Summer 2017

How do lizards use behavior and physiology to inhabit different climate zones?

Matthew R. McTernan
Western Washington University, mcternm@wwu.edu

HOW DO LIZARDS USE BEHAVIOR AND PHYSIOLOGY TO INHABIT DIFFERENT CLIMATE ZONES?

By

Matthew R. McTernan

Accepted in Partial Completion
of the Requirements for the Degree
Master of Science

Kathleen L. Kitto, Dean of the Graduate School

ADVISORY COMMITTEE

Chair, Dr. Roger A. Anderson

Dr. Benjamin Miner

Dr. Deborah Donovan

MASTER'S THESIS

In presenting this thesis in partial fulfillment of the requirements for a master's degree at Western Washington University, I grant to Western Washington University the non-exclusive royalty-free right to archive, reproduce, distribute, and display the thesis in any and all forms, including electronic format, via any digital library mechanisms maintained by WWU.

I represent and warrant this is my original work, and does not infringe or violate any rights of others. I warrant that I have obtained written permissions from the owner of any third party copyrighted material included in these files.

I acknowledge that I retain ownership rights to the copyright of this work, including but not limited to the right to use all or part of this work in future works, such as articles or books.

Library users are granted permission for individual, research and non-commercial reproduction of this work for educational purposes only. Any further digital posting of this document requires specific permission from the author.

Any copying or publication of this thesis for commercial purposes, or for financial gain, is not allowed without my written permission.

Matthew R. McTernan
July 21st, 2017

HOW DO LIZARDS USE BEHAVIOR AND PHYSIOLOGY TO INHABIT DIFFERENT CLIMATE ZONES?

A Thesis Presented to
The Faculty of
Western Washington University

In Partial Fulfillment
Of the Requirements for the Degree
Master of Science

By
Matthew R. McTernan
July 2017

ABSTRACT

Rapid climatic change is expected to pose extreme ecological and physiological challenges on many ectothermic vertebrates. Some ectothermic species are notable, however, for inhabiting wide geographic ranges and variety of climate zones. Studying how exemplars among ectotherms can behaviorally and physiologically accommodate differing temperature ranges should provide useful mechanistic perspectives on climate change challenges for less accomplished ectotherms. The western fence lizard (*Sceloporus occidentalis*) is one such exemplar, ranging from southern California to northern Washington. In Washington State, a single subspecies of this lizard occupies strongly contrasting climate zones. Thus, the focus of this thesis was to determine how this subspecies uses behavior and physiology to successfully inhabit these very different habitats within these climate zones.

I chose to study *Sceloporus occidentalis* populations from the Sondino Ponds Unit in the Columbia River Gorge ("CRG"; mean max air = 38.9°C), Goat Wall in the North Cascades ("GW"; mean max air = 33°C), and along the coastal shores of the Salish Sea just north of Marysville ("CS"; mean max air = 27.7°C). In summer 2015 and 2016, to compare thermoregulatory capacity in the field among lizards at each of these contrasting climate zones, I measured field-active body temperatures (field-active T_b) of lizards immediately upon capture. To determine whether lizards may have needed to accept field-active T_b that were suboptimal — presumably due to suboptimal thermal conditions — I compared the distribution of a) field-active T_b among the three locales, and b) field-active T_b with preferred

body temperatures of alert-and-active lizards in the lab (lab T_b) where they were free to select precise body temperatures in a thermal gradient.

To test for presence of temperature-dependent physiological differences among the three populations of lizards, I used a flow-through respirometry system in lab to measure whole-animal resting metabolic rates (RMR) — lizards with digesting and assimilating food in their guts — at three ecologically and physiologically relevant body temperatures (20°C, 28°C, and 36°C), as well as standard metabolic rates (SMR) — lizards that were fasted and empty of foodstuff — at 28°C T_b.

Lizards at the warmest locale, CRG, had significantly higher field-active T_b than those at the cool coastal locale, CS (ANOVA, $p=0.05$; *post hoc*, $p=0.045$), but field-active T_b of lizards at the high-elevation, northern population, GW, were not significantly different from those of lizards at the other two locales. The distribution of field-active T_b of lizards from CRG skewed warmer than lab T_b (t-tests comparing upper quartile, $p<0.05$), whereas field-active T_b of lizards from GW and CS skewed cooler than those selected in lab (t-test comparing mid and lower quartiles, $p<0.05$). Lab T_b of lizards from GW were significantly higher than lab T_b of lizards from CRG (ANOVA, $p=0.02$; *post hoc* $p=0.025$), whereas lab T_b of CS lizards did not differ from those of lizards at the other two locales.

At 20°C T_b and 36°C T_b, RMRs of lizards from CRG and GW were similar, but RMRs and SMRs of lizards from these two inland sites were significantly lower than the RMRs and SMRs of lizards from the cool coastal site, CS, at all body temperatures (ANCOVAs, all $p<0.05$; *post hoc* all $p<0.015$). Furthermore, RMRs of lizards from CRG measured in the late spring-to-early summer (when metabolism may be highest due to

reproductive effort in adult lizards) were still significantly lower at 28°C T_b and 36°C T_b when compared to RMR of lizards from CS measured during the post-reproductive season in mid-summer (t-tests, both $p<0.05$).

Sceloporus occidentalis in the field at all sites are able to thermoregulate within the preferred T_b range of this species (34-36°C) during activity, in spite of the different climates these populations inhabit. Lizards from CRG, however, may be forced by higher environmental temperatures to maintain field-active T_b near the upper limit of their acceptable range of body temperatures. Furthermore, lizards along the coast may mitigate the retarding effects of cooler environmental temperatures (and the resulting cooler T_b the lizards must accept during inactivity) by elevating their temperature-dependent metabolism. Additionally, this RMR of lizards from the coast may also serve to increase food energy assimilation and growth rates during a shorter activity season than experienced by the inland populations. Thus, although lizards at each of these contrasting climate zones could behaviorally thermoregulate within the acceptable range of field-active Tb during mid-summer, physiological adjustment by increase in RMR — presumably related to increasing catabolism and anabolism of foodstuffs — also occurred in lizards inhabiting CS, the coolest climate zone.

ACKNOWLEDGEMENTS

I would like to first and foremost thank Dr. Roger A. Anderson for the invaluable guidance and help he provided me as a graduate student, and for the wisdom and perspective he imparted to me as a young adult. Furthermore, I would like to thank Dr. Deb Donovan and Dr. Ben Miner for agreeing to be on my committee, and for the advice and support they provided me during this process. I am especially indebted to Frank and Kate Slavens, who not only permitted me to work on the Sondino Ponds Unit, but even went so far as to house me during one of my research visits. I am also thankful to the Tulalip Indian Tribe for permitting me to conduct research on their lands. Furthermore, I am grateful to the Society for the Study of Amphibians and Reptiles as well as Western Washington University for providing me the funding to do my research.

I would also like to thank all the undergraduate volunteers who helped me in lab. In particularly, special thanks go to Arthur Tenebrae, Christian Olsen, Quinn McMahon, all of whom were critically important assistants in both the field and the lab. To Arthur especially, thanks for sticking with me for two years and always being willing to help, regardless of the task. I am also grateful for my fellow graduate students, who not only helped keep me through the rigors of graduate school, but also become close friends with whom I drank many craft beers in the process. Lastly, I would like to thank my brother, who provided me with advice and guidance early in my graduate student career, and my mom, whose love and support has helped me achieve my goals in life every step of the way.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

INTRODUCTION

Rationale for Research

Predicting the effects of climate change on the spatial and temporal patterns of individuals and populations of any species, much less that of multiple species comprising a community or biome, is a complex of challenges facing ecologists and evolutionary biologists (Huey and Kingsolver 1993, Huey *et al.* 2012, Quintero and Wiens 2013, Sutherland *et al.* 2013, Clusella-Trullas and Chown 2014). Rising daily mean and maximum temperatures, more extreme events of high temperatures, and more intense droughts are expected to impose formidable physiological and ecological challenges on terrestrial ectotherms (Clusella-Trullas *et al.* 2011). Lizards are a major taxonomic exemplar of how species diversity is expected to plummet in response to a warming climate, as lizards are particularly sensitive to changes in temperature (Porter and Gates 1969, Dutton and Fitzpatrick 1975, Brown and Griffin 2005, Luo *et al.* 2010). As such, current models (e.g., Sinervo *et al.* 2010) predict that changes in climate will cause about 20% of all lizard species to become extinct by 2080. Presumably, understanding individual-level behavioral and physiological responses to temperature changes in a successful, geographically wide-spread lizard would offer useful mechanistic perspective needed to predict population-level and species-level changes for other ectothermic taxa — including threatened and endangered species — under different climate change scenarios. Thus, my thesis research attempts to answer the following question: "How are individuals of *Sceloporus occidentalis*, a geographically wide-spread lizard species, able to thrive in different climate zones with considerably different annual and diurnal temperature regimes?"

Temperature and Terrestrial Ectotherms

The temperature of the ambient environment strongly affects the physiology and ecology of individual animals. Consequently, environmental temperatures appear to govern, at least in part, the global distribution of most animal species (Stevens 1989, Clarke and Gaston 2006). That is, the global distribution of an animal species may largely be a consequence of how environmental temperatures influence that animal's body temperature (hereby denoted at "T_b"), particularly if it is not capable of maintaining a T_b independently from that of its environment. If an animal is not capable of independently maintaining a relatively constant T_b, especially in variable environmental temperatures, then its body may become nearly the same temperature as that of its environment (*i.e.* a *poikilotherm*). But to gain some control over the mean or variation of its T_b during activity, some terrestrial poikilotherms change body location to utilize spatial variation in environmental temperatures to either raise or lower their T_b relative to the air temperature. That is, when under conditions that permit, a poikilotherm — for example, a day-active lizard during its activity season — can become an *ectotherm*. That is, it uses its behavior to be where it can gain or lose heat to its environment via conduction with objects, convection with air, or radiation to maintain itself at a relatively narrow zone of body temperatures during its activity period. Thus, it thermoregulates.

When thermoregulating to a self-selected T_b, ectothermic animals are typically attempting to achieve a temperature to which their physiology is maximized (Huey and Bennett 1987, Huey and Kingsolver 1989, Clarke and Fraser 2004). For example, reproductive output (Luo *et al.* 2010), rate of digestion (Brown and Griffin 2005, Du *et al.*

2007), food acquisition (Andrews 1984, Grigaltchik *et al.* 2012), muscle function (James *et al.* 2012), body size (Kingsolver and Huey 2008, Ohlberger 2013), locomotor performance (Miles 1994, Artacho *et al.* 2013), and activity (Adolph 1990, Adolph and Porter 1993) are all known to be directly influenced by T_b, with maximal performance occurring within a specific, often narrow, T_b range. Most fundamental, however, is likely the effect that temperature has on an animal's metabolism, as metabolic rates of an animal vary directly with T_b, and metabolism is the critical link between an organism and both the mass and chemical energy it requires from its environment (Gillooly *et al.* 2001, Brown *et al.* 2004).

Therefore, to inhabit a certain climate zone, an ectotherm must be able to meet the thermoregulatory challenges posed by the spatiotemporal patterns, means, and extremes of environmental temperatures within that climate zone. More specifically, it must be able to maintain a T_b range that permits sufficiently functional physiological performance, allowing for activity and food processing for enough hours per day and days per year to enable the animal to survive, thrive, and reproduce (Angilletta *et al.* 2006, Levy *et al.* 2017). Two mechanisms by which ectotherms can meet these thermoregulatory challenges — behavioral thermoregulation or physiological adjustments — are discussed in further detail in the following sections.

Behavioral Thermoregulation of Ectotherms

While endothermic birds and mammals can be active over a relatively broad range of ambient temperature and sunlight conditions, ectotherms are more limited. But when the air is cool-to-warm (neither cold nor hot), an active ectotherm may use spatiotemporal variation in direct and reflected light intensity, as well as in air and substratum temperatures, to maintain a near-optimum, narrow range body temperatures (Cowles and Bogert 1944, Sears *et al.* 2016). For example, it is common for lizards in the genus *Sceloporus*, which are typically seen in open, sunlit habitats — and regarded by vertebrate biologists to be among the paragons of behavioral thermoregulation — to thermoregulate at about 35˚C during their daily activity periods, even when air temperatures are only 20˚C. Despite weather-induced periods of inactivity, lizards can still be active enough long enough annually to thrive in a range of climate zones (Garrick 1979, Seebacher and Franklin 2005, López-Alcaide *et al.* 2014, Sunday *et al.* 2014).

In response to particularly challenging environmental temperatures, lizards can show flexibility in their thermoregulatory behavior; either by limiting or adjusting their habitat use to utilize only the segments of their habitat in which their "operative temperatures" (*i.e.* potential temperatures an ectothermic animal's body may achieve while in various locations of its habitat) permit effective thermoregulation, or by limiting their activity to times of day when environmental temperatures are more accommodating (Adolph and Porter 1993, Sears and Angilletta 2015). This flexibility allows ectothermic animals, such as lizards, to inhabit geographic ranges that encompass an array of warm to temperate climates. Within lizard species living in locales with sufficient sunny weather,

conspecific individuals from different climate zones are able to achieve the same narrow range of optimal, field-active body temperatures by varying activity times, locations, and thermoregulatory behavior (Grant and Dunham 1990, Andrews 1998, Schwarzkopf 1998, Gvozdík and Castilla 2001). Furthermore, this effective, flexible behavioral thermoregulatory capacity may reduce the need to physiologically adjust to differing climatic conditions. Indeed, it is believed that behavioral thermoregulation may limit, or even prevent, differences in thermal physiology from developing among populations (Huey *et al.* 2003, Muñoz *et al.* 2013, Buckley *et al.* 2015).

Behavioral thermoregulation, however, is a task that can be curtailed if the fitness-associated costs in a given habitat at a given time are too great (Huey 1974, 1991). For example, lizards that leave their refugia to thermoregulate also, in turn, increase their exposure to predators (Huey and Montgomery 1976, Adolph and Porter 1993). Therefore, when predation risk is too high, moving to a position to thermoregulate (or remaining exposed while thermoregulating) may be too risky. Lizards also may allow their T_b to drift away from the optimal range when pursuing or handling prey in thermally suboptimal microhabitats, and the resulting suboptimal T_b may subsequently enhance predation risk (Sears 2005).

Furthermore, the thermoregulatory costs of different climates can influence both a lizard's body size at adulthood and age at first reproduction (Kingsolver and Huey 2008). In cooler climates, activity seasons are shorter. As such, lizards in these cooler climates often have more limited time to eat and grow compared to those born into a warmer climate. Hence, cooler-climate lizards may not reach the apparent minimum body size at first

reproduction as quickly as warmer-climate lizards, and the cooler climate lizards may reproduce a year later (Sears and Angilletta 2004, Horváthová *et al.* 2013). Thus, cooler-climate lizards must allocate the requisite time and energy to growth to reach the minimum size for first reproduction, but it is also possible that they may grow even larger in that "missed reproductive season" and will then produce more eggs than a smaller-bodied lizard could when the next reproductive season arrives. In stark contrast, where warm seasons are too hot and dry for too long, the lizard activity is severely curtailed and embryos (produced either oviparous or viviparous lizards) and juveniles may die (Du and Ji 2006, Du and Shine 2014).

Physiological Thermoregulation in Terrestrial Ectotherms

Behavioral thermoregulation is energetically cheap (in contrast to endo-thermoregulation) and spatiotemporally plastic, but is effective only when the ectotherm is in a suitable thermal environment. When the environment does not allow for viable behavioral thermoregulation, or when an ectotherm becomes inactive, the erstwhile etho-thermoregulator is then again poikilothermic (Kingsbury 1994, Catenazzi *et al.* 2005, Cadena and Tattersall 2009, Guizado-Rodriguez *et al.* 2011). If so, temporary changes in physiology (*i.e.* reversible plasticity) with respect to body temperature may be beneficial (Seebacher 2005). For example, a lizard may adjust its preferred body temperature or its metabolic rate to compensate for increasing or decreasing body temperature. Such temporary physiological adjustments have been observed in ectothermic animals as a response to seasonal temperature changes (Dutton and Fitzpatrick 1975, Tsuji 1988, Hadamová and

Gvoždík 2011, Ortega and Pérez-Mellado 2016), differences in temperature among climate zones (Schwarzkopf 1998, Angilletta 2001, Artacho *et al.* 2017), or increasing temperatures caused by climate change (Seebacher *et al.* 2015, Caruso *et al.* 2014).

