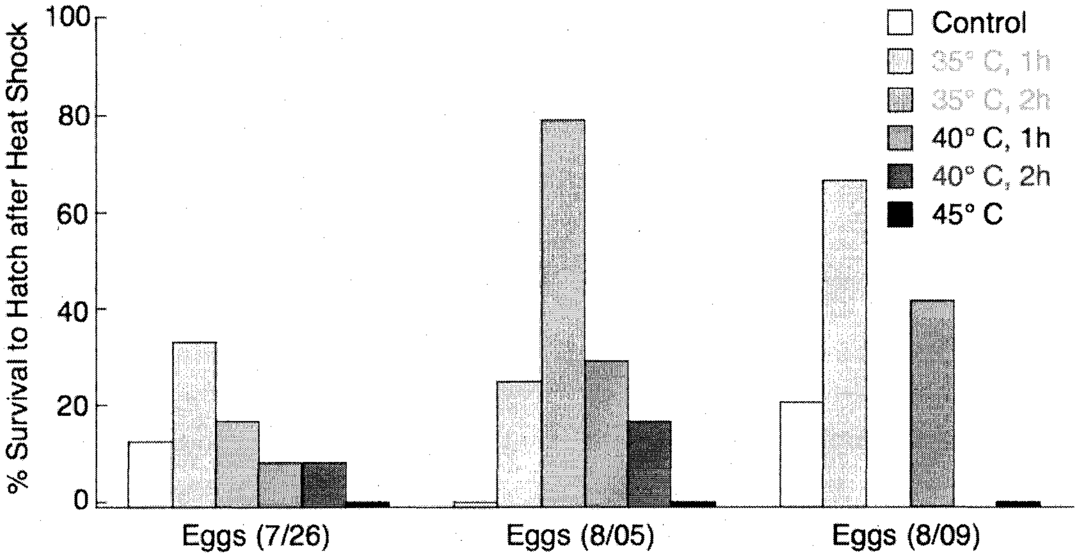

Figure 5.3.1 Percent survival of eggs to hatch after heat shock for three trial groups

Preliminary data suggest an advantage to being thermally shocked as eggs at 35° C. Unfortunately, a large die-off event occurred in every control group, disallowing analysis in comparison to the control. These data present many questions and will likely spur continued work on spring and early summer clutches.

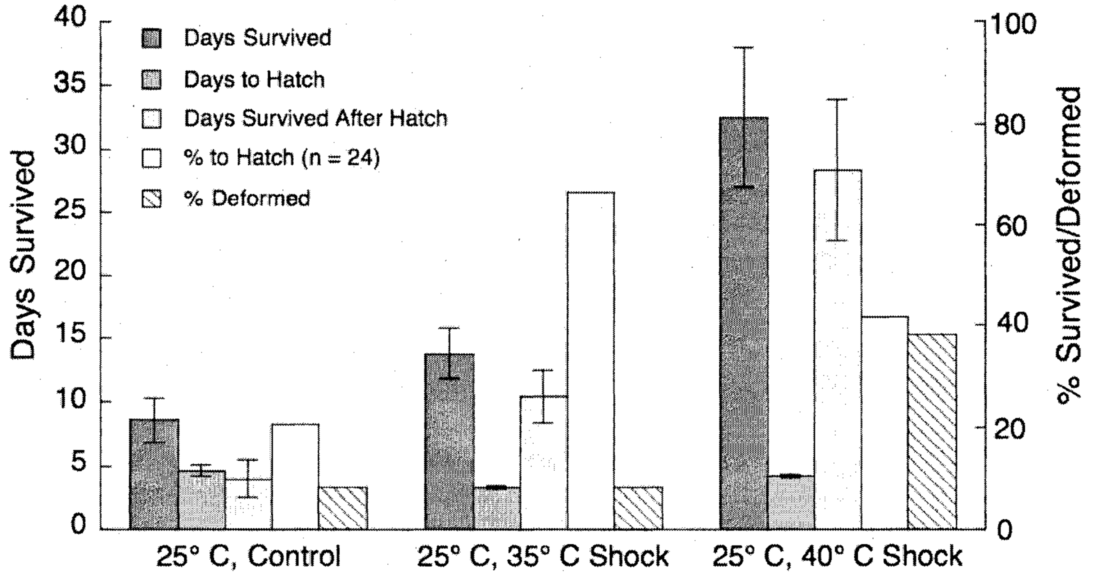

Figure 5.3.2 Average days survived beyond hatch and rates of deformity, of heat shocked developing embryos (eggs), reared at 25° C

