

2-2010

# Functional Consequences of Acute Temperature Stress in the Western Fence Lizard, *Sceloporus Occidentalis*

David Michael McMillan  
*University of Massachusetts Amherst*, [dmcmill@gmail.com](mailto:dmcmill@gmail.com)

Follow this and additional works at: [https://scholarworks.umass.edu/open\\_access\\_dissertations](https://scholarworks.umass.edu/open_access_dissertations)



Part of the [Ecology and Evolutionary Biology Commons](#)

---

## Recommended Citation

McMillan, David Michael, "Functional Consequences of Acute Temperature Stress in the Western Fence Lizard, *Sceloporus Occidentalis*" (2010). *Open Access Dissertations*. 156.  
[https://scholarworks.umass.edu/open\\_access\\_dissertations/156](https://scholarworks.umass.edu/open_access_dissertations/156)

This Open Access Dissertation is brought to you for free and open access by ScholarWorks@UMass Amherst. It has been accepted for inclusion in Open Access Dissertations by an authorized administrator of ScholarWorks@UMass Amherst. For more information, please contact [scholarworks@library.umass.edu](mailto:scholarworks@library.umass.edu).

FUNCTIONAL CONSEQUENCES OF ACUTE TEMPERATURE STRESS IN THE  
WESTERN FENCE LIZARD, *SCeloporus occidentalis*

A Dissertation Presented

by

DAVID MICHAEL MCMILLAN

Submitted to the Graduate School of the

University of Massachusetts Amherst

in partial fulfillment of the requirements for the degree of

DOCTOR OF PHILOSOPHY

February 2010

Program in Organismic and Evolutionary Biology

© Copyright by David M. McMillan 2010

All rights reserved

FUNCTIONAL CONSEQUENCES OF ACUTE TEMPERATURE STRESS IN THE  
WESTERN FENCE LIZARD, *SCeloporus occidentalis*

A Dissertation Presented

by

DAVID MICHAEL MCMILLAN

Approved as to style and content by:

---

Duncan J. Irschick, Chair

---

Stephen D. McCormick, Member

---

Bernard B. Rees, Member

---

Paul R. Sievert, Member

---

Joseph S. Elkinton, Director  
Organismic and Evolutionary Biology

## ACKNOWLEDGMENTS

I would like to begin by thanking my dissertation advisor, Duncan Irschick for his guidance and support throughout my graduate experience, first at Tulane University and then at the University of Massachusetts Amherst. Without his guidance and support, this dissertation would not have been possible.

I would also like to thank my collaborators, Barney Rees and Steve Adolph. I began working with Barney while still a student at Tulane during which time he welcomed me, and continues to welcome me, into his laboratory. He has challenged me to think about my research in new ways and many of the analyses I present here are a direct consequence of my many conversations with him. Without his support, both in time and equipment, I would not have been able to successfully complete my dissertation. Steve, whose continuous mentoring and logistical support while at Harvey Mudd every summer have allowed my trips to Southern California to be both productive and fun. I also extend my thanks to my other committee members, Steve McCormick and Paul Sievert who have provided me with valuable advice at crucial points in my graduate education and continue to be supportive with their time and effort.

I must also thank my fellow graduate students in Amherst and New Orleans. With his inquisitive nature, Justin Henningsen has been a valuable colleague in the lab and brewing companion in the kitchen. Philip Bergmann, with his ability to excel at any task to which he dedicates himself has been a valuable friend and discussion partner in the lab. Tara Massad and Kevin Simpson, whom I have known since my time in New Orleans have always been willing to house a visitor from the north with an open couch

and a friendly smile. Finally, Christine Buckley's assistance in the field and the laboratory has been crucial to my graduate career while her friendship has been valuable beyond measure.

I am also deeply indebted to my undergraduate advisors, Elizabeth Dahlhoff and Nathan Rank. Their willingness to invite me into their research program gave me the experience necessary to pursue a career as a scientist while also allowing me to experience the infinite beauty of the Eastern Sierra. Their commitment to their students, in the classroom and the laboratory is immeasurable and continues to serve as a model of inspiration for my career.

I am also thankful for the assistance of the many people who have helped me in the field and the lab: Michelle Staudinger, Simon Minor, Marc Badger, Hector Valtierra, Sunil Akkina, Ian Townley, and Elena Skripnikova. I am also thankful to John Smiley and the staff of the White Mountain Research Station in Bishop who has provided a home and lab for me year after year during my travels to California.

Finally, I must acknowledge the support of my family. My brother and sister, Christopher and Kristina, who always cheer me up and my parents, Richard and Kathy, who have taught me to not sweat the small stuff and have encouraged me to do what makes me happy and have always supported me as I have done so.

## ABSTRACT

### FUNCTIONAL CONSEQUENCES OF ACUTE TEMPERATURE STRESS IN THE WESTERN FENCE LIZARD, *SCELOPORUS OCCIDENTALIS*

FEBRUARY 2010

DAVID MICHAEL MCMILLAN, B.S., SANTA CLARA UNIVERSITY

Ph.D., UNIVERSITY OF MASSACHUSETTS AMHERST

Directed by: Professor Duncan J. Irschick

Understanding the effects of natural variation in environmental temperature on organisms and how those organisms evolve to live in different thermal environments is a central tenet of evolutionary physiology. Phenotypic differences among populations are the result of local adaptation, innate genetic differences between populations, and phenotypic plasticity, differential responses to the environment. Although not mutually exclusive, distinguishing between these paradigms can help illuminate species boundaries resulting from thermal limitations in physiology. For my dissertation, I examined geographic variation in measures of thermal physiology of the western fence lizard, *Sceloporus occidentalis* to understand the relative role of adaptation and acclimation in determining the thermal biology of populations of this species living in different thermal environments.

To achieve this goal I measured three indices of physiological function; body temperature, thermal tolerance and heat shock protein (Hsp70) abundance, across geographic and seasonal variation in temperature. Furthermore, I examined variation in sprint speed performance before and after heat stress and its relationship to Hsp70 to

determine if stress protein expression is a reliable indicator of whole organism physiological stress.

I found that geographic location can have a major effect on thermal physiology and performance in *S. occidentalis* in that thermal tolerance, Hsp70, and sprint speed varied with site and season with warmer southern sites typically more heat adapted than cooler northern sites. I also found a trade off in thermal tolerance suggesting that specialization to temperature was occurring in these lizards. Finally, lizards with increased Hsp70 were typically slower after heat stress indicating that Hsp70 is a reliable indicator of organism stress. Despite these findings, there was no difference in body temperature among sites and seasonal patterns in thermal tolerance suggest that during certain times of the year plastic responses to temperature may mask adaptive differences.

Here, I argue that temperature differences between sites has resulted in temperature adaptation at these sites, but that plastic responses to seasonal variation in temperature can become more important during certain times of the year. Although these relationships have been thoroughly studied in invertebrate organisms, further research should examine whether these patterns exist in other vertebrate ectotherm species.



**TABLE OF CONTENTS**

ACKNOWLEDGMENTS ..... iv

ABSTRACT ..... vi

LIST OF TABLES ..... ix

LIST OF FIGURES ..... x

I. GEOGRAPHIC VARIATION IN ENVIRONMENTAL TEMPERATURE AND ITS EFFECTS ON BODY TEMPERATURE IN THE WESTERN FENCE LIZARD, *SCELOPORUS OCCIDENTALIS* ..... 1

Abstract ..... 1

Introduction ..... 2

Methods ..... 7

Results ..... 11

Discussion ..... 12

Tables ..... 19

Figures ..... 22

II. PATTERNS OF GEOGRAPHIC VARIATION IN THERMAL TOLERANCE IN THE WESTERN FENCE LIZARD *SCELOPORUS OCCIDENTALIS* ..... 27

Abstract ..... 27

Introduction ..... 28

Methods ..... 32

Results ..... 37

Discussion ..... 39

Tables ..... 49

Figures ..... 52

III. GEOGRAPHIC VARIATION IN SPRINT SPEED AND HEAT SHOCK PROTEIN ABUNDANCE BEFORE AND AFTER HEAT STRESS IN THE WESTERN FENCE LIZARD ..... 58

Abstract ..... 58

Introduction ..... 59

Methods ..... 65

Results ..... 69

Discussion ..... 72

Tables ..... 78

Figures ..... 81

IV. CONCLUDING REMARKS ..... 85

BIBLIOGRAPHY ..... 90

## LIST OF TABLES

Table	Page
1. ANOVA results for variation in environmental temperature at four sites in the western United States.....	19
2. Summary of F-statistics for 2-way ANOVAs conducted on mean, minimum, and maximum daily temperatures measured at four study sites from June 2008 until September 2008 .....	20
3. Summary of F-statistics for ANOVAs conducted on field body temperature and preferred body temperature in <i>S. occidentalis</i> at four study sites .....	21
4. Two-way ANOVA showing the effects of site and season on (A) critical thermal maximum (CTMax) and (B) critical thermal minimum (CTMin) in <i>S. occidentalis</i> .....	49
5. Site and seasonal effects on thermal breadth in <i>S. occidentalis</i> using two-way ANOVA .....	50
6. Effects of collection year (1983 vs 2008) on CTMax and CTMin in <i>S. occidentalis</i> for the Joshua Tree site.....	51
7. Effects of site and time on mean maximum sprint speed for <i>Sceloporus occidentalis</i> before and after exposure to experimental heat stress Variation in sprint speed before and after heat stress .....	78
8. Effects of collection site and Hsp70 abundance on sprint speeds in <i>Sceloporus occidentalis</i> from four different sites in California.....	79
9. Effects of collection site on abundance of total and constitutive levels of Hsp70 in <i>S. occidentalis</i> after experimental heat stress .....	80

## LIST OF FIGURES

Figure	Page
1. Map of California showing locations of study sites in Eastern Sierra and San Gabriel mountain ranges.....	22
2. Variation in environmental temperature at <i>S. occidentalis</i> collection sites.....	23
3. Maximum, mean, and minimum daily temperatures during summer 2008 for each site used for this study .....	24
4. Relationship between ambient air temperature ( $T_A$ ) and body temperature ( $T_B$ ) of lizards collected in the field during 2007 and 2008 at each of the four study sites ...	25
5. Site differences in A) field body temperatures and B) preferred body temperatures for <i>S. occidentalis</i> at four sites in California.....	26
6. Site differences in A) critical thermal maximum (CTMax) and B) critical thermal minimum (CTMin) for <i>S. occidentalis</i> lizards measured during May / June and September 2008 .....	52
7. Site and seasonal differences in CTMax and CTMin in <i>S. occidentalis</i> from four California sites during early and late summer months.....	53
8. Correlations between Hsp70 abundance and A) CTMax and B) CTMin in <i>S. occidentalis</i> collected during May and June 2008.....	54
9. Correlation between CTMax and CTMin in <i>S. occidentalis</i> from four sites during May, June, and September 2008.....	55
10. Effects of site and season on thermal breadth (CTMax – CTMin) in <i>S. occidentalis</i> .....	56
11. Long-term changes in A) CTMax and B) CTMin in <i>S. occidentalis</i> from Joshua Tree during 1983 and 2008.....	57
12. Sprint speeds for <i>S. occidentalis</i> from four sites before and after exposure to experimental heat stress (40°C) or control temperature (20°C) for four hours.....	81
13. Mean maximum sprint speeds after experimental treatment and recovery period in <i>S. occidentalis</i> from four different sites.....	82

14. Abundance levels of Hsp70 in <i>S. occidentalis</i> from four sites in California.....	83
15. Correlation between total Hsp70 abundance and A) sprint speed at T3 and B) the amount of reduction in sprint speed between T1 and T3 .....	84

## CHAPTER I

### GEOGRAPHIC VARIATION IN ENVIRONMENTAL TEMPERATURE AND ITS EFFECTS ON BODY TEMPERATURE IN THE WESTERN FENCE LIZARD, *SCELOPORUS OCCIDENTALIS*

#### Abstract

One of the ongoing goals of evolutionary biology is to understand geographic variation in organism phenotypes along environmental gradients. Phenotypic differences among populations can reflect local adaptation, which results from genetic differentiation among populations, phenotypic plasticity, which results from phenotypic variation in a genotype along an environmental gradient, or, a combination of both local adaptation and phenotypic plasticity. Environmental temperature is one factor which is known to be highly variable across geographic space and has been shown to affect nearly all aspects of organism development and function in ectotherms, such as growth, feeding, respiration, locomotion, and reproduction. As a result, species with wide geographic distributions that encompass different thermal habitats may experience differing pressures from natural selection and may become locally adapted to their environment so that organismal function is locally maximized. Understanding whether individual populations are locally adapted can help us to better understand the thermal limits of individual species and how those species may respond in changing thermal environments. For this chapter of my dissertation I examined geographic variation in body temperature of the western fence lizard, *Sceloporus occidentalis*, which is distributed throughout the western United States across a wide range of thermal habitats. My goal was to explore whether or not temperature adaptation has occurred in this species. To do this, I measured field body

temperatures of *S. occidentalis* caught in the field as well as preferred body temperatures in the laboratory. In addition, I also placed thermal loggers at each of the collection sites to empirically test whether or not environmental temperature varied among sites. I found that air temperature was variable within each site both diurnally and seasonally and that there was significant differences in temperature across sites so that mean temperature decreased with increasing altitude and latitude. However, despite these differences in environmental temperature among these sites, there was little difference in lizard body temperatures from the field or the lab which provided little evidence to support my hypothesis that local adaptation has occurred in this species.

### Introduction

Organisms living in nature face physiological challenges due to constantly changing environments. The ability of an individual organism or a species to respond to these challenges can be a critical determinant of fitness. For widely distributed species, geographic variation in abiotic factors can lead to populations of organisms that are specifically suited to their local environment, either through behavioral modifications or genetic adaptations. However, these effects are not limited to differences between populations because individual populations can also experience diurnal or seasonal variation in environmental conditions that can create physiological challenging conditions that may impact fitness. Understanding the way organisms respond to environmental temperature variation can help us determine how species have come to occupy their current distributions and allow us to better predict future distributions in the face of a changing climate.

Temperature has been shown to affect virtually all aspects of organism function, especially in ectotherms (Clarke 2003). Previous studies on the effects of temperature in natural populations have found that variation in body temperature affects many organism processes, including respiration rates (Nielsen 1961, Seymour and Bradford 1995, Dahlhoff et al. 2001), growth and development rates (Conover and Present 1990, Adolph and Porter 1996, Buckley et al. 2007), food assimilation rates (Ji et al. 1996), offspring sex (Janzen and Paukstis 1991), as well as frequency and intensity of social displays (Brooke et al. 2000, D. McMillan, personal observation). In addition, temperature has been shown to have a profound effect on components of fitness such as survival (Portner 2001a, McMillan et al. 2005, Huang et al. 2006), fecundity (Watt 1992, Dahlhoff et al. 2008), predator escape (Hertz et al. 1982, Bergmann and Irschick 2006), and locomotion (Bennett 1980, Angilletta et al. 2002a, Lailvaux and Irschick 2007, Rank et al. 2007). Finally, environmental temperature has been shown to closely correlate with body temperature in ectotherms, (Dahlhoff and Rank 2000), and examining populations of the same species over a thermal gradient can give us a picture of thermal evolution at work.

The distribution of a species is often defined by geographic variation in temperature across latitudes and altitudes (Pagel et al. 1991, Clarke 1996, Parmesan et al. 1999, Pounds et al. 1999, Austin 2002). Populations living near the latitudinal or altitudinal edge of a distribution may be more likely to experience physiological stress due to a greater frequency of extreme temperatures relative to centrally distributed populations. However, the effects of extreme temperatures are not limited to edge populations. Centrally located populations can also experience thermal stress due to diurnal or seasonal variation in environmental temperatures (Kingsolver and Watt 1983).

When faced with these stressful temperatures, a population of organisms can respond in one of three ways. The first way is to limit exposure to stressful temperatures through behavioral modification of microhabitat use (Adolph 1990, Asbury and Adolph 2007) or by migration to a new habitat (Parmesan et al. 1999, Davis and Shaw 2001). The second option is for populations to become physiologically adapted to environmental temperatures in a way that minimize the effects of extreme temperatures (Somero 1978, Ayres and Scriber 1994, Dahlhoff and Rank 2000, Hochachka and Somero 2002). These approaches are not mutually exclusive and natural populations may actually implement both strategies to various degrees. However, for any population that is unable to effectively minimize stress from the thermal environment face a third option, the possibility of extinction (Hochachka and Somero 2002, Sorensen et al. 2003).

One way in which temperature has been shown to affect organisms in natural environments is through its effects on cellular physiology. These effects include changes in the viscosity of cellular and organelle membranes, which become leaky at high temperatures and rigid at low temperatures. These membrane changes can alter diffusion rates across the membrane, effectively increasing the amount of cellular energy (ATP) necessary to maintain homeostasis (Hazel 1995, Hochachka and Somero 2002). Furthermore, extreme temperatures can also lead to a reduction in the binding efficiency of many enzymes, which are only marginally stable at normal physiological temperatures due to a balance of stabilizing and destabilizing interactions (Jaenicke 1991). These increased ATP needs coupled with a decreased ability to store and utilize energy can result in diminished performance at higher-level organism functions such as feeding, reproduction, and locomotion (Dahlhoff and Rank 2000, Portner 2001b, Biewener 2003).



Organisms or populations living in environments which are frequently stressful may thus accumulate substitutions in amino acid sequences and changes in membrane permeability which help to maintain overall thermal physiology (Dahlhoff and Somero 1993, Fields and Houseman 2004, Elderkin and Klerks 2005, Dong and Somero 2009). Populations of species that are distributed over large, thermally variable, geographic areas may experience differing frequencies of stressful temperatures and as a result, differing selection pressures. Over evolutionary time, populations can become genetically distinct as they become adapted to local environmental temperatures (Duman et al. 1991, Hochachka and Somero 2002, Dahlhoff et al. 2008).

Recently, growing concerns over the effects of climate change have renewed interest in understanding the effects of temperature variation in natural populations. Historically, in addition to physiological adaptations, populations have responded to climatic changes by poleward shifts in distribution (Parmesan et al. 1999, Davis and Shaw 2001, Pearson and Dawson 2003, Parmesan 2006). However, current anthropogenic barriers can prevent population migration and can restrict range shifts as an option for populations living in extreme environments (Parmesan 2006). Current models of climate change predict that rates of the global increase in temperature will be greater in the near future (Intergovernmental 2007), which may further limit the ability of species to migrate and accelerate the rate of species loss (Midgley et al. 2001, Hooper et al. 2005, Thuiller et al. 2005). In addition, at smaller scales, such as high latitudes or altitudes, this problem may be exacerbated and there is the potential for greater changes in environmental temperature (Walther et al. 2002). Given these predictions, it is especially important to understand the limits of populations' ability to respond to extreme

environmental temperatures. Knowing these limits will help to identify those populations that are more likely to be sensitive to rapid fluctuations in temperature and allow us to take appropriate actions to mitigate the effects of these changes (Dahlhoff and Rank 2000, Hochachka and Somero 2002, Helmuth et al. 2005, Parmesan 2006, Chown and Terblanche 2007, Chown et al. 2009).

The study of thermal adaptation in natural populations is especially useful in ectotherms because most species must utilize environmental temperatures to maintain body temperature and, as a result, virtually all aspects of physiology, behavior, and performance (Huey and Slatkin 1976, Angilletta et al. 2002b). Due to the critical nature of body temperature for an ectotherm and the physiological effects of temperature in determining organism performance, it is reasonable to expect that over evolutionary time variation in preferred body temperature will mimic thermal heterogeneity of the environment such that organisms living in warmer habitats will have higher body temperatures relative to individuals from cooler environments, but that performance capacities should be maintained across populations at environmentally appropriate temperatures (Dahlhoff and Somero 1993). However, support for this hypothesis has been mixed; suggesting that variation in body temperature is due to environmental or behavioral changes as well as adaptive differences between populations (Hertz et al. 1982, Hertz et al. 1993, Angilletta et al. 2002b, Youssef et al. 2008).

The western fence lizard, *Sceloporus occidentalis*, is ideal for studies of the effects of temperature variation because this species is widely distributed throughout western North America, from Washington State to northern Mexico. This species occupies many different habitats, from semi-dry scrub to riparian areas to forested areas,

at elevations from sea level to approximately 2500 m (Behler 2000, Stebbins 2003). Adults average 60-90 mm in snout-vent length (SVL) and are semi-arboreal habitat generalists, utilizing several substrates as perches, from boulders to trees (Adolph 1990, Asbury and Adolph 2007). Previous studies in this species have shown links between body temperature and organismal traits such as development rate, morphology, growth rate, microhabitat use, and sprint speed (Adolph 1987, Sinervo and Adolph 1989, Adolph 1990, Asbury and Adolph 2007, Buckley et al. 2007).

With this study, I extend upon previous work by examining whether geographic variation in environmental temperature has resulted in population-level differences in body temperature in *S. occidentalis*. Specifically, I wish to address the following questions. First, do measures of environmental temperature, such as mean, maximum, and minimum temperature vary with altitude and latitude across collection sites of *S. occidentalis*? This first step is necessary to show that it is in fact temperature adaptation that is occurring in this species. Second, if environmental temperatures do vary, do body temperatures of *S. occidentalis* vary in a manner that is consistent with environmental temperature? A better understanding of the effects of geographic variation in environmental temperature within this species can help us to better predict the effects future climate change will have in this and other species.

## Methods

Site descriptions and temperature profiles:

To examine the effects of geographic variation in temperature on *S. occidentalis*, I measured body temperatures of lizards caught in the field as well as preferred body

temperature in the lab. Individuals were collected at 4 locations in California: 1) Bishop Creek (BC), 7.9 km southwest of Bishop, Inyo County (37°17'34"N, 118°33'05"W, Elev. 2135 m); 2) Keough Hot Springs (KH), 12.5 km south of Bishop, Inyo County (37°15'4"N, 118°22'20"W, Elev. 1220 m); 3) Table Mountain (TM), 3.5 km northwest of Wrightwood, San Bernardino County (34°22'43"N, 117°39'33"W, Elev. 2230 m); and 4) Joshua Tree (JT), 11.2 km west of Pinion Hills, Los Angeles County (34°26'30"N, 117°45'42"W, Elev. 1370 m). At these sites, lizards are typically active from mid- April until September or October (Adolph 1990, Asbury and Adolph 2007). These four sites occur at paired latitudes and altitudes, with both a low and high elevation represented at each latitude (Figure 1) and were chosen because of predicted differences in environmental temperature at each site (Jacobson 2005).

To test for differences in thermal profiles at each site I placed a HOBO pendant logger (Onset Computer Corp, Pocasset, MA) at each site from August 2005 until September 2008. The placement of each logger was standardized across sites to best measure the variation in air temperatures between the study sites. All loggers were housed in 350 ml inverted white plastic cups punched with 1-mm diameter holes to minimize heating due to direct radiation. Cups were suspended in a tree at each site approximate 75 cm above the ground in a location that was selected to be approximately equal in exposure to direct sunlight across all sites. The loggers measured ambient air temperature every 20 minutes to an accuracy of  $\pm 0.1^{\circ}\text{C}$ . Air temperatures at the TM logger were not recorded after June 2007 due to a brush fire that destroyed the logger. As a result, a second logger was placed at the Table Mountain site (as well as each of the

other sites) to measure air temperature during the summer of 2008. All loggers were removed from the field during September 2008 and downloaded for analysis.