Furthermore, this capacity for reversible phenotypic plasticity in individuals may reduce challenges imposed by changing environmental conditions on a population, thus buffering populations against evolutionary change (Lorenzon *et al.* 2001, Seebacher *et al.* 2015). Alternatively, some physiological traits may be evolutionarily constrained, preventing the development of population-specific adaptations (Somero 2010). For example, an inference drawn from recent modeling studies is that thermal tolerance may be phylogenetically constrained within any lizard species, thus preventing evolutionary change among populations (Grigg and Buckley 2013).

If, however, generations of lizards encounter a change in environmental temperatures that is different enough and persists long enough, then it seems likely (barring any limitations regarding the genetic adaptability of the animal) that selective pressure on thermal physiology would result in evolutionary change in the thermal responses of the population (Huey and Kingsolver 1993, Crawford *et al.* 1999). Thus, there may be selection for physiological processes (*e.g.,* adjusted thermal sensitivity of metabolic machinery) that are favored in a given thermal environment (Huey and Kingsolver 1993, Scheers and Van Damme 2002, Clarke and Fraser 2004, Somero 2010). Although rates of evolution vary among animal species — as related to genetic variance and generation time — evolution in small vertebrates may occur rapidly, particularly if selection is strong. For example, changes in morphotypes have evolved in the lizard *Anolis lemurinus* over the

course of only 14,000 years, where males of two separated populations developed longer toe pads and nails, as well as larger dewlaps than females, in response to different habitat structures among island (Logan *et al.* 2012). Similarly, two separate islet populations of the lizard *Podarcis gaigeae*, these with an estimated divergence time of ~8,700 years, show considerable differences in body mass and GI tract length relative to body mass, as related to protein digestion efficiency (Pafilis *et al.* 2016). Furthermore — and more germane to my thesis question — differences in preferred T_b between insular and continental populations of the Balkan green lizard (*Lacerta trilineata*) seem have evolved as a function of the different thermal and environmental conditions experienced by these separate populations (Sagonas *et al.* 2013).

Study System and Hypotheses

I chose to investigate whether the behavioral thermoregulatory and metabolic performances of the western fence lizard, *Sceloporus occidentalis* (Baird and Girard 1852) varied among populations inhabiting distinct climate zones. *Sceloporus occidentalis* is a relatively small, insectivorous lizard in the family *Phrynosomatidae* (*Appendix A*, Photos 1-4), with a geographic distribution from northern Washington southward through Oregon and California, into northern Mexico, and eastward into Nevada and portions of Utah and Idaho (Hollingsworth and Hammerson 2007). Because the species has a relatively large latitudinal geographic range that includes a number of mountain ranges, the regional climates vary among the many populations. Nevertheless — weather and habitat structure permitting — temperate populations of *S. occidentalis* all attempt to behaviorally

thermoregulate so that individuals are active with a mean internal body temperature of about 35°C (Andrews 1998). Lizards in the genus *Sceloporus* are typically effective behavioral thermoregulators, achieving a narrow range of field-active body temperatures by exploiting the availability of warm substrates and solar radiation in their habitats — individuals move between sunny or warm spots and shady or cool spots as needed to regulate internal body temperature (McGinnis 1970, McGinnis and Falkenstein 1971).

After the last glaciers receded and during a warm period about 3000 to 8000 years ago, coinciding with the northward migration of oak woodlands west of the Cascade Mountains, one (unnamed) subspecies of *Sceloporus occidentalis* is presumed to have expanded from the Willamette Valley of Oregon, northward across the Columbia River, along the east and west sides of the Cascade Range (Adam Leaché and James Archie, personal communication). This one subspecies now inhabits a wide variety of climate types. Three exemplars in Washington are 1) the forest edge of southwest facing slopes along beaches of the Washington coast in the Salish Sea region, 2) the warm, dry ecotone between the pine-oak woodlands and shrub-steppe in southern WA, and 3) the highlands of the fir-pine ecotone on the east slopes of the Cascade Mountains in northern Washington (Figure 1). Thus, I chose to compare aspects of behavioral thermoregulation and temperature-related, whole-animal physiology of individuals from the populations of *S. occidentalis* located within each of these climate zones.

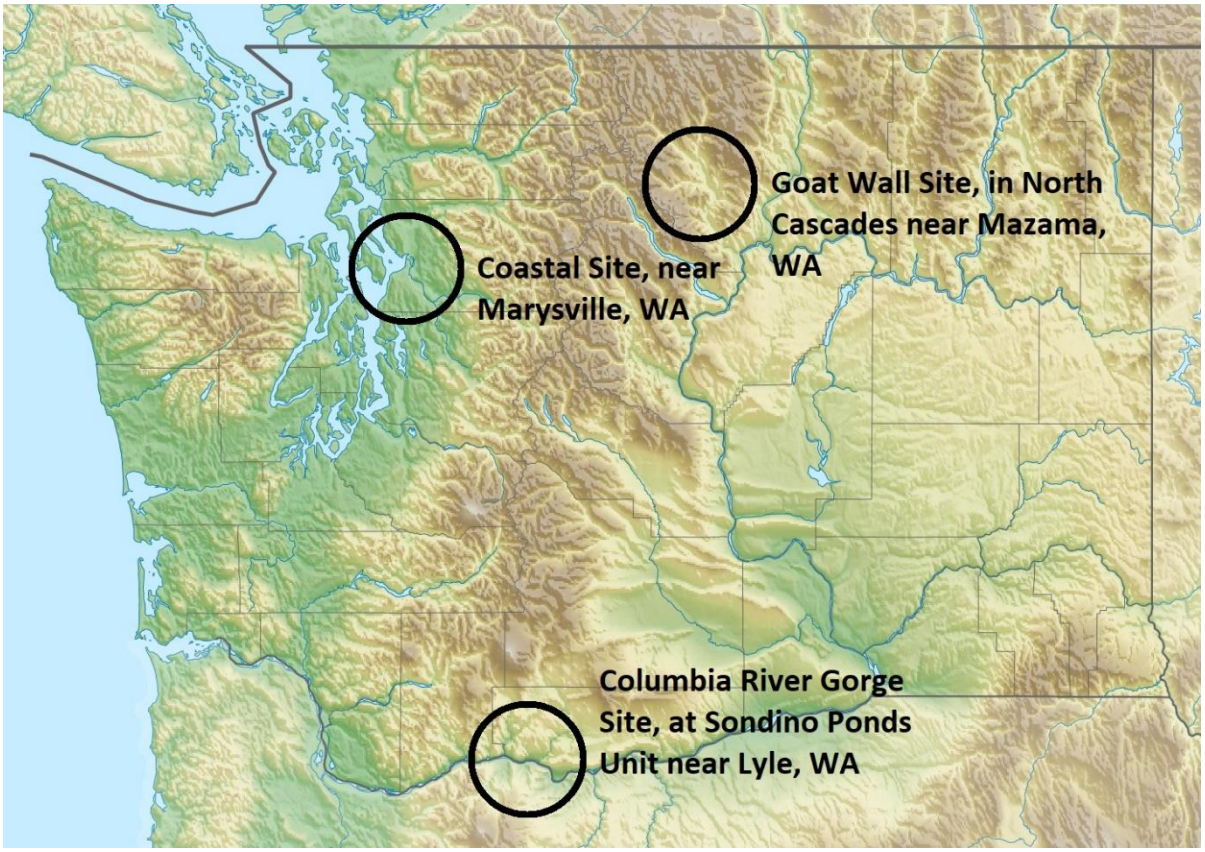


Figure 1. The location of the three study sites within Washington State. See *Appendix A* for photos of the habitat at each site (Photos 5, 6 and 7). The coastal site, west of the North Cascades is the coolest site, whereas the higher elevation, inland site on the east side of North Cascade Mountains site is comparatively warm during the day, and the low elevation inland site in the southern extreme of Washington, in the Columbia River Gorge, is the hottest site.

The Washington coast habitats ("coastal shores", CS; *Appendix A*, Photo 5), north of Marysville, WA, generally have shorter summers and mild temperatures, so not only are the coastal lizards probably cooler than lizards at the other two locales during inactivity periods in the summer, but coastal lizards also may need to accept relatively low body temperatures during activity. In contrast, at the drier, hotter inland habitats at the Sondino Ponds Unit near the Columbia River Gorge ("CRG"; *Appendix A*, Photo 6), it is reasonable to infer that lizards either must accept, or actually prefer, higher body temperatures during their daily activity period. The high-latitude, high-elevation inland habitat at Goat Wall near Mazama, WA, ("GW"; *Appendix A*, Photo 7) combines the cooler nighttime temperatures of the coastal shores with the warm afternoons of an inland site, creating another unique habitat type. Because of these differing climatic conditions amongst sites, I expected that *S. occidentalis* at these three localities provided a chance to investigate how ectothermic animals use behavior or physiology to adjust to widely varying thermal conditions.

I formulated hypotheses to assess whether there were differences in behavioral thermoregulation and temperature-dependent metabolic rates amongst these climatically different populations of *S. occidentalis*. I chose to test three hypotheses about thermoregulation:

H_{T0}) *Effective behavioral thermoregulation permits S. occidentalis at all locales to achieve similar body temperatures during activity,*

H_{T1}) *Temperature conditions at each locale force S. occidentalis to thermoregulate at the most easily achieved body temperature within its temperature preference range during activity, and*

H_{T2}) *Behavioral thermoregulation does not effectively allow for S. occidentalis at any locale to maintain a narrow range preferred body temperatures during activity (graphical representations of these hypotheses can be found in Appendix A, Figure 10).*

Similarly, I chose to test three hypotheses to compare the temperature-dependent metabolic rates amongst populations (a graphical example of how this to evaluate these hypotheses is provided by Figure 2):

H_{M0}) *Temperature-dependent metabolic rates do not differ among S. occidentalis from different climate zones when measured across a range of ecologically relevant body temperatures (20°C, 28°C when inactive and 36°C when active),*

H_{M1}) *Temperature-dependent metabolic rates of lizards resting at daily-relevant inactive body temperatures (20°C, 28°C) vary among S. occidentalis from different climate zones, and*

H_{M2}) *Temperature-dependent metabolic rates differ among S. occidentalis from different climate zones when measured across a range of ecologically relevant body temperatures (20°C, 28°C and 36°C).*

Summary of Project

I investigated how *Sceloporus occidentalis* meets the challenge of thermally variable habitats by conducting a whole-organism, macro-physiological study (Gaston *et al.* 2011, Chown and Gaston 2016). I characterized the thermal physiology and thermoregulatory behavior of each population of *Sceloporus occidentalis* during the peak of the activity season. I compared field-active body temperatures, where thermoregulation may be challenging, with lab-active body temperatures where thermoregulation is easy. I also compared temperature-dependent resting metabolic rates among lizards from each locale. Thus, I asked: *How do individuals of a single lizard species vary among populations in how they utilize behavioral and physiological mechanisms to survive and thrive in distinct, different climates?*

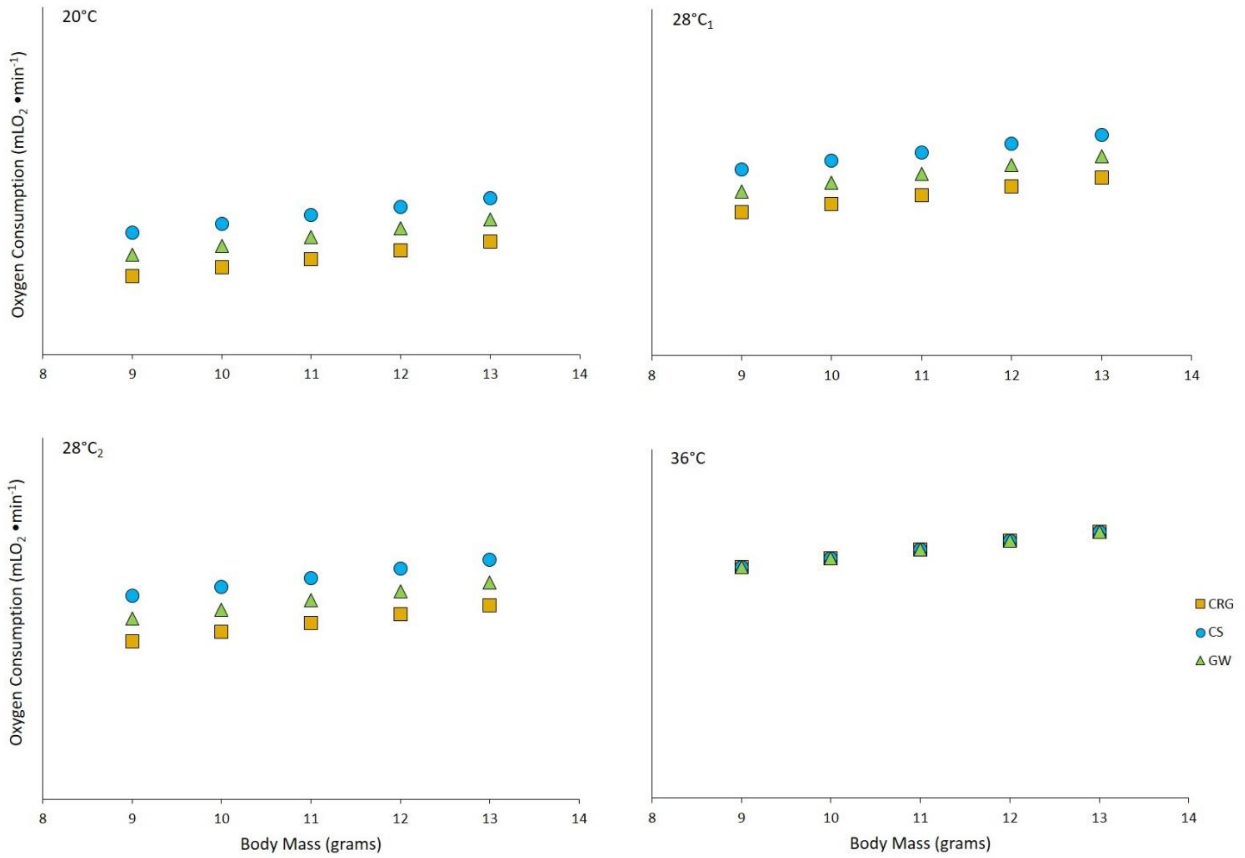


Figure 2. Graphical depiction of five hypothetical *Sceloporus occidentali*s of varying body masses from each locale (CRG, GW, and CS), with oxygen consumption measures being measured in fed lizards at 20°C, 28°C (28°C_2), and 36°C and in unfed lizards at 28°C (28°C_1). Here the rates of oxygen consumption of *S. occidentalis* differ at the inactive body temperatures (*i.e.* 20°C and 28°C), but are similar at the field-active body temperature, 36°C. Thus, these hypothetical data would support H_{M1}, the hypothesis that lizards from all three locales differ in temperature-dependent rates of oxygen consumption at inactive body.

METHODS

Permits and ACUC

Prior to conducting my research, I attained Scientific Collecting Permit (#239) from the Washington State Department of Wildlife and Fisheries. I also received permission from the Animal Care and Use Committee at WWU to conduct noninvasive physiological experiments on live lizards for this thesis.

Schedule

I studied populations during their post-reproductive periods in mid-to-late summer to avoid the direct effects of reproduction on lizard behavior and physiology. Locales that are warmer earlier in the year are expected to have earlier phenological progression — that is, lizards that have an earlier reproductive season and earlier post-reproductive season should be studied before lizards in the cooler locales. The Sondino Ponds Unit in the Columbia River Gorge is warmer earlier, so I collected field and lab data from that site first: in mid-to-late July of 2015 and 2016. The next site in the phenological progression is Goat Wall, west of Mazama, in the Methow Valley along the east side of the North Cascades. There, data were collected on lizards in early August 2016 (better timing) and late August to mid-September 2015 (poorer timing, caused by equipment-related logistical difficulties). The coolest locality, and the last of the three site-comparisons, was along the Pacific Coast near Marysville, WA, which was studied in late August 2016. Equipment failure in summer 2015 delayed research on lizards at that locale until 2016, as the only option for study in

2015 was in late September and early October, which was unacceptable because it would have been the time in which transition into hibernation occurs for these lizards.

I also gained some preliminary data for testing the hypothesis that any potential differences in metabolic rates among lizards from each climate zone may be due to phenotypic plasticity induced by season-related temperatures. I measured resting metabolism of *Sceloporus occidentalis* from the hottest site, CRG, in spring 2016 (late April through early June) when conditions onsite are relatively mild. I could then compare these metabolic measures to those collected from lizards from the cool, coastal site in the summer, which has mild conditions similar to those found at CRG in the spring.