The days survived, days to hatch and days survived after hatch are represented (reference left axis) for each heat shock group (n = 24) reared at 25° C (mean ± SE). The percent of individuals from each group to hatch is displayed in white and the percent of all individuals who exhibited deformity during development is represented by diagonal lines (reference right axis).

development. Of the embryos that developed at 25° C, the 35° C shocked individuals exhibited deformation (evidenced by bilateral asymmetry) in proportions equivalent to those demonstrated in the surviving control animals, 8.3%. In contrast, 40° C shocked eggs exhibited a 38% deformity rate (5.3.2).

Eggs shocked at 35° C or 40° C and reared at 25° C developed much faster and out lived control (no treatment) eggs (Table 5.1). In one experiment, no control eggs survived to hatch. However, in two other experiments, a few eggs developed to hatch, but died promptly. Eggs that had been heat shocked at 35° C showed the highest rate of survival to hatch. 40° C shock groups showed high hatch rates and similar times to hatch as 35° C shock groups, while having much longer survival times, but still none reached adulthood (Figures 5.3.2, 5.3.3, 5.3.4). There did not appear to be a clear advantage of 1-hour or 2-hour heat shocks. Some experiments demonstrated a survival advantage for 2-hour thermal shocks, while others exhibited an advantage after 1 hour.

Hatchlings (stage 23) and tadpoles (stage 35) shocked at 35° C demonstrated similar survival rates to controls. Hatchlings also exhibited similar survival rates following a 40° C shock, while stage 35 tadpoles did not survive the 40° C shock. Survival declined drastically in 40° C shocked hatchlings after a period of several days. Late stage tadpoles (> stage 39), all died during 35° C and 40° C heat shocks; this stage was not shocked at 45° C.

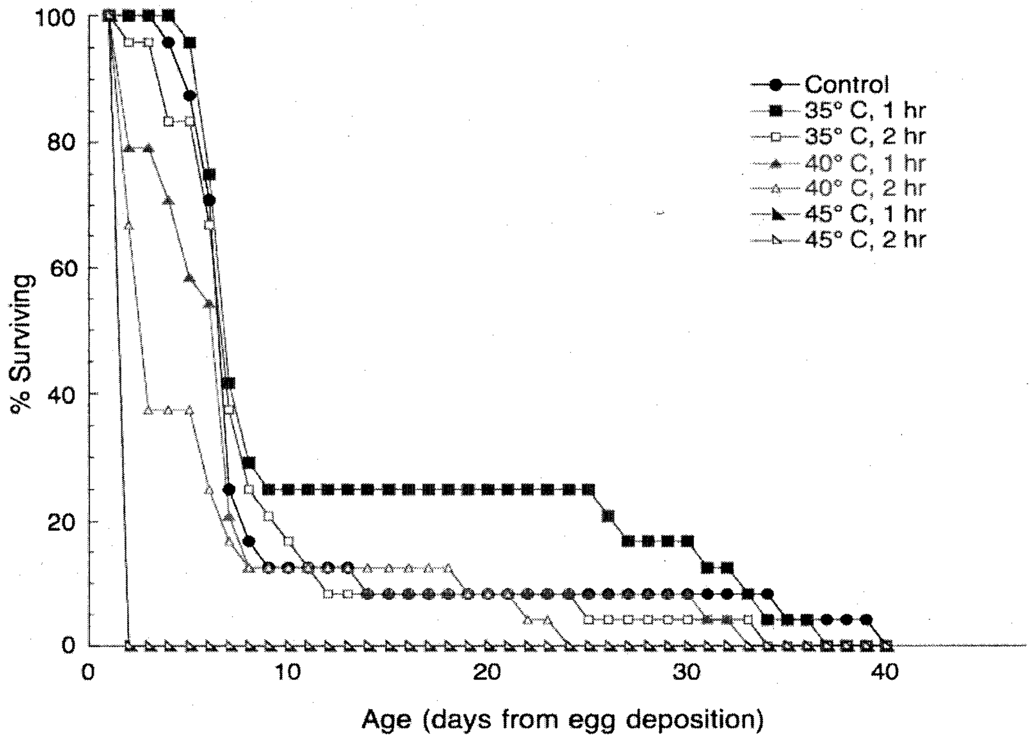

Figure 5.3.3 Percent survival of heat shocked developing embryos (eggs), reared at 15° C

Percent survival of developing embryos (eggs) after no treatment (control, closed black circles), after being heat-shocked at 35° C (squares), 40° C (triangles), or 45° C (right triangles), for 1 (closed shapes) or 2 hours (open shapes). Heat-shock was applied within 24-hours of egg deposition and no tadpoles had developed beyond embryonic stages. No eggs survived a thermal shock of 45° C, for any period. No eggs developed to metamorphosis.