#### Animal care and field body temperature measurements:

Adult male and female individuals of *S. occidentalis* (N=227) were collected by noose during late May and early June in 2007 and 2008 and again in September 2008 at all four locations (46 to 68 lizards per site). All individuals were collected between 0800 and 1400 hours. Upon capture, field body temperature (T<sub>b</sub>) was measured using a cloacal thermometer (Miller and Webber, Inc.) within 5 seconds of capture, and mass was measured using a spring scale (Pesola, Baar, CH). Of the total number of lizards collected during May and June 2008, a random sample (N = 85) were returned to the lab within 8 hours of capture for measurement of preferred body temperatures. In the lab, lizards were housed individually in custom-made 25 L enclosures with sand substrate and a rock perch. Heat lamps (125 W) were suspended above one end of each cage to allow for thermoregulation and attached to a digital timer to create a diurnal cycle (12 hours light: 12 hours dark). Water was provided *ad libitum* using a small dish in the cage and a garden sprayer. Crickets dusted with vitamin powder were provided as food every other day.

#### Preferred body temperature measurements:

Preferred body temperature was measured using custom-made wood enclosures within 5 days of capture. Enclosures measured 40.5 cm x 40.5 cm x 122 cm and contained 2 cm of sand as a substrate. A single 250 W infrared bulb was suspended

16cm above the substrate at one end of the enclosure to allow lizards to thermo regulate. These enclosures generated a thermal gradient from 25° C - 50° C and have been used in previous studies on this species (Buckley et al. 2007). Lizards were placed in the enclosure and not disturbed for two hours to allow time for a preferred body temperature to be reached. At the end of two hours, body temperature was measured using a cloacal thermometer.

#### Statistical Analysis:

All statistical tests were conducted using JMP statistical software (version 8.0, SAS Institute, Cary, NC). Mean ambient temperatures for each 4 hour interval were calculated for all temperature data (6 per day) and used for subsequent analysis. To test for overall site differences in air temperature across multiple months and years I performed an ANOVA to test the effects of site, year, month, day, and time interval on environmental air temperature. Interactive effects could not be tested because there was insufficient replication in months and years as a result of the lost Table Mountain logger.

In addition to testing for overall site differences in ambient temperature, I also tested for differences among sites in mean, maximum, and minimum daily temperature within a single summer (June 2008 to September 2008, the only summer for which air temperature measurements at all sites were complete) using the secondary loggers placed at each site after the fire at Table mountain. I used ANOVA to test for the effects of month and site on differences in daily mean, maximum, and minimum temperatures at each site. Finally, to test for site effects on body temperature for lizards in the field and

preferred body temperatures in the lab I used ANOVA with site as a fixed factor and either field or preferred body temperature as the response variable.

## Results

Temperature variation across sites:

Air temperature was highly variable across sites and seasons (Figure 2A). However, despite seasonal variation, mean temperatures were consistently higher at both Joshua Tree and Keough Hot Springs than at either Table Mountain or Bishop Creek (Figure 2B, table 1). Mean temperature at Joshua Tree was highest overall, followed by Keough Hot Springs and then Table Mountain. Finally, Bishop Creek was the coolest site during most months and had the lowest mean temperature of all sites (Figure 2, table 1).

A similar pattern was observed for differences in daily site temperature within a single season (summer 2008). Maximum daily temperature was higher at both of the low elevation sites, Joshua Tree and Keough Hot Springs, than it was at either of the high elevation sites (Figure 3A, Table 2). Mean daily temperature exhibited a similar pattern (Figure 3B, Table 2). However, minimum daily temperature exhibited a different pattern: Minimum daily temperatures were colder at the high elevation sites but the magnitude of the difference between high and low elevation sites was smaller for minimum temperature relative to either maximum or mean daily temperature (Figure 3C, and Table 2).

### Geographic variation in body temperature:

Despite the range of environmental temperatures present at each of the four sites, lizard body temperatures did not correlate with measurements of environmental air temperature measure at time of capture for most of the sites (Figure 4). The only exception was Bishop Creek, where lizard body temperatures were positively correlated with ambient temperatures ( $T_B = 0.1182 * T_A + 31.37$ ,  $p < 0.0002$ ,  $R^2 = 0.14$ ). The lack of a relationship between body temperature and ambient air temperature at these sites (or slight, albeit significant relationship at Bishop Creek) is due to the ability of *S. occidentalis* to maintain body temperature from 30 - 40°C over corresponding air temperatures from 9 - 43°C. Furthermore, there were no observed differences among sites in field body temperatures for Bishop Creek, Table Mountain or Joshua Tree. Yet, lizards collected at Keough Hot Springs had a lower body temperatures relative to the other three sites ( $F_{3,223} = 19.88$ ,  $p < 0.0001$ , Figure 5A). However, there were no observed differences in preferred body temperatures among the three sites ( $F_{3,81} = 0.26$ ,  $p = 0.851$ , Figure 5B).

### Discussion

In this study, I found differences in environmental temperature across sites but not a corresponding difference in preferred body temperature of *S. occidentalis*. There was a difference in body temperatures of field-caught lizards, but that was due to lower body temperatures at one site (Keough Hot Springs). During the course of the study, mean monthly temperatures were highest at Joshua Tree, the southern desert site, and lowest at Bishop Creek, the northern mountain site. This difference in temperature between the



sites persisted despite seasonal fluctuations in temperature. Monthly mean, maximum, and minimum temperatures during the summer months of 2008 showed a similar pattern in that Joshua Tree was the warmest and Bishop Creek was the coolest. However, these differences in environmental temperatures did not translate into differences in lizard body temperature, either in the field or preferred temperatures in the lab.

Geographic variation in air temperature:

Environmental temperature showed significant variation across the four sites chosen for this study. This supports the hypothesis that these populations of *S. occidentalis* across different altitudes and latitudes experience different thermal environments making them ideal to study the potential for temperature adaptation. Also, differences in temperature between the sites matched predictions for geographic variation in temperature, decreasing with latitude and altitude. The environmental lapse rate is one predictive model that accounts for altitudinal changes in temperature across different latitudes (Jacobson 2005). According to this model, differences in environmental temperature between low altitude and high altitude locations is expected to decrease with latitude with a global average of  $6.49^{\circ}\text{C} \cdot 1000 \text{ m}^{-1}$  in elevation change (Glickman 2000, Jacobson 2005). The findings for this study were consistent with the model in that temperature at both the northern and southern population decreased with altitude at a rate of  $6.6^{\circ}\text{C} \cdot 1000 \text{ m}^{-1}$  and  $4.3^{\circ}\text{C} \cdot 1000 \text{ m}^{-1}$  respectively.

There is a long history of research into the effects of temperature on organismal processes in a wide range of organisms (Beuchat 1988, Burger 1991, Ackerman 1994, Loeschke et al. 1994, Smith and Ballinger 1994, Pounds et al. 1999, Addo-Bediako et al.

2000, Andrews et al. 2000, Hansen and Petersen 2001, Hare et al. 2004, Huang et al. 2006, Lailvaux 2007). In addition, temperature has been shown to be highly variable in nature, especially over small areas in structurally complex environments (Pagel et al. 1991, France 1992, Helmuth et al. 2006). However, many studies that examine geographic variation in temperature adaptation often assume temperature variation between sites and fail to empirically measure whether or not a difference actually exists (Gaston and Chown 1999, Addo-Bediako et al. 2000). The data presented here show that temperature varies across these sites, making them ideal to examine the potential effects of temperature adaptation.

Initial predictions about temperature variation across the sites were that temperature would decrease with increasing altitude and latitude within this part of the state of California. Due to the inclusion of only four sites in this study, it was not possible to directly test the effects of latitude and altitude on temperature. However, the thermal profiles for the four sites included in this study support this idea because temperature decreased from the southern to northern sites and from the low altitude to high altitude sites. Furthermore, across these sites, altitude was a greater determinant of environmental temperature, with the desert sites being warmer than either of the mountain sites. It is important to note that there is a possibility that these differences in environmental temperature between sites are the result of a statistical artifact that is a byproduct of studying large sample sizes (Sokal and Rohlf 1997, Zar 2009). However, this is not likely to be the only explanation because the pattern observed here, decreasing temperature with increasing latitude and altitude, is consistent with previous research which has found similar results (Stouffer et al. 1994, Hughes 2000, Smith and Reynolds

2003). Using this information of geographic differences in temperature at these sites can help us understand whether adaptation is occurring in *Sceloporus occidentalis*.

Geographic variation in body temperature:

The role of body temperature in maintaining organismal function in many ectotherms necessitates that these species are able to regulate their body temperature in highly variable environments. Previous work in *S. occidentalis* has shown that these lizards are able to precisely regulate its body temperature within a structurally complex habitat across a range of environmental temperatures (Adolph 1990, Asbury and Adolph 2007). The present study builds upon previous research by extending these findings to multiple environments. At three out of the four sites there was no relationship between lizard body temperature and ambient air temperature at the time of capture. The exception was Bishop Creek, where there was a positive relationship between body temperature and air temperature. Previous studies in other lizards have found that ectotherms at higher altitudes and latitudes have shorter activity periods due to limited availability or duration of adequate thermal environments (Wilson 1991, Morrison and Hero 2003, Sears 2005). The cooler temperatures at Bishop Creek coupled with the potential for shorter activity times may mean that fewer lizards are able to achieve preferred body temperature during thermoregulation, or are able to reach these temperatures less frequently. Further studies should incorporate measurements of thermoregulatory behavior to determine whether high-latitude and high-altitude individuals spend a greater proportion of their time thermo regulating relative to other sites, which would further support this hypothesis.

I also found no difference among the sites in preferred body temperatures of *S. occidentalis*. Previous studies in other lizards have found that aspects of thermal physiology, such as preferred body temperature, optimal body temperature, and critical thermal limits, are important for organismal performance. These measures are often conserved across populations of the same species and between closely related species, even across continental boundaries in similar environmental conditions (Angilletta et al. 2002b, Youssef et al. 2008). However, although there is no difference among sites in preferred body temperature for *S. occidentalis*, other research on this species has shown that critical thermal limits for *S. occidentalis* vary with both altitude and latitude with changes in environmental temperature (Chapter 2). The observed decoupling between critical thermal limits and preferred body temperature may be due to the frequency at which individual populations are exposed to extreme temperatures. Preferred body temperature has been shown to be maintained across thermally heterogeneous environments, whereas critical thermal limits can vary between similar populations that vary in the frequency of exposure to extreme temperature (McGinnis 1966, Greer 1980). This means that for a population that migrates to a new habitat, brief periods of extreme temperature can alter critical thermal limits while preferred body temperatures remain unchanged. However, the adaptive relationship between preferred body temperature and critical thermal limits has not been tested within a single species and requires further investigation.

Although preferred body temperature of lizards from the different sites did not vary, field body temperatures of lizards caught at Keough Hot Springs were lower than body temperatures of lizards from the other sites. Body temperatures of individuals from

natural populations have been shown to vary diurnally with environmental temperature in several species of ectotherms (Mellette et al. 1951, Dahlhoff and Rank 2000, Sabo 2003, Sinclair et al. 2006). It could be argued that site differences in body temperature observed for *S. occidentalis* are the result of diurnal variation in temperature. Yet, collection times did not significantly vary between the sites. In addition, lizard body temperature at Keough Hot Springs was not correlated with ambient air temperature. Furthermore, body temperatures of field-caught lizards were highly variable and the observed differences in field body temperatures between the sites may be a result of two individual lizards from Keough Hot Springs that had lower body temperatures than any other individuals caught for this study (Figure 5). There was no *a priori* reason to exclude these individuals, which appear as statistical outliers, so they were included in the analysis. Additional sampling at all sites may help to illustrate whether body temperatures among the sites persist or whether or not the observed results are a consequence of these two individuals.

One interesting result of this study was that lizards were able to maintain relatively consistent body temperature over such a wide range of environmental temperatures. Lizards from the low elevation sites (KHS and JT) were effectively able to maintain a relatively constant body temperatures between 32°C and 35°C at both warmer and cooler ambient temperatures. This is not surprising, and has been reported in previous studies (Asbury and Adolph 2007). However, at the high-elevation sites, although no lizards collected had body temperatures above 25°C, some individuals were able to maintain high body temperatures even when environmental temperatures were as low as 9°C. Given that lizards collected at Bishop Creek had on average cooler body

temperatures than lizards collected at other sites, and that Bishop Creek is the only site that showed a significant relationship between body temperature and environmental air temperature, it is possible to infer that the thermal boundary for *S. occidentalis* to maintain a consistent body temperature is between 9 - 15°C. Further work is necessary to verify this hypothesis, which may be useful to predict current or future range limits for *S. occidentalis*.

I tested the ambient air temperature as well as preferred and actual body temperatures in *S. occidentalis* and found that although ambient temperature varied among the sites, variation in lizard body temperature was not consistent with the variation in environmental temperature. This finding suggests that if temperature adaptation has occurred in this species, it has not resulted in behavioral changes in thermoregulatory behavior in *S. occidentalis* lizards.

## Tables

Table 1: ANOVA results for variation in environmental temperature at four sites in the western United States. Sites included Bishop Creek, Keough Hot Springs, Table Mountain and Joshua Tree. Only main effects were included due to insufficient replication at Table Mountain during 2007 and 2008.

Source	df	SS	F
Site	3	189180.8	2598.5****
Year	3	5410.7	74.32****
Month	11	1452071.7	5439.48****
Time Interval	5	526525.4	4339.33****
Day of month	30	6989.5	9.6****
Error	24230	595008.5	

\* $p < 0.05$ , \*\* $p < 0.01$ , \*\*\* $p < 0.001$ , \*\*\*\* $p < 0.0001$

Table 2: Summary of F-statistics for 2-way ANOVAs conducted on mean, minimum, and maximum daily temperatures measured at four study sites from June 2008 until September 2008. Min = minimum daily temperature, Mean = mean daily temperature, Max = maximum daily temperature.

Source	df	Min	Mean	Max
Site	3	50.2****	174.9****	146.0****
Month	3	46.7****	55.3****	16.9****
S x M	9	6.1****	4.9****	4****
Error	401			

\*p<0.05, \*\*p<0.01, \*\*\*p<0.001, \*\*\*\*p<0.0001



Table 3: Summary of F-statistics for ANOVAs conducted on field body temperature and preferred body temperature in *S. occidentalis* at four study sites.

Field Body Temperature				
Source	df	SS	F	P
Site	3	194.80	19.88	<0.0001
Error	223	728.40		

Preferred Body Temperature				
Source	df	SS	F	P
Site	3	3.26	0.26	0.851
Error	81	333.70		

Figures

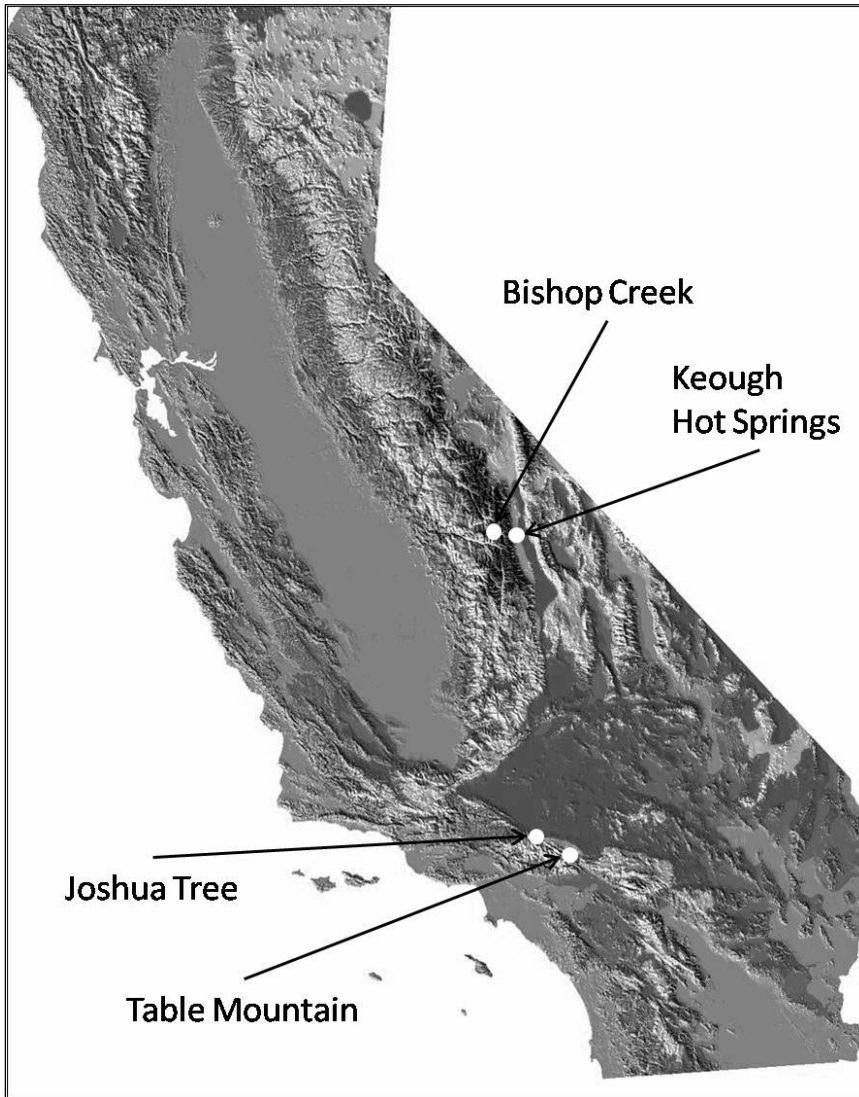


Figure 1. Map of California showing locations of study sites in Eastern Sierra and San Gabriel mountain ranges. At each latitude, one site is at a high elevation (Bishop Creek and Table Mountain) and one site is at a low elevation (Keough Hot Springs and Joshua Tree). Map reproduced courtesy of USGS.

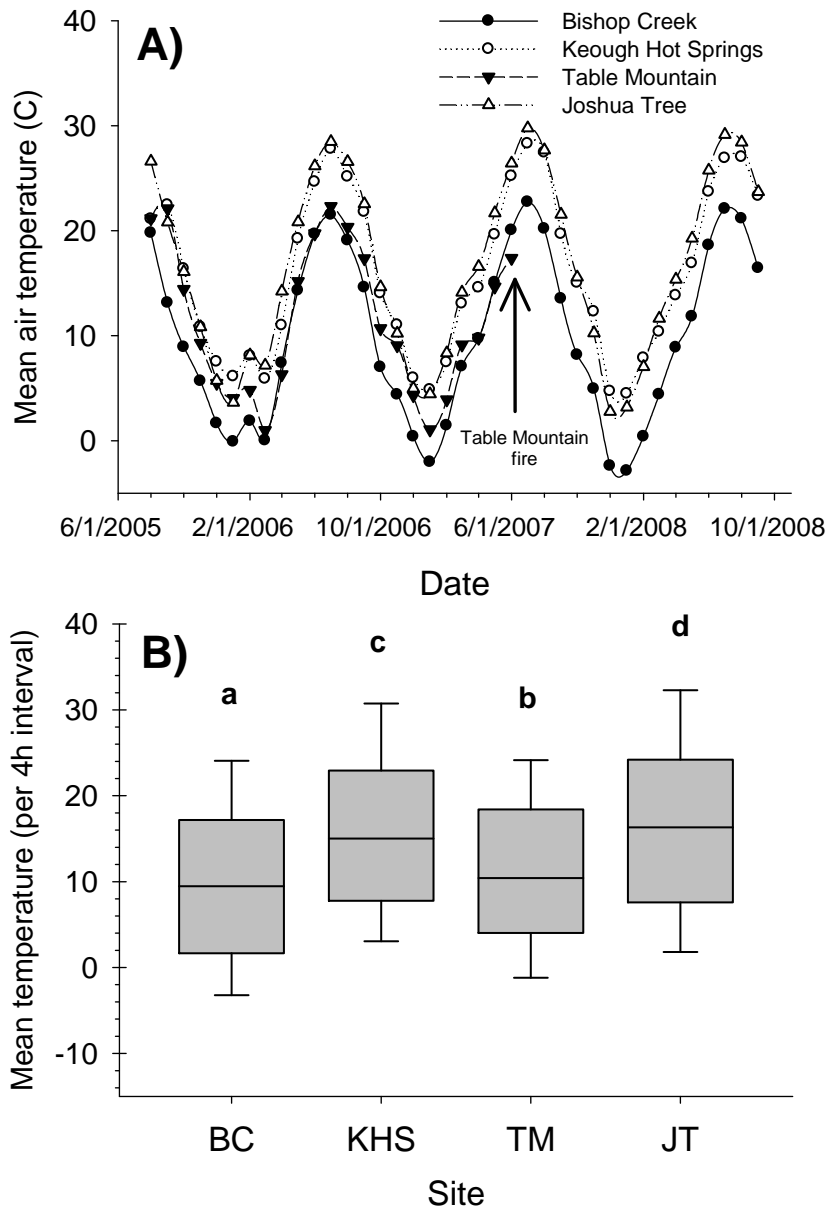


Figure 2. Variation in environmental temperature at *S. occidentalis* collection sites. A) Site variation in monthly mean temperatures. Points represent monthly mean temperature (°C) connected by a smoothing spline for each site from August 2005 until October 2008. B) Box and whisker plots for mean interval temperature. Boxes represent the median, lower and upper quantiles. Whiskers represent the 10<sup>th</sup> and 90<sup>th</sup> percentile. Letters represent significant differences. See Table 1 for statistical analysis.

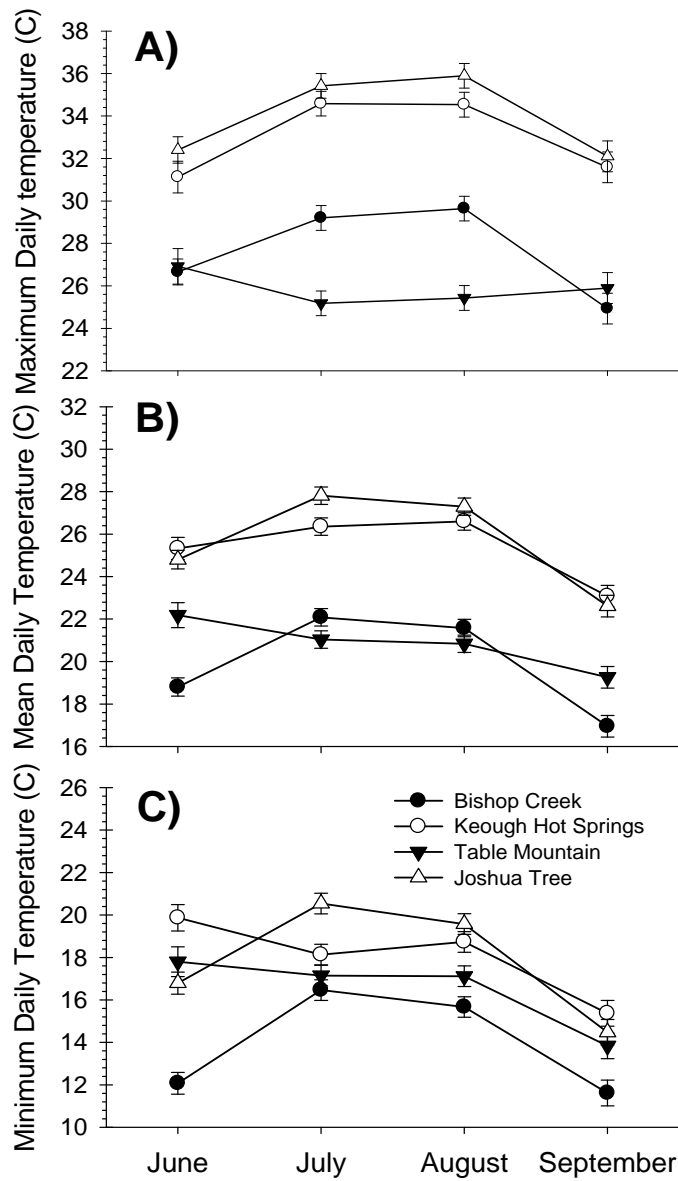


Figure 3. Maximum, mean, and minimum daily temperatures during summer 2008 for each site used for this study. Data points represent monthly values ( $\pm$ SE) in  $^{\circ}$ C. See Table 2 for statistical analysis.