Field Work

Measuring Air and Soil Temperatures

When active, *Sceloporus occidentalis* shuttles between sunny and shaded locations while attempting to thermoregulate within a T_b range of 34-36°C. When inactive, however, the lizards are presumably the temperature of the soil or crevice in which they use as a refugium. Therefore, I characterized the air and substratum temperatures in the sun and shade to compare among sites, as well as measured temperatures at multiple potential refugia depths in soil.

I deployed iButtons (Maxim Integrated) and HOBO data loggers (Onset Computer Corporation) at each field site to characterize substratum temperatures and air temperatures (see Appendix B for model numbers and specifications). Substratum temperature measurements occurred from June through August of 2015, with data

collection occurring concurrently amongst the sites from June 17th through August 4th. Air temperature measurements were done within the same time, with data collection occurring concurrently amongst sites from June 22nd to July 26th. These data could be compared to data from nearby weather stations so that microclimate and nanoclimate conditions at these sites could be estimated with future weather station records alone.

I measured substratum temperatures with pairs of iButtons buried in the soil of flat ground at both a sunny and a shady location within each site. Pairs of iButtons in sunny locations were tethered to a wooden dowel and buried at soil depths of 0 cm, 5 cm, 10 cm, 20 cm, 30 cm, and 40 cm. iButtons in shady locations were buried similarly at soil depths of 0 cm, 5 cm, 10 cm, 20 cm, and 40 cm. I set the iButtons to record temperature measurements every 40 minutes, with each iButton in a pair being offset from the other of the pair by 20 minutes; two iButtons at one depth both reduced consequences of iButton failure and provided more resolution of temporal dynamics of temperature change.

I measured the air temperature at each site within a few meters of where iButtons were buried. HOBO data loggers were set to record air temperatures every 24 minutes, and were secured in perforated containers attached to the north side of trees at about 1.5 meters above the ground. The location and container shielded the logger from direct sunlight, wind and rain, whereas the perforations allowed air flow, thus making the logger more likely to represent the ambient air temperature accurately.

Lizard Sightings and Captures

Upon first sighting, we recorded data on the behavior of the lizard and the features of the habitat within proximity of the lizard. Data variables recorded include time of sighting, distance at which lizard was seen, lizard behavior when first seen (*e.g.*, basking, pursuing prey, displaying to conspecific, changing perch-search positions, moving to avoid humans), the mesohabitat (*e.g.*, boulder field, log field, rock slope, etc.), microhabitat (*e.g.*, tree, shrub, shrub, grass patch, log, boulder), nanohabitat (*e.g.*, top, near top, side, near bottom, below) and substratum (*e.g.*, rock, bark, bare wood, woody debris, sand, soil, leaf litter) used by the lizard, current sunlight conditions (*e.g.*, sunny, cloudy, hazy) and the kind of lighting to which the lizard was exposed (*e.g.*, full sun, full shade, dappled, filtered), as well as the precise lizard body position (*e.g.*, relative to vertical v. horizontal and relative to perpendicular rays of sunlight). The foregoing habitat and behavioral categories provided confidence in the thermo-ecological relevance of the measured body temperature. Added comments or clarifying notes were recorded as deemed necessary.

Lizards were captured primarily with a noose attached to the distal eyelet of a 2-meter-long spin-casting fishing rod, but on rare occasions when the lizards were hiding under a small log or rock, we moved the cover and captured the lizards by hand. Immediately upon capture of the lizard, we measured its deep-body temperature with a quick-reading, thin-bulb mercury cloacal thermometer inserted at least 1 cm deep into the cloaca, and tilted dorsally (into the body core and away from the ventral skin). The lizard's sex, toe clip status (toe clips are discussed later in *Laboratory Work* section) and apparent size/age class (juvenile v. adult) were then stated by me and scribed either by me or an

assistant who verified to me that which was scribed. The lizard was placed into a uniquely numbered cloth bag which I clipped to my field belt until we returned to the field vehicle. At the lizard's location where first seen, and within a few minutes of first sighting the lizard, we measured substratum temperature, air temperature and wind speed using an infrared thermometer gun, thermocouple thermometer, and a hand-held wind speed meter, respectively. Upon return to the field vehicle, the cloth bag containing the lizard was placed in a standard 10-quart plastic cooler with a perforated lid, and transported to Western Washington University. If not already toe-clipped, a unique toe-clip order was assigned to the lizard in the laboratory.

Laboratory Work

Housing Conditions

After each field visit, lizards were transported back to the laboratory at Western Washington University. I housed lizards individually in glass terraria with a heating lamp, heating stone, small refugium (upturned bowl, 12 cm diameter, with a hole in the 3 cm high side), and water bowl (*Appendix A*, Photo 8). I used a random number generator (random.org) to select the terrarium location (which shelf and which location on the shelf) where each lizard would be housed. Heating lamps and heating stones were scheduled to be on from 0900 hours to 1900 hours, replicating the approximate diurnal heating cycle of summer in temperate latitudes

All lizards were provided water *ad libitum*. Unless they were being intentionally fasted for research purposes, lizards were provided with 2-3 medium-sized (small adult

males and subadult females) crickets (*Acheta domestica*) per day. Crickets were purchased at Clarke's Feed and Seed in downtown Bellingham. Once or twice per week we removed fecal pellets and wiped that location with a clean moist cloth), and any dead crickets or shed skin. Most lizards were returned to the field within two weeks, shortly after all data were collected. On a couple of occasions the logistics with respect to data collection on lizards from another locality necessitated holding the lizards for a week or two longer. All lizards were released at their exact sites of capture.

Body Data Measurements

Lizards in lab were fasted for three days to completely clear their guts of any foodstuff. I measured the body mass of fasted lizards by placing them in a weight-tared, plastic container on a digital laboratory balance, weighed to the nearest 0.01g. Then either Dr. Anderson or I would measure the lizard's snout-vent length (SVL) and tail length to the nearest half millimeter using a standard ruler. Unique traits (*e.g.*, a scar, bite marks, abnormal coloration, etc.) were also recorded for each lizard.

Lizards were toe-clipped prior to leaving the lab (Perry *et al.* 2011) as a long-term identifying marker (thus also preventing me from using the same individual if I had to return for a larger sample size). Toe clips were administered as one toe clipped per foot, with three or four toes being clipped total. I assigned a specific toe clip pattern for each lizard I captured. The two longest toes on the back feet were not clipped, as they may serve an important function in climbing for this species (Dr. Roger Anderson, personal observation).

Respirometry System Design

I measured whole-animal metabolic rates of *Sceloporus occidentalis* using a flow-through, push-respirometry set up (Lighton 2008, Lighton and Halsey 2011). A push system (*i.e.* the pump is upstream from the sample chamber and oxygen sensor) is preferred when measuring oxygen consumption of small animals such as *Sceloporus occidentalis*, as the positive pressure inside the sample pathway prevents outside air from seeping into the pathway and diluting the gas sample (Lighton and Halsey 2011). Since different respirometry methods may produce different oxygen consumption values for the same individual (Kristín and Gvoždík 2012), I used the same flow-through push set-up for all of my measurements.

Sample chambers (180 ml internal volume, see Appendix B) were housed in a large temperature-controlled cabinet (Percival Scientific, Incorporated). A small port on the side of the cabinet allowed incurrent and excurrent tubing to enter and exit the cabinet. Up to four sample chambers containing lizards were in the incubator during a set of measurements. Only one chamber could be connected to the sample pathway at a time (see Figure 3), so I used an electronic pump (Model DOA-P161-AA, Gast Manufacturing, Inc.) and a SideTrak 840 mass flow meter (Sierra Instruments, Inc.) to provide the chambers off the sample pathway with flowing air. A five-way gang valve allowed equal amounts of air to flow from the mass flow meter through these sample chambers. With this design, I could easily switch sample chambers from the gang valve onto the sample pathway, allowing me to measure the metabolism of multiple lizards in quick succession. An empty chamber was used as the baseline measure during trials.

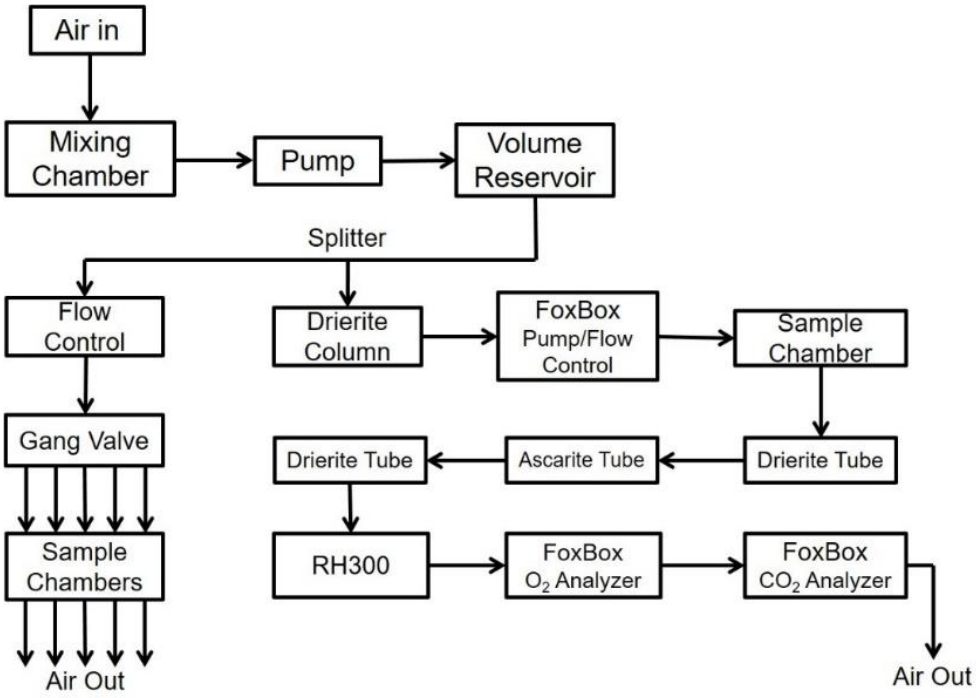


Figure 3. A box schematic showing the movement of air through the flow-through, push respiration system used in my study.

I measured oxygen consumption using a FoxBox Field Respirometry System (Sable System International). Incurrent air, pulled from a vent leading to outside of the building by the electronic pump, was first homogenized by a Plexiglass mixing chamber and desiccated by a Drierite column. Flow rate through the sample pathway was then controlled by the pump and flow meter on the FoxBox. I chose a flow rate of 125 mL min^{-1}, which is known to be appropriate for lizards that average 10 grams in body mass (respirometry.org) and is similar to what has been used in other *Sceloporus* species (Angilletta 2001). After passing through the sample chamber containing the lizard, the air was again desiccated by Drierite. The relative humidity, percent O_2, and percent CO_2 were then measured using an RH-300 Water Vapor Analyzer (Sable Systems International), and the O_2 and CO_2 sensors of the FoxBox. I recorded and analyzed all respiration data using Expedata Data Analysis Software (Sable Systems International). For photos and specifications of the equipment described above, see Appendix B.

Metabolic Measurements

I measured the resting metabolic rate (*i.e.* non-moving, absorptive lizards; "RMR") and standard metabolic rate (*i.e.* non-moving, post-absorptive lizards; "SMR") of *Sceloporus occidentalis* from all three locales. To avoid any effects of circadian rhythm on metabolism (Roe *et al.* 2005), I measured the RMR of the lizards during their normal hours of daily activity (between 1000 and 1930 hours). Lizards in lab were fed store-bought "domestic crickets" for a minimum of two days prior to having their RMR being measured. I also

occasionally measured the RMR of lizards the day after they were captured and brought to lab if they already had ample food in their gut.

Baseline O_2 measurements were recorded for two minutes using the empty sample chamber before and after each lizard measurement. Lizard metabolism was measured for 10 minutes, with only the stable plateaus in oxygen content (indicating a non-active, resting lizard) being used (Lighton 2008).

I measured resting metabolic rate (RMR) at three ecologically relevant body temperatures: 20 ˚C (as if in refugium), 28 ˚C (as if emerging from refugium for daily activity), and at 36 ˚C (as if the lizard is field-active), and I measured standard metabolic rate (SMR) at 28°C. The RMR includes the metabolism of digestion and assimilation of food (absorption), the metabolism of maintenance of the tissue, and perhaps metabolism of storage or growth. Thus, RMR is useful as an ecologically-relevant metabolism of an ectotherm during the field season. Lizard metabolism is higher in fed, resting lizards than in post-absorptive, resting lizards (Benabib and Congdon 1992, Roe *et al.* 2005). As such, I used SMR measurements to compare with RMR to confirm my tactile determination (by palpating the entire gut from stomach to cloaca) that there was food in gut during RMR trials.

In 2015, the body temperature order was randomized during metabolic measurements, but no effect of order was detected. Thereafter, in 2016, oxygen consumption measures were made in a consistent order, 20°C, 36°C, and 28°C, because this order was the most time-efficient for changing temperatures of the temperature control cabinet.

After the RMR measures, the lizards were fasted for three or four days, then SMR was measured at 28°C. On the third day of fasting, I checked for food mass and fecal by palpating the lizard's abdomen. If I felt a food bolus in its gastrointestinal tract, it was fasted for another day, then checked again.

Oxygen content of the air sample was recorded as percent oxygen (% O_2), adjusted for barometric pressure. The data were corrected for dilution via water vapor pressure and corrected for drift using tools and equations available on Expedata. I calculated the oxygen consumption of each lizard (VO_2) using the following equation:

$$VO_2 = (BL - Or) \times FL$$

where "*BL*" is the weighted mean of the % O_2 of the first and second baselines, "*Or*" is the mean of the % O_2 of the organism's oxygen consumption measurement, and "*FR*" is the flow rate (mL min^{-1}). If body mass data ranges were disparate among sites, data were corrected for body mass (mLO_2 min^{-1} g^{-1}) for statistical comparisons (see *Analysis of Metabolism* section, page 29).

Thermal Gradient Measurements

To measure preferred body temperature, I placed lizards in a thermal gradient designed by Dr. Anderson and built by Scientific Technical Services at WWU (Appendix A, Photo 9). The 4 cm thick aluminum floor surface (substratum), which was cooled by refrigerated ethylene glycol pumped through two round tunnels that penetrated the plate

within a few cm of the end and parallel to it, had temperatures of about 10°C at one end. At the other end of the aluminum floor an electric heating coil penetrated the aluminum plate, causing the floor surface to be about 50°C. A 50 watt spot-floodlight 15 cm from the end added heat from above, thus allowing the hot end to approximate the effect of a sunlit surface. Air temperature at 1 cm above substratum ranged from about 20°C at the cold end to about 35°C in the hot end. I confirmed these temperatures routinely for each trial with two HH81A thermocouple thermometers (accurate to 1% of reading + 0.7°C; Omega Engineering Incorporated), each attached to five thermocouple wires (using a switch box) at evenly spaced points along the gradient. A 10 cm deep shelf runs along the back of the gradient at 7 cm above the substratum, which both provided a shaded hiding place as well as a place for lizards to perch. The walls on either end of the gradient are covered in a fine metal mesh, which allowed lizards that were more intent on escaping than thermoregulating to reveal that behavior by leaving the substratum and climbing up the vertical surface. Furthermore, occasionally during a trial a lizard would venture into the cool end of the gradient to hide in the back, dark corner opposite of where the light was located. I considered this to be a hiding, non-thermoregulatory behavior, and T_b measures taken from a lizard behaving such a way were not used when calculating preferred T_b.

Lizards could move freely throughout the gradient. Every 20 minutes I recorded the lizard location in the gradient and the lizard's behavior; these notes helped me determine whether the lizard was calmly thermoregulating or hiding or trying to escape. I then opened the Plexiglas door that spanned the entire front of the gradient, grabbed the lizard and quickly measured its deep-body temperature with a quick-read cloacal thermometer,

used in the same manner as I did in the field. I then quickly and gently placed the lizard either in the middle of the gradient if it had been on the shelf or on the wall, or back to the substratum location where it had been resting. Trials lasted anywhere from 1.5 to 2 hours, with a total of 6-8 measurements being taken. I required a minimum of three body temperatures per trial (most had more) that were associated with a behavior of a relaxed thermoregulating lizard (i.e., not trying to climb out). I then averaged these measurements to produce a mean preferred T_b for that lizard.