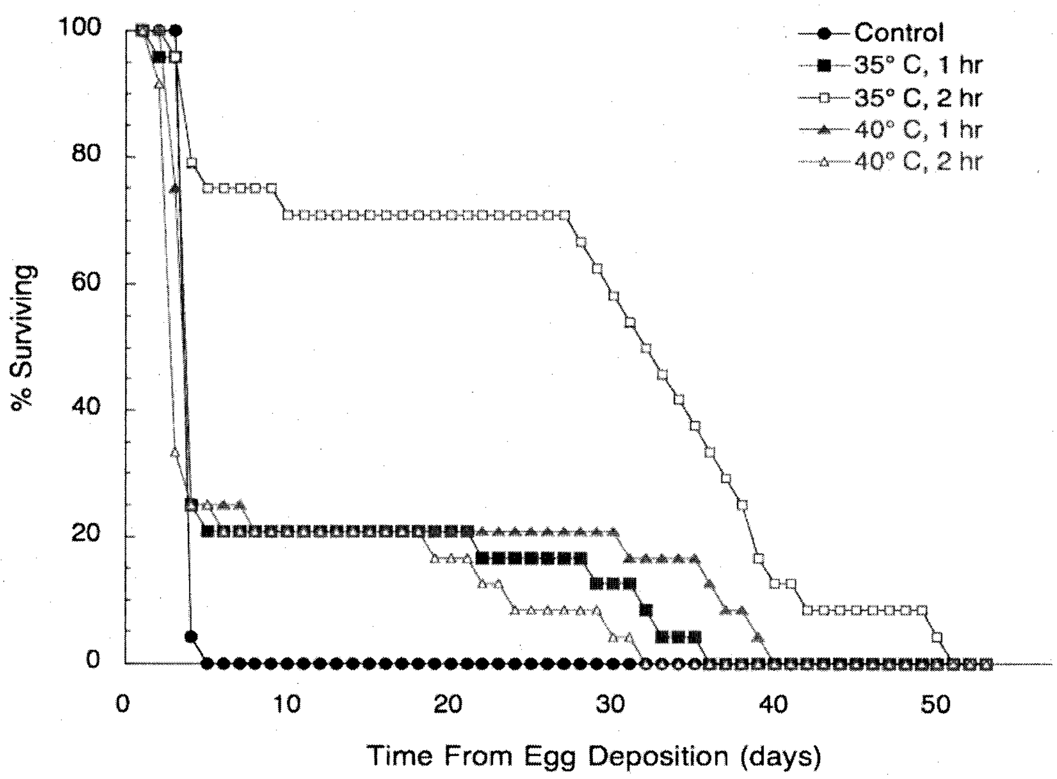

Figure 5.3.4 Percent survival of heat shocked developing embryos (eggs), reared at 25° C

Percent survival of developing embryos (eggs) after no treatment (Control, closed black circles), after being heat-shocked at 35° C (squares) or 40° C (triangles), for 1 (closed shapes) or 2 hours (open shapes). Heat-shock was applied within 24-hours of egg deposition and no tadpoles had developed beyond embryonic stages. Eggs were raised at 25° C, and were carefully observed to characterize time to hatch and survival after hatch. No eggs developed to metamorphosis.

### 5.3.3 Trade-offs of Water Loss, Drinking & Thermal Avoidance

Evaporative cooling is crucial to terrestrial staged anurans' maintenance of moderate body temperature. However, this cooling method does not come without cost. In order to acquire enough water for metabolism and cooling, amphibians must absorb water and have enough either available in the environment or stored in the bladder and tissues to cool the body to reasonable temperatures. Anurans acquire most of their body water through cutaneous absorption, this method of water uptake requires increase capillary dilation and substantial increases in blood flow across the seat patch (Viborg & Hillyard, 2005).

In future efforts, I would like to look at the physiological cost of water absorption using indirect calorimetry, and establish an energy budget model addressing the interplay between increased body temperature, water loss and cost of water uptake. At what body water will amphibians down-regulate water loss in the face of increasing body temperature?