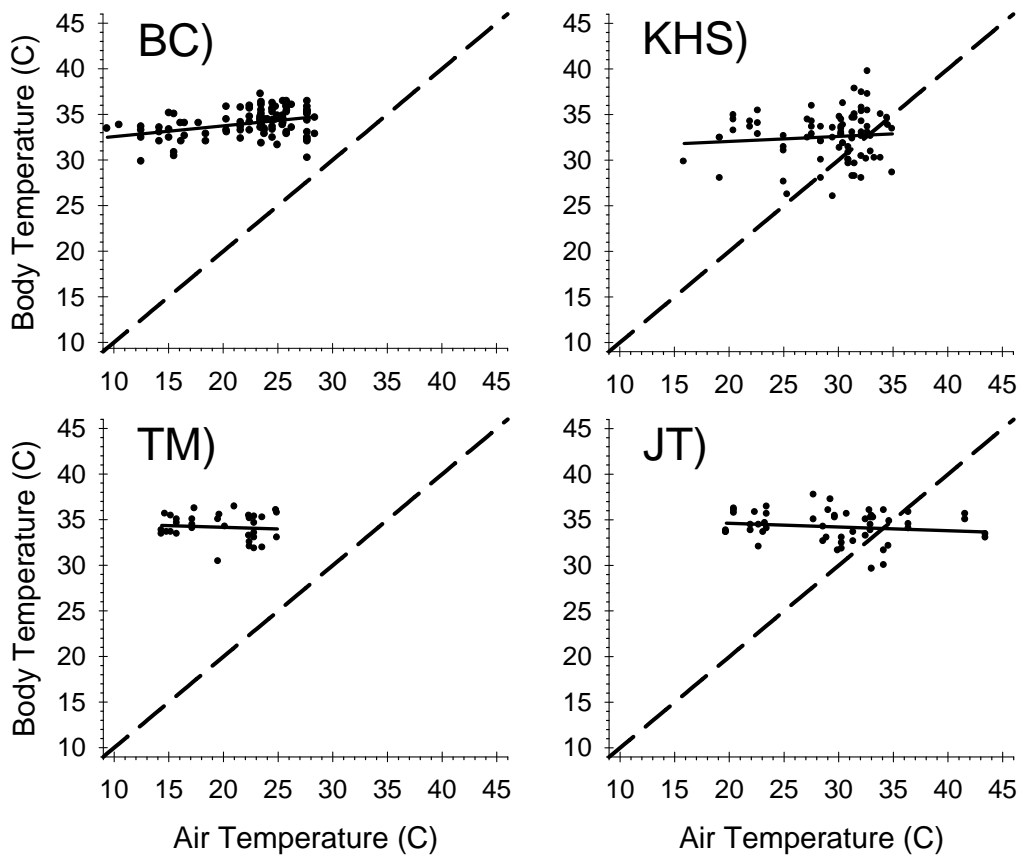


Figure 4. Relationship between ambient air temperature ( $T_A$ ) and body temperature ( $T_B$ ) of lizards collected in the field during 2007 and 2008 at each of the four study sites. Points represent individual lizards and solid lines are least square means fit lines. The relationship between air temperature and lizard body temperature was not significant at all sites except for Bishop Creek (BC,  $T_B = 0.1182 \cdot T_A + 31.37$ ,  $p < 0.0002$ ). Dashed lines represents the isoclines when  $T_A = T_B$

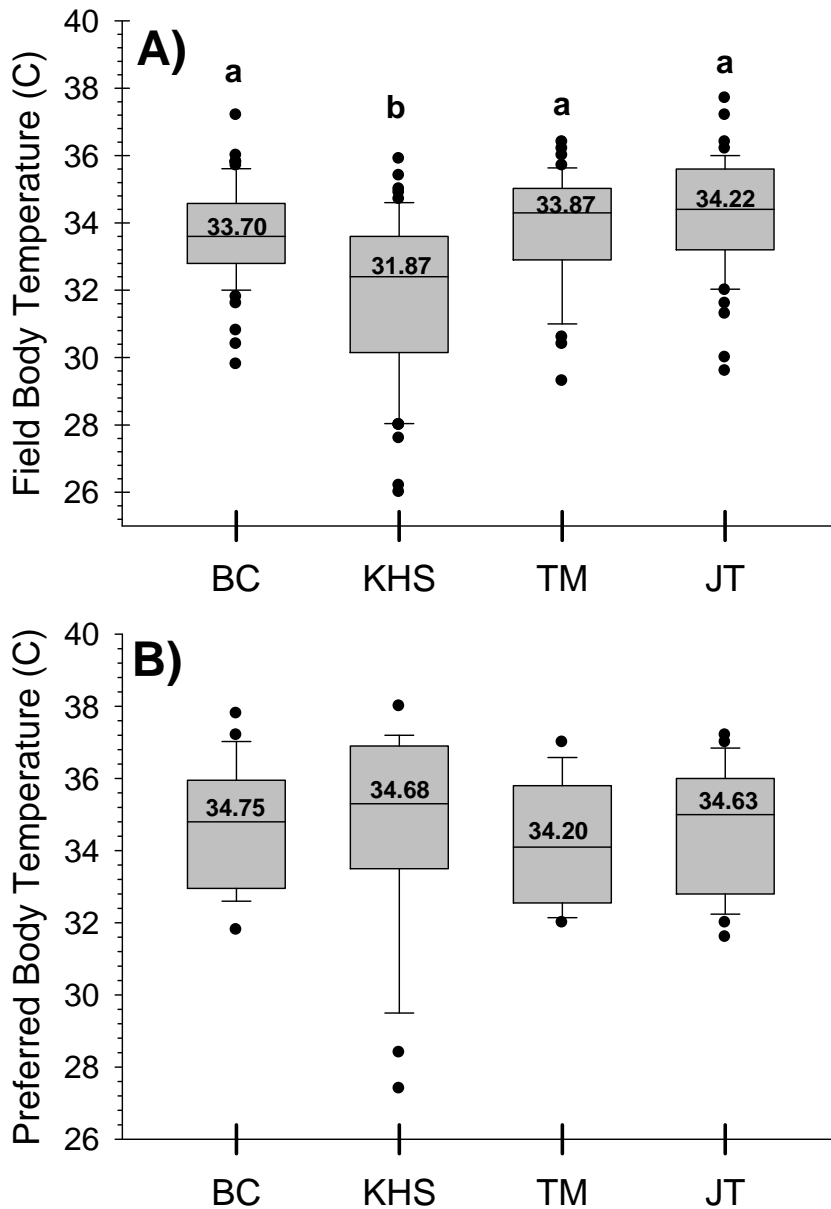


Figure 5. Site differences in A) field body temperatures and B) preferred body temperatures for *S. occidentalis* at four sites in California. Numbers represent mean body temperature for each site while boxes represent the median, lower and upper quantiles. Whiskers represent the 10<sup>th</sup> and 90<sup>th</sup> percentile. Outliers are represented by individual points and statistical differences are represented by letters. See table 3 for statistical analysis.

## CHAPTER II

### PATTERNS OF GEOGRAPHIC VARIATION IN THERMAL TOLERANCE IN THE WESTERN FENCE LIZARD *SCELOPORUS OCCIDENTALIS*

#### Abstract

In recent years, ecologists have emphasized the need for studies that examine the effects of global climate change on naturally occurring populations. While one consequence of climate change is an increase in mean global temperature, another consequence is an increase in daily, monthly, and seasonal temperature variation resulting from an increase in the number of days in which there are uncharacteristically hot or cold temperatures. As a result, it is necessary to understand the consequences of these extreme temperatures in natural populations to be able to predict how these populations will respond to climatic changes. One way of predicting the thermal limits of a population is by measuring thermal tolerance, the ability of an organism to withstand acute periods of extreme temperature. Common measures of thermal tolerance include the critical thermal maximum (CTMax) and critical thermal minimum (CTMin). For this chapter of my dissertation I used CTMax and CTMin to determine whether or not the western fence lizard, *Sceloporus occidentalis*, differed in its ability to withstand extreme temperatures among populations living in distinct thermal environments across seasonal fluctuations in temperature. To do this, I collected *S. occidentalis* from four sites in California over two years (2007 and 2008) at two different times during the year (May and Sept). In the laboratory I measured the CTMax and CTMin of each individual. In addition, I also measured abundance levels of Hsp70, a molecular chaperone, to determine whether or not the presence of Hsp70 increased thermal tolerance in this

species, as it has been shown to do in some invertebrate species. Finally, I compared my results from one site with previous research conducted during 1983 at the same location in an effort to determine whether or not changes in thermal tolerance during the past 25 years are the result of climatic changes in California during the same period. I found that although site temperature seemed to be a significant predictor of thermal tolerance in this species, there were substantial differences between seasons in thermal tolerance among the sites, possibility resulting from genetic similarities between individual populations. Furthermore, I found that there was no relationship between Hsp70 abundance and increased thermal tolerance. Finally, there was no relationship between temperature changes over the past 25 years and change in thermal tolerance during the same time. These results suggest that although *S. occidentalis* is able to withstand a wide variety of environmental temperatures, there are limits in the ability of individual populations to tolerate elevated temperatures, especially populations living in cooler environments.

### Introduction

Geographic variation in organismal traits can occur when natural populations of organisms living along an environmental gradient experience differential selective pressures (Endler 1977, Mitton 1997). Our understanding of the effects of geographic variation on traits among individuals and between species has provided a foundation for evolutionary theory and our understanding of natural selection (Gould and Johnston 1972). Variation in traits can occur at both the phenotypic and genotypic level and may be differentially adaptive across populations. Furthermore, this variation among populations and the resultant differences in selection pressure due to differences in the



environment can represent the first step in the formation of new species (Garcia Ramos and Kirkpatrick 1997, Herrel et al. 2001, Gavrillets 2003). By studying these differences, we can better understand how future environmental changes will affect individual populations or entire species, which may be especially important to help mediate current anthropogenic changes to natural systems.

Environmental temperature is one factor that varies among natural environments and has been shown to affect physiological function (Huey 1991, Feder et al. 2000, Hochachka and Somero 2002). Temperature can vary with geographic location or season and therefore can also create differential selective pressures within or across populations (Johnston and Bennett 1996, Hochachka and Somero 2002). For either geographic or seasonal temperature variation, the physiological effects of temperature can be especially pronounced in ectotherms, which utilize thermal energy from the environment to maintain body temperature and, as a result, physiological function. Individuals exposed to extreme environmental temperatures and are unable to maintain target body temperatures may be unable to perform organismal functions such as feeding, locomotion, or mating. For this reason, the ability of an organism to maintain activity across a variety of environmental temperatures can be a major component of fitness (Huey and Stevenson 1979, Kristensen et al. 2007, Loeschcke and Hoffmann 2007, Chown et al. 2009). As a result, biologists have long been interested in studying the thermal limits of activity and how temperature affects organismal performance (Huey and Stevenson 1979, Huey and Kingsolver 1993, Angilletta et al. 2002b, Hochachka and Somero 2002).

In addition, growing concern over the effects of climate change have renewed interest in understanding thermal limits of organismal activity (Hochachka and Somero 2002, Helmuth et al. 2005, Parmesan 2006, Chown and Terblanche 2007, Chown et al. 2009). Greater understanding of the degree to which genetic (adaptive) and environmental (plastic) processes determine the thermal limits of species, and how fast these processes can respond to climatic changes can help us to better predict range shifts that may occur as a result of global warming (Hoffmann and Blows 1993, Loeschcke et al. 1997, Parmesan et al. 1999, Davis and Shaw 2001, Klok and Chown 2003).

One way to characterize an organism's thermal limits is by using measures of thermal tolerance, the high and low temperatures at which an animal reaches a predetermined thermal limit. These limits can be determined in a variety of ways. Two of the most common methods include determining the lethal temperature for 50% of a given population (LT<sub>50</sub>) and the critical thermal temperature for a specific individual (CTT). The LT<sub>50</sub> is a more extreme measure of thermal tolerance while the CTT is the upper (CTMax) and lower (CTMin) temperatures at which an organism is able to maintain whole organism activities in a given environment. These measures are of interest because of their potential for determining species ranges because outside of this range of temperatures, an individual loses the ability to respond to an environmental stimulus (Stevens 1989, Chown and Gaston 1999, Jenkins and Hoffmann 1999, Addo-Bediako et al. 2000, Klok and Chown 2003, Nearing et al. 2003).

In this study I determined critical thermal maximum temperature (CTMax) and the critical thermal minimum temperature (CTMin) for multiple populations of *Sceloporus occidentalis* across multiple seasons and years. These measures were first defined by

Cowles and Bogert (1944) as “the thermal point at which locomotory activity becomes disorganized and the animal loses its ability to escape from conditions that will promptly lead to its death”. This definition has proved useful in determining the ability of an organism to withstand extreme temperatures in invertebrates, anurans, reptiles, and fish (Smith and Ballinger 1994, Nearing et al. 2003, Ravaux et al. 2003, Chown et al. 2009, Cross and Rawding 2009). Variation in CTMax and CTMin between individuals and populations has been shown to be adaptive in some species and plastic in other species but usually closely related to survival (Greer 1980, Klok and Chown 2001, Nearing et al. 2003, Huang et al. 2006).

Another way to characterize the thermal physiology of an organism is through the measurement of heat shock proteins (Hsps), which are used to protect the cell from damage due to short periods of thermal stress (Lindquist 1986, Lindquist and Craig 1988, Ulmasov et al. 1992, Sorensen et al. 2003). Expression of heat shock proteins, specifically HSP70 (70 kDa) has been shown to enhance survival and fitness in a wide range of organisms, from individual cells (Arya et al. 2006) to insects (Krebs and Feder 1997a, Edgerly-Rooks et al. 2005, Rinehart et al. 2006) to reptiles (Ulmasov et al. 1992). Heat shock protein expression has also been shown to increase thermal tolerance in many invertebrates (Sanders 1993, Klok and Chown 2003, Ravaux et al. 2003), yet few studies have examined the relationship between heat shock protein expression and thermal tolerance in a vertebrate system (Ulmasov et al. 1992, Kassahn et al. 2009).

This study had four main objectives: 1) To better understand the effects of geographic and seasonal variation in temperature on thermal tolerance in *S. occidentalis*. I expected to find that thermal tolerance varies geographically and seasonally in a manner that is

consistent with differences in environmental temperature across four study sites. 2) To examine whether levels of heat shock protein are correlated with thermal tolerance. In this case I expected to find increased Hsp70 abundance correlated with an increase in heat and cold tolerance. 3) To examine potential trade-offs between heat and cold tolerance at the level of an individual. and 4) To determine if, on a longer timescale, thermal tolerance in *S. occidentalis* has responded to recent changes in environmental temperature. By addressing these objectives, my overall goal is to understand how thermal tolerance in *S. occidentalis* varies with environmental temperature and whether or not this species may be able to withstand the climatic changes that are predicted to result from global warming.

## Methods

Site descriptions and climate data:

I examined geographic and seasonal variation in thermal tolerance in *S. occidentalis* at four locations in California; two in the Eastern Sierra and two in the San Gabriel Mountains. These four sites occur at paired latitudes (north and south) and altitudes (high and low) and have been shown to be variable in mean temperature throughout the year (Chapter 1). The study sites included: 1) Bishop Creek (BC), 7.9 km southwest of Bishop, Inyo County (elevation 2135 m); 2) Keough Hot Springs (KHS), 12.5 km south of Bishop, Inyo County (elevation 1220 m); 3) Table Mountain (TM), 3.5 km northwest of Wrightwood, San Bernardino County (elevation 2230 m); and finally 4) Joshua Tree (JT), 11.2 km west of Pinion Hills, Los Angeles County (elevation 1370 m).

#### Study organism and collection:

The western fence lizard, *Sceloporus occidentalis*, is geographically widespread lizard ranging from southern Washington State south into Baja California, Mexico and from sea level up to 2500 m elevation (Behler 2000, Stebbins 2003). This small lizard (adult size: 60-90 mm SVL, 15-30 g) is typically active from April until October at these study sites and utilizes a wide range of substrates for intra-specific displays and thermoregulation (Adolph 1990, Asbury and Adolph 2007).

For this study I collected lizards during May and June of 2007 and 2008 and again in September 2008. All lizards were collected by noose (N = 23 - 39 per site) and transported to the laboratory within eight hours of capture. Lizards were housed in a custom 25 L enclosure with a sandy substrate and a single rock for perching. In addition, a heat lamp was provided on a 12-hr light: 12-hr dark cycle to create a thermal gradient and allow lizards to thermoregulate. Lizards were fed crickets dusted with commercially available vitamin powder every other day and water was provided *ad libitum*.

#### Thermal tolerance measurements:

Captured lizards were held in the laboratory between five and seven days prior to laboratory experiment to minimize effects of recent thermal exposure prior to capture. After laboratory acclimatization, CTMax and CTMin for each lizard was measured using the loss of righting response (LRR) method. This method, which is defined as the body temperature at which a lizard is no longer able to right itself after being turned onto its back, is less invasive than other methods of measuring critical thermal tolerance that can

result in permanent physiological changes such as heat hardening (Lutterschmidt and Hutchison 1997a, b).

Lizards were randomly assigned to treatment groups that differed in whether CTMax or CTMin was measured first. CTMax was determined by placing each lizard in a 20 L bucket with a thin layer of moistened sand on the bottom. A 150-watt heat lamp was suspended 10 cm above the bucket to heat lizards at approximately 1°C/minute (determined empirically by preliminary trials). All lizards were held at room temperature (~21°C) for a minimum of one hour before heat stress and placed in the bucket at the beginning of the heat trial. Activity in these lizards was typically minimal at the beginning of the trial but increased steadily until peaking approximately halfway through the trial, before decreasing again. As activity decreased, lizards began to show visible signs of heat stress, such as panting and disorientation. Once voluntary activity ceased, lizards were placed on their backs to test their ability to turn themselves over (righting response). This was repeated every 15 seconds until LRR was achieved. At that point, cloacal temperature was taken within 5 seconds and was recorded as the CTMax.

CTMin was determined in a similar fashion by placing lizards in 38 L aquarium that contained a custom metal tray with a thin layer of sand suspended in an ice bath. Lizards were placed in the tray at the start of CTMin trials and voluntary activity quickly diminished after trials began. Once voluntary activity ceased, lizards were flipped on their back to test for righting response. This test was repeated every 15 seconds until loss of righting response was achieved. As before, body temperature was measured using a cloacal thermometer within 5 seconds and recorded as CTMin.

## Biochemical analysis of heat shock proteins:

I also tested whether baseline levels of Hsp70 were correlated with increased thermal tolerance by measuring Hsp70 abundance in all lizards collected during May and June in 2007 and 2008. After thermal tolerance measurements, all lizards were returned to the enclosures for 48 h, at which point they were sacrificed. Hind leg tissue was dissected and flash frozen at  $-80^{\circ}\text{C}$  until Hsp70 expression levels were quantified.

Expression levels of total Hsp70 were quantified following methods modified from previously published studies (McMillan et al. 2005, Rank et al. 2007). Dissected tissue was homogenized in buffer [100 mM Tris-HCL, 0.5% SDS, 0.5% Triton-X, 1% Protease inhibitor cocktail (Sigma-Aldrich Co., St. Louis, MO), pH 7.2] using ground glass tissue homogenizers (Kontes #20) and centrifuged at  $15,000 \times g$  for 15 minutes at  $4^{\circ}\text{C}$ . Total protein concentration was determined in the supernatant using a commercially available kit (Thermo Fisher Scientific, Rockford, IL, USA) that utilized a modified BCA approach (Smith et al. 1985). Samples were then diluted with water to achieve an equal concentration and combined with sample buffer (Invitrogen Corp., Carlsbad, CA) and a reducing agent (50 mM DTT final concentration). Twenty micrograms of protein from each lizard was loaded into a single well of a 4-12% pre-cast gradient Bis-Tris gel (Invitrogen Corp.). In order to quantify Hsp70 abundances, two wells of each gel were loaded with between 10 and 80 ng pure human Hsp70 (NSP-555; Assay Designs, Ann Arbor MI) as positive controls. To standardize these controls across gels, each control was loaded on multiple gels. Gels were electrophoresed at 150 V for 90 minutes to ensure decent band separation. After separation, bands were transferred to a  $0.45 \mu\text{m}$  PVDF membrane using constant amperage (0.4 Amperes) for 1 hour (Towbin et al. 1979). Blots

were probed for Hsp70 using an Hsp70-specific antibody (MA3-006, Affinity BioReagents, Golden CO) and a peroxidase conjugated anti-rat secondary antibody. Bands were then visualized using chemi-luminescence and quantified using Quantity One<sup>®</sup> software (Bio-Rad).

Historical changes in thermal tolerance:

I also examined the long-term effects of environmental temperature on thermal tolerance by comparing thermal tolerance in lizards from Joshua Tree measured during September 1983 to thermal tolerance for lizards from Joshua Tree measure during September 2008. Critical thermal maximum and minimum temperature from 1983 were provided by Steve Adolph, Harey Mudd College, Claremont, CA. These lizards were collected during September 1983 for a similar study and were returned to the lab within 7 days for determination of critical thermal limits. CTMax and CTMin were determined using the LRR method described above.

To model thermal conditions for both time periods, I used historical weather data available from the National Climate Data Center (<http://1wf.ncdc.noaa.gov/oa/ncdc.html>). I chose a single weather station (Fox Field, Lancaster, CA: Id# 723816 03159, elevation 713 km) which was located closest to Joshua Tree, approximately 50 km from Joshua Tree, and for which hourly temperatures were available from January, 1980 until December, 2008. To build a thermal profile of Joshua Tree during each year (1983 and 2008), I compared daily means and standard deviations for each 4 hour interval from April 15<sup>th</sup> until September 30<sup>th</sup>, the approximate activity period of *S. occidentalis*.



## Statistical Analysis:

I performed all tests using JMP statistical software (version 7.0.2, SAS Institute, Cary, NC). To begin, I correlated CTMax with CTMin in order to test for a trade-off between individual tolerance of heat and cold. To test for geographic and seasonal variation in thermal tolerance, I used a two-way analysis of variance (ANOVA) with site and season as fixed factors. I also used a two-way ANOVA to test the effects of site and season on thermal breadth (CTMax – CTMin). A correlation analysis was used to test for a relationship between Hsp70 abundance and thermal tolerance (CTMax and CTMin). In addition, I tested for seasonal changes in thermal breadth across the sites using a two-way ANOVA with site and season as fixed effects. Finally, I tested for changes in CTMax and CTMin from 1983 to 2008 using a one-way ANOVA with year as the single fixed effect.