Statistical Analyses

All statistical analyses were done using the open source software R (The R Project), version 3.3.2, and Excel (Microsoft Corporation), from Office 2016. A p-value of 0.05 was used to determine significance for all analyses. Before running any F-tests, I first tested the data for normality and equal variance using Shapiro-Wilks tests and Levene's tests, respectively. I used a nonparametric test such as a Kruskal-Wallis one-way analysis of variance test if the data were not normally distributed and the "degree" of non-normality was high enough to suspect that the robustness of the parametric test was in doubt. Because many F-tests assume linear data are being compared, the distributions of body mass, SVL, and oxygen consumption data were linearized by way of a natural logarithm transformation prior to these analyses. Furthermore, covariate data were tested for equal range of values using an analysis of variance test (ANOVA) prior to being used in any analysis of covariance tests (ANCOVA).

Statistical Analyses of Body Condition

Body condition data (mass SVL^{-1}) of *Sceloporus occidentalis* from the Columbia River Gorge site, the Goat Wall site, and the coastal shores site (CRG n=55, GW n=79, CS n=52) were compared among sites with an ANCOVA: body mass of gut-empty lizards was the response variable, the locality from which the individual was collected and sex were the predictor variables, and SVL was the covariate. Sex was found to be a significant factor, so I then ran two separate ANCOVAs — one comparing the body condition of male lizards among sites (CRG n=36, GW n=37, CS n=31) and the other comparing the body condition of females among sites (CRG n=19, GW n=42, CS n=21).

Statistical Analyses of Thermoregulation

After confirming the normality and homoscedasticity of the data, I used ANOVAs to test for differences among localities in both field-active (CRG n=40, GW n=58, CS n=89) and laboratory body temperatures (CRG n=33, GW n=39, CS n=34). I then compared field-measured to lab-measured body temperatures within each locality using either a Student's t-test (if data were normally distributed) or Mann-Whitney U test (if data were not normally distributed). I divided the range of field-active body temperature data into quartiles for these comparisons, with 25% of the data in the upper quartile and lower quartile (CRG n=10, GW n=15, CS n=22), and 50% of the data in the middle two quartiles (CRG n=20, GW n=28, CS n=45), which I then compared to the lowest, mean, and highest T_b values of thermoregulating lizards on the thermal gradient (CRG n=33, GW n=39, CS n=34). Since we may expect lizards from differing climate zones to "bump into" either the hotter or cooler

ends of their preferred T_b range because of the thermoregulatory challenges posed by their hotter or cooler environments, I judged it best to examine these upper and lower quartiles specifically. I then compared the middle two quartiles of the field active T_b data to the calculated mean T_b values found using the thermal gradient.

Statistical Analyses for Metabolism

Oxygen consumption rates among sites were compared with ANCOVAs (CRG n=15, GW n=16, CS n=13 at 20°C T_b; CRG n=14, GW n=26, CS n=15 at 28°C T_b fasted; CRG n=15, GW n=17, CS n=11 at 28°C T_b fed; CRG n=16, GW n=19, CS n=9 at 36°C T_b). Oxygen consumption rate, recorded in μLO_2/minute, was the response variable. The locality from which the lizard was obtained was the predictor variable, and the lizard body mass was the covariate. When an ANCOVA resulted in significance, I used a *post hoc* Fisher's Least Significant Difference test (Fisher's LSD) to determine which sites were significantly different from the others.

To test for seasonal changes in metabolism among sites, I also made comparisons of oxygen consumption rates between *Sceloporus occidentalis* from the cooler CS site measured in the summer and a set of *S. occidentalis* from the hotter, inland CRG site measured in the spring (CRG n=7, CS n=13 at 20°C T_b; CRG n=7, CS n=15 at 28°C T_b fasted; CRG n=6, CS n=11 at 28°C T_b fed; CRG n=7, CS n=9 at 36°C T_b). Since body sizes of lizards were found to be dissimilar between sites (limiting the effectiveness of using an ANCOVA), oxygen consumption rates of CS lizards measured

during summer months and of CRG lizards measured during spring months were corrected to a standard body mass (9.5 grams, which was near the mean body mass of the lizards being used in this comparison) using the following equation:

$$LN(V_{O2}/min)_{CORR} = LN(V_{O2}/min) + (b)(LN(M_{STD}) - LN(M))$$

where "$(V_{O2}/min)_{CORR}$" is the oxygen consumption rate corrected to the standard mass, "(V_{O2}/min)" is the observed whole animal oxygen consumption rate, "(b)" is the slope from the regression of oxygen consumption rate over body mass for each site at each temperature, "M_{STD}" is the standard body mass, and "M" is the observed body mass of the animal. I then compared standard mass-corrected oxygen consumption rates between the two sites for each body temperature and gut-load status using Student's t-tests.

RESULTS

Environmental Temperature Conditions

At the three study sites in general, daily maximum temperatures at the soil surface averaged 8.2°C hotter in the open than at nearby shaded soil surface (30.7°C versus 22.5°C, respectively). Maximum air temperatures in open locations were, on average, 3.5°C hotter than in shady locations at each site (32.8°C versus 29.3°C, respectively). The mean maximum soil temperature decreased with soil depth (Figure 4, Graph "a"), whereas the mean minimum soil temperature increased (Figure 4, Graph "b"). Likewise, the difference in mean maximum soil temperature among sites decreased with soil depth, though such a pattern is not apparent with mean minimum soil temperatures.

Comparing mean maximum temperatures among sites, the Columbia River Gorge (CRG) had hotter maximum soil temperatures than Goat Wall (GW) or the coastal shores (CS) in both open and shaded locations at every soil depth, except for GW having hotter mean surface soil temperature in the shaded location (34.5°C for GW versus 33.9°C for CRG; full list of averages shown in Table 1). In open locations, the mean maximum surface soil temperature at CRG was 6.65°C hotter than the combined mean of the other two sites (64.0°C at CRG vs 58.1°C at GW and 56.6°C at CS, respectively). GW and CS had similar maximum soil temperatures in the open, with a mean difference of only 0.4°C across all soil depths. In the shaded locations, however, surface soil temperatures at GW were 12.1°C hotter on average than CS (34.5°C vs 22.4°C, respectively). CS had the coolest minimum subsurface soil temperatures (*i.e.* 5 cm depth and below), averaging 0.9°C cooler temperatures than CRG and GW in the sunny location, and 1.7°C cooler temperatures in the

shaded location. In contrast, CRG had the hottest minimum soil temperatures across all subsurface depths, averaging 1.5°C hotter temperatures than GW and CS in the sunny location, and 1.9°C hotter temperatures in the shaded location.

Regarding air temperatures, CRG had the hottest maximum air temperature in the open, with an average temperature of 38.9°C. GW had the next hottest average maximum temperature at 33°C, followed by CS at 27.7°C (Figure 5, Graph “a”). GW had the highest average minimum temperature in the open at 17.1°C, followed by CRG at 15.5°C, and CS at 14.0°C (Figure 5, Graph “b”). CRG again had the hottest maximum air temperatures in the shade, with an average temperature of 32.1°C, followed by GW at 31.7°C, and CS at 24.8°C. GW again had the highest minimum average minimum temperature in the shade at 17.3°C, followed by CRG at 16.4°C, and CS at 14.7°C.

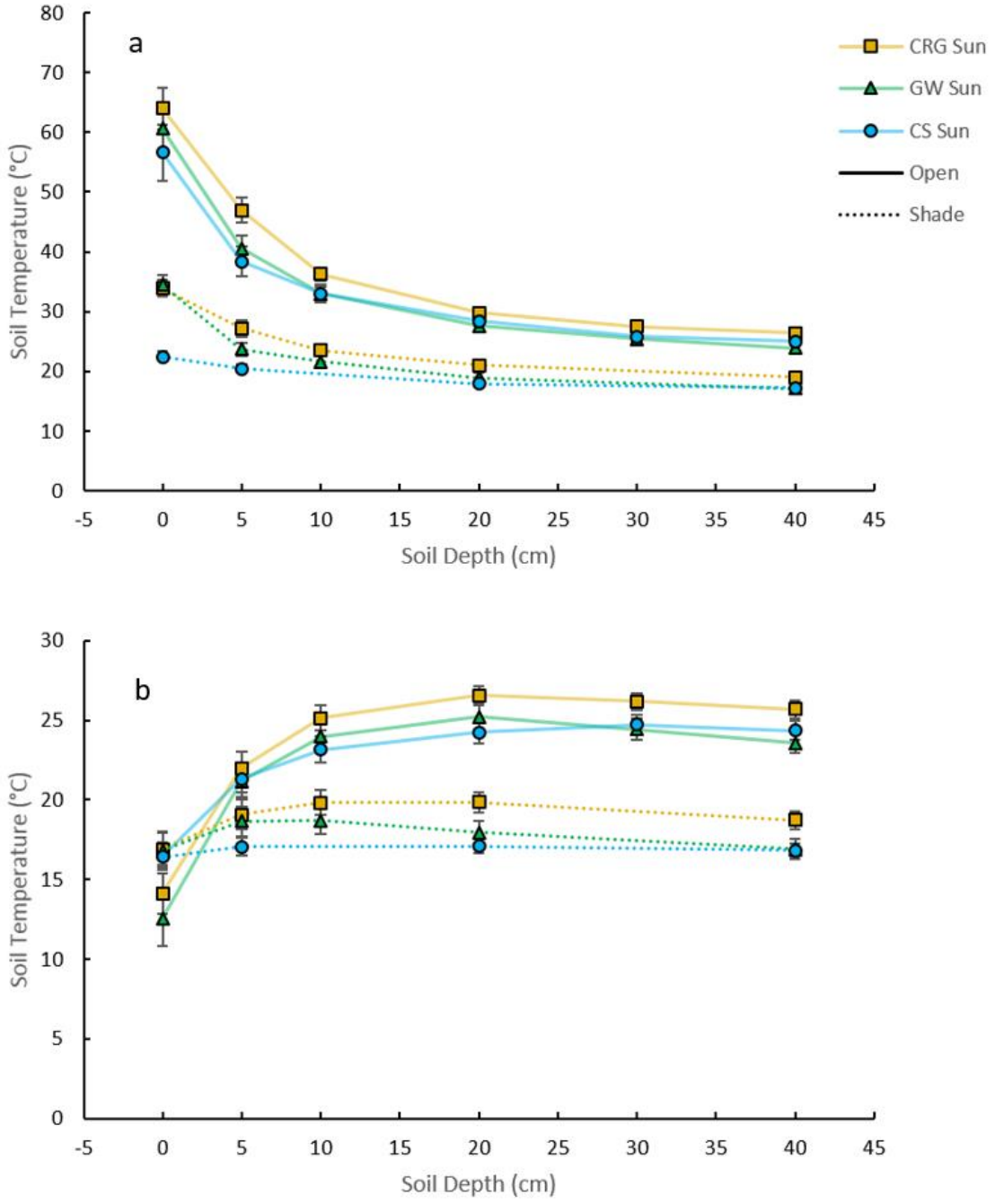


Figure 4. The mean maximum (a) and minimum (b) soil temperatures (°C) at each depth from three climate zones: the warm, inland site at Columbia River Gorge (CRG), the more moderate summer climate inland site on the east side of the Cascade Mountains at Goat Wall (GW), and cool, maritime climate of the coastal shores (CS) sites. Data were recorded from 6/17/2015 through 8/4/2015.

Table 1. Listed are means ± SE of daily mean, maximum and minimum soil temperatures (°C) at different soil depths in representative sunny and shaded locations at the warm inland site at Columbia River Gorge (CRG), the moderate summer climate at northern-and-high elevation site east of the Cascade Mountains, at Goat Wall (GW), and the maritime climate coastal shores (CS) sites.

		Soil Temperature (°C) ± Standard Deviation					
Soil Depth	**Site**	**Mean Sun**	**Mean Shade**	**Mean Max Sun**	**Mean Min Sun**	**Mean Max Shade**	**Mean Min Shade**
0 cm	CRG	31.9 ± 16.7	24.9 ± 5.60	64.0 ± 6.94	14.1 ± 2.57	33.9 ± 2.95	16.9 ± 2.05
	GW	31.3 ± 16.9	24.0 ± 5.83	58.1 ± 14.6	12.3 ± 3.82	34.5 ± 3.24	16.8 ± 2.39
	CS	29.0 ± 13.9	18.7 ± 2.30	56.6 ± 9.29	16.5 ± 1.63	22.4 ± 2.06	16.4 ± 1.18
5 cm	CRG	31.9 ± 8.22	23.1 ± 3.33	47.0 ± 4.15	22.0 ± 2.01	27.2 ± 2.88	19.1 ± 1.92
	GW	29.9 ± 6.91	20.9 ± 2.45	40.6 ± 4.18	21.2 ± 1.94	23.7 ± 2.14	18.7 ± 1.87
	CS	28.2 ± 6.32	18.4 ± 1.60	38.4 ± 4.87	21.3 ± 1.74	20.4 ± 1.50	17.1 ± 1.14
10 cm	CRG	30.1 ± 3.94	21.8 ± 2.04	36.2 ± 2.21	25.2 ± 1.59	23.5 ± 1.87	19.8 ± 1.60
	GW	28.2 ± 3.43	19.9 ± 1.89	33.0 ± 2.31	24.0 ± 1.70	21.6 ± 1.83	18.7 ± 1.72
	CS	27.3 ± 3.82	-	33.0 ± 2.92	23.2 ± 1.61	-	-
20 cm	CRG	28.1 ± 1.51	20.4 ± 1.37	29.8 ± 1.09	26.6 ± 1.14	21.1 ± 1.38	19.9 ± 1.32
	GW	26.4 ± 1.67	18.4 ± 1.42	27.6 ± 1.51	25.2 ± 1.55	18.9 ± 1.50	18.0 ± 1.45
	CS	26.1 ± 1.90	17.5 ± 0.961	28.4 ± 1.55	24.2 ± 1.43	17.9 ± 0.95	17.1 ± 0.94
30 cm	CRG	26.9 ± 1.14	-	27.5 ± 1.09	26.2 ± 1.06	-	-
	GW	24.9 ± 1.34	-	25.4 ± 1.29	24.4 ± 1.32	-	-
	CS	25.3 ± 1.29	-	25.8 ± 1.26	24.7 ± 1.25	-	-
40 cm	CRG	26.1 ± 1.18	18.9 ± 1.07	26.5 ± 1.16	25.7 ± 1.14	19.1 ± 1.09	18.7 ± 1.11
	GW	23.7 ± 1.21	17.0 ± 1.24	23.9 ± 1.24	23.6 ± 1.25	16.8 ± 2.75	16.6 ± 2.71
	CS	24.7 ± 1.25	17.1 ± 0.863	23.9 ± 5.08	23.9 ± 3.69	17.2 ± 0.85	16.8 ± 0.91

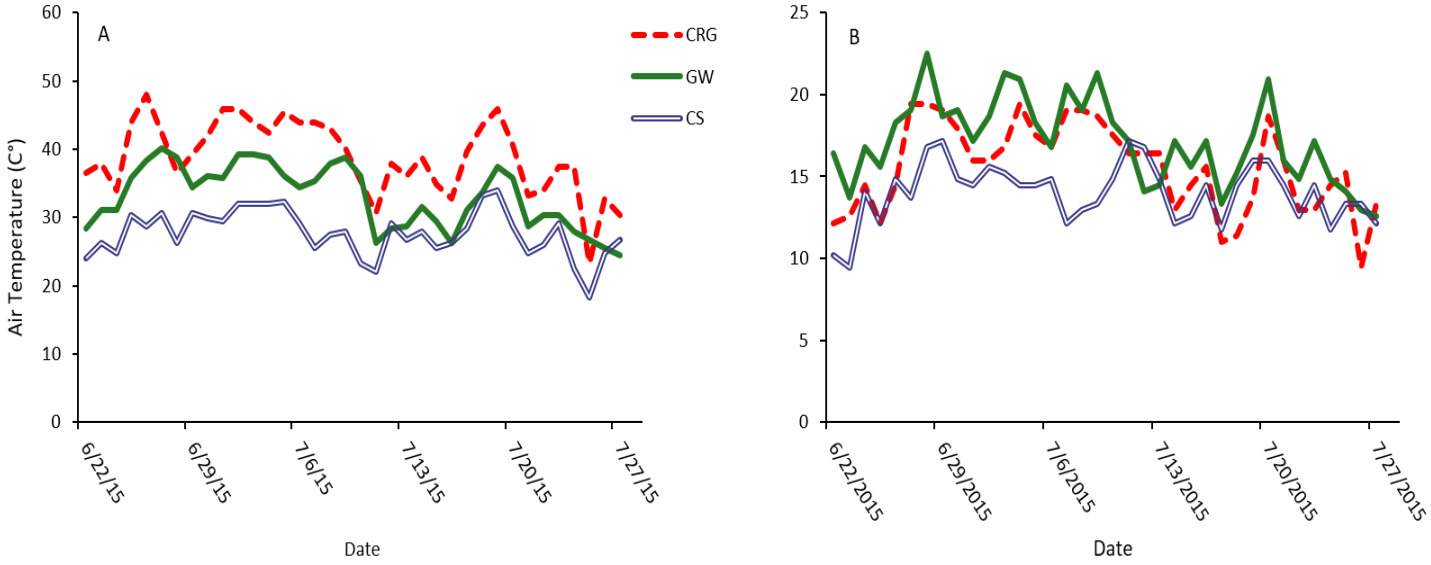


Figure 5. Maximum (a) and minimum (b) daily air temperatures (°C) measured approximately two meters above the ground in open areas at the Columbia River Gorge (CRG), Goat Wall (GW), and the coastal shores (CS) sites.