# APPENDIX I

## SABLE SYSTEMS THERMAL ACTIVITY GRADIENT

The thermal gradient from Sable Systems Inc., Las Vegas, NV, was used in all thermal preference experiments. The gradient uses two independently operated Peltier devices, controlled by a dual Peltier regulator, to establish a linear thermal gradient along a 2 cm thick aluminum surface 66 cm x 6 cm (Sable Systems Inc., Las Vegas, NV, USA). 6 cm high Plexiglas walls, lined with 64 motion triggered LED light gates allow for observation of animals from the side and with a computer operated location logging device. Once the gradient was established, the temperature was measured at each of the gates.

The consistency of the gradient was verified between each trial of the experiment by measuring temperature at 8 cm increments. The temperature of each animal-selected location at the conclusion of the experiment was verified with a thermocouple. Location data were logged using a Linear Activity Detector, LAD (Sable Systems Inc., Las Vegas, NV, USA), interfaced with LadScan software (Sable Systems Inc., Las Vegas, NV, USA). When an individual LED beam was interrupted, the LAD transmitted a signal to LadScan corresponding to the location of the animal. All data of location and time spent were integrated in LadScan.

The gradient was covered to reduce stress to animals. Though accommodations were attempted to record the locations of smaller tadpoles, several trials were removed from the data set as the tadpoles failed to trigger the light gates on their own. Initially, thermal gradients of no more than 10 degrees were used, however the gradient proved so effective at forming linear gradients even at much broader thermal ranges, the gradient was set to allow animals the high and low limits of their preferences in a single gradient. This change in method also reduced edge effects to the point of being undetectable.

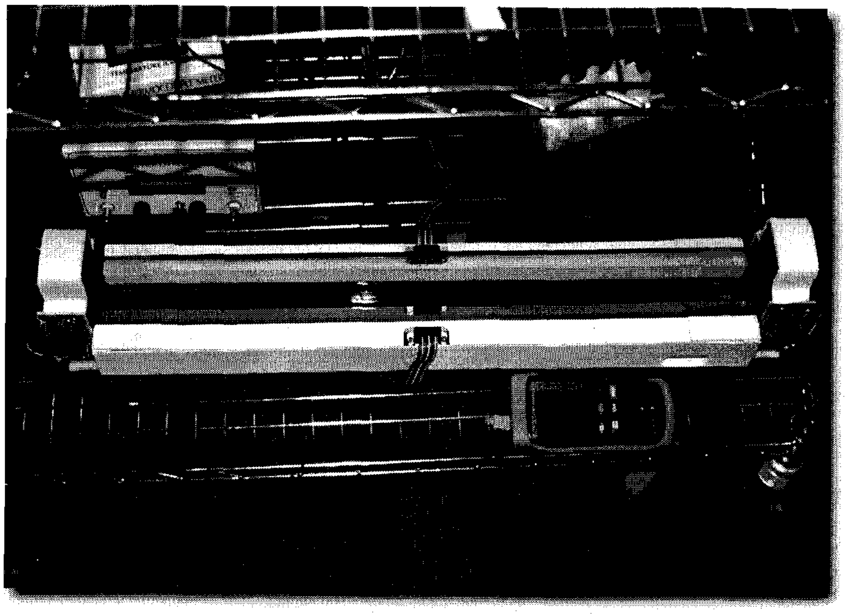

**Figure A.1 Sable Systems Thermal Gradient**

# APPENDIX II

## LIST OF ABBREVIATIONS AND ACRONYMS

$T_b$ – Body temperature
$CT_{max}$ – Critical Thermal Maxima
$T_p$ – Preferred body temperature
PBT – Preferred Body Temperature
$T_{air}$ – Air temperature
$T_a$ – Ambient temperature
$T_{water}$ & $T_w$ – Water temperature
$T_{water\ column}$ – Water temperature when measured mid water column
$T_{rock\ surface}$ – Rock surface temperature
TB – Total Body length, the length of a tadpole's body from snout to tip of tail
BL – Body Length, the length of a tadpole's body excluding tail, from snout to the base of tail musculature
DO – Dissolved Oxygen, the amount of oxygen dissolved in solution, often expressed by percent
$PO_2$ – Partial Pressure of Oxygen
h – Hour(s)
min – Minute(s)
ANCOVA – ANalysis of COVAriance
SE – Standard Error
n – sample size, number of individuals in a sample or trial

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