## Results

### Site and seasonal differences in thermal tolerance:

Critical thermal tolerance in *S. occidentalis* varied among the four sites for both maximum and minimum temperature. Altitude was a significant predictor of CTMax while both altitude and latitude were significant predictors of CTMin (Figure 6A, Table 4A). Individuals from the desert sites (Joshua Tree and Keough Hot Springs) had higher CTMax than individuals from either of the mountain sites (Bishop Creek and Table Mountain). However, the same pattern was not observed for CTMin (Figure 6B, Table 4). Lizards from Bishop Creek had the lowest CTMin, while lizards from Joshua Tree

had the highest CTMin. Lizards from Keough Hot Springs and Table Mountain were intermediate (Figure 6B, Table 4).

There was also a season by site interaction for both CTMax and CTMin (Figure 7, Table 4). During the early summer, CTMax was greatest in individuals from Joshua Tree and lowest at Bishop Creek, with Keough Hot Springs and Table Mountain having intermediate values (Figure 7A). Over the course of the summer months, CTMax increased for lizards at all sites, however, the only site where CTMax increased significantly was at Keough Hot Springs. The pattern for CTMin was more complex. During the early summer months, the northern sites had higher cold tolerance (lower CTMin) than the southern sites. However, by the end of the summer, cold tolerance improved the most at Table Mountain, and also (to a lesser extent) at Joshua Tree, such that lizards at both high altitude sites (BC and TM) had higher cold tolerance than lizards at either of the low altitude site (KHS and JT) (Figure 7B).

Trade-offs between CTMax and CTMin and the effects of Hsp70 expression:

The relationships between thermal tolerance and Hsp70 abundance observed in this study were not expected and differed between hot and cold tolerance. There was no relationship between constitutive Hsp70 abundance and CTMax (Figure 8A,  $R^2 = 5.18 \times 10^{-5}$ ,  $p = 0.9596$ ). However, increased Hsp70 abundance was positively correlated with an increase in CTMin (Figure 8B,  $R^2 = 0.08$ ,  $p = 0.0360$ ).

Independent of the site and seasonal differences found for CTMax and CTMin, I found that there was a trade-off in thermal tolerance such that individuals with high heat tolerance had relatively poor cold tolerance (indicated by a higher value for CTMin,

Figure 9,  $R^2 = 0.11$ ,  $p < 0.0001$ ,  $N = 102$ ). In addition to a trade-off in thermal tolerance, there was also a site by season effect on thermal breadth (CTMax- CTMin) (Figure 10, Table 5). Early in the season, the northern latitude sites had greater thermal breadth. However, over the summer months, thermal breadth increased the most at Table Mountain and Joshua Tree, primarily due to a decrease in CTMin, so that by the end of the summer there was no statistical difference in the tolerance breadth for lizards at these four sites.

Historical changes in thermal tolerance:

Thermal tolerance measured at Joshua Tree changed between 1983 and 2008. *Sceloporus occidentalis* individuals at Joshua Tree measured in 1983 had a greater thermal breadth with a higher CTMax (Figure 11A, Table 6) and a lower CTMin (Figure 11B, Table 6) than individuals measured during the summer of 2008. The corresponding mean daily summer temperatures during each year at Fox Field, the proxy for Joshua Tree, increased from 22.1°C to 24.9°C from 1983 to 2008 (ANOVA:  $F_{1,1977} = 70.71$ ,  $p < 0.0001$ , not shown). However, standard deviation for daily temperature during the same period increased at this site from 7.53 °C in 1983 to 7.96°C in 2008 (ANOVA:  $F_{1,1971} = 24.98$ ,  $p < 0.0001$ , not shown).

## Discussion

Site and seasonal differences in thermal tolerance:

Results from this study indicate that thermal tolerance varies among geographic locations and that this variation is consistent with environmental temperature. However,

the patterns for CTMax and CTMin were different among study sites. CTMax tended to be greater for individuals from the desert sites than those from the mountain sites, and was also higher at the end of the summer. However, cold tolerance, independent of altitude, tended to be higher at the northern sites and decreased over the summer months. Furthermore, although there is a trade-off between CTMax and CTMin, thermal breadth increased at the southern sites from May until September to levels similar to the northern sites.

One unexpected result is that there was no relationship between Hsp70 and CTMax as originally expected. Yet, surprisingly, there was a positive correlation between Hsp70 and CTMin. Finally, although there were changes in thermal tolerance for Joshua Tree lizards from 1983 until 2008 with tolerance to both heat and cold decreasing during this time period, there was no associated change in air temperature. These results expand upon previous studies that have investigated thermal tolerance in the context of either geographic or seasonal variation in temperature. However, by studying these factors together we can better understand how thermal tolerance in natural populations can contribute to species distributions.

Previous studies have examined variation among populations in thermal tolerance and its relationship with environmental temperature. However, many of these studies have only examined one type of thermal tolerance (hot or cold) or have only focused on a single variable, such as altitude (Smith and Ballinger 1994, Brown 1996), season (Fry 1947, Hu and Appel 2004, Sinclair et al. 2006) or species (Moore 1949, Cuculescu et al. 1998). This study has also expanded upon previous studies by incorporating site and season simultaneously, to better account for the relative roles that the local environment

and population structure play in determining hot and cold thermal tolerance in a single species. For example, CTMax was greatest at Joshua Tree and lowest at Bishop Creek with a similar pattern observed for CTMin. Lizards from Bishop Creek, the coolest site, had the lowest CTMin, or best cold tolerance, while lizards from Joshua Tree had the highest CTMin, or worst cold tolerance. The other two sites were intermediate. However, alone, these results may be misleading because, by incorporating seasonal effects of thermal tolerance, it becomes clear that much of the variation among sites in CTMax and CTMin is the result of differing levels of the strength of a seasonal effect on thermal tolerance.

Although there was relatively little difference in CTMax among the four sites during May, during the summer there was a marked increase in heat tolerance at Keough Hot Springs and Joshua Tree, the two desert sites, such that by the end of the summer there were differences in CTMax between altitudes. This pattern is not surprising because environmental temperature was similar between the two desert sites and was higher at both sites when compared to the mountain sites during the summer months (Chapter 1). Cold tolerance in *S. occidentalis* showed a different pattern than heat tolerance when site and season was considered simultaneously. At the beginning of the summer, there was a clear latitudinal division in cold tolerance, with both northern sites having better cold tolerance than either southern site, independent of altitude. Over the course of the summer months, cold tolerance improved at the southern sites, especially at Table Mountain, and by September lizards at both high altitude sites had better cold tolerance than lizards at the low altitudes sites, similar to the pattern observed for CTMax during September.

Previous work in these populations of *S. occidentalis* has shown genetic similarity between the Table Mountain and Joshua Tree sites relative to the Keough Hot Springs site suggesting that gene flow has occurred along an altitudinal gradient but not across latitudes (S. Adolph, personal communication). Unfortunately, genetic similarity has not been measured between the Bishop Creek site and Keough Hot Springs. However, given the similar geographic distance and landscape features between each pair of sites in the north and south, it is reasonable to conclude that there is also gene flow between Bishop Creek and Keough Hot Springs. The genetic differences between the northern and southern sites in conjunction with the environmental variability in temperature between the high and low altitude sites suggest that there are both adaptive (genetic) and plastic (environmental) determinants for thermal tolerance in *S. occidentalis*. Furthermore, these forces are not static throughout the year and can vary independently for both hot and cold tolerance in *S. occidentalis* depending on the season.

The relative importance of both genetic and environmental factors in determining thermal tolerance is evident from the observed patterns among the sites and from the change in these patterns during the course of this study. At the beginning of the summer, there was relatively little difference in CTMax for individuals at all four sites, with the exception of a comparison between Bishop Creek and Joshua Tree. This suggests that lizards at these two sites are locally adapted to the temperature of their environment and that the other two sites are intermediates in both thermal environment (Chapter 1) and heat tolerance. During the summer, however, CTMax increased for individuals only at the two desert sites, which suggests that there is indeed a plastic component to CTMax that can vary within seasons. A similar pattern was observed for cold tolerance. At the

beginning of the summer, there was a latitudinal division in CTMin among the sites suggesting that cold tolerance in *S. occidentalis* is better at the cooler northern sites as a result of local adaptation. However, by the end of the study period, cold tolerance differed by altitude. Surprisingly, cold tolerance remained unchanged or improved at all four sites and did not decrease as originally expected. In summary, critical thermal tolerance in *S. occidentalis* may be under selection at the most extreme thermal sites (JT for heat; BC for cold) and these sites may act as a buffer for the matching latitudinal site against local adaptation to temperature. As a result, even though Keough Hot Springs is a desert site, lizards here have better cold tolerance than expected from environmental temperature because of the site's proximity to Bishop Creek. In a similar fashion, lizards at Table Mountain have better tolerance of heat than predicted by environmental temperature at the site because of the site's proximity to Joshua Tree. In order to test this hypothesis, however, further additional sampling points at different times throughout the year need to be added to the data collected in this study. Unfortunately, this may not be possible because of the lack of lizard activity at these sites during the winter months, especially at the high altitude sites which are covered in snow.

The range of temperatures over which an individual lizard is able to function can often vary between individuals and populations with changes in environmental temperature. Evolutionary physiologists often argue that these trade-offs in organismal performance are centered on the specialist / generalist barrier, which states that as an organism becomes increasingly adapted to tolerance extreme temperatures, the range of temperatures over which that organism is able to function decreases (Huey and Kingsolver 1993, Gilchrist 1995, Angilletta et al. 2003). These trade-offs typically arise

from the structure / function limitation of enzymes (Hochachka and Somero 2002). Enzymes with higher conformational stability function better at higher temperatures, while enzymes with less conformational stability function better at lower temperatures (Somero 1978, Gilchrist 1995, Hochachka and Somero 2002). This means that for a given organismal trait, an organism should be unable to perform better at one temperature extreme without sacrificing performance at the other temperature extreme. Using CTMax and CTMin as the thermal boundaries of organism performance, *S. occidentalis* is an example of an organism in which a trade-off has occurred between heat and cold tolerance. However, this trade-off does not seem to be present at all sites as cold tolerance improved over the course of the summer months at some of the sites in this study (Table Mountain and Joshua) while heat tolerance was improving at the same time (Joshua). Furthermore, other work in *S. occidentalis* has found no evidence for a trade-off between hot and cold tolerance; instead, there was a positive relationship between heat and cold tolerance (S. Adolph, unpublished data). These contradictory findings illustrate a high level of population variability in thermal tolerance for *S. occidentalis*. This variation is likely due to both genetic differentiation among the populations of *S. occidentalis* and recent patterns of environmental air temperature experienced by each population. Either way these results emphasize the need for further studies to better understand the adaptive or plastic limits of extreme temperature tolerance, especially in a geographically widespread species such as *S. occidentalis*.

Given the presence of a trade-off among individuals in cold versus heat tolerance, it could be expected that a similar trade-off would occur across populations of *S. occidentalis* so that sites with more extreme temperatures, such as Bishop Creek and



Joshua Tree may have a smaller range of temperatures over which individuals are able to be active. On the other hand, sites with milder temperatures may have a wider range of temperatures over which individuals are active. However, despite this expectation, there is little evidence for a population level trade-off in thermal tolerance. Instead, thermal breadth was higher in the northern populations than it was in the south during the first part of the summer, but that difference disappeared by the end of the summer. This latitudinal difference in thermal breadth is most likely attributed to Rapoport's rule, which states that species ranges will increase with latitude due to the ability of organisms to withstand greater variability in climatic conditions at higher latitudes (Stevens 1989, 1992, Blackburn and Gaston 1996, Johnson 1998, Gaston and Chown 1999). Much of this latitudinal variation in Rapoport's rule is attributed to variation in cold tolerance (Gaston and Chown 1999), which can be seen in the large variation among sites in cold tolerance during the May sampling. However, Rapoport's rule can only partially explain the latitudinal differences in thermal breadth as there was no difference in thermal breadth among the sites by the end of the summer. Instead, it is possible that the relative importance of Rapoport's rule in explaining the latitudinal variation in thermal breadth can diminish during seasons with more extreme temperatures, such as the summer and winter. Either way, further investigation into the geographic patterns of thermal breadth are necessary to better understand how flexible different populations of *S. occidentalis* are and how they may respond to an increase or decrease in variability in their thermal environment.

Relationship between thermal tolerance and Hsp70 abundance:

In many invertebrate organisms, critical thermal tolerance has been shown to increase with the production of Hsp70 (Nearing et al. 2003, Ravaux et al. 2003, Rinehart et al. 2007, Sorensen et al. 2009). Yet, few studies have examined the relationship between Hsp70 and heat and cold resistance in vertebrates, and none have examined the relationship between Hsp70 and critical thermal tolerance in reptiles (Ulmasov et al. 1992, Yu et al. 1998, Sorensen et al. 2009). For *S. occidentalis*, I found no relationship between levels of Hsp70 abundance and thermal tolerance as expected. In fact, CTMin was positively correlated with Hsp70 abundance in *S. occidentalis*, indicating that as Hsp70 abundance increased cold tolerance decreased. This result has not been reported elsewhere and is surprising given that Hsp70 has been shown to enhance cold tolerance in addition to heat tolerance in insects (Yocum 2001, Rinehart et al. 2007). One possible explanation for this relationship between Hsp70 abundance and cold tolerance in this species is that the expression of Hsp70 has been shown to incur a high metabolic cost for many organisms leading to possible reductions in fitness (Hofmann and Somero 1995, Krebs and Bettencourt 1999, Sorensen et al. 2003, Dahlhoff and Rank 2006, Rinehart et al. 2006). However, much of this work has centered on organisms that are thermo conformers with body temperature that closely track air temperature. As a result, the link between Hsp70 and thermal tolerance may be decoupled in organisms that are able to behaviorally maintain body temperatures within critical thermal limits at which point the expense of producing Hsp70 is not beneficial to enhancing thermal tolerance (Sorensen et al. 2009).

Historical changes in thermal tolerance:

At Joshua Tree there was a decrease in both heat and cold tolerance measured during September 2008 relative 1983. This result was not expected given that from April to September it was warmer in 2008 than it was in 1983. Instead, thermal tolerance for both heat and cold was expected to increase given the seasonal increases in thermal tolerance observed elsewhere in this study. However, this comparison is limited in its ability to detect long term patterns of thermal tolerance as a result of changing temperatures because there were only two time points available for analysis. In addition, the Fox Field data logger, which was used as a thermal proxy for Joshua Tree, may not reflect the actual thermal patterns of temperature at Joshua Tree due to the geographic distance separating the two sites (50km). Despite the lack of a relationship with environmental temperature in this portion of the study, the significant change in CTMax and CTMin from 1983 to 2008 warrants further investigation to determine the source of these changes. If long term changes in thermal tolerance for *S. occidentalis* are due to environmental temperature, understanding the mechanism of those changes can help better predict the consequences of further changes in temperature that can result as a consequence of climate change.

Conclusion:

In this study, I found that the relative importance of environmental temperature, a consequence of geographic location, varied seasonally in determining critical thermal tolerance to both hot and cold temperatures in *S. occidentalis*. This is likely caused by selection working on thermal tolerance in habitats with more extreme temperatures, while

in other habitats, recent environmental temperature has a greater role in determining critical thermal tolerance. In addition, there was a trade-off between hot and cold tolerance for individual lizards, but there was no trade-off for populations of organisms. Furthermore, the relationship between Hsp70 and thermal tolerance appears to be decoupled in this species even though it has been shown in several other species, possibly because *S. occidentalis* is able to thermoregulate. Finally, although there were changes in thermal tolerance over the past 25 years for Joshua Tree lizards, my results show that it was not due to differences in mean summer temperature during that time. In all of these studies, there was a significant amount of individual level variation and future studies on the genetic basis of thermal tolerance in *S. occidentalis* may help clarify the relative roles that environmental temperature and local adaptation play in determining the critical thermal tolerance in *Sceloporus occidentalis*.

## Tables

Table 4: Two-way ANOVA showing the effects of site and season on (A) critical thermal maximum (CTMax) and (B) critical thermal minimum (CTMin) in *S. occidentalis*.

### A) CTMax:

Source	df	SS	F
Site	3	16.085	10.06****
Season	1	14.150	26.5619****
Site x Season	3	6.126	3.83*
Error	139	74.049	

\* $p < 0.05$ , \*\* $p < 0.01$ , \*\*\* $p < 0.001$ , \*\*\*\* $p < 0.0001$

### B) CTMin:

Source	df	SS	F
Site	3	16.412	14.69****
Season	1	5.232	14.05***
Site x Season	3	11.638	10.41****
Error	102	35.386	

\* $p < 0.05$ , \*\* $p < 0.01$ , \*\*\* $p < 0.001$ , \*\*\*\* $p < 0.0001$

Table 5: Site and seasonal effects on thermal breadth in *S. occidentalis* using two-way ANOVA.

Source	df	SS	F
Site	3	7.056	4.53**
Season	1	15.795	30.42****
Site by Season	3	7.871	5.05**
Error	94	48.812	

\*p<0.05, \*\*p<0.01, \*\*\*p<0.001, \*\*\*\*p<0.0001

Table 6: Effects of collection year (1983 vs 2008) on CTMax and CTMin in *S. occidentalis* for the Joshua Tree site.  $N = 24$  (1983) and 10 (2008).

A) CTMax

Source	df	SS	F
Year	1	18.688	37.54****
Error	32	15.930	

\* $p < 0.05$ , \*\* $p < 0.01$ , \*\*\* $p < 0.001$ , \*\*\*\* $p < 0.0001$

B) CTMin

Source	df	SS	F
Year	1	74.444	62.54****
Error	32	38.090	

\* $p < 0.05$ , \*\* $p < 0.01$ , \*\*\* $p < 0.001$ , \*\*\*\* $p < 0.0001$

## Figures

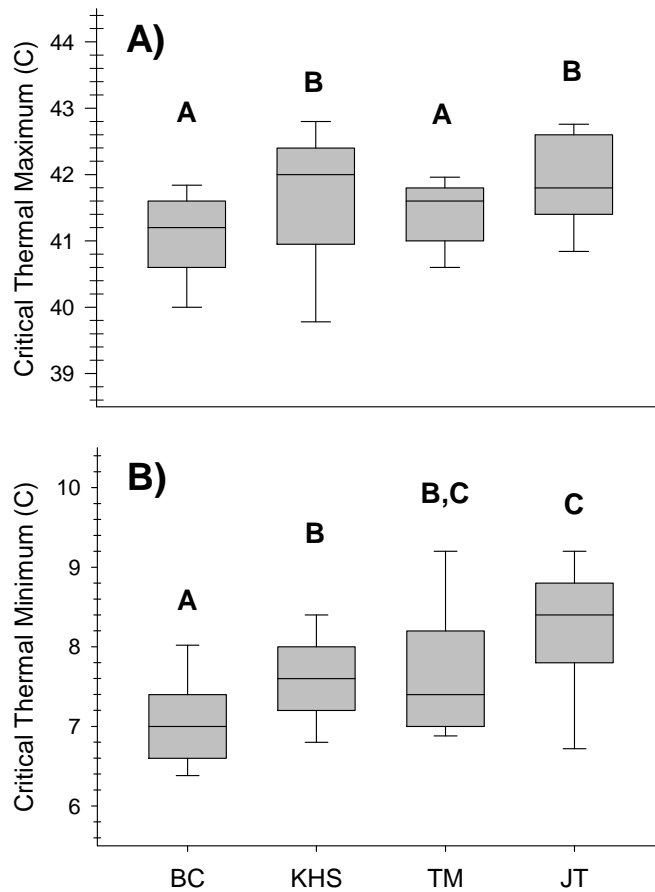


Figure 6. Site differences in A) critical thermal maximum (CTMax) and B) critical thermal minimum (CTMin) for *S. occidentalis* lizards measured during May / June and September 2008. Sites included are BC, KHS, TM, and JT ( $N_{CTMax} = 31 - 47$  per site and  $N_{CTMin} = 23 - 28$  per site). Boxes represent the median, lower and upper quartiles. Whiskers represent the 10<sup>th</sup> and 90<sup>th</sup> percentile. Letters represent significant difference in pair wise comparisons. See Table 4 for statistical analysis.



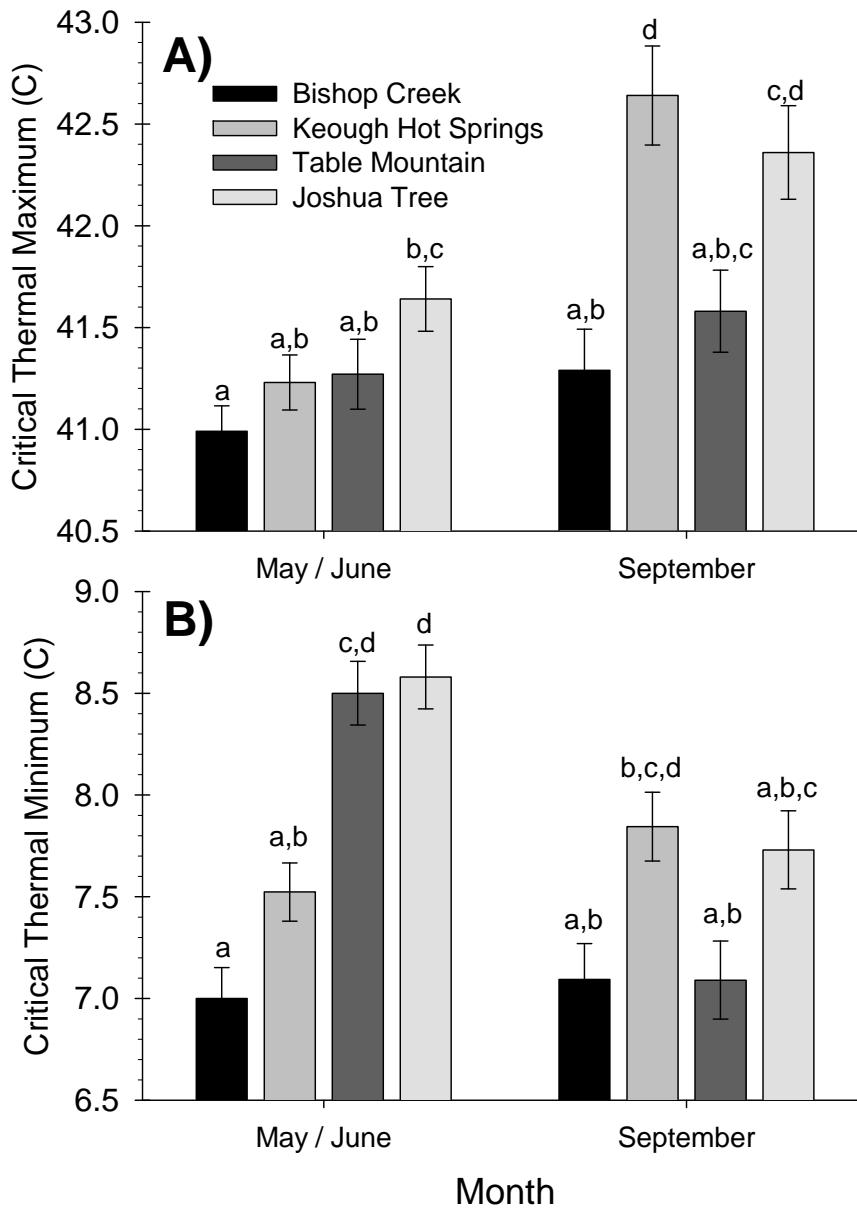


Figure 7. Site and seasonal differences in CTMax and CTMin in *S. occidentalis* from four California sites during early and late summer months. Data points represent site means ( $\pm$ SE) in  $^{\circ}$ C for A) CTMax and B) CTMin. Bars not connected by the same letter are significantly different. See Table 4 for statistical analysis.