Body Condition Analyses

Regardless of sex, the largest adult lizards, by both average snout-vent length (SVL) and body mass (no food in gut), were found at GW (Table 2). For the two inland sites, the hotter CRG site and the warm GW site, females had larger SVLs and body masses than males. The females from these two sites in fact had the largest mean SVL and mass values of all lizards measured at those sites, but GW females had higher body condition indices (BCI) than those at CRG. Males from CRG had the smallest average SVL and body masses, and had significantly lower BCI measures than males from the coastal CS site. At the cool, coastal CS site, females were smaller than females at the inland sites, and thus close to the body size of coastal males, which were intermediate in size compared to the other two sites.

I tested for differences in BCI amongst sites by way of a two-way analysis of covariance test, with mass as the response variable, site and sex (males n=104, females n=82) as the predictor variables, and snout-vent length as the covariate. Both site and sex were found to be significant factors ($ANCOVA_{1,179}$: $p_{site} < 0.001$, $p_{sex} < 0.001$).

Table 2. Body measurements of adult lizards from three the Columbia River Gorge (CRG), Goat Wall (GW), and the coastal shores (CS) sites. Listed are the means ± SD for snout-vent length (SVL), body mass and body condition index (BCI, as mass per unit SVL).

Site	Sex	SVL (mm)	Mass (g)	BCI (g/mm)
CRG	Male (n=28)	65.5 ± 5.8	8.96 ± 1.84	0.137
	Female (n=17)	71.6 ± 4.3	11.0 ± 2.10	0.153
GW	Male (n=33)	68.9 ± 3.7	10.73 ± 1.96	0.156
	Female (n=32)	71.6 ± 4.0	12.66 ± 2.85	0.177
CS	Male (n=28)	66.8 ± 4.4	10.56 ± 2.07	0.158
	Female (n=12)	66.9 ± 5.0	10.69 ± 2.25	0.160

Since sex was found to be a significant factor, I conducted BCI analyses for each sex independently (Figure 6). I tested for an interaction between SVL and site on body mass using ANOVAs, which showed no significant interactions. I then ran ANCOVAs with body mass as the response variable, site as the predictor variable, and SVL as the covariate. The result of the ANCOVAs showed that body mass differed significantly amongst sites, and that the relationship between body mass and SVL also differed significantly amongst sites (male data $ANCOVA_{2,100}$: $p_{site} < 0.001$, $p_{SVL} < 0.001$; female data $ANCOVA_{2,78}$: $p_{site} < 0.001$, $p_{SVL} < 0.001$). A *post hoc* Tukey HSD revealed a significant difference in body mass of males between the CRG and CS sites (Tukey HSD, p=0.04). Regression equations for the BCI of male and female lizards from each site are shown in Table 3.

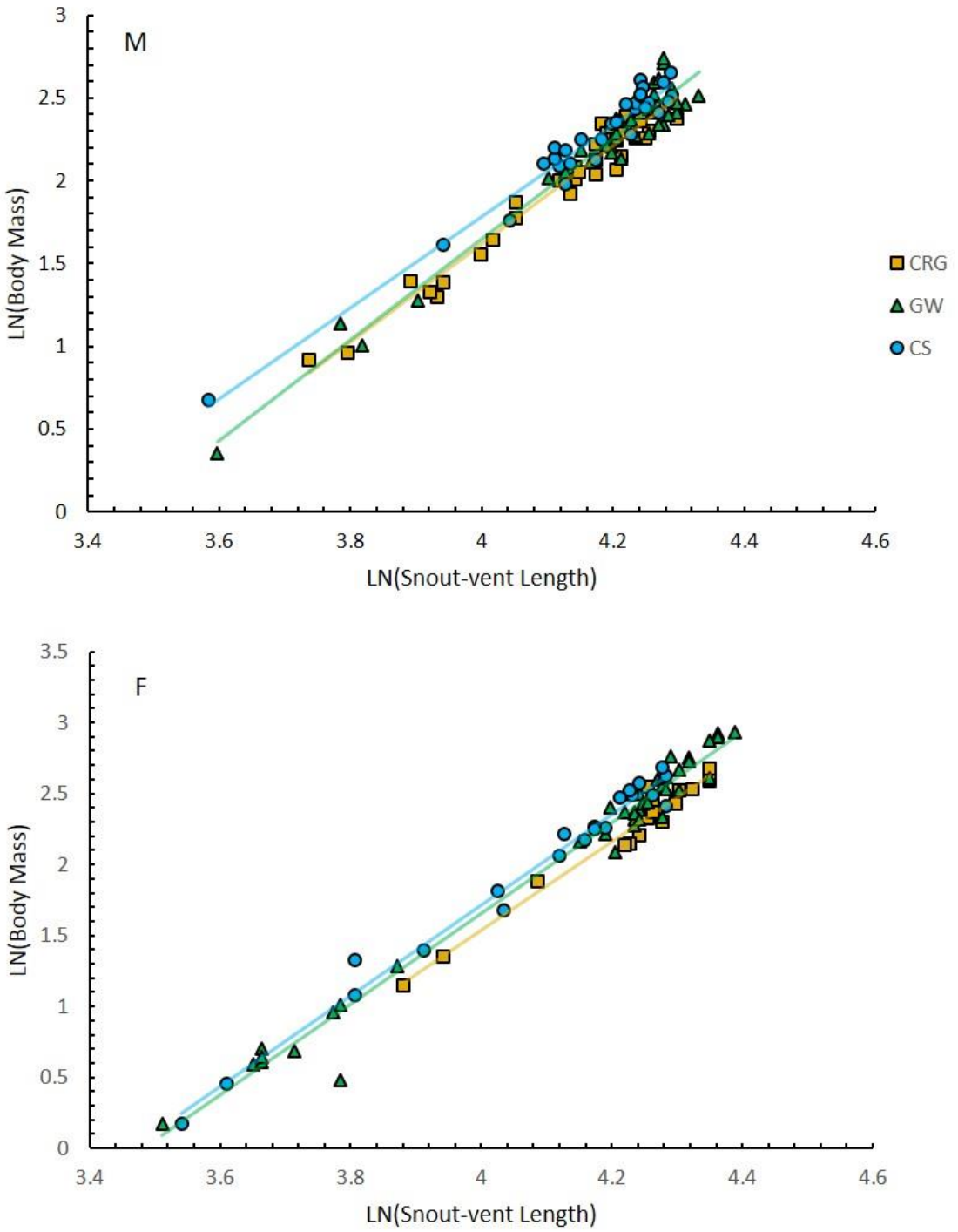


Figure 6. The natural log of body mass (grams) plotted as a function of the natural log of snout-vent length (millimeters) for male (M) and female (F) lizards from the Columbia River Gorge (CRG), Goat Wall (GW), and coastal shores (CS) sites.

Table 3. Regression equations for the body condition index (BCI) comparisons among males and females from each site, derived using natural log transformed body mass (y) and SVL (x) data.

Regression Equations - BCI			
Sex	Site	Equation	R^2
Male	CRG	y = 2.9691x - 10.257	0.9606
	GW	y = 3.0444x - 10.531	0.9499
	CS	y = 2.7526x - 9.2264	0.9545
Female	CRG	y = 3.1092x - 10.899	0.9656
	GW	y = 3.2015x - 11.154	0.9788
	CS	y = 3.1957x - 11.07	0.9825

Analyses of Thermoregulation

Mean field-active T_b of lizards from CRG, GW, and CS were 35.9°C, 35.7°C, and 35.4°C, respectively, whereas mean lab-measured T_b of lizards from CRG, GW, and CS were 35.8°C, 36.4°C, and 35.9°C, respectively (Figure 7).

I tested the hypothesis that lizards at all three locales can regulate to the same known preferred T_b during activity (H_{T0}) by comparing T_b data among sites using a one-way ANOVAs, with site as the predictor variable. The ANOVAs showed a significant difference in both lab-measured T_b among sites ($ANOVA_{2,103}$; p=0.02) and field active T_b ($ANOVA_{2,184}$; p=0.05). I then conducted *post-hoc* Tukey HSD tests, which resulted in a significant difference in the lab-measured T_b between the CRG versus GW comparison (p=0.025), and in the field active T_b between the CRG versus CS comparison (p=0.045).

Within each site, I compared the upper 25%, middle 50%, and lower 25% quartiles of the field active to the highest, mean, and lowest lab-measured T_b of thermoregulating lizards using either Student's t-tests or Mann-Whitney U tests. The lower quartile field T_b data and minimum lab-measured T_b data were normally distributed for every site except CS (Wilks-Shapiro test, $p_{Field}<0.001$). The upper quartile field T_b and maximum lab-measured T_b data were normally distributed in the CRG site, but not GW or CS (Wilks-Shapiro $test_{GW}$, $p_{Field}<0.019$, $p_{Lab}<0.001$; Wilks-Shapiro $test_{CS}$, $p_{Lab}<0.026$). The middle quartiles of the field T_b and mean lab-measured T_b data were normally distributed for every site except CS (Wilks-Shapiro test, p=0.017). The lower temperatures T_b data from neither GW nor CS showed equal variance (Levene's $test_{1,52}$, $p_{GW}=0.003$; Levene's $test_{1,55}$, $p_{CS}<0.001$), while the data from CRG did show equal variance. The T_b data from the higher quartile exhibited

equal variance for every site except CS (Levene's $test_{1,55}$, p=0,005). The variance of the median T_b data was unequal for every site (Levene's $test_{1,51}$, p_{CRG}=0.047; Levene's $test_{1,65}$, p_{GW}<0.001; Levene's $test_{1,77}$, p_{CS}=0.003). The heteroscedasticity of the data sets could not be normalized by any transform, so the raw data were used for the following comparisons.

There was no significant difference between the lower quartile field-active T_b and the minimum lab-measured T_b data in either the CS or GW sites. The hotter CRG site, however, had significantly higher T_b values in the field when compared to the lab measures (t-test, p=0.004). The warm, inland GW and the cool, coastal CS site both had significantly lower field-active T_b in the upper quartile when compared to the maximum lab-measured values (Mann-Whitney U test, p_{GW}=0.001; Mann-Whitney U test, p_{CS}=0.001), unlike for CRG in which there was no difference. For the median T_b data, again both GW and CS had significantly lower field-active T_b values when compared to the mean lab-measured T_b values (t-test, p_{GW}=0.001; Mann-Whitney U test, p_{CS}=0.001), while CRG showed no difference.

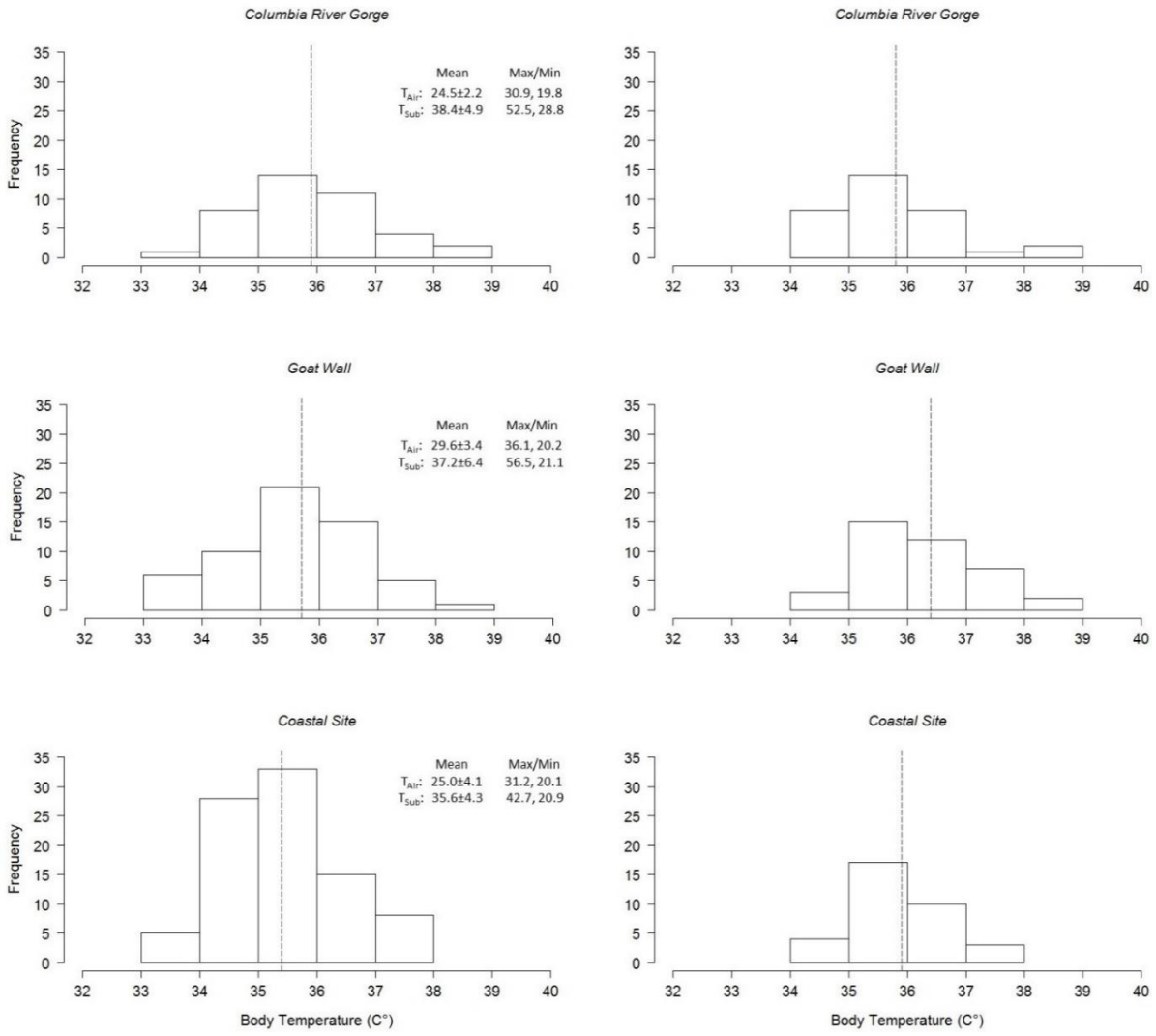


Figure 7. Left: Field-active body temperatures (°C) of lizards from the Columbia River Gorge (CRG), Goat Wall (GW), and the coastal shores (CS) sites. Listed on the figures are the mean, standard deviation, maximum, and minimum air and substratum temperatures at time of capture. Right: Lab-measured body temperatures of lizards from CRG, GW, and CS. Slashed lines represent the mean body temperature of each group.

Analysis of Metabolism

The lizards used in these comparisons all had similar body masses (Table 4; ANOVAs all $p>0.05$), and therefore could be compared directly using ANCOVAs. Some data sets still exhibited unequal variance amongst sites even after the natural log transformation (RMRs of lizards at 28°C and 36°C T_b, Levene's $test_{2,40}$, $p_{28°C}=0.04$; Levene's $test_{2,41}$, $p_{36°C}=0.01$, respectively). These data sets were still compared by way of ANOVA or ANCOVA.