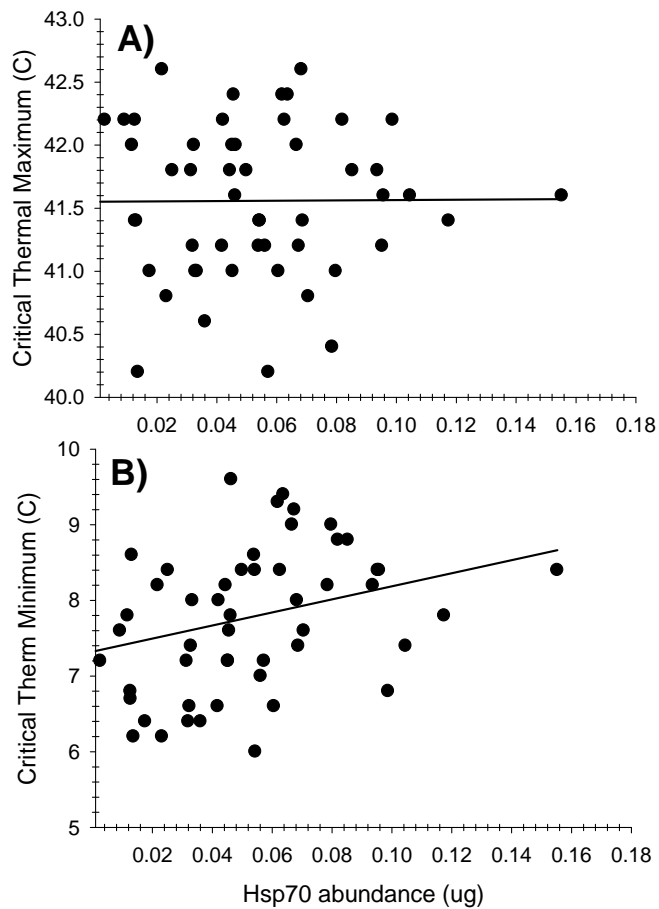


Figure 8. Correlations between Hsp70 abundance and A) CTMax and B) CTMin in *S. occidentalis* collected during May and June 2008. Data represent individual lizards ( $N = 52$  for each). There was no relationship between Hsp70 and CTMax ( $R^2=5.18 \times 10^{-5}$ ,  $p=0.9596$ ), however there was a positive relationship between CTMin and Hsp70 abundance ( $R^2=0.08$ ,  $p=0.0360$ ).

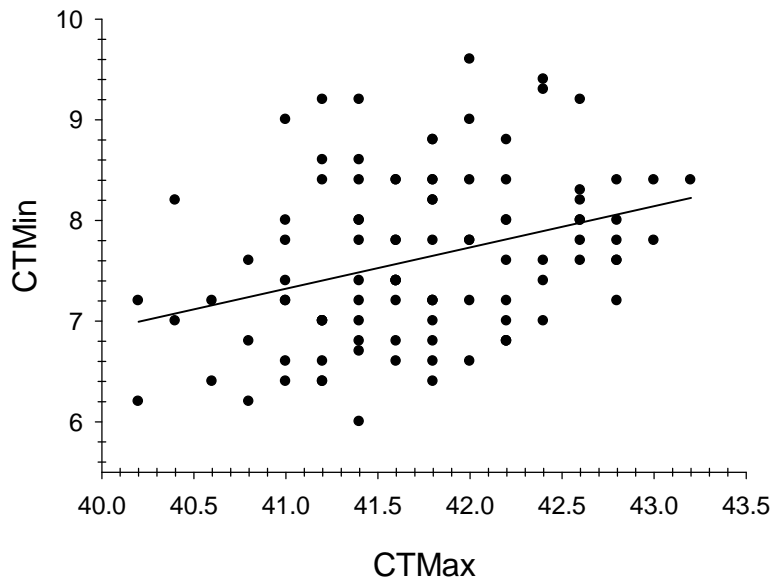


Figure 9. Correlation between CTMax and CTMin in *S. occidentalis* from four sites during May, June, and September 2008. Points are individual lizards for which both CTMax and CTMin were measured (N=102). CTMin increased linearly with CTMax ( $Y = 0.410X - 9.492$ ,  $R^2 = 0.11$ ,  $p < 0.0001$ ).

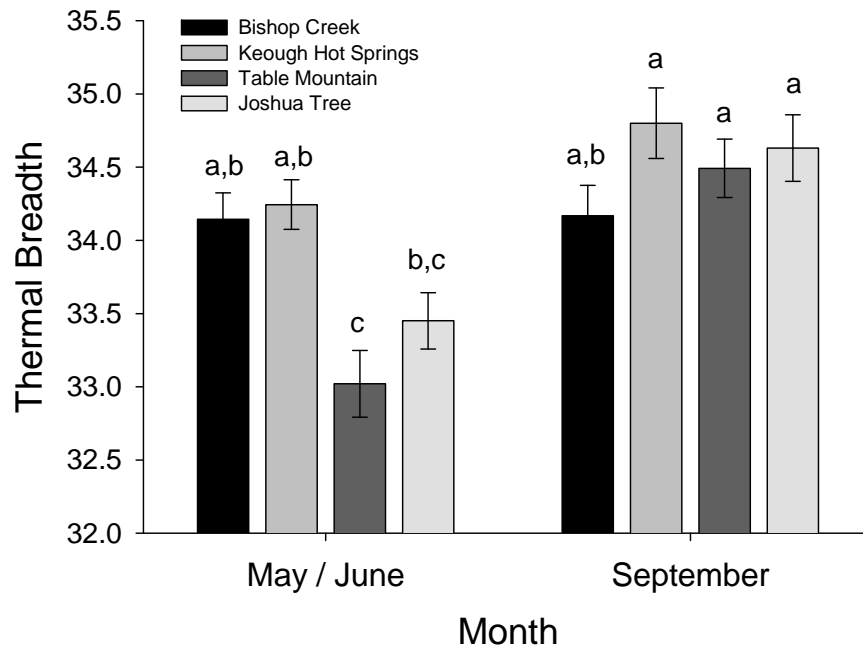


Figure 10. Effects of site and season on thermal breadth (CTMax – CTMin) in *S. occidentalis*. Data points represent means ( $\pm$ SE). Columns not connected by the same letter are significantly different. See Table 5 for statistical analysis.

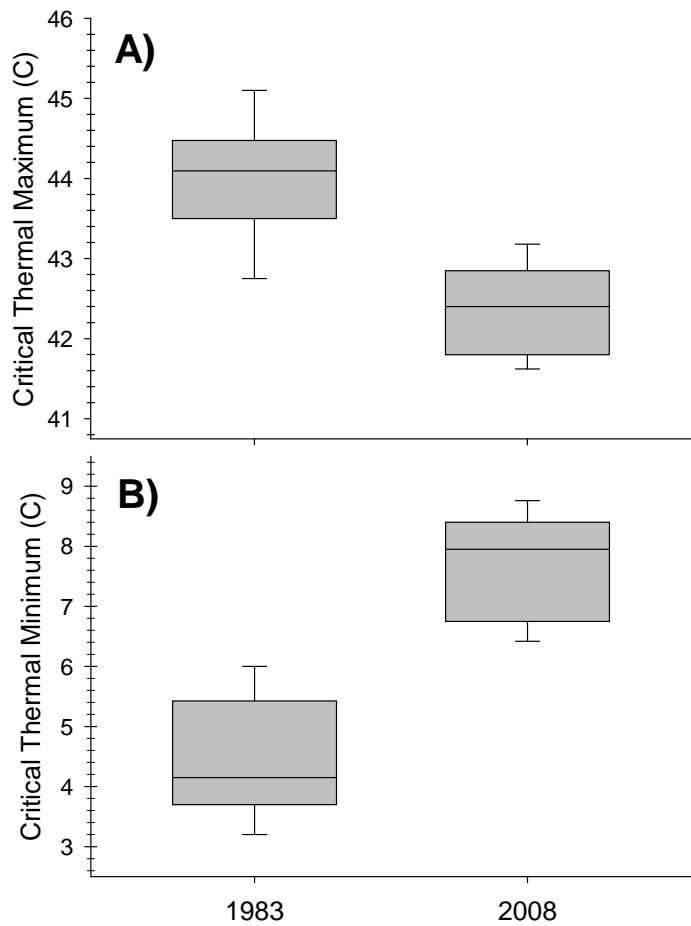


Figure 11. Long-term changes in A) CTMax and B) CTMin in *S. occidentalis* from Joshua Tree during 1983 and 2008. Sample sizes are  $N = 24$  (1983) and  $N = 10$  (2008). Boxes represent the median, lower and upper quartiles. Whiskers represent the 10<sup>th</sup> and 90<sup>th</sup> percentile. See Table 6 for statistical analysis.

## CHAPTER III

### GEOGRAPHIC VARIATION IN SPRINT SPEED AND HEAT SHOCK PROTEIN ABUNDANCE BEFORE AND AFTER HEAT STRESS IN THE WESTERN FENCE LIZARD

#### Abstract

The Western fence lizard, *Sceloporus occidentalis*, is a geographically widespread lizard occurring throughout the western United States. This lizard occurs in many diverse thermal environments and can be exposed to potentially stressful temperatures in these natural habitats. The production of molecular chaperone proteins, specifically heat shock proteins (Hsps) can aid in recovery from thermal stress by restoring proteins that have become denatured. In addition to the physiological effects on cellular processes, environmental temperature has also been shown to affect locomotor ability, especially sprint speed, an important determinant of predator escape ability in lizards. Therefore, production of Hsps during periods of thermal stress should be a reliable indicator of physiological stress and should be correlated with reduced sprint speeds in *S.*

*occidentalis*. In this chapter of my dissertation I measured sprint speeds in *S. occidentalis* from four populations during June 2007 and June 2008 before and after exposure to experimental heat stress (or a control temperature). After sprinting trials were complete, I dissected hind limb muscle tissue to quantify abundance of Hsp70, one member of the heat shock protein family. I found that lizard sprint speed from each of the four sites differentially decreased as a result of the heat stress. I also found that while there was no difference among sites in abundance of Hsp70 for the experimental treatment or control group treatment, the difference between these two groups (experimental and control) was

greater at the Northern sites, suggesting that lizards from the northern sites were more physiologically stressed after heat shock. Finally, I found that Hsp70 abundance was negatively correlated with sprint speed, meaning that it is a reliable indicator of organism stress in this species. These results, along with the measured thermal variation among these study sites (Chapter 1) indicate the potential for natural selection to act within these populations and may lead to local adaptation to temperature in *S. occidentalis*.

### Introduction

Understanding the diversity of physiological processes that allow organisms to exist in extreme environments can help elucidate the current and historical limits of species ranges (Feder et al. 1987, Bennett and Huey 1990). Typically, geographic variation in intraspecific physiological function has been attributed to adaptive genetic differences among populations that allow organisms living in different environmental conditions to maintain and optimize physiological function (Endler 1977, Huey and Kingsolver 1993, Dahlhoff and Rank 2000, Hochachka and Somero 2002). However, geographic variation in physiological function can also be environmentally based due to individual acclimation to environmental conditions (Sinervo and Adolph 1994, Crill et al. 1996, Dufty et al. 2002). Understanding how these two processes interact can allow us to better understand how natural selection acts to shape natural populations.

Environmental temperature is one factor that varies across geographic area and has been found to result in range shifts and local extinctions of populations (Parmesan et al. 1999, Clarke 2003, Bradshaw et al. 2004, Rank et al. 2007). Current models of climate change predict that the average global temperature will increase by more than

0.15°C per decade, and that the increase in temperature at some local environments, especially higher latitudes and elevations may be greater (Walther et al. 2002, IPCC 2007). In addition to this overall warming, many models predict an increase in the frequency and intensity of extreme temperature events, which may increase selection pressure on organisms due to these extreme temperatures (Jayco and Millar 2001). Together, these changes in the thermal environment, both in average temperature and the frequency of extreme temperatures, have the potential to drastically alter species distributions, especially for populations living at the edge of their range.

Even if species distributions remain unchanged, population densities or composition may suffer if only certain individuals are able to survive the changes in the thermal environment. For this reason, it is necessary to take a wide range of approaches in order to understand the effects of temperature on natural populations, from a biochemical understanding of the limits of thermal tolerance to understanding the ecological consequences of thermal plasticity and adaptation (Hochachka and Somero 2002, Angilletta 2009). By understanding how extreme environmental conditions, and temperature in particular, lead to physiological variation, and whether this variation is adaptive, we are better equipped to predict future potential shifts in species ranges, especially in the face of changing climatic conditions (Clarke 1996, Parmesan et al. 1999, Feder et al. 2000, Angilletta et al. 2002b).

One effect of extreme temperature is that it can cause many organisms to experience physiological stress as they attempt to maintain homeostasis (Hochachka and Somero 2002, Bradshaw et al. 2004, Dahlhoff and Rank 2006). Consequently, species living in environments that experience thermal stress are faced with three options: 1)



move to a new environment, 2) adapt to the stress or, 3) face the risk of extinction (Sorensen et al. 2003). Individual populations of a species can experience differing pressures from selection as a result of geographic variation in environmental temperature which has been shown to not only affect only an organism's physiology but also behavior and performance (Endler 1977, Huey et al. 1990, Dahlhoff and Rank 2000, Sears and Angilletta 2003). This phenotypic variation results from variation in selection pressures among populations and represents the first step in genetic divergence that may ultimately lead to speciation (Garcia Ramos and Kirkpatrick 1997, Herrel et al. 2001, Gavrilets 2003). Understanding the effects of geographic variation represent an excellent opportunity to study evolution in the wild (Feder 1999, Dahlhoff et al. 2001, McMillan et al. 2005).

One of the reasons that temperature tolerance is an ideal trait to better understand selection is that temperature affects nearly all organismal processes, including energy utilization, locomotion, feeding, and reproduction (Johnston and Bennett 1996, Clarke 2003). The effects of temperature can be especially pronounced in ectotherms, which utilize the ambient thermal environment to maintain body temperature and physiological function. Temperature has been shown to limit species distributions in ectotherms through its effects on reproduction and survival (Helmuth and Hofmann 2001, Rank and Dahlhoff 2002, McMillan et al. 2005). Furthermore, thermal stress has also been shown to limit an ectotherm's ability to perform functions such as feeding, reproduction, and locomotion (Huey and Slatkin 1976, Bennett 1980, Ji et al. 1996, Lailvaux 2007). As a result, populations of ectotherms from different thermal environments may experience differing levels of temperature stress but, over evolutionary time, natural selection should

act on this variation to maintain overall organismal function within a given population (Bennett 1990, Ji et al. 1996, Johnston and Bennett 1996, Angilletta 2001, Angilletta et al. 2002a).

Natural selection can help to maintain organismal function across different thermal environments by mitigating the effects of temperature on cellular structures and processes such as protein structure, membrane composition and fluidity, and biochemical reaction rates in order to maintain homeostasis (Hazel 1995, Somero 1995, Dahllhoff and Rank 2000, Hochachka and Somero 2002). An increasing number of studies have demonstrated that changes in environmental temperature, and the resulting effects on cellular processes, can be one of the factors that lead to range shifts and local extinctions among populations (Parmesan et al. 1999, Clarke 2003, Bradshaw et al. 2004, Dahllhoff et al. 2008). This makes understanding the relationship between environmental temperature and physiological function especially important to better predict how future changes in climate may affect the distribution of species living near a thermal boundary, such as a geographic or altitudinal edge of the current species distribution.

Temperature can be highly variable in natural environments (Dahllhoff and Rank 2000, Jacobson 2005). During periods of elevated temperatures, cellular proteins can become denatured due to perturbation of the electrostatic interactions that maintain the tertiary and quaternary structures of many proteins. This can also lead to inappropriate interactions with other proteins or the formation of protein aggregates (Hofmann 1999, Hochachka and Somero 2002). To mitigate the damage from these stressful temperatures, many organisms synthesize a specific class of proteins called molecular chaperones that aid in preventing denaturation of proteins or restoring function to already

denatured proteins. One class of molecular chaperones, the heat shock proteins (Hsps), is used to refold denatured proteins and prevent the accumulation of denatured or partially unfolded proteins in the cell (Lindquist 1986, Parsell and Lindquist 1993, Dahlhoff et al. 2008). The production of Hsps, specifically Hsp70 (70 kDa) have been shown to increase thermal tolerance and enhance survivorship for a variety of organisms living in fluctuating thermal environments (Krebs and Bettencourt 1999, Sorensen et al. 2003).

Whether or not the expression of Hsps enhances thermal tolerance, the synthesis and expression of Hsps require energy that may be diverted from other metabolic or behavioral processes, such as feeding and reproduction (Lindquist 1986, Krebs and Holbrook 2001, Sorensen et al. 2003, Dahlhoff et al. 2008). As a result, individuals that necessarily produce more Hsps to survive or recover from periods of thermal stress may suffer a relative fitness cost over their lifetime in comparison to other individuals that are better adapted to extreme temperatures (Krebs and Feder 1998, Krebs and Holbrook 2001). Over evolutionary time, however, biochemical adaptations should accumulate that minimize the production of Hsps but still maintain adequate protection from periods of thermal stress (Hofmann and Somero 1995, Hochachka and Somero 2002).

Even though natural selection should minimize over-expression of Hsps, selection acts upon the whole organism and not individual traits. Therefore, it becomes necessary to propose a mechanism through which selection can act upon heat shock protein expression and use a reductionist approach to test each step in the proposed mechanism, from cellular protein expression to the ecological and evolutionary consequences of expression (Hochachka and Somero 2002). One way to examine the effects of natural selection on heat shock protein expression is to examine variation in a measure of whole

organism performance, such as sprinting speed. The sprinting ability of an organism has been shown to be highly correlated with survivorship (Irschick and Garland 2001, Calsbeek and Irschick 2007, Irschick and Le Galliard 2008). By examining the relationship between sprint speed performance and heat shock protein expression, we can begin to understand whether or not there are tradeoffs between whole-organism performance and heat shock protein synthesis, which will help us to better predict how different populations in different thermal regimes will respond to a change in their thermal habitat and whether that will lead to an increase in thermal stress.

In this study, I examined the effects of temperature stress on performance and stress protein expression in the western fence lizard, *Sceloporus occidentalis*. This species has a wide geographic range along western North America that encompasses a diversity of thermal habitats, making *S. occidentalis* ideal to study the effects of temperature variation on stress protein expression and its relationship to locomotion. Heat shock protein abundance levels have not been examined in *S. occidentalis*, but previous studies have found that there is geographic variation in sprint speed within this species (Sinervo and Losos 1991, Buckley et al. 2007). In the laboratory, sprint speed in *S. occidentalis* has been shown to increase gradually with temperature up to approximately 35°C and then decline sharply (Adolph 1987, Vandamme et al. 1989, Hertz et al. 1993, Angilletta et al. 2002a, Du et al. 2007). I hypothesize that the sharp decline in the temperature-performance curve is due to the denaturing of critical locomotor proteins and a corresponding increase in Hsp70 expression to repair the damaged proteins. As a result, abundance of Hsp70, which can be rapidly altered in response to temperature stress (Lindquist 1986, Sorensen et al. 2003, Dahlhoff and Rank

2006), will serve as an effective proxy for physiological stress in this species and as a reliable indicator of the relative levels of selection pressure between populations in different thermal environments.

This study is one of the first to examine geographic variation in heat shock protein abundance and sprint speed within *S. occidentalis* across sites that vary in environmental temperature (Chapter 1). In this study, I address two questions. First, are there inter-population differences in heat shock protein abundance and sprint speed in *S. occidentalis* after exposure to heat stress? Second, do the negative effects of heat stress on sprint speeds correlate with increased abundance of heat shock proteins? By answering these questions, I hope to understand whether or not increased abundance of a molecular chaperone in response to heat stress is indicative of increased thermal stress for a given population and whether or not temperature adaptation has, or is, occurring in this species.

## Methods

### Study Organism:

The western fence lizard, *Sceloporus occidentalis*, occurs throughout western North America from Washington state to central Baja California, Mexico, and from sea level to approximately 2500 m (Behler 2000, Stebbins 2003). Adults average 60 – 90 mm in snout-vent length (SVL) and are semi-arboreal habitat generalists, utilizing perches from boulders to bushes and trees. This species is typically active at the sites used for this study from April to October (Adolph 1990, Asbury and Adolph 2007).

Specimen collections and site descriptions:

I collected adult male *S. occidentalis* by noose at 4 locations in California during late May and early June in 2007 and 2008, and September in 2008: 1) Bishop Creek (BC), 7.9 km southwest of Bishop, Inyo County (37°17'34"N, 118°33'05"W, Elev. 2135 m); 2) Keough Hot Springs (KH), 12.5 km south of Bishop, Inyo County (37°15'4"N, 118°22'20"W, Elev. 1220 m); 3) Table Mountain (TM), 3.5 km northwest of Wrightwood, San Bernardino County (34°22'43"N, 117°39'33"W, Elev. 2230 m); and 4) Joshua Tree (JT), 11.2 km west of Pinion Hills, Los Angeles County (34°26'30"N, 117°45'42"W, Elev. 1370 m). These four sites occur at paired latitudes and altitudes, with both a low and high elevation represented at each of the south and north latitudes.

At each site, I collected 13-15 lizards during 2007 and six during 2008. All lizards were transported back to the laboratory within 8 hours of capture. Lizards were housed in custom-made 25 L enclosures with a single rock for a perch and sand as a substrate. Heat lamps suspended above each cage provided a 12-h light and 12-h dark cycle and provided a thermal gradient that lizards could use to thermoregulate. Water was provided *ad libitum* in a small dish. Crickets dusted with vitamin powder were provided as food every other day. The presence of food in the stomach has been shown to affect locomotor performance in other species (Huey et al. 1984, Kelehear and Webb 2006), so lizards were not fed within 24 hours of the beginning of experimental treatment.

Heat stress and sprint speed trials:

Sprint speed performance trials were conducted within 72 hours of capture. Lizards were assigned to one of two experimental temperature treatments: 40°C (heat stress) or 20° (control). Of the lizards caught during 2008, five were randomly assigned to the control treatment and one was assigned to the heat stress, while all lizards from 2007 were assigned to the heat stress. For all lizards sprint speed was measured using a custom built 2 m racetrack held at 22°C. Sprint speed was initially measured in all lizards before exposure to experimental temperature treatment (0 hours, time point T1). To measure maximum sprint speed, each lizard was raced three times along the racetrack. Speed for each 25 cm interval was determined using photogates connected to a computer. To correct for individual variation in maximum sprint speed, I calculated a mean maximum sprint speed for all three trials using the fastest 25 cm interval in each replicate.

After initial sprinting trials, each lizard was placed in a small plastic container in a temperature incubator at either 40°C or 20°C for four hours. At the end of the four hour treatment, sprint speed was measured again (4 hours, time point T2) after which the lizards were given a one hour rest period to allow for induction of Hsp70 synthesis (Hofmann and Somero 1995, Dahlhoff et al. 2001, McMillan et al. 2005). At the end of the rest period, a final sprint speed was measured (5 hours, time point T3). After final sprinting trials all lizards were sacrificed and hind limb muscle tissue was dissected for Hsp70 analysis. All dissected tissue was flash frozen on dry ice and stored at -80°C for less than six month until biochemical analysis could be performed.