I tested the hypothesis that *Sceloporus occidentalis* from all three sites had similar whole-animal metabolic rates when measured at similar body temperatures (H_{M0}) by comparing oxygen consumption amongst sites within each T_b using ANCOVAs, with oxygen consumption as the response variable, site and the site*body mass interaction as the predictor variables, and body mass as the covariate (Figure 8). Site, body mass, and the site*mass interaction were all found to be significant for every comparison (20°C, $p_{site}<0.001$, $p_{mass}<0.001$, $p_{interaction}=0.03$; 28°C fasted, $p_{site}<0.001$, $p_{mass}<0.001$, $p_{interaction}=0.04$; 28°C fed, $p_{site}<0.001$, $p_{mass}<0.001$, $p_{interaction}=0.009$; 36°C, $p_{site}<0.001$, $p_{mass}<0.001$, $p_{interaction}=0.02$). Fisher LSD tests revealed that 1) CS lizards had a significantly higher RMR than lizards from the other two sites at 20°C T_b (CS vs. CRG, $p<0.001$; CS vs. GW, $p<0.001$), 2) RMR and SMR differed at 28°C T_b at each site, with CS lizards being the highest in both comparisons ($p<0.015$ for all comparisons), and 3) CS lizards had a significantly higher RMR at 36°C T_b than lizards from the other two sites (CS vs. CRG, $p<0.001$; CS vs. GW, $p<0.001$). The power regression equations are listed in Table 5. To check for potential skews in body mass among sites, I evaluated the residuals of oxygen consumption versus body mass and site within each body temperature using an ANCOVA with residuals as the response

variable, site as the predictor variable, and body mass as the covariate. None of the residuals were found to be significantly different based on site or mass at any T_b.

During data collection, I had the strong impression that lizards from CRG seemed more skittish, and were more likely to move in the sample chambers than lizards from the other two sites (thereby raising their oxygen consumption rates due to exercise). I was careful to only include measures that I was confident were of resting, stationary lizards, though the seemingly jittery nature of lizards from CRG may have led to increased variance in RMR measures for this population at 36°C T_b.

Table 4. Body mass data (g) *Sceloporus occidentalis* measured by respirometry during summer from the warm site at Columbia River Gorge (CRG), the cooler site Goat Wall (GW), and the coolest site at coastal shores (CS).

Mean Body Mass (grams) ± SD				
Site	**20°C**	**28°C Fasted**	**28°C**	**36°C**
CRG	n = 15	n = 14	n = 15	n = 16
	10.47 ± 1.41	10.59 ± 1.37	10.47 ± 1.41	10.51 ± 1.37
GW	n = 16	n = 26	n = 17	n = 19
	11.15 ± 2.11	11.03 ± 2.22	11.24 ± 2.15	11.02 ± 2.19
CS	n = 13	n = 15	n = 11	n = 9
	11.24 ± 2.52	11.31 ± 2.28	11.37 ± 2.47	10.02 ± 1.98

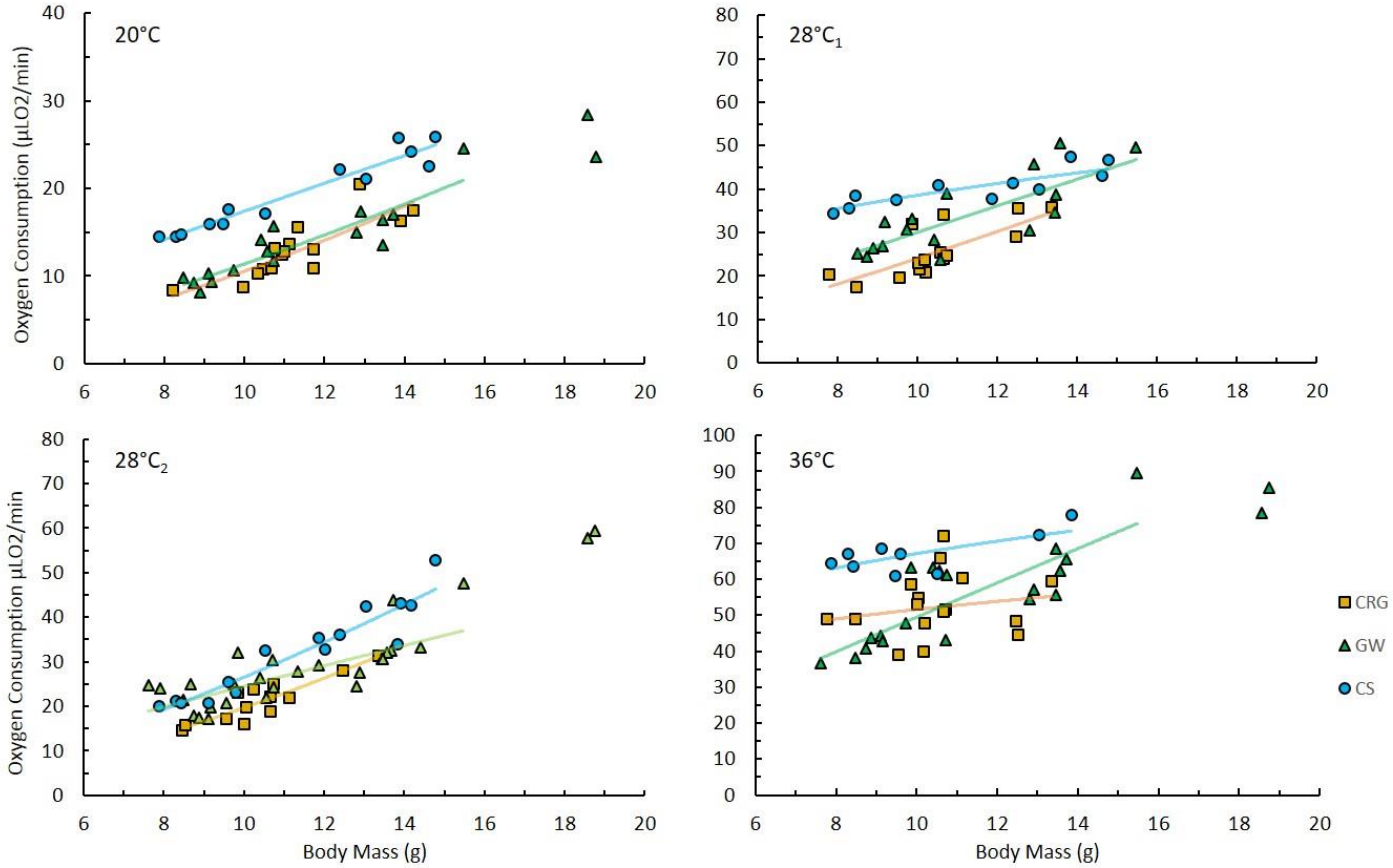


Figure 8. RMR (metabolism of fed, resting lizards, measured as microliters of oxygen per minute) of *Sceloporus occidentalis* from all three sites, plotted with respect to body mass (grams) for each of three body temperatures (20°C 28°C and 36°C). The SMR (metabolism of post-absorptive, resting lizards) was also measured at 28°C (28°C_2). Power trendlines were used to show the relationship between body mass and whole-animal rate of oxygen consumption for lizards at each site. For y-axes with standardized ranges, see Figure 11 in *Appendix A*.

Table 5. Power regression equations of oxygen consumption rate (r), of standard and resting metabolic rates, versus body mass (m) for each site at all three body temperatures (°C).

Power Regressions Equations				
Site	T_b	Metabolism	Equation	R^2
CRG	20	RMR	$r = 0.3393m^{1.5452}$	0.7365
	28	SMR	$r = 0.4963m^{1.5978}$	0.7982
	28	RMR	$r = 1.3260m^{1.2585}$	0.5933
	36	RMR	$r = 29.978m^{0.2358}$	0.0369
GW	20	RMR	$r = 0.4518m^{1.4007}$	0.827
	28	SMR	$r = 2.7923m^{0.9431}$	0.5754
	28	RMR	$r = 2.2193m^{1.1352}$	0.6402
	36	RMR	$r = 5.3103m^{0.9689}$	0.6834
CS	20	RMR	$r = 2.0686m^{0.9247}$	0.9474
	28	SMR	$r = 0.9931m^{1.4264}$	0.9135
	28	RMR	$r = 16.385m^{0.3718}$	0.7328
	36	RMR	$r = 35.625m^{0.2751}$	0.4719

Seasonal Effect on Metabolism

Spring oxygen consumption data of *Sceloporus occidentalis* from the hotter, inland CRG site were compared to summer oxygen consumption data of *S. occidentalis* from the cool, coastal CS site to test for a potential seasonal effect on metabolism of the inland versus coastal populations (Figure 9). The body mass data of the two groups of lizards being compared were not similar (Table 6; $ANOVA_{1,20}$, p=0.006), rendering the body mass data inapplicable as a covariate for an ANCOVA. Therefore, I instead adjusted the oxygen consumption data to a standard mass value (9.5 grams). All data groupings being compared were found to be normally distributed and equally variable, except for oxygen consumption at 36°C T_b, which was heteroscedastic (Levene's $test_{1,14}$, p=0.026). A natural log transformation of the data corrected this, however (Levene's $test_{1,14}$, p=0.07).

Oxygen consumption data corrected to the standard mass were compared amongst sites within each T_b using Student's t-tests. There were significant differences in RMR at T_b 28°C (df=15, p<0.001, CS>CRG), and in RMR at 36°C T_b (df=14, p<0.001, CS>CRG). There was no significant difference in mass-specific oxygen consumption rates of lizards at a T_b of 20°C (df=18, p=0.13). Power regression equations for the data are listed in Table 7. Spring CRG SMR at 28°C T_b data were nonsensical, as they were higher than RMR for spring CRG lizards. Therefore, this data were not used (see "*Discussion of Metabolism"* in Discussion, pg. 55).

Table 6. Body mass data (g) of *Sceloporus occidentalis* measured by respirometry from the Columbia River Gorge site (CRG) in spring compared with the corresponding data of lizards from the coastal shores site (CS) in summer.

	Mean Body Mass (grams) ± SD			
Site	**20°C**	**28°C Fasted**	**28°C**	**36°C**
CRG	n = 7	n = 7	n = 6	n = 7
	8.02 ± 2.40	8.02 ± 2.40	7.80 ± 2.52	8.02 ± 2.40
CS	n = 13	n = 15	n = 11	n = 9
	11.24 ± 2.52	11.31 ± 2.28	11.37 ± 2.47	10.02 ± 1.98

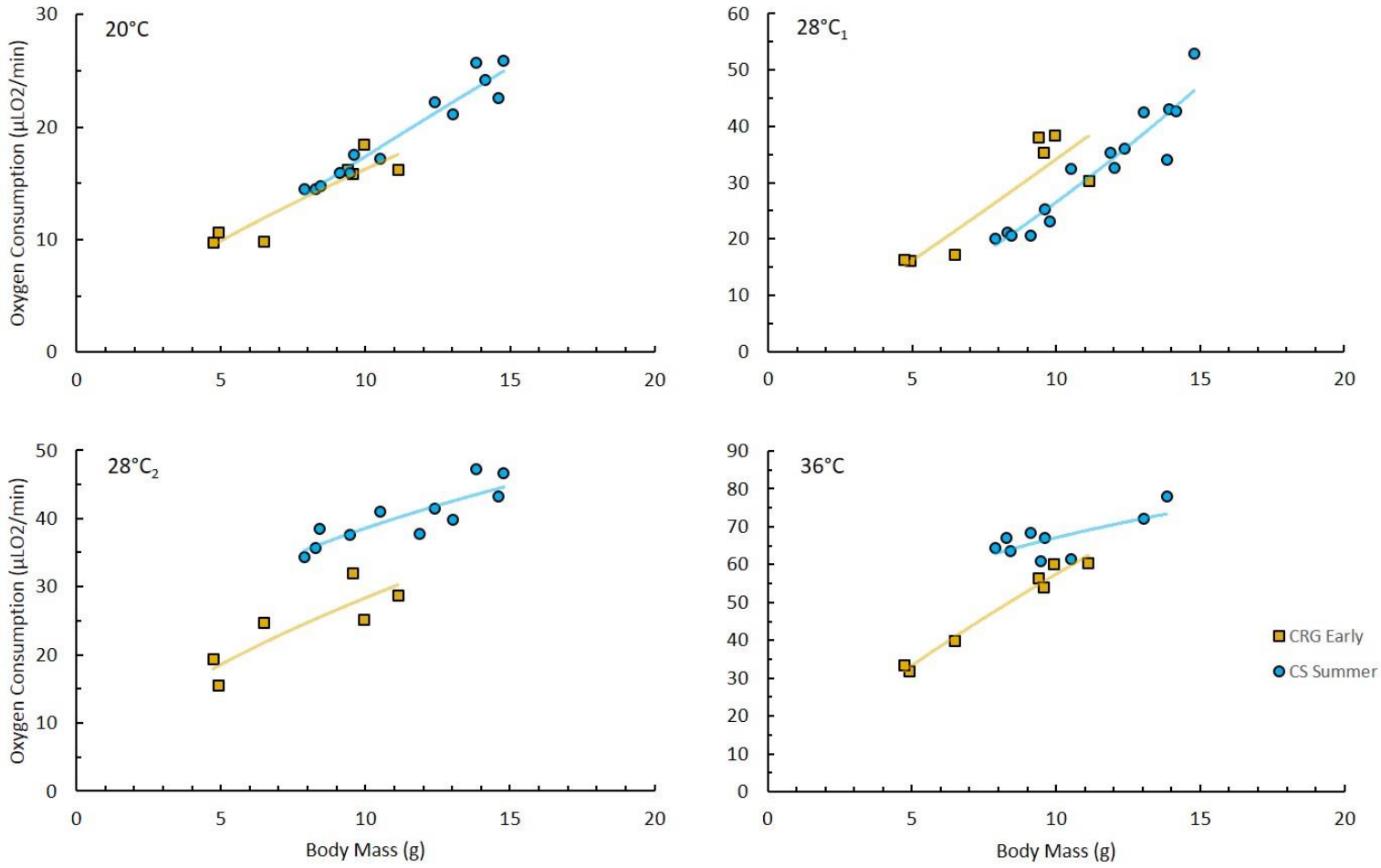


Figure 9. RMR of CRG lizards in late spring-to-early summer at 20°C, 28°C and 36°C T_b, compared with RMR of CS lizards in mid-summer at the same temperatures, plotted as a function of body mass. SMRs also were compared at 28°C. Power trendlines were used to show the relationship between body mass and whole-animal rate of oxygen consumption for lizards each site.

Table 7. The power regression equations of oxygen consumption rate (r), of standard and resting metabolic rates, versus body mass (m) for the CRG lizards from spring and the CS lizards from summer all three body temperatures.

Power Regression Equations – Spring CRG versus Summer CS				
Site	T_b	Metabolism	Equation	R^2
CRG	20	RMR	$r = 3.1187m^{0.7173}$	0.8466
	28	SMR	$r = 2.8578m^{1.0771}$	0.8439
	28	RMR	$r = 6.9751m^{0.6091}$	0.7459
	36	RMR	$r = 9.4534m^{0.7837}$	0.9813
CS	20	RMR	$r = 2.0685m^{0.9248}$	0.9474
	28	SMR	$r = 0.9930m^{1.4265}$	0.9135
	28	RMR	$r = 16.385m^{0.3718}$	0.7328
	36	RMR	$r = 35.625m^{0.2751}$	0.4719

DISCUSSION

Discussion of Thermoregulation

Sceloporus occidentalis from the hottest site, the Columbia River Gorge (CRG), thermoregulated at significantly warmer field-active body temperatures (field-active T_b) than those at the cool, coastal shores site (CS). Furthermore, the distribution of field active T_b of lizards from CRG skewed hotter than the preferred T_b measures selected on the thermal gradient in the lab (lab T_b). Thus, one may infer that the first alternative hypothesis "H_{T1}" cannot be wholly refuted; that is, high ambient temperatures at CRG and GW may have resulted in *S. occidentalis* accepting body temperatures nearer the upper end of the accepted T_b range (35.7°C at GW and 35.9°C at CRG), despite weather conditions at CRG being atypically cool during my time there (Figure 7). Corroborating the hypothesis is 1) the CS lizards thermoregulated near the upper limit of the preferred T_b range when in lab (35.9°C), but had a relatively low mean field-active Tb, at 35.4°C and 2) the CRG lizards thermoregulated at cooler temperatures in the lab than in the field. Moreover, mean field-active T_b measures from all three sites rank in order, hottest to coolest, directly with the average field temperatures at each site: CRG, GW, CS.

Due to the inability to perfectly utilize the thermal heterogeneity of their environment, as well as other pressures such as predation or interspecific competition, lizards are known to carefully thermoregulate either at, or below, their optimal T_b (*i.e.* "suboptimal is optimal"; Martin and Huey 2008). That is, because the physiological consequences of a lizard's T_b dropping below the preferred range are less severe than if it drifts hotter, lizards should tend to thermoregulate towards the cooler end of their

preferred T_b range if environmental conditions permit. That lizards at CRG and GW thermoregulated at near the upper limit of their preferred T_b range, despite this notion that suboptimal T_b during activity may be preferred (*e.g.*, 34-35°C), further corroborates the prevailing hypothesis that *S. occidentalis* at these hotter sites are forced to accept higher T_b during activity. Although the temperature conditions at all three sites do appear to be influencing the thermoregulatory effectiveness of lizards, the mean T_b achieved by lizards among sites differ between the hottest (CRG) and coolest (CS) sites by only 0.5°C. As such, the physiological consequences of such a small difference in T_b may be negligible.

Interestingly, *Sceloporus occidentalis* from GW selected warmer T_b in the thermal gradient than lizards from the other two sites. Indeed, the laboratory T_b measures of *S. occidentalis* from GW are actually significantly warmer than those of lizards from CRG, where lizards are exposed to the hottest climatic conditions. This result is somewhat surprising and difficult to explain. The ability to behaviorally thermoregulate tends to negate the inverse association of T_b with elevation (Adolph 1990, Andrews 1998, Huey *et al.* 2003, Zamora-Camacho *et al.* 2016), as seen in the similar field-active T_b of lizards at CRG and GW. One potential hint toward an explanation for high lab-preferred T_b of GW lizards is that the GW locale is at a much higher elevation than the other two sites, such that compensating for low food processing rates during cool nights may be offset by thermoregulating at higher temperatures during the day. There has been another instance where lizards from cooler environments have selected hotter preferred T_b than other populations inhabiting warmer climates when measured on a thermal gradient (Artacho *et al.* 2017), and their speculation for this outcome is similar. It is possible, however, that the

GW lizards are more willing to accept warmer T_b than the other two populations, particularly when exploring a novel environment. Lizards at GW are often found on large boulders in relatively open, sun-exposed areas (Photo 6, Appendix A), and thus may be forced to accept higher T_b when moving from location to location. If so, it would not be surprising for them to also accept higher T_b when exploring the thermal gradient as well.

Ultimately, I was interested in studying how these three populations of *Sceloporus occidentalis* were able use behaviorally thermoregulation to successfully inhabit three very different climate types in Washington. Within each climate, *S. occidentalis* achieved mean field active T_b that were within the known preferred T_b range for this species (*i.e.* 34-36°C). Furthermore, lizards brought into the lab from the cool, coastal CS site and the hot, inland CRG site both chose lab T_b within this preferred T_b range. Therefore, due to the apparent thermoregulatory success of *S. occidentalis* at each site, and because the lizard population densities appear to be robust at each site, it is clear that all three climate zones are suitable for this species to persist.

Discussion of Metabolism

The resting metabolic rates (RMR) and standard metabolic rates (SMR) at each body temperature during summer were significantly higher in *Sceloporus occidentalis* from the cool, coastal shores site (CS) than in lizards from the two inland sites, CRG and GW. Thus, all three hypotheses presented about metabolism were refuted. That is, 1) null hypothesis H_{M0}, predicting no differences among lizards from the three climate zones in metabolism at any of the three body temperatures was refuted because of the higher metabolism of CS

lizards and 2) H_{M1} and H_{M2} were refuted because the RMR of lizards from CRG and GW did not differ at 20°C and 28°C T_b. It can be argued, however, that there is partial support for H_{M2} — which states that *S. occidentalis* from all three sites will differ in RMR at every T_b measured — because RMR of lizards from the cool, coastal CS site is significantly higher at all three temperatures than RMR of lizards from the two inland locales, CRG and GW, which have warmer daytime temperatures. When inactive and in refugia (several centimeters or more below ground surface) either in leaf litter or under logs (Dr. Roger Anderson, personal observation), the lizards at CS are probably passively achieving cooler T_b during the inactivity period T_b (*i.e.* poikilothermic) than inactive lizards at the warmer inland sites. It is well known that cooler T_b results in slower physiological processes, including the sum total of metabolism, as is RMR. Therefore, it seems that *S. occidentalis* at the coast may exhibit elevated metabolism to compensate for the retarding effects of cooler T_b on physiological processes, such as the catabolism and anabolism of food in the gut. Such increases of metabolism in response to exposure to cooler environmental temperatures has been recorded in other species of *Sceloporus* populations in response to both seasonal and climatic differences among separate populations (Dutton and Fitzpatrick 1975, Tsuji 1988, Angilletta 2001).

If elevated resting metabolism of *Sceloporus occidentalis* along the coast does compensate for cooler body temperatures in summer, then either of two mechanisms for this compensation may have occurred. First is the phenomenon of phenotypic plasticity within a genotype, such as can be seen in many poikilotherms — especially fish and amphibians — living in temperate climates (Seebacher and Wilson 2006, Hadamová and

Gvoždík 2011, Caruso *et al.* 2014). Since *S. occidentalis* are essentially poikilothermic when inactive in refugia, it is possible that the cooler temperatures at CS may have induced a similar phenotypically plastic response. The second possible method of temperature compensation is that the elevated RMR of CS lizards could be a result of an evolutionary response to cooler summer temperatures. Based on prevailing opinions about time since last glaciation west of the Cascade Range (Porter and Swanson 1998, Mood and Smith 2015), and the resulting time frame for arrival of the CS population to the shores of the Salish Sea during a hypsithermal period (Kuchta and Tan 2005, Leopold *et al.* 2016), the CS population may have had enough time to evolve higher metabolic rate (3,000 — 8,000 years).

I have some data that indirectly can compare the two causes for higher RMR in CS lizards. It is known that the metabolic rates of *S. occidentalis* from inland WA may have higher RMR during the cool of spring in late May through early June than they do in late summer (Tsuji 1988). Thus, one may expect that if the cool summer conditions of CS are similar to the spring conditions at CRG, then *S. occidentalis* along the coast exhibited higher RMR during their relatively cool summer in a similar way to the CRG lizards during their relatively cool spring. When I compared RMRs of CS lizards from the summer to that of CRG lizards from the spring, although there was no significant difference at 20°C T_b, the RMR of CS lizards were significantly greater at higher T_b (*e.g.*, 28°C and 36°C). Thus, it is clear that further evaluation will be needed to determine whether the difference in RMR between the inland and coastal populations are a function of seasonal plasticity, or if the coastal site has indeed evolved elevated RMR in response to cooler climatic conditions. To effectively begin

addressing whether the CS population of *S. occidentalis* indeed evolved elevated metabolic rate, or are simply exhibiting increased metabolic rate as a form of metabolic temperature compensation, a simple acclimation study using coastal and inland lizards should be conducted. Furthermore, to evaluate whether evolutionary change has occurred in the coastal population, a common garden experiment using lizards from both inland and coastal populations should be conducted, while also considering the historical climatic conditions to which these separate populations of *S. occidentalis* have been exposed.

A factor corroborating high metabolic rates of *Sceloporus occidentalis* along the coast may be related to the higher availability of food. Lizards along the coast have shorter activity seasons than those inland, but consume significantly more food energy and have heavier BCIs (Powers 2010). That is, because *S. occidentalis* along the coast are consuming larger quantities of food and should be converting that food energy into a larger body mass in a shorter period than apparently occurs in the lizards at the drier, warmer inland CRG site, then perhaps the coastal lizards have a higher specific dynamic action (SDA) — they may have increased metabolic effort at digesting, absorbing, and assimilating food stuffs, and may also include building tissue as in growth and storage. A comparison of SMR to RMR within each site supports this claim. When compared at a body mass of 9.5g, lizards from the two inland sites, CRG and GW, increase oxygen consumption rates 24.5% and 22.5% from unfed SMR to fed RMR. However, lizards from the coastal site increase oxygen consumption rates 53.6% when comparing unfed SMR to red RMR. Furthermore, because the only significant differences in metabolism I found between spring CRG lizards and summer CS lizards were in RMR at higher body temperatures, there may be differences in

the SDA between lizards in these two populations, despite having presumably similar amounts of food in their guts. The SMR data for spring CRG lizards is considered unusable and should be ignored because the unfed CRG lizards had the absurd outcome of higher SMR than the fed lizards did for RMR, indicating the CRG lizards were not calm enough during the SMR trials. Recall that the RMR data from CRG lizards at 36°C suffered the same activity-induced data error (see "*Seasonal Effect on Metabolism*" in Results). Note also that when compared within-season (*i.e.* in the summer) both RMR and SMR were higher in CS lizards than CRG lizards. Because there does appear to be seasonally related variation in RMR of CRG lizards (*i.e.*, spring CRG lizards have elevated RMR), then a greater response of SDA in summer CS lizards could be a reversible acclimation response similar to that seen in spring CRG lizards.

Conclusions

In summary, lizards from all three sites were capable of effectively thermoregulating within the known preferred T_b range for *Sceloporus occidentalis* while active in the field during summer. However, lizards at the hotter sites (CRG and GW) had to accept hotter body temperatures during activity than those along the coast. Furthermore, more research is needed to understand the cause for lizards from the GW population thermoregulating at unexpectedly high body temperatures on the thermal gradient. The coastal population of *S. occidentalis* had higher RMR than lizards from the two inland sites, which may allow the lizards from the cooler coastal site to digest and assimilate larger amounts of food more quickly and at cooler body temperatures than those at the inland sites. Further research is required to assess whether the higher RMR and SMR of lizards along the coast is related to a higher SDA per gram of food, and whether higher RMR, SDA and SMR are a function of seasonal acclimation, evolution, or a combination of these factors. Determining and understanding the mechanistic causes of metabolic rate differences and lab T_b differences among lizards from these different climate zones may further our understanding of the thermal ecophysiology of terrestrial ectotherms, and may provide useful perspectives when investigating how ectotherms with broader geographic ranges will respond to changing climatic conditions as compared to the responses of ectotherms with much narrower geographic ranges.

LITERATURE CITED

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. 1993. Temperature, activity, and lizard life histories. The American Naturalist 142:273—295.

Andrews, R. M. 1984. Energetics of sit-and-wait and widely-searching lizard predators. Vertebrate ecology and systematics - A Tribute to Henry S. Fitch:137—145.

Andrews, R. M. 1998. Geographic variation in field body temperature of *Sceloporus* lizards. Journal of Thermal Biology 23:329—334.

Angilletta, M. J. 2001. Variation in metabolic rate between populations of a geographically widespread lizard. Physiological and Biochemical Zoology 74:11—21.

Angilletta, M. J., A. F. Bennett, H. Guderley, C. a Navas, F. Seebacher, and R. S. Wilson. 2006. Coadaptation: a unifying principle in evolutionary thermal biology. Physiological and Biochemical Zoology 79:282—294.

Artacho, P., I. Jouanneau, and J.-F. Le Galliard. 2013. Interindividual variation in thermal sensitivity of maximal sprint speed, thermal behavior, and resting metabolic rate in a lizard. Physiological and Biochemical Zoology 86:458—469.

Artacho, P., J. Saravia, S. Perret, J. L. Bartheld, and J.-F. Le Galliard. 2017. Geographic variation and acclimation effects on thermoregulation behavior in the widespread lizard *Liolaemus pictus*. Journal of Thermal Biology 63:78—87.

Baird, and Girard. 2003. (Case 3140). *Sceloporus occidentalis* Baird & Girard, 1852 (Reptilia, Sauria): Rediscovered Syntypes Replaced By A Neotype. The Bulletin of Zoological Nomenclature 60:1.

Benabib, M., and J. D. Congdon. 1992. Metabolic and water-flux rates of free-ranging tropical lizards *Sceloporus variabilis*. Physiological Zoology 65:788—802.

Brown, J. H., J. F. Gillooly, A. P. Allen, M. van Savage, and G. B. West. 2004. Toward a metabolic theory of ecology. Ecology 85:1771—1789.

Brown, R. P., and S. Griffin. 2005. Lower selected body temperatures after food deprivation in the lizard *Anolis carolinensis*. Journal of Thermal Biology 30:79—83.

Buckley, L. B., J. C. Ehrenberger, and M. J. Angilletta. 2015. Thermoregulatory behaviour limits local adaptation of thermal niches and confers sensitivity to climate change. Functional Ecology 29:1038—1047.

Cadena, V., and G. J. Tattersall. 2009. The effect of thermal quality on the thermoregulatory behavior of the bearded dragon *Pogona vitticeps*: influences of methodological assessment. Physiological and Biochemical Zoology 82:203—217.

Caruso, N. M., M. W. Sears, D. C. Adams, and K. R. Lips. 2014. Widespread rapid reductions in body size of adult salamanders in response to climate change. Global Change Biology 20:1751—1759.

Catenazzi, A., J. Carrillo, and M. A. Donnelly. 2005. Seasonal and geographic eurythermy in a coastal Peruvian lizard. Copeia 2005:713—723.

Chown, S. L., and K. J. Gaston. 2016. Macrophysiology - progress and prospects. Functional Ecology 30:330—344.

Clarke, A., and K. P. P. Fraser. 2004. Why does metabolism scale with temperature? Functional Ecology 18:243—251.
Clarke, A., and K. J. Gaston. 2006. Climate, energy and diversity. Proceedings of the Royal Society B 273:2257—2266.
Clusella-Trullas, S., T. M. Blackburn, and S. L. Chown. 2011. Climatic predictors of temperature performance curve parameters in ectotherms imply complex responses to climate change. The American naturalist 177:738—51.
Clusella-Trullas, S., and S. L. Chown. 2014. Lizard thermal trait variation at multiple scales: a review. Journal of Comparative Physiology. B, Biochemical, Systemic, and Environmental Physiology 18:5—21.
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 83:261—296.
Crawford, D. L., V. A. Pierce, and J. A. Segal. 1999. Evolutionary physiology of closely related taxa: Analyses of enzyme expression. American Zoologist 39:389—400.
Du, W.-G., and X. Ji. 2006. Effects of constant and fluctuating temperatures on egg survival and hatchling traits in the northern grass lizard (*Takydromus septentrionalis*, Lacertidae). Journal of Experimental Zoology 305:47—54.
Du, W. G., and R. Shine. 2014. The behavioural and physiological strategies of bird and reptile embryos in response to unpredictable variation in nest temperature. Biological Reviews 90:19—30.
Du, W., Y. Lu, L. I. N. Shu, and Y. Bao. 2007. Thermal dependence of food assimilation and locomotor performance in juvenile blue-tailed skinks, *Eumeces elegans*. Animal Biology 57:29—38.
Dutton, R. H., and L. C. Fitzpatrick. 1975. Metabolic compensation to seasonal temperatures in the rusty lizard, *Sceloporus olivaceus*. Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology 5:309—318.
Garrick, L. D. 1979. Lizard thermoregulation: Operant responses for heat at different thermal intensities. Copeia 1979:258—266.
Gaston, K. J., S. L. Chown, P. Calosi, J. Bernardo, D. T. Bilton, A. Clarke, S. C. Trullas, C. K. Ghalambor, M. Konarzewski, S. Lloyd, W. P. Porter, H. O. Pörtner, E. L. Rezende, P. M. Schulte, I. John, J. H. Stillman, J. S. Terblanche, M. Van Kleunen, S. Clusella-trullas, L. S. Peck, H. O. Po, and J. I. Spicer. 2011. Macrophysiology: A Conceptual Reunification. The American Naturalist 174:595—612.
Gillooly, F. J., H. J. Brown, B. G. West, M. V. Savage, and L. E. Charnov. 2001. Effects of size and temperature on metabolic rate. Science 293:2248—2251.
Grant, B. W., and A. E. Dunham. 1990. Elevational covariation in environmental constraints and life histories of the desert lizard *Sceloporus merriami*. Ecology 71:1765—1776.
Grigaltchik, V. S., A. J. W. Ward, and F. Seebacher. 2012. Thermal acclimation of interactions: differential responses to temperature change alter predator−prey relationship. Proceedings of the Royal Society B 279:4058—4064.
Grigg, J. W., and L. B. Buckley. 2013. Conservatism of lizard thermal tolerances and body temperatures across evolutionary history and geography. Biology Letters 9.

Guizado-Rodriguez, A., U. O. García-Vazquez, and I. Solano-Zavaleta. 2011. Thermoregulation by a population of *Sceloporus palaciosi* from Sierra del Ajusco, Distrito Federal, Mexico. The Southwestern Naturalist 56:120—124.

Gvozdík, L., and A. M. Castilla. 2001. A comparative study of preferred body temperatures and critical thermal tolerance limits among populations of *Zootoca vivipara* (Squamata: Lacertidae) along an altitudinal gradient. Journal of Herpetology 35:486—492.

Hadamová, M., and L. Gvoždík. 2011. Seasonal acclimation of preferred body temperatures improves the opportunity for thermoregulation in newts. Physiological and Biochemical Zoology 84:166—174.

Hollingsworth, B. & Hammerson, G.A. 2007. *Sceloporus occidentalis*. The IUCN Red List of Threatened Species. Version 2014.3. <www.iucnredlist.org>. Downloaded on 08 February 2015.