## Biochemical analysis:

Levels of total Hsp70 were quantified following methods modified from previously published studies (McMillan et al. 2005, Rank et al. 2007). Dissected tissue was homogenized in buffer [100 mM Tris-HCl, 0.5% SDS, 0.5% Triton-X, 1% Protease inhibitor cocktail (Sigma-Aldrich Co.), pH 7.2] using ground glass tissue homogenizers (Kontes #20) and centrifuged at 15,000 x g for 15 min 4°C. Total protein concentration was determined in the supernatant using a commercially available kit (Thermo Fisher Scientific, Rockford, IL, USA) that utilized a modified BCA approach (Smith et al. 1985). Samples with equal protein concentration were made using homogenized tissue, commercially available sample buffer (Invitrogen Corp., Carlsbad, CA), reducing agent (50 mM DTT final concentration), and ultrapure water. Twenty micrograms of total protein from each lizard was loaded into a 4-12% gradient pre-cast gel (Invitrogen Corp.) and separated using poly-acrylamide gel electrophoresis (150 V, 90 min, MOPS buffer). Quantification of Hsp70 abundance was achieved by loading two wells of each gel with between 10 and 80 ng pure human Hsp70 (NSP-555; Assay Designs, Ann Arbor MI) as positive controls. To standardize quantification of positive controls across gels, each control volume was loaded on multiple gels. After separation, bands were transferred to a 0.45 µm PVDF membrane using a constant current (0.4 amperes) for 60 minutes according to previously published methods (Towbin et al. 1979, McMillan et al. 2005). Blots were then probed for Hsp70 using an Hsp70 specific antibody (MA3-006, Affinity BioReagents, Golden CO) and a peroxidase conjugated anti-rat secondary antibody. Bands were visualized using chemi-luminescence and quantified using Quantity One<sup>®</sup> software (Bio-Rad).



## Statistical Analysis:

All tests were conducted using JMP statistical software (version 7.0.2, SAS Institute, Cary, NC). To detect differences in sprint speed among sites over time I used two separate repeated-measures ANCOVA on each of the control and experimental temperature groups. For both tests I used site as a between-subjects factor, SVL as a covariate, and time (0, 4, 5 hours) as a within-subjects factor.

To test for site differences in total Hsp70 abundance, I performed an ANCOVA on Hsp70 expression with site as the factor and SVL as a covariate. I also tested site differences in abundance of Hsp70 for non-stressed individuals using a similar design. Finally, to examine the relationship between Hsp70 abundance and sprint speed, I performed an ANCOVA on site differences in sprint speed at the end of sprint trials, as well as the difference in sprint speed from the beginning of the experiment until the end (T1-T3). For this model, I used site as a factor and then included Hsp70 expression and SVL as covariates.

## Results

### Sprint Trials:

Sprint speed in *S. occidentalis* was highly variable across all sites independent of experimental treatment. Individuals from the Joshua Tree site, one of the warm weather sites, had the fastest sprint speeds (1.70 m/s), independent of time or treatment temperature. Lizards from Keough Hot Springs were second fastest (1.28 m/s), followed by Table Mountain (1.17 m/s), and finally lizards from Bishop Creek (1.02 m/s), which were slowest ( $F_{3,75} = 5.983$ ,  $p < 0.001$ , not shown). Tukey's pairwise comparisons also

revealed that sprint speeds at Joshua Tree were significantly faster than Bishop Creek and Table Mountain and that sprint speeds at Keough Hot Springs are intermediate to the other three sites.

A similar pattern was observed for individuals assigned to the experimental heat stress treatment (40°,  $N = 15$  per site). Joshua Tree lizards had the fastest sprint speeds and lizards from Table Mountain, one of the cool sites, were slowest ( $F_{3,55} = 3.370$ ,  $p = 0.025$ , Figure 12). Lizards assigned to the control temperature treatment (22°C,  $n = 5$  per site) did not differ in sprint speed across sites at any point before or after heat stress (time X site:  $F_{6,28} = .2230$ ,  $p = 0.9660$ , Figure 12). As a result, control treatment lizards from all sites were pooled at each time point for subsequent analysis.

Sprint speed in *S. occidentalis* was significantly reduced after exposure to the heat stress treatment with the exception of individuals from Joshua Tree which were consistently the fastest group and exhibited the least amount of variation in sprint speed from the beginning of the time trial until the end of the recovery period. However, all other sites suffered significant reductions in sprint speeds as a result of the heat treatment (Figure 12, Table 7). Table Mountain and Keough Hot Springs lizards exhibited similar patterns of reduction: sprint speeds decreased slightly during sprinting trials and then showed a greater decline in sprint speed measured after the 1 hour recovery period. Bishop Creek was unique in that there was a rapid decrease in sprint speed during the stress treatment, however, there was only a slight additional decrease in sprint speed from the end of the stress treatment to the end of the recovery period (Time X Site:  $F_{6,102} = 3.72$ ,  $p = 0.0021$ , Figure 12).

Final sprint speeds also differed among the sites. Absolute sprint speed for *S. occidentalis* at the end of recovery period were fastest at Joshua Tree, intermediate at Keough Hot springs and Table Mountain, and lowest at Bishop Creek (Figure 13A, Table 8). In addition, the amount of reduction in sprint speed from initial measurement to sprint speeds measured after recovery also differed between sites (Figure 13B, Table 8B). Lizards from Bishop Creek had the greatest reduction in sprint speed from the beginning of the experiment to the end, followed by Keough Hot springs, Table Mountain, and Joshua Tree (Figure 13b, Table 8).

Hsp70 abundance:

There were no statistical differences observed in total Hsp70 abundance levels across sites in *S. occidentalis* for lizards that were heat stressed at 40°C for 4 hours (Figure 14, Table 9). In addition, there were no differences in Hsp70 abundance across sites for the lizards in the control temperature treatment (Figure 14, Table 9). Although there were no statistical differences among sites in Hsp70 abundance, for all lizards, sprint speed at the end of the recovery period was negatively correlated with Hsp70 abundance (Figure 15A, Table 8,  $p = 0.0149$ ), such that lizards with higher Hsp70 abundance were poorer sprinters. Furthermore, the magnitude of the reduction in sprint speed was positively correlated with increased Hsp abundance, such that individuals with higher Hsp70 abundances suffered a greater reduction in sprint speed from the beginning of heat trial to the end (Figure 15B, Table 8,  $p = 0.0226$ ).

## Discussion

In this study, I found that lizards from all four populations differed in sprint speed independent of exposure to heat stress and that heat stress had very little effect on Joshua Tree lizards. These results suggest that Joshua Tree lizards are the most physiologically resistant to heat-induced stress. Conversely, lizards from Bishop Creek appeared to be the most susceptible because they had the largest reduction in sprint speed after heat stress. These findings suggest that animals chronically exposed to higher temperatures, like those experienced by the low-latitude populations such as in Joshua Tree and Keough Hot springs, are better adapted to higher temperatures and may be better able to withstand increases in environmental temperature at their site.

### Geographic differences in sprint speed:

Exposure to heat stress also led to variation in sprint speeds among the sites. Joshua Tree lizards were only marginally faster than lizards from other sites at the beginning of the experiment, but by the end of the trials, the difference among sites was much more pronounced, indicating sprint speeds at the Joshua Tree site were the most resilient to the experimental thermal stress. The other three sites did not statistically vary in sprint speed at the beginning or the end of the sprinting trials, but did vary at the end of the stress treatment before the recovery period. Bishop Creek lizards seemed to be the most susceptible to heat stress because the decrease in sprint speed during sprinting trials was greatest at Bishop Creek.

In addition to being the fastest lizards at each time point, lizards from Joshua Tree also had the least reduction in sprint speed as a result of heat stress. Bishop Creek

suffered the greatest reduction in sprint speed while Keough Hot Springs and Table Mountain were intermediate, with lizards from Keough Hot Springs having a slightly higher, yet non-significant, reduction in sprint speed from the heat stress than at Table Mountain. Given that environmental temperature is higher at both Keough Hot Springs and Joshua Tree than it is at Bishop Creek or Table Mountain, it is surprising that sprint speed at Keough Hot Springs was more susceptible to heat than sprint speed at Table Mountain. This suggests that sprint speeds in this species are not determined solely by adaptation to local environmental temperatures, but a combination of adaptive and environmental factors.

Hsp70 abundance after exposure to heat stress also varied across these sites. Although these differences were not significant, lizards from the southern sites tended to be more resistant to heat stress, as observed from their lower abundance of Hsp70, while both northern sites had higher abundance levels. Control lizards exhibited a different trend in that northern sites had lower expressed levels of Hsp70 than southern sites. One caveat is that total Hsp70 abundance levels measured in this study represent the sum of both constitutive and inducible abundance levels. Constitutive Hsp70 levels are present in the cell at all times and help to fold proteins as they are synthesized, while inducible Hsp70 levels are expressed only in response to periods of thermal stress (Lindquist and Craig 1988, Sorensen et al. 2003).

We were unable to directly measure induced levels of Hsp70 due to the lack of availability of a lizard specific antibody. However, given that control lizards were not exposed to heat stress at any point during the experiment, it is reasonable to assume that Hsp70 abundance in the control individuals is representative of constitutive Hsp70

abundance levels at each site. Thus, the difference between total Hsp70 abundance levels and constitutive Hsp70 abundance levels should approximate induced Hsp70 expression levels at each site. From this, it is clear there was a clear latitudinal difference in inducible heat shock protein because although the southern sites had a higher abundance of constitutive Hsp70, the amount induced in response to heat stress was relatively little. Conversely, induced abundance at the northern site was much higher.

Previous studies in other ectotherms have found that Hsp70 expression is a plastic physiological reaction that can be adjusted in response to environmental temperature (Krebs and Feder 1997b, Feder 1999, Feder and Hofmann 1999, Tomanek and Somero 1999). Results from this study are consistent with previous results in that Hsp70 seems to be a plastic response. Although total Hsp70 expression did not vary among our sites, the relative proportion due to induced and constitutive levels did vary with population. This is consistent with the idea that temperature adaptation has occurred between the northern and southern sites because, while the warmer temperatures at the southern sites necessitate a higher sustained (constitutive) levels of Hsp70, these populations are better able to withstand periods of thermal stress. Across all sites, total abundance levels are conserved, which is consistent with previous work that suggest that overall thermal physiology should be conserved across populations to maintain organism function (Bennett 1990, Ji et al. 1996, Johnston and Bennett 1996, Angilletta 2001, Angilletta et al. 2002a).

I also found a negative correlation between Hsp70 abundance and sprint speed after heat stress and a positive correlation between a reduction in sprint speed and Hsp70 levels. This finding is consistent with the hypothesis that Hsp70 abundance is an

important indicator of physiological stress in *S. occidentalis* and that there is a trade-off between Hsp70 expression and maximum sprint speed. However, given that differences in air temperature at these sites was greatest between altitudes (Chapter 1), and that that difference in hsp70 abundance and sprint speed was greatest between latitudes in this study, the results of this study contradict the hypothesis of temperature adaptation in this species.

One possible explanation for these results is that underlying genetic differences among these populations can explain the difference between sprint speed and environmental temperature. Although I did not measure gene flow across our sites in the current study, previous work has shown that there is a much higher level of gene flow between the two southern populations than there is between the southern populations and the northern desert site (S. Adolph, personal communication). This finding can be extended to conclude that there may be gene flow between the two northern sites. As a result, it is possible that temperature adaptation has occurred for *S. occidentalis* at the thermally extreme sites, Bishop Creek and Joshua Tree. However, at the corresponding intermediate sites, Keough Hot Springs and Table Mountain, the relationship between temperature and sprint speed may be partially determined by adaptive differences between the populations as well as recent thermal history (hot temperatures at Keough Hot Springs and cool temperatures at Table Mountain). However, additional work is needed to determine the relative importance of these two factors in determining sprint speed in *S. occidentalis*.

*Sceloporus occidentalis* has been shown to be extremely effective at regulating its body temperature in nature by utilizing the microhabitat structure of the environment

(Adolph 1990, Asbury and Adolph 2007). Given this, it is surprising that populations of *S. occidentalis* vary in sprint speeds after heat stress and in Hsp70 expression. Since they are able to accurately regulate their body temperature, one would expect that this would remove or at least reduce the selection pressure to withstand increased temperature in this species. However, slight variations in microhabitat can create a mosaic of environmental temperatures in habitats with very little structure (Helmuth et al. 2006). Structurally complex habitats, such as those where *S. occidentalis* is found, may include areas where individuals become stressed if they are unable to leave, or other areas where an individual never becomes stressed. If an individual is unable to escape an unsuitable thermal habitat, this can lead to thermal stress that may result in lower sprinting speeds which may make them more prone to predation, and have lower fitness relative to unstressed individuals (Irschick et al. 2000, Irschick et al. 2008).

#### Conclusion:

In conclusion, I found that my hypothesis of temperature adaptation was supported by these populations of *S. occidentalis* because overall lizards from warmer sites were more resilient to higher temperatures and had higher sprint speeds and lower Hsp70 induced in response to heat stress. However, suspected gene flow within latitudes makes it difficult to predict patterns solely based on environmental temperature. Also, individuals with higher Hsp70 abundances exhibited lower sprint speeds, supporting the hypothesis that Hsp70 serves as protections for a protein or a group of proteins critical to locomotion. As a result, individuals that express more Hsp70 may have lower survivorship due to increased predation on slower individuals. Additional studies are



needed to better quantify the effects of stressful temperatures on locomotion performance, so that we can better predict the future distribution of this species in response to a changing climate.

## Tables

Table 7: Effects of site and time on mean maximum sprint speed for *Sceloporus occidentalis* before and after exposure to experimental heat stress. Repeated measures Analysis of covariance (ANCOVA) was used with site as a fixed factor and snout-vent length (SVL) as covariate. Time since the beginning of the experiment (0, 4, 5 hours) is included as the repeated measure.

Source	df	F	p
<b>Between-subjects factors</b>			
Site	3	7.652	0.0002*
SVL	1	0.786	0.3791
Error	55		
<b>Within-subject factors</b>			
Time	2	1.441	0.2458
Time x Site	6	3.720	0.0021*
Time x SVL	2	0.796	0.4562
Error	108		

\* significant

Table 8: Effects of collection site and Hsp70 abundance on sprint speeds in *Sceloporus occidentalis* from four different sites in California. Sprint speeds used in this analysis were measured at A) T3 (end of experimental trials) and B) the difference in sprint speed from the beginning of the trial to the end of the trial (T1-T3). I performed an ANCOVA with collection site as a fixed effect and Hsp70 abundance and SVL as covariates. The interaction between site and Hsp70 was included to test for differing relationships across sites.

A) Sprint Speed at T3

Source	df	SS	F	p
Site	3	4.156	6.316	0.0010*
Hsp70	1	1.393	6.352	0.0149*
Site*Hsp70	3	0.620	0.941	0.4274
SVL	1	0.224	1.021	0.3169
Error	51	11.187		

\* significant

B) Reduction in Sprint Speed (T1-T3)

Source	df	SS	F	p
Site	3	2.135	7.087	0.0005*
Hsp70	1	0.556	5.532	0.0226*
Site*Hsp70	3	0.361	1.198	0.3198
SVL	1	0.186	1.856	0.1798
Error	51	5.122		

\* significant

Table 9: Effects of collection site on abundance of total and constitutive levels of Hsp70 in *S. occidentalis* after experimental heat stress. Total Hsp70 abundance was measured in lizards exposed to experimental heat stress (4 hours @ 40°C) and constitutive Hsp70 abundance was measured for lizards in the control treatment (4 hours @ 22°C). I performed an ANCOVA with site as a fixed factor and SVL as a covariate.

Total Hsp70 expression					Constitutive Hsp70 Expression				
Source	df	SS	F	p	Source	df	SS	F	p
Site	3	5.73E-03	1.276	0.2913	Site	3	4.41E-03	1.222	0.3085
SVL	1	1.08E-03	0.725	0.3981	SVL	1	1.55E-03	1.79	0.2601
Error	56	0.084			Error	67	0.0805		

\* significant

Figures

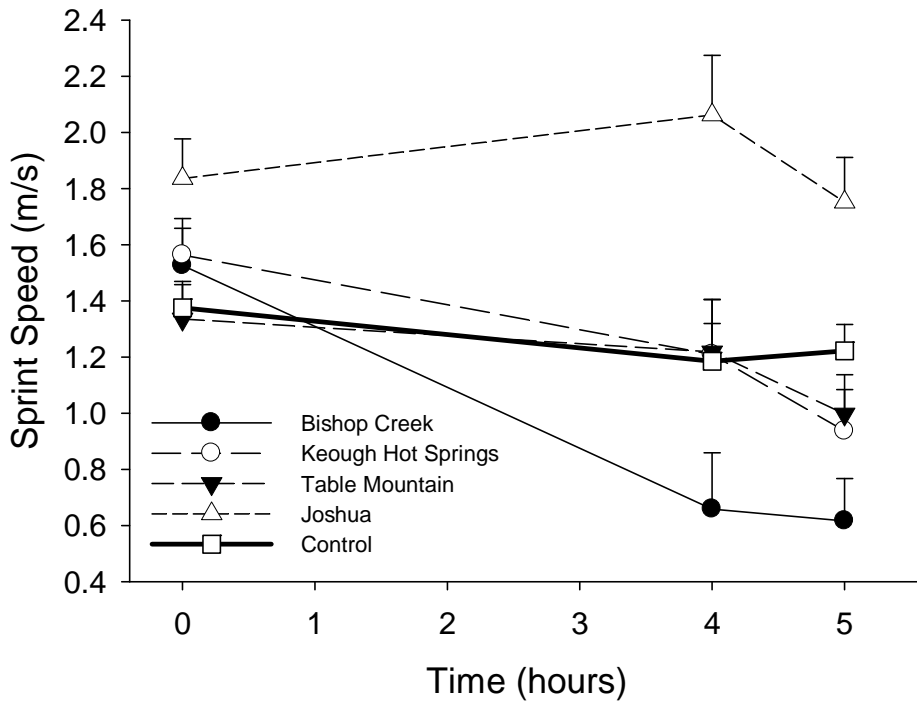


Figure 12. Sprint speeds for *S. occidentalis* from four sites before and after exposure to experimental heat stress (40°C) or control temperature (20°C) for four hours. Sprint speeds were measured at three time intervals for each lizard; 0 hours (before heat stress, T1), 4 hours (immediately after heat stress, T2), and 5 hours (after heat stress and 1 hour recovery period, T3). Data are means ( $\pm$ SE) for each site (N = 14 – 16 per site). Control treatment values represents data pooled across sites (N = 5 per site, 20 total). See Table 7 and text for statistical analysis.

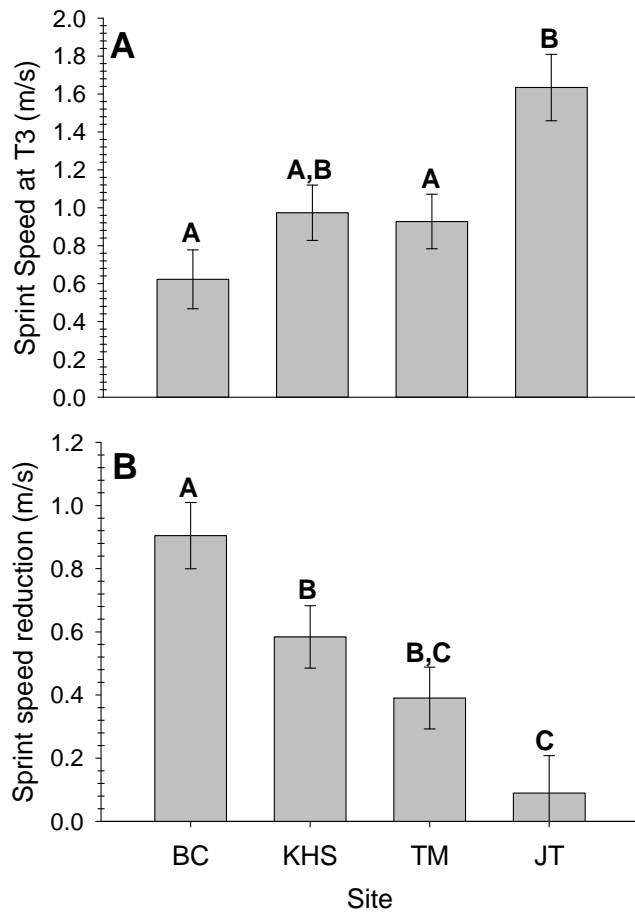


Figure 13. Mean maximum sprint speeds after experimental treatment and recovery period in *S. occidentalis* from four different sites. Data are means ( $\pm$ SE) for BC, KHS, TM, and JT (N = 14-16 for each site). Letters denote statistical differences among pairwise site comparisons. A) Sprint speed at each site after 4 hour heat stress at 40°C and 1 hour recovery at 20°C (T3). B) Reduction in sprint speed between T1 (before heat stress) and T3 (after heat stress and recovery) at each site. See Table 8 and text for statistical analysis.

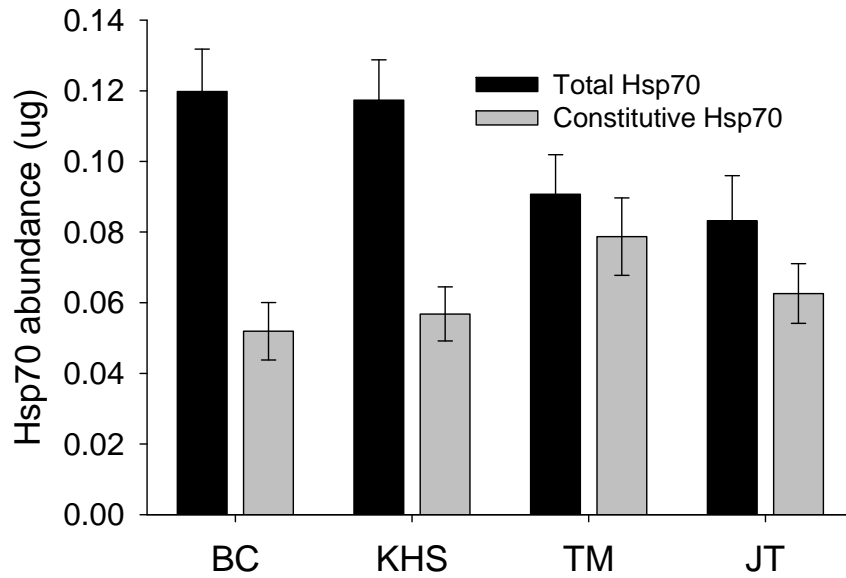


Figure 14. Abundance levels of Hsp70 in *S. occidentalis* from four sites in California. Data are means ( $\pm$ SE) for lizards from BC, KH, TM, and JT (N=14-16 each site). Black bars represent total Hsp70 abundance levels after 4 hour heat stress at 40°C and one hour recovery period. Shaded bars represent constitutive Hsp70 abundance in control individuals (20°C for four hours). See Table 9 and text for statistical analysis.

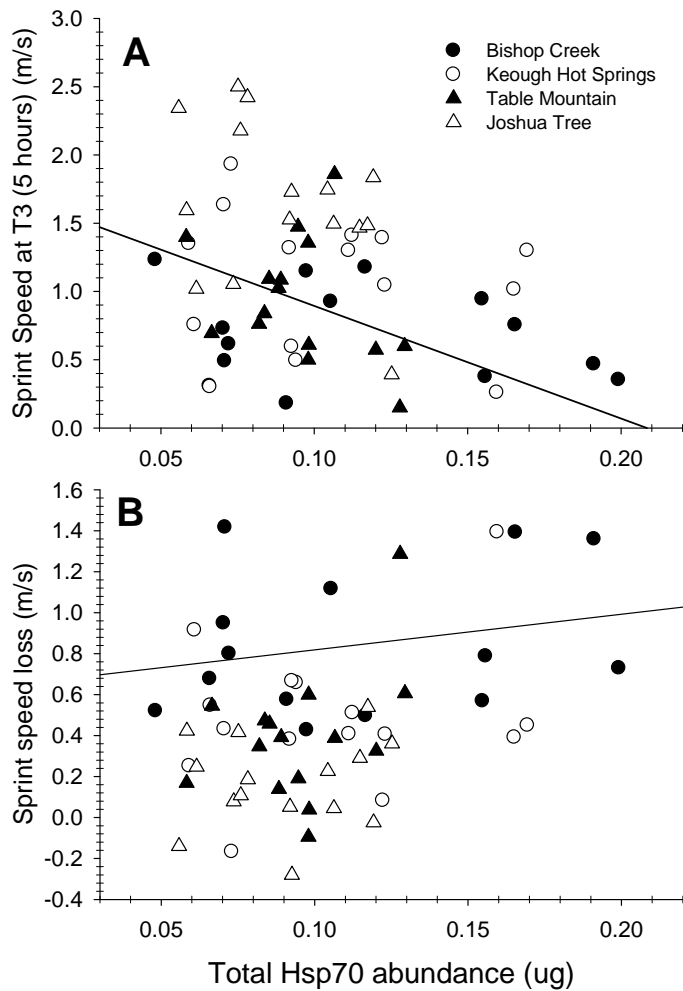


Figure 15. Correlation between total Hsp70 abundance and A) sprint speed at T3 and B) the amount of reduction in sprint speed between T1 and T3. Data points represent individual lizards from Bishop Creek (filled circles), Keough Hot Springs (open circles), Table Mountain (filled triangles), and Joshua Tree (open, triangles). Sprint speed at T3 decreases with increased Hsp70 abundance and the difference between sprint speed at T1 and T3 increases with Hsp70 abundance. See Table 8 and text for statistical analysis.



## CHAPTER IV

### CONCLUDING REMARKS

My dissertation examined three different aspects of temperature adaptation in the western fence lizard, *Sceloporus occidentalis*. Chapter 1 examined variation in environmental and lizard temperature at different latitudes and altitudes. Chapter 2 addressed geographic, seasonal and historical differences in thermal tolerance and thermal breadth of *S. occidentalis* and the role of Hsp70 in thermal tolerance of this species. Finally, Chapter 3 examined geographic variation in maximum sprint speed and abundance of Hsp70 for *S. occidentalis* in order to assess whether or not Hsp70 abundance is a reliable indicator of whole-organism stress after exposure to a heat shock. Together these studies illustrate variation in the relationship between environmental temperature and organism function, from physiology, to whole organism performance, to behavior. Here I summarize and integrate these studies into a common framework relating these different measures of temperature physiology to the ability of *S. occidentalis* to withstand further changes in environmental conditions.

In chapter 1, I began by making the case that given the importance of temperatures in ectotherm processes, individuals living in different thermal environments should become locally adapted to the temperature of their environment (Dahlhoff and Somero 1993, Hochachka and Somero 2002). I did this by first establishing that my study sites did indeed differ in mean annual temperatures and mean temperature during the active period for the lizards. This finding corroborates previous work that found that low-elevation populations of *S. occidentalis* utilized higher perches to escape the hotter

ambient temperatures closer to the ground (Adolph 1990, Buckley 2008). Despite finding thermal differences at these sites, there was no evidence for temperature adaptation in this species as both field and preferred body temperature did not vary among sites. One possible explanation is that thermal specialization is actually not favored for this species which can occupy many different niches in its environment and may survive equally well as a thermal generalist. Similar reasoning has been used to explain a lack of specialization in morphology – performance relationships (Alfaro et al. 2005, Young et al. 2007) but may apply to physiological specialization as well. An additional possibility is that *S. occidentalis* at these sites are extremely effective at utilizing microhabitat to regulate their body temperature which may effectively negate thermal difference across sites (Asbury and Adolph 2007). Although body temperatures did not vary with thermal environment, providing evidence local adaptation among these populations, this does not rule out geographic variation in the response of individual physiological mechanisms to temperature, which I explored in subsequent chapters.

In the second chapter I examined the effects of an acute thermal stress in *S. occidentalis* in an attempt to understand how individual populations are able to tolerate extreme temperatures. I presented evidence that heat tolerance was highest among the hotter, low elevation sites and lower at the cooler, high elevation sites while cold tolerance was highest at northern sites independent of elevation. In addition, I also presented evidence that there is a tradeoff in heat versus cold tolerance and the range of temperatures over which an individual lizard is able to function varied with site and season. Furthermore, I found no protective effect of Hsp70 abundance on thermal tolerance as I had hypothesized which is contradictory to previous research in

invertebrates (Nearing et al. 2003, Tomanek and Sanford 2003). My working hypothesis is that lizards are able behaviorally regulate body temperatures more effectively than invertebrates, which effectively decouples pressure for selection on Hsp70 and thermal tolerance due to the energetic cost of Hsp70 expression and the delay in expression after the onset of a thermal stress (Sorensen et al. 2009). Finally, I found no evidence of a long term relationship between thermal tolerance and air temperature which is likely due to a strong influence of recent thermal history in determining thermal tolerance (Fry 1947, Sinclair et al. 2006).

In chapter 3, I studied how expression of Hsp70 is related to whole organism performance after heat stress. I presented data showing that in the absence of heat stress, lizards from all sites had similar sprinting performance. However, after heat stress, lizards from warmer sites sprinted faster than lizards from cooler sites and lizards with higher levels of Hsp70 also had lower sprint speeds and were more susceptible to heat stress. The temperature – performance relationship has been well studied in lizards (Vanhooydonck et al. 2001, Angilletta et al. 2002a, Du et al. 2007) but this relationship is rarely studied in multiple populations of the same species [but see Buckley (2008)]. My study is unique in that it examined the effects of geographic variation in sprinting performance of a single species after exposure to a potentially stressful temperature. Furthermore, northern populations had higher levels of induced Hsp70 suggesting that these populations are under greater thermal stress, supporting the hypothesis of local adaptation in this species (Dahlhoff and Rank 2000, Tomanek and Somero 2000, Rank et al. 2007).

My results, taken as a whole, suggest that although there appear to be adaptive differences among these populations (which could not be verified for northern latitude sites) there is a large component of performance and thermal physiology in this species that is determined by recent thermal history. Most likely, selection pressure is greatest at the thermally extreme sites (Bishop Creek and Joshua Tree) and gene flow within latitudes creates a buffering effect for the two intermediate sites. This means that during milder seasons, such as the spring and fall, sites with more genetic similarity will exhibit similar patterns in thermal physiology and performance. However, during the seasons with more extreme temperatures, summer and winter, local environmental temperature will play a greater role in determining the thermal physiology and performance capabilities of *S. occidentalis*. In order to test this hypothesis further it would be ideal to examine changes in heat and cold tolerance at other point during the year, especially at the beginning and the end of the winter season. However, currently this is not possible at all of these sites because of the absence of lizards during winter months.

Understanding the effects of environmental temperature and its ability to limit species distributions are necessary in order to be able to predict future changes in distributions that occur as a result of climate change (Clarke 1996, Chamaillé-Jammes et al. 2006, Deutsch et al. 2008). The responses of individual species will vary as some species migrate to new habitats while others will adapt to climatic changes (Hoffmann and Blows 1993, Bradshaw and Holzapfel 2006, Parmesan 2006). Ectotherms, specifically those that are able to behaviorally thermoregulate, are one group of organisms that are predicted to be buffered against climatic changes (Kearney et al. 2009). However, in order to verify this prediction it is important to understand the effects

of environmental temperature on individual species, especially the effects of extreme temperatures. My dissertation addressed this idea using *S. occidentalis* because of the range of thermal environments that different populations of this species occupy.

This dissertation is one of the first studies to examine the relationship between geography, thermal tolerance, stress protein expression and sprinting performance all within a single vertebrate species and found that the relative importance of adaptive and plastic factors can vary with season and geography. Further research into developing a greater understanding of this relationship in *Sceloporus occidentalis* and understanding whether similar patterns exist in other species can be beneficial better predicting the effects of climate change in natural populations.

## BIBLIOGRAPHY

- Ackerman, R. A. 1994. Temperature, Time, and Reptile Egg Water Exchange. *Israel Journal of Zoology* **40**:293-306.
- Addo-Bediako, A., S. L. Chown, and K. J. Gaston. 2000. Thermal tolerance, climatic variability and latitude. *Proceedings of the Royal Society of London Series B-Biological Sciences* **267**:739-745.
- Adolph, S. C. 1987. Physiological and behavioral ecology of the lizards *Sceloporus occidentalis* and *Sceloporus graciosus*. Dissertation. University of Washington, Seattle.
- Adolph, S. C. 1990. Influence of behavioral thermoregulation on microhabitat use by two *Sceloporus* lizards. *Ecology* **71**:315-327.
- Adolph, S. C. and W. P. Porter. 1996. Growth, seasonality, and lizard life histories. *Oikos* **77**:267-278.
- Alfaro, M. E., D. I. Bolnick, and P. C. Wainwright. 2005. Evolutionary consequences of many-to-one mapping of jaw morphology to mechanics in labrid fishes. *American Naturalist* **165**:E140-E154.
- Andrews, R. M., T. Mathies, and D. A. Warner. 2000. Effect of incubation temperature on morphology, growth, and survival of juvenile *Sceloporus undulatus*. *Herpetological Monographs* **14**:420-431.
- Angilletta, M. J. 2001. Thermal and physiological constraints on energy assimilation in a widespread lizard (*Sceloporus undulatus*). *Ecology* **82**:3044-3056.
- Angilletta, M. J. 2009. Looking for answers to questions about heat stress: researchers are getting warmer. *Functional Ecology* **23**:231-231.
- Angilletta, M. J., T. Hill, and M. A. Robson. 2002a. Is physiological performance optimized by thermoregulatory behavior?: a case study of the eastern fence lizard, *Sceloporus undulatus*. *Journal of Thermal Biology* **27**:199-204.
- Angilletta, M. J., P. H. Niewiarowski, and C. A. Navas. 2002b. The evolution of thermal physiology in ectotherms. *Journal of Thermal Biology* **27**:249-268.
- Angilletta, M. J., R. S. Wilson, C. A. Navas, and R. S. James. 2003. Tradeoffs and the evolution of thermal reaction norms. *Trends in Ecology & Evolution* **18**:234-240.
- Arya, R., M. Mallik, and S. C. Lakhotia. 2006. Heat shock genes - integrating cell survival and death. Pages 595-610 *in* International Symposium on Environmental Factors, Cellular Stress and Evolution, Varanasi, INDIA.

- Asbury, D. and S. C. Adolph. 2007. Behavioural plasticity in an ecological generalist: microhabitat use by western fence lizards. *Evolutionary Ecology Research* **9**:801-815.
- Austin, M. P. 2002. Spatial prediction of species distribution: an interface between ecological theory and statistical modelling. *Ecological Modelling* **157**:101-118.
- Ayres, M. P. and J. M. Scriber. 1994. Local adaptation to regional climate in *Papilio canadensis* (*Lepidoptera, Papilionidae*). *Ecological Monographs* **64**:465-482.
- Behler, J. L., editor. 2000. National Audubon Society Field Guide to North American Amphibians and Reptiles. Alfred A. Knopf, New York.
- Bennett, A. F. 1980. The thermal dependence of lizard behavior. *Animal Behaviour* **28**:752-762.
- Bennett, A. F. 1990. Thermal dependence of locomotor capacity. *American Journal of Physiology* **259**:R253-R258.
- Bennett, A. F. and R. B. Huey. 1990. Studying the evolution of physiological performance. Pages 251-284 in J. Futuyma and J. Antonovics, editors. *Oxford Surveys in Evolutionary Biology*. Oxford University Press, Oxford.
- Bergmann, P. and D. J. Irschick. 2006. Effects of temperature on maximum acceleration, deceleration and power output during vertical running in geckos. *Journal of Experimental Biology* **209**:1404-1412.
- Beuchat, C. A. 1988. Temperature effects during gestation in a viviparous lizard. *Journal of Thermal Biology* **13**:135-142.
- Biewener, A. A. 2003. *Animal Locomotion*. Oxford, New York.
- Blackburn, T. M. and K. J. Gaston. 1996. Spatial patterns in the geographic range sizes of bird species in the New World. *Philosophical Transactions of the Royal Society of London Series B-Biological Sciences* **351**:897-912.
- Bradshaw, W. E. and C. M. Holzapfel. 2006. Climate change - Evolutionary response to rapid climate change. *Science* **312**:1477-1478.
- Bradshaw, W. E., P. A. Zani, and C. M. Holzapfel. 2004. Adaptation to temperate climates. *Evolution* **58**:1748-1762.
- Brooke, P. N., R. A. Alford, and L. Schwarzkopf. 2000. Environmental and social factors influence chorusing behaviour in a tropical frog: examining various temporal and spatial scales. *Behavioral Ecology and Sociobiology* **49**:79-87.

- Brown, R. P. 1996. Thermal biology of the gecko *Tarentola boettgeri*: Comparisons among populations from different elevations within Gran Canaria. *Herpetologica* **52**:396-405.
- Buckley, C. R. 2008. Functional morphology, phenotypic plasticity, and geographic variation in two terrestrial ectotherms. Dissertation. University of Massachusetts Amherst, Amherst.
- Buckley, C. R., M. Jackson, M. Youssef, D. J. Irschick, and S. C. Adolph. 2007. Testing the persistence of phenotypic plasticity after incubation in the western fence lizard, *Sceloporus occidentalis*. *Evolutionary Ecology Research* **9**:169-183.
- Burger, J. 1991. Effects of incubation temperature on behavior of hatchling pine snakes: implications for reptilian distribution. *Behavioral Ecology and Sociobiology* **28**:297-303.
- Calsbeek, R. and D. J. Irschick. 2007. The quick and the dead: Correlational selection on morphology, performance, and habitat use in island lizards. *Evolution* **61**:2493-2503.
- Chamaille-Jammes, S., M. Massot, P. Aragon, and J. Clobert. 2006. Global warming and positive fitness response in mountain populations of common lizards *Lacerta vivipara*. *Global Change Biology* **12**:392-402.
- Chown, S. L. and K. J. Gaston. 1999. Exploring links between physiology and ecology at macro-scales: the role of respiratory metabolism in insects. *Biological Reviews* **74**:87-120.
- Chown, S. L., K. R. Jumbam, J. G. Sorensen, and J. S. Terblanche. 2009. Phenotypic variance, plasticity and heritability estimates of critical thermal limits depend on methodological context. *Functional Ecology* **23**:133-140.
- Chown, S. L. and J. S. Terblanche. 2007. Physiological diversity in insects: Ecological and evolutionary contexts. Pages 50-152 *Advances in Insect Physiology*, Vol 33.
- Clarke, A. 1996. The influence of climate change on the distribution and evolution of organisms. *in* I. A. Johnston and A. F. Bennett, editors. *Animals and Temperature: Phenotypic and Evolutionary Adaptation*. Cambridge University Press, New York, NY.
- Clarke, A. 2003. Costs and consequences of evolutionary temperature adaptation. *Trends in Ecology & Evolution* **18**:573-581.



- Conover, D. O. and T. M. C. Present. 1990. Countergradient variation in growth rate: compensation for length of the growing season among Atlantic silversides from different latitudes. *Oecologia* **83**:316-324.
- Cowles, R. B. and C. M. Bogert. 1944. A preliminary study of the thermal requirements of desert reptiles. *Bulletin of the American Museum of Natural History* **83**:263-296.
- Crill, W. D., R. B. Huey, and G. W. Gilchrist. 1996. Within- and between-generation effects of temperature on the morphology and physiology of *Drosophila melanogaster*. *Evolution* **50**:1205-1218.
- Cross, E. E. and R. S. Rawding. 2009. Acute thermal tolerance in the round goby, *Apollonia melanostoma* (*Neogobius melanostomus*). *Journal of Thermal Biology* **34**:85-92.
- Cuculescu, M., D. Hyde, and K. Bowler. 1998. Thermal tolerance of two species of marine crab, *Cancer pagurus* and *Carcinus maenas*. *Journal of Thermal Biology* **23**:107-110.
- Dahlhoff, E. and G. N. Somero. 1993. Kinetic and structural adaptation of cytoplasmic malate dehydrogenase of Eastern pacific abalone (genus *Haliotis*) from different thermal habitats - Biochemical correlates of biogeographical patterning. *Journal of Experimental Biology* **185**:137-150.
- Dahlhoff, E. P., B. A. Buckley, and B. A. Menge. 2001. Physiology of the rocky intertidal predator *Nucella ostrina* along an environmental stress gradient. *Ecology* **82**:2816-2829.
- Dahlhoff, E. P., S. L. Fearnley, D. A. Bruce, A. G. Gibbs, R. Stoneking, D. M. McMillan, K. Deiner, J. T. Smiley, and N. E. Rank. 2008. Effects of Temperature on Physiology and Reproductive Success of a Montane Leaf Beetle: Implications for Persistence of Native Populations Enduring Climate Change. *Physiological and Biochemical Zoology* **81**:718-732.
- Dahlhoff, E. P. and N. E. Rank. 2000. Functional and physiological consequences of genetic variation at phosphoglucose isomerase: Heat shock protein expression is related to enzyme genotype in a montane beetle. *Proceedings of the National Academy of Sciences of the United States of America* **97**:10056-10061.
- Dahlhoff, E. P. and N. E. Rank. 2006. The role of stress proteins in responses of a montane willow leaf beetle to environmental temperature variation. Pages 477-488 *in* International Symposium on Environmental Factors, Cellular Stress and Evolution, Varanasi, INDIA.

- Davis, M. B. and R. G. Shaw. 2001. Range shifts and adaptive responses to Quaternary climate change. *Science* **292**:673-679.
- Deutsch, C. A., J. J. Tewksbury, R. B. Huey, K. S. Sheldon, C. K. Ghalambor, D. C. Haak, and P. R. Martin. 2008. Impacts of climate warming on terrestrial ectotherms across latitude. *Proceedings of the National Academy of Sciences of the United States of America* **105**:6668-6672.
- Dong, Y. W. and G. N. Somero. 2009. Temperature adaptation of cytosolic malate dehydrogenases of limpets (genus *Lottia*): differences in stability and function due to minor changes in sequence correlate with biogeographic and vertical distributions. *Journal of Experimental Biology* **212**:169-177.
- Du, W. G., Y. W. Lu, L. Shu, and Y. X. Bao. 2007. Thermal dependence of food assimilation and locomotor performance in juvenile blue-tailed skinks, *Eumeces elegans*. *Animal Biology* **57**:29-38.
- Duffy, A. M., J. Clobert, and A. P. Moller. 2002. Hormones, developmental plasticity and adaptation. *Trends in Ecology & Evolution* **17**:190-196.
- Duman, J. G., D. W. Wu, L. Xu, D. Tursman, and T. M. Olsen. 1991. Adaptations of insects to subzero temperatures. *Quarterly Review of Biology* **66**:387-410.
- Ederly, J. S., A. Tadimalla, and E. P. Dahlhoff. 2005. Adaptation to thermal stress in lichen-eating webspinners (*Embioptera*): habitat choice, domicile construction and the potential role of heat shock proteins. *Functional Ecology* **19**:255-262.
- Elderkin, C. L. and P. L. Klerks. 2005. Variation in thermal tolerance among three Mississippi River populations of the zebra mussel, *Dreissena polymorpha*. *Journal of Shellfish Research* **24**:221-226.
- Endler, J. A. 1977. *Geographic Variation, Speciation, and Clines*. Princeton University Press, Princeton, NJ.
- Feder, M. E. 1999. Organismal, ecological, and evolutionary aspects of heat-shock proteins and the stress response: Established conclusions and unresolved issues. Pages 857-864 in *Annual Meeting of the Society-for-Comparative-and-Integrative-Biology*, Denver, Colorado.
- Feder, M. E., A. F. Bennett, W. Burggren, and R. B. Huey, editors. 1987. *New Directions in Ecological Physiological Ecology*. Cambridge University Press, Melbourne, Australia.
- Feder, M. E., A. F. Bennett, and R. B. Huey. 2000. Evolutionary physiology. *Annual Review of Ecology and Systematics* **31**:315-341.

- Feder, M. E. and G. E. Hofmann. 1999. Heat-shock proteins, molecular chaperones, and the stress response: Evolutionary and ecological physiology. *Annual Review of Physiology* **61**:243-282.
- Fields, P. A. and D. E. Houseman. 2004. Decreases in activation energy and substrate affinity in cold-adapted A(4)-lactate dehydrogenase: Evidence from the Antarctic notothenioid fish *Chaenocephalus aceratus*. *Molecular Biology and Evolution* **21**:2246-2255.
- France, R. 1992. The north american latitudinal gradient in species richness and geographical range of fresh water crayfish and amphipods. *American Naturalist* **139**:342-354.
- Fry, F. E. J. 1947. Effects of the environment on animal activity. Univeristy of Toronto Studies, Biological Services, No. 55. Publication of the Ontaria Fisheries Research Laboratory **68**:1-62.
- Garcia Ramos, G. and M. Kirkpatrick. 1997. Genetic models of adaptation and gene flow in peripheral populations. *Evolution* **51**:21-28.
- Gaston, K. J. and S. L. Chown. 1999. Elevation and climatic tolerance: a test using dung beetles. *Oikos* **86**:584-590.
- Gavrilets, S. 2003. Perspective: Models of speciation: What have we learned in 40 years? *Evolution* **57**:2197-2215.
- Gilchrist, G. W. 1995. Specialists and generalists in changing environments. 1. Fitness lanscapes of thermal sensitivity. *American Naturalist* **146**:252-270.
- Glickman, T. S. 2000. Glossary of Meterology. 2nd edition. American Meterological Society, Boston, MA.
- Gould, S. J. and R. F. Johnston. 1972. Geographic variation. *Annual Review of Ecology and Systematics* **3**:457-498.
- Greer, A. E. 1980. Critical thermal maximum temperature in Australian scincid lizards - Their ecological and evolutionary significane. *Australian Journal of Zoology* **28**:91-102.
- Hansen, T. K. and I. B. Petersen. 2001. The influence of rearing temperature on early development and growth of spotted wolffish *Anarhichas minor* (Olafsen). *Aquaculture research* **32**:369-378.