Horváthová, T., C. R. Cooney, P. S. Fitze, T. A. Oksanen, D. Jelić, I. Ghira, T. Uller, and D. Jandzik. 2013. Length of activity season drives geographic variation in body size of a widely distributed lizard. Ecology and Evolution 3:2424—2442.

Huey, R. B. 1974. Behavioral thermoregulation in lizards: importance of associated costs. Science 184:1001—1003.

Huey, R. B. 1991. Physiological consequences of habitat selection. The American Naturalist 137:S91—S115.

Huey, R. B., and A. F. Bennett. 1987. Phylogenetic studies of coadaptation: preferred temperatures versus optimal performance temperatures of lizards. Evolution 41:1098—1115.

Huey, R. B., P. E. Hertz, and B. Sinervo. 2003. Behavioral drive versus behavioral inertia in evolution: a null model approach. The American Naturalist 161:357—366.

Huey, R. B., M. R. Kearney, A. Krockenberger, J. a M. Holtum, M. Jess, and S. E. Williams. 2012. Predicting organismal vulnerability to climate warming: roles of behaviour, physiology and adaptation. Philosophical transactions of the Royal Society of London. Series B, Biological Sciences 367:1665—79.

Huey, R. B., and J. G. Kingsolver. 1989. Evolution of thermal sensitivity of ectotherm performance. Trends in Ecology and Evolution 4:131—135.

Huey, R. B., and S. Montgomery. 1976. Cost and benefits of lizard thermoregulation. The Quarterly Review of Biology 51:363—384.

Huey, R., and J. Kingsolver. 1993. Evolution of resistance to high temperature in ectotherms. The American Naturalist 142:S21-S46.

James, R. S., J. Tallis, A. Herrel, and C. Bonneaud. 2012. Warmer is better: thermal sensitivity of both maximal and sustained power output in the iliotibialis muscle isolated from adult *Xenopus tropicalis*. Journal of Experimental Biology 215:552—558.

Kingsbury, B. A. 1994. Thermal constraints and eurythermy in the lizard *Elgaria multicarinata*. Herpetologica 50:266—273.

Kingsolver, J. G., and R. B. Huey. 2008. Size, temperature, and fitness: three rules. Evolutionary Ecology Research 10:251—268.

Kristín, P., and L. Gvoždík. 2012. Influence of respirometry methods on intraspecific variation in standard metabolic rates in newts. Comparative Biochemistry and Physiology - A Molecular and Integrative Physiology 163:147—151.

Kuchta, S. R., and A. N. M. Tan. 2005. Isolation by distance and post-glacial range expansion in the rough-skinned newt, *Taricha granulosa*. Molecular Ecology 14:225—244.
Leopold, E. B., P. W. Dunwiddie, C. Whitlock, R. Nickmann, and W. A. Watts. 2016. Postglacial vegetation history of Orcas Island, northwestern Washington. Quaternary Research (United States) 85:380—390.
Levy, O., J. Borchert, T. Rusch, and L. Buckley. 2017. Diminishing returns limit energetic costs of climate change. Ecology 98:1217—1228.
Lighton, J. R. B., and L. G. Halsey. 2011. Flow-through respirometry applied to chamber systems: pros and cons, hints and tips. Comparative Biochemistry and Physiology, Part A 158:265—275.
Lighton, J.R.B. 2008. Measuring metabolic rates: a manual for scientists. Oxford University Press, United States.
Logan, M. L., C. E. Montgomery, S. M. Boback, R. N. Reed, and J. a. Campbell. 2012. Divergence in morphology, but not habitat use, despite low genetic differentiation among insular populations of the lizard *Anolis lemurinus* in Honduras. Journal of Tropical Ecology 28:215—222.
López-Alcaide, S., M. Nakamura, R. Macip-Ríos, and E. Martínez-Meyer. 2014. Does behavioural thermoregulation help pregnant *Sceloporus adleri* lizards in dealing with fast environmental temperature rise? Herpetological Journal 24:41—47.
Lorenzon, P., J. Clobert, and M. Massot. 2001. The contribution of phenotypic plasticity to adaptation in *Lacerta vivipara*. Evolution 55:392—404.
Luo, L.-G., G.-H. Ding, and X. Ji. 2010. Income breeding and temperature-induced plasticity in reproductive traits in lizards. The Journal of experimental biology 213:2073—8.
Martin, T. L., and R. B. Huey. 2008. Why "suboptimal" is optimal: Jensen's inequality and ectotherm thermal preferences. The American Naturalist 171:E102—E118.
McGinnis, S. M. 1970. Flexibility of thermoregulatory behavior in the western fence lizard *Sceloporus occidentalis*. Herpetologica 26:70—76.
McGinnis, S. M., and M. Falkenstein. 1971. Thermoregulatory behavior in three sympatric species of iguanid lizards. Copeia 1971:552—554.
Miles, D. B. 1994. Population differentiation in locomotor performance and the potential response of a terrestrial organism to global environmental change. American Zoologist 34:422—436.
Mood, B. J., and D. J. Smith. 2015. Holocene glacier activity in the British Columbia Coast Mountains, Canada. Quaternary Science Reviews 128:14—36.
Muñoz, M. M., M. A. Stimola, A. C. Algar, A. Conover, A. J. Rodriguez, M. A. Landestoy, G. S. Bakken, and J. B. Losos. 2013. Evolutionary stasis and lability in thermal physiology in a group of tropical lizards. Proceedings of the Royal Society B 281:DOI: 10.1098/rspb.2013.2433.
Ohlberger, J. 2013. Climate warming and ectotherm body size — from individual physiology to community ecology. Functional Ecology 27:991—1001.
Ortega, Z., and V. Pérez-Mellado. 2016. Seasonal patterns of thermoregulation & microhabitat selection in a lizard. Acta Oecologica 77:201—206.
Pafilis, P., S. Meiri, D. Karakasi, E. Kourelou, and E. D. Valakos. 2016. Body size affects digestive performance in a Mediterranean lizard. Herpetological Journal 26:199—205.

Perry, G., M. C. Wallace, D. Perry, H. Curzer, and P. Muhlberger. 2011. Toe clipping of amphibians and reptiles: science, ethics, and the law. Journal of Herpetology 45:547—555.

Porter, S. C., and T. W. Swanson. 1998. Radiocarbon Age Constraints on Rates of Advance and Retreat of the Puget Lobe of the Cordilleran Ice Sheet during the Last Glaciation. Quaternary Research 50:205—213.

Porter, W., and D. Gates. 1969. Thermodynamic equilibria of animals with environment. Ecological Monographs 39:227—244.

Powers, S.D. 2010. How does spatial variation in climate cause spatiotemporal patterns in lizard energetics? Thesis, Western Washington University, Bellingham, Washington, USA.

Quintero, I., and J. J. Wiens. 2013. Rates of projected climate change dramatically exceed past rates of climatic niche evolution among vertebrate species. Ecology Letters 16:1095—1103.

Roe, J. H., W. A. Hopkins, and L. G. Talent. 2005. Effects of body mass, feeding, and circadian cycles on metabolism in the lizard *Sceloporus occidentalis*. Journal of Herpetology 39:595—603.

Sagonas, K., E. D. Valakos, and P. Pafilis. 2013. The impact of insularity on the thermoregulation of a Mediterranean lizard. Journal of Thermal Biology 38:480—486.

Scheers, H., and R. Van Damme. 2002. Micro-scale differences in thermal habitat quality and a possible case of evolutionary flexibility in the thermal physiology of lacertid lizards. Oecologia 132:323—331.

Schwarzkopf, L. 1998. Evidence of geographic variation in lethal temperature but not activity temperature of a lizard. Journal of Herpetology 32:102—106.

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. 2004. Body size clines in *Sceloporus* lizards: proximate mechanisms and demographic constraints. Integrative and Comparative Biology 44:433—442.

Sears, M. W., and M. J. Angilletta. 2015. Costs and benefits of thermoregulation revisited: both the heterogeneity and spatial structure of temperature drive energetic costs. The American Naturalist 185:E94—E102.

Sears, M. W., M. J. Angilletta Jr., M. S. Schuler, J. Borchert, K. F. Dilliplane, M. Stegman, T. W. Rusch, and W. A. Mitchell. 2016. Configuration of the thermal landscape determines thermoregulatory performance of ectotherms. Proceedings of the National Academy of Sciences of the United States of America 113:10595—10600.

Seebacher, F. 2005. A review of thermoregulation and physiological performance in reptiles: what is the role of phenotypic flexibility? Journal of Comparative Physiology B 175:453—461.

Seebacher, F., and C. E. Franklin. 2005. Physiological mechanisms of thermoregulation in reptiles: a review. Journal of Comparative Physiology B 175:533—541.

Seebacher, F., C. R. White, and C. E. Franklin. 2015. Physiological plasticity increases resilience of ectothermic animals to climate change. Nature 5:61—66.

Seebacher, F., and R. S. Wilson. 2006. Fighting fit: thermal plasticity of metabolic function and fighting success in the crayfish Cherax destructor. Functional Ecology 20:1045—1053.

Sinervo, B., F. Méndez-de-la-Cruz, D. B. Miles, B. Heulin, E. Bastiaans, M. Villagrán-Santa Cruz, R. Lara-Resendiz, N. Martínez-Méndez, M. L. Calderón-Espinosa, R. N. Meza-Lázaro, H. Gadsden, L. J. Avila, M. Morando, I. J. De la Riva, P. Victoriano Sepulveda, C. F. D. Rocha, N. Ibargüengoytía, C. Aguilar Puntriano, M. Massot, V. Lepetz, T. a Oksanen, D. G. Chapple, A. M. Bauer, W. R. Branch, J. Clobert, and J. W. Sites. 2010. Erosion of lizard diversity by climate change and altered thermal niches. Science (New York, N.Y.) 328:894—899.

Somero, G. N. 2010. The physiology of climate change: how potentials for acclimatization and genetic adaptation will determine “winners” and “losers”. The Journal of Experimental Biology 213:912—920.

Stevens, G. C. 1989. The latitudinal gradient in geographic range: how so many species coexist in the tropics. The American Naturalist 130:526—543.

Sunday, J. M., A. E. Bates, M. R. Kearney, R. K. Colwell, N. K. Dulvy, J. T. Longino, and R. B. Huey. 2014. Thermal-safety margins and the necessity of thermoregulatory behavior across latitude and elevation. Proceedings of the National Academy of Sciences of the United States of America 111:5610—5615.

Sutherland, W. J., R. P. Freckleton, H. C. J. Godfray, S. R. Beissinger, T. Benton, D. D. Cameron, Y. Carmel, D. a. Coomes, T. Coulson, M. C. Emmerson, R. S. Hails, G. C. Hays, D. J. Hodgson, M. J. Hutchings, D. Johnson, J. P. G. Jones, M. J. Keeling, H. Kokko, W. E. Kunin, X. Lambin, O. T. Lewis, Y. Malhi, N. Mieszkowska, E. J. Milner-Gulland, K. Norris, A. B. Phillimore, D. W. Purves, J. M. Reid, D. C. Reuman, K. Thompson, J. M. J. Travis, L. a. Turnbull, D. a. Wardle, and T. Wiegand. 2013. Identification of 100 fundamental ecological questions. Journal of Ecology 101:58—67.

Tsuji, J. S. 1988. Seasonal profiles of standard metabolic rate of lizards (*Sceloporus occidentalis*) in relation to latitude. Physiological Zoology 61:230—240.

Zamora-Camacho, F. J., S. Reguera, and G. Moreno-Rueda. 2016. Thermoregulation in the lizard *Psammodromus algirus* along a 2200-m elevational gradient in Sierra Nevada (Spain). International Journal of Biometeorology 60:687—697.

APPENDIX A — PHOTOS AND SUPPLEMENTAL FIGURES

Photos 1-4. Pictured about are photos (starting from the top left) of a 1) male *Sceloporus occidentalis* captured in the field, 2) the ventral coloration of a male *Sceloporus occidentalis*, 3) a *Sceloporus occidentalis* housed in the laboratory at WWU, and 4) *Sceloporus occidentalis* lizard perched on a substrate with its back to the sun (basking).

Photo 5. Rock beaches, driftwood, and cliffs characterize the Puget Sound habitat. *Sceloporus occidentalis* typically inhabits areas where driftwood is located next to a sandy, open patch of south-facing cliff.

Photo 6. The Columbia River Gorge habitat is characterized by the transition from pine-oak woodlands to shrub-steppe. *Sceloporus occidentalis* inhabit open areas with enough fallen logs to provide cover.

Photo 7. The North Cascades habitat is characterized by the transition from pine-to-fir forests, steep southern-facing slopes, and abundant boulders/rocks. *Sceloporus occidentalis* typically inhabit open areas with amble boulders or logs to provide cover.

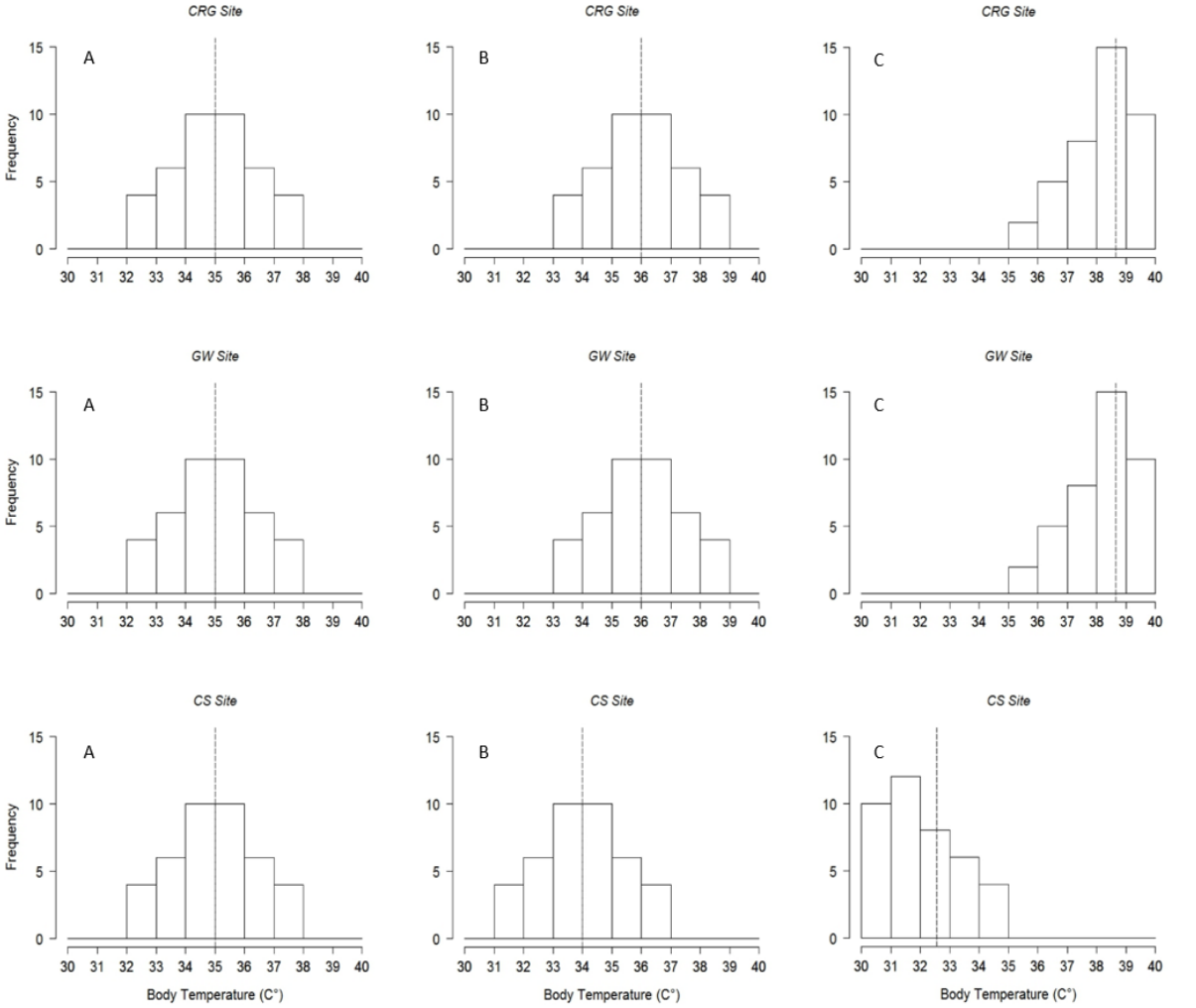