- Hare, K. M., C. G. Longson, S. Pledger, and C. H. Daugherty. 2004. Size, growth, and survival are reduced at cool incubation temperatures in the temperate lizard *Oligosoma suteri* (Lacertilia : Scincidae). *Copeia*:383-390.
- Hazel, J. R. 1995. Thermal adaptation in biological membranes - Is homeoviscous adaptation the explanation? *Annual Review of Physiology* **57**:19-42.
- Helmuth, B., B. R. Broitman, C. A. Blanchette, S. Gilman, P. Halpin, C. D. G. Harley, M. J. O'Donnell, G. E. Hofmann, B. Menge, and D. Strickland. 2006. Mosaic patterns of thermal stress in the rocky intertidal zone: Implications for climate change. *Ecological Monographs* **76**:461-479.
- Helmuth, B., J. G. Kingsolver, and E. Carrington. 2005. Biophysics, physiological ecology, and climate change: Does mechanism matter? *Annual Review of Physiology* **67**:177-201.
- Helmuth, B. S. T. and G. E. Hofmann. 2001. Microhabitats, thermal heterogeneity, and patterns of physiological stress in the rocky intertidal zone. *Biological Bulletin* **201**:374-384.
- Herrel, A., J. J. Meyers, and B. Vanhooydonck. 2001. Correlations between habitat use and body shape in a phrynosomatid lizard (*Urosaurus ornatus*): a population-level analysis. *Biological Journal of the Linnean Society* **74**:305-314.
- Hertz, P. E., R. B. Huey, and E. Nevo. 1982. Fight versus flight - Body temperatures influence defensive responses in lizards. *Animal Behaviour* **30**:676-&.
- Hertz, P. E., R. B. Huey, and R. D. Stevenson. 1993. Evaluating temperature regulation by field active ectotherms - The fallacy of the inappropriate question. *American Naturalist* **142**:796-818.
- Hochachka, P. W. and G. N. Somero. 2002. *Biochemical Adaptation: mechanism and process in physiological evolution*. Oxford University Press, New York.
- Hoffmann, A. A. and M. Blows. 1993. Evolutionary Genetics and climate change: will animals adapt to global warming? *in* P. Kareiva, J. G. Kingsolver, and R. B. Huey, editors. *Biotic interactions and global change*. Sinauer Associates, Inc., Sunderland, MA.
- Hofmann, G. E. 1999. Ecologically relevant variation in induction and function of heat shock proteins in marine organisms. Pages 889-900 *in* Annual Meeting of the Society-for-Comparative-and-Integrative-Biology, Denver, Colorado.

- Hofmann, G. E. and G. N. Somero. 1995. Evidence for protein damage at environmental temperatures - Seasonal changes in levels of ubiquitin conjugates and Hsp70 in the intertidal mussel *Mytilus trossulus*. *Journal of Experimental Biology* **198**:1509-1518.
- Hooper, D. U., F. S. Chapin, J. J. Ewel, A. Hector, P. Inchausti, S. Lavorel, J. H. Lawton, D. M. Lodge, M. Loreau, S. Naeem, B. Schmid, H. Setälä, A. J. Symstad, J. Vandermeer, and D. A. Wardle. 2005. Effects of biodiversity on ecosystem functioning: A consensus of current knowledge. *Ecological Monographs* **75**:3-35.
- Hu, X. P. and A. G. Appel. 2004. Seasonal variation of critical thermal limits and temperature tolerance in Formosan and eastern subterranean termites (*Isoptera* : *Rhinotermitidae*). *Environmental Entomology* **33**:197-205.
- Huang, S. P., Y. Y. Hsu, and M. C. Tu. 2006. Thermal tolerance and altitudinal distribution of two *Sphenomorphus* lizards in Taiwan. *Journal of Thermal Biology* **31**:378-385.
- Huey, R. B. 1991. Physiological consequences of habitat selection. *American Naturalist* **137**:S91-S115.
- Huey, R. B., A. F. Bennett, H. Johnalder, and K. A. Nagy. 1984. Locomotor capacity and foraging behavior of Kalahari lacertid lizards. *Animal Behaviour* **32**:41-50.
- Huey, R. B., A. E. Dunham, K. L. Overall, and R. A. Newman. 1990. Variation in locomotor performance in demographically known populations of the lizard *Sceloporus merriami*. *Physiological Zoology* **63**:845-872.
- Huey, R. B. and J. G. Kingsolver. 1993. Evolution in resistance to high temperature in ectotherms. *American Naturalist* **142**:S21-S46.
- Huey, R. B. and M. Slatkin. 1976. Cost and benefits of lizard thermoregulation. *Quarterly Review of Biology* **51**:363-384.
- Huey, R. B. and R. D. Stevenson. 1979. Integrating thermal physiology and ecology of ectotherms - Discussion of approaches. *American Zoologist* **19**:357-366.
- Hughes, L. 2000. Biological consequences of global warming: is the signal already apparent? *Trends in Ecology & Evolution* **15**:56-61.
- Intergovernmental Panel on Climate Change. 2007. *Climate Change 2007: Synthesis Report*.
- Irschick, D. J. and T. Garland. 2001. Integrating function and ecology in studies of adaptation: Investigations of locomotor capacity as a model system. *Annual Review of Ecology and Systematics* **32**:367-+.

- Irschick, D. J. and J. F. Le Galliard. 2008. Studying the evolution of whole-organism performance capacity: sex, selection, and haiku - an introduction. *Evolutionary Ecology Research* **10**:155-156.
- Irschick, D. J., T. E. Macrini, S. Koruba, and J. Forman. 2000. Ontogenetic differences in morphology, habitat use, behavior, and sprinting capacity in two west Indian *Anolis* lizards. *Journal of Herpetology* **34**:444-451.
- Irschick, D. J., J. J. Meyers, J. F. Husak, and J. F. Le Galliard. 2008. How does selection operate on whole-organism functional performance capacities? A review and synthesis. *Evolutionary Ecology Research* **10**:177-196.
- Jacobson, M. Z. 2005. *Fundamentals of Atmospheric modeling*. Cambridge University Press, Cambridge, MA.
- Jaenicke, R. 1991. Protein stability and molecular adaptation to extreme conditions. *European Journal of Biochemistry* **202**:715-728.
- Janzen, F. J. and G. L. Paukstis. 1991. Environmental sex determination in reptiles - Ecology, evolution, and experimental design. *Quarterly Review of Biology* **66**:149-179.
- Jayco, A. S. and C. I. Millar. 2001. Impacts of climate change on landscapes in the eastern Sierra Nevada and western Great Basin. *U.S. Geological Survey Surface Processes and Climate Change.*, Bishop, CA.
- Jenkins, N. L. and A. A. Hoffmann. 1999. Limits to the southern border of *Drosophila serrata*: Cold resistance, heritable variation, and trade-offs. *Evolution* **53**:1823-1834.
- Ji, X., W. G. Du, and P. Y. Sun. 1996. Body temperature, thermal tolerance and influence of temperature on sprint speed and food assimilation in adult grass lizards, *Takydromus septentrionalis*. *Journal of Thermal Biology* **21**:155-161.
- Johnson, C. N. 1998. Rarity in the tropics: latitudinal gradients in distribution and abundance in Australian mammals. *Journal of Animal Ecology* **67**:689-698.
- Johnston, I. A. and A. F. Bennett, editors. 1996. *Animals and Temperature: Phenotypic and Evolutionary Adaptation*. Cambridge University Press, New York, NY.
- Kassahn, K. S., R. H. Crozier, H. O. Portner, and M. J. Caley. 2009. Animal performance and stress: responses and tolerance limits at different levels of biological organization. *Biological Reviews* **84**:277-292.

- Kearney, M., R. Shine, and W. P. Porter. 2009. The potential for behavioral thermoregulation to buffer "cold-blooded" animals against climate warming. *Proceedings of the National Academy of Sciences of the United States of America* **106**:3835-3840.
- Kelehear, C. and J. K. Webb. 2006. Effects of tail autotomy on anti-predator behavior and locomotor performance in a nocturnal Gecko. *Copeia*:803-809.
- Kingsolver, J. G. and W. B. Watt. 1983. Thermoregulatory strategies in *colias* butterflies - thermal stress and the limits to adaptation in temporally varying environments. *American Naturalist* **121**:32-55.
- Klok, C. J. and S. L. Chown. 2001. Critical thermal limits, temperature tolerance and water balance of a sub-Antarctic kelp fly, *Paractora dreuxi* (Diptera : Helcomyzidae). *Journal of Insect Physiology* **47**:95-109.
- Klok, C. J. and S. L. Chown. 2003. Resistance to temperature extremes in sub-Antarctic weevils: interspecific variation, population differentiation and acclimation. *Biological Journal of the Linnean Society* **78**:401-414.
- Krebs, R. A. and B. R. Bettencourt. 1999. Evolution of thermotolerance and variation in the heat shock protein, Hsp70. Pages 910-919 *in* Annual Meeting of the Society for Integrative and Comparative Biology, Denver, Co.
- Krebs, R. A. and M. E. Feder. 1997a. Natural variation in the expression of the heat-shock protein Hsp70 in a population of *Drosophila melanogaster* and its correlation with tolerance of ecologically relevant thermal stress. *Evolution* **51**:173-179.
- Krebs, R. A. and M. E. Feder. 1997b. Tissue-specific variation in Hsp70 expression and thermal damage in *Drosophila melanogaster* larvae. *Journal of Experimental Biology* **200**:2007-2015.
- Krebs, R. A. and M. E. Feder. 1998. Hsp70 and larval thermotolerance in *Drosophila melanogaster*: how much is enough and when is more too much? *Journal of Insect Physiology* **44**:1091-1101.
- Krebs, R. A. and S. H. Holbrook. 2001. Reduced enzyme activity following Hsp70 overexpression in *Drosophila melanogaster*. *Biochemical Genetics* **39**:73-82.
- Kristensen, T. N., V. Loeschcke, and A. A. Hoffmann. 2007. Can artificially selected phenotypes influence a component of field fitness? Thermal selection and fly performance under thermal extremes. *Proceedings of the Royal Society B-Biological Sciences* **274**:771-778.

- Lailvaux, S. P. and D. J. Irschick. 2007. Effects of temperature and sex on jump performance and biomechanics in the lizard *Anolis carolinensis*. *Functional Ecology* **21**:534-543.
- Lindquist, S. 1986. The heat shock response. *Annual Review of Biochemistry* **55**:1151-1191.
- Lindquist, S. and E. A. Craig. 1988. The heat shock proteins. *Annual Review of Genetics* **22**:631-677.
- Loeschcke, V. and A. A. Hoffmann. 2007. Consequences of heat hardening on a field fitness component in *Drosophila* depend on environmental temperature. *American Naturalist* **169**:175-183.
- Loeschcke, V., R. A. Krebs, and J. S. F. Barker. 1994. Genetic variation for resistance and acclimation to high temperature stress in *Drosophila buzzatii*. *Biological Journal of the Linnean Society* **52**:83-92.
- Loeschcke, V., R. A. Krebs, J. Dahlgard, and P. Michalak. 1997. High-temperatures stress and the evolution of thermal resistance in *Drosophila*. Pages 175-190 in R. Bijlsma and V. Loeschcke, editors. *Environmental stress, adaptation and evolution*. Birkhäuser, Basel.
- Lutterschmidt, W. I. and V. H. Hutchison. 1997a. The critical thermal maximum: data to support the onset of spasms as the definitive end point. *Canadian Journal of Zoology-Revue Canadienne De Zoologie* **75**:1553-1560.
- Lutterschmidt, W. I. and V. H. Hutchison. 1997b. The critical thermal maximum: history and critique. *Canadian Journal of Zoology-Revue Canadienne De Zoologie* **75**:1561-1574.
- McGinnis, S. M. 1966. *Sceloporus occidentalis* - Preferred body temperature of the western fence lizard. *Science* **152**:1090-&.
- McMillan, D. M., S. L. Fearnley, N. E. Rank, and E. P. Dahlhoff. 2005. Natural temperature variation affects larval survival, development and Hsp70 expression in a leaf beetle. *Functional Ecology* **19**:844-852.
- Mellette, H. C., B. K. Hutt, S. I. Askovitz, and S. M. Horvath. 1951. Diurnal variations in body temperature. *Journal of Applied Physiology* **3**:665-675.
- Midgley, G. F., L. Hannah, D. Millar, M. C. Rutherford, and L. W. Powrie. 2001. Assessing the vulnerability of species richness to anthropogenic climate change in a biodiversity hotspot. Pages 445-451 in *Meeting on Climate Change and Conservation*, Cambridge, England.



- Mitton, J. B. 1997. Selection in natural populations. Oxford University Press, New York, NY.
- Moore, J. A. 1949. Geographic variation of adaptive characters in *Rana pipiens schreber*. *Evolution* **3**:1-24.
- Morrison, C. and J. M. Hero. 2003. Geographic variation in life-history characteristics of amphibians: a review. *Journal of Animal Ecology* **72**:270-279.
- Neargarder, G., E. P. Dahlhoff, and N. E. Rank. 2003. Variation in thermal tolerance is linked to phosphoglucose isomerase genotype in a montane leaf beetle. *Functional Ecology* **17**:213-221.
- Nielsen, B. 1961. Regulation of respiration in reptiles. 1. Effects of temperature and CO<sub>2</sub> on respiration of lizards (*Lacerta*). *Journal of Experimental Biology* **38**:301-&.
- Pagel, M. D., R. M. May, and A. R. Collie. 1991. Ecological aspects of the geographical distribution and diversity of mammalian species. *American Naturalist* **137**:791-815.
- Parmesan, C. 2006. Ecological and evolutionary responses to recent climate change. *Annual Review of Ecology Evolution and Systematics* **37**:637-669.
- Parmesan, C., N. Ryrholm, C. Stefanescu, J. K. Hill, C. D. Thomas, H. Descimon, B. Huntley, L. Kaila, J. Kullberg, T. Tammaru, W. J. Tennent, J. A. Thomas, and M. Warren. 1999. Poleward shifts in geographical ranges of butterfly species associated with regional warming. *Nature* **399**:579-583.
- Parsell, D. A. and S. Lindquist. 1993. The function of heat shock proteins in stress tolerance - Degradation and reactivation of damaged proteins. *Annual Review of Genetics* **27**:437-496.
- Pearson, R. G. and T. P. Dawson. 2003. Predicting the impacts of climate change on the distribution of species: are bioclimate envelope models useful? *Global Ecology and Biogeography* **12**:361-371.
- Portner, H. O. 2001a. Climate variations and the physiological basis of temperature dependent biogeography: systemic to molecular hierarchy of thermal tolerance in animals. Pages 739-761 *in* Annual Meeting of the Society-for-Experimental-Biology, Canterbury, England.
- Portner, H. O. 2001b. Environmental and functional limits to muscular exercise and body size in marine invertebrate athletes. Pages 303-321 *in* Annual Meeting of the Society-for-Experimental-Biology, Canterbury, England.

- Pounds, J. A., M. P. L. Fogden, and J. H. Campbell. 1999. Biological response to climate change on a tropical mountain. *Nature* **398**:611-615.
- Rank, N. E., D. A. Bruce, D. M. McMillan, C. Barclay, and E. P. Dahlhoff. 2007. Phosphoglucose isomerase genotype affects running speed and heat shock protein expression after exposure to extreme temperatures in a montane willow beetle. *Journal of Experimental Biology* **210**:750-764.
- Rank, N. E. and E. P. Dahlhoff. 2002. Allele frequency shifts in response to climate change and physiological consequences of allozyme variation in a montane insect. *Evolution* **56**:2278-2289.
- Ravaux, J., F. Gaill, N. Le Bris, P. M. Sarradin, D. Jollivet, and B. Shillito. 2003. Heat-shock response and temperature resistance in the deep-sea vent shrimp *Rimicaris exoculata*. *Journal of Experimental Biology* **206**:2345-2354.
- Rinehart, J. P., S. A. L. Hayward, M. A. Elnitsky, L. H. Sandro, R. E. Lee, and D. L. Denlinger. 2006. Continuous up-regulation of heat shock proteins in larvae, but not adults, of a polar insect. *Proceedings of the National Academy of Sciences of the United States of America* **103**:14223-14227.
- Rinehart, J. P., A. Li, G. D. Yocum, R. M. Robich, S. A. L. Hayward, and D. L. Denlinger. 2007. Up-regulation of heat shock proteins is essential for cold survival during insect diapause. *Proceedings of the National Academy of Sciences of the United States of America* **104**:11130-11137.
- Sabo, J. L. 2003. Hot rocks or no hot rocks: overnight retreat availability and selection by a diurnal lizard. *Oecologia* **136**:329-335.
- Sanders, B. M. 1993. Stress proteins in aquatic organisms - An environmental perspective. *Critical Reviews in Toxicology* **23**:49-75.
- Sears, M. W. 2005. Geographic variation in the life history of the sagebrush lizard: the role of thermal constraints on activity. *Oecologia* **143**:25-36.
- Sears, M. W. and M. J. Angilletta. 2003. Life-history variation in the sagebrush lizard: Phenotypic plasticity or local adaptation? *Ecology* **84**:1624-1634.
- Seymour, R. S. and D. F. Bradford. 1995. Respiration of amphibian eggs. *Physiological Zoology* **68**:1-25.
- Sinclair, B. J., J. S. Terblanche, M. B. Scott, G. L. Blatch, C. J. Klok, and S. L. Chown. 2006. Environmental physiology of three species of *Collembola* at Cape Hallett, North Victoria Land, Antarctica. *Journal of Insect Physiology* **52**:29-50.

- Sinervo, B. and S. C. Adolph. 1989. Thermal sensitivity of growth-rate in hatchling *Sceloporus* lizards - environmental, behavioral and genetic aspects. *Oecologia* **78**:411-419.
- Sinervo, B. and S. C. Adolph. 1994. Growth plasticity and thermal opportunity in *Sceloporus* lizards. *Ecology* **75**:776-790.
- Sinervo, B. and J. Losos. 1991. Walking the tight rope: arboreal sprint performance among *Sceloporus occidentalis* lizard populations. *Ecology* **72**:1225-1233.
- Smith, G. R. and R. E. Ballinger. 1994. Thermal tolerance in the tree lizard (*Urosaurus ornatus*) from a desert population and a low montane population. *Canadian Journal of Zoology-Revue Canadienne De Zoologie* **72**:2066-2069.
- Smith, P. K., R. I. Krohn, G. T. Hermanson, A. K. Mallia, F. H. Gartner, M. D. Provenzano, E. K. Fujimoto, N. M. Goeke, B. J. Olson, and D. C. Klenk. 1985. Measurement of Protein using Bicinchoninic acid. *Analytical Biochemistry* **150**:76-85.
- Smith, T. M. and R. W. Reynolds. 2003. Extended reconstruction of global sea surface temperatures based on COADS data (1854-1997). *Journal of Climate* **16**:1495-1510.
- Sokal, R. R. and F. J. Rohlf. 1997. *Biometry*. 3 edition. W. H. Freeman and Company, New York.
- Somero, G. N. 1978. Temperature and adaptation of enzymes - Biological optimization through structure-function compromises. *Annual Review of Ecology and Systematics* **9**:1-29.
- Somero, G. N. 1995. Proteins and temperature. *Annual Review of Physiology* **57**:43-68.
- Sorensen, J. G., T. N. Kristensen, and V. Loeschcke. 2003. The evolutionary and ecological role of heat shock proteins. *Ecology Letters* **6**:1025-1037.
- Sorensen, J. G., M. Pekkonen, L. C. Beatrice, V. Loeschcke, A. Laurila, and J. Merila. 2009. Complex patterns of geographic variation in heat tolerance and Hsp70 expression levels in the common frog *Rana temporaria*. *Journal of Thermal Biology* **34**:49-54.
- Stebbins, R. C. 2003. *A Field Guide to Western Reptiles and Amphibians*. 3 edition. Houghton Mifflin Company, New York.
- Stevens, G. C. 1989. The latitudinal gradient in geographical range - How so many species coexist in the tropics. *American Naturalist* **133**:240-256.

- Stevens, G. C. 1992. The elevational gradient in altitudinal range - An extension of Rapoport's rule to altitude. *American Naturalist* **140**:893-911.
- Stouffer, R. J., S. Manabe, and K. Y. Vinnikov. 1994. Model assessment of the role of natural variability in recent global warming *Nature* **367**:634-636.
- Thuiller, W., S. Lavorel, M. B. Araujo, M. T. Sykes, and I. C. Prentice. 2005. Climate change threats to plant diversity in Europe. *Proceedings of the National Academy of Sciences of the United States of America* **102**:8245-8250.
- Tomanek, L. and E. Sanford. 2003. Heat-shock protein 70 (Hsp70) as a biochemical stress indicator: An experimental field test in two congeneric intertidal gastropods (Genus : *Tegula*). *Biological Bulletin* **205**:276-284.
- Tomanek, L. and G. N. Somero. 1999. Evolutionary and acclimation-induced variation in the heat-shock responses of congeneric marine snails (genus *Tegula*) from different thermal habitats: Implications for limits of thermotolerance and biogeography. *Journal of Experimental Biology* **202**:2925-2936.
- Tomanek, L. and G. N. Somero. 2000. Time course and magnitude of synthesis of heat-shock proteins in congeneric marine snails (Genus *Tegula*) from different tidal heights. *Physiological and Biochemical Zoology* **73**:249-256.
- Towbin, H., T. Staehelin, and J. Gordon. 1979. Elephoretic transfer of proteins from polyacrylamide gels to citrocellulose sheets - Procedures and some applications. *Proceedings of the National Academy of Sciences of the United States of America* **76**:4350-4354.
- Ulmasov, K. A., S. Shammakov, K. Karaev, and M. B. Evgenev. 1992. Heat shock proteins and thermoresistance in lizards. *Proceedings of the National Academy of Sciences of the United States of America* **89**:1666-1670.
- Vandamme, R., D. Bauwens, A. M. Castilla, and R. F. Verheyen. 1989. Altitudinal variation of the thermal biology and running performance in the lizard *Podarcis tiliguerta*. *Oecologia* **80**:516-524.
- Vanhooydonck, B., R. Van Damme, T. J. M. Van Dooren, and D. Bauwens. 2001. Proximate causes of intraspecific variation in locomotor performance in the lizard *Gallotia galloti*. *Physiological and Biochemical Zoology* **74**:937-945.
- Walther, G. R., E. Post, P. Convey, A. Menzel, C. Parmesan, T. J. C. Beebee, J. M. Fromentin, O. Hoegh-Guldberg, and F. Bairlein. 2002. Ecological responses to recent climate change. *Nature* **416**:389-395.

- Watt, W. B. 1992. Eggs, enzymes, and evolution - Natural genetic variants change insect fecundity. *Proceedings of the National Academy of Sciences of the United States of America* **89**:10608-10612.
- Wilson, B. S. 1991. Latitudinal variation in activity season mortality rates of the lizard *Uta stansburiana*. *Ecological Monographs* **61**:393-414.
- Yocum, G. D. 2001. Differential expression of two HSP70 transcripts in response to cold shock, thermoperiod, and adult diapause in the Colorado potato beetle. *Journal of Insect Physiology* **47**:1139-1145.
- Young, R. L., T. S. Haselkorn, and A. V. Badyaev. 2007. Functional equivalence of morphologies enables morphological and ecological diversity. *Evolution* **61**:2480-2492.
- Youssef, M. K., S. C. Adolph, and J. Q. Richmond. 2008. Evolutionarily conserved thermal biology across continents: The North American lizard *Plestiodon gilberti* (*Scincidae*) compared to Asian *Plestiodon*. *Journal of Thermal Biology* **33**:308-312.
- Yu, Z. M., R. Dickstein, W. E. Magee, and J. R. Spotila. 1998. Heat shock response in the salamanders *Plethodon jordani* and *Plethodon cinereus*. *Journal of Thermal Biology* **23**:259-265.
- Zar, J. H. 2009. *Biostatistical Analysis*. 5 edition. Prentice H, New Jersey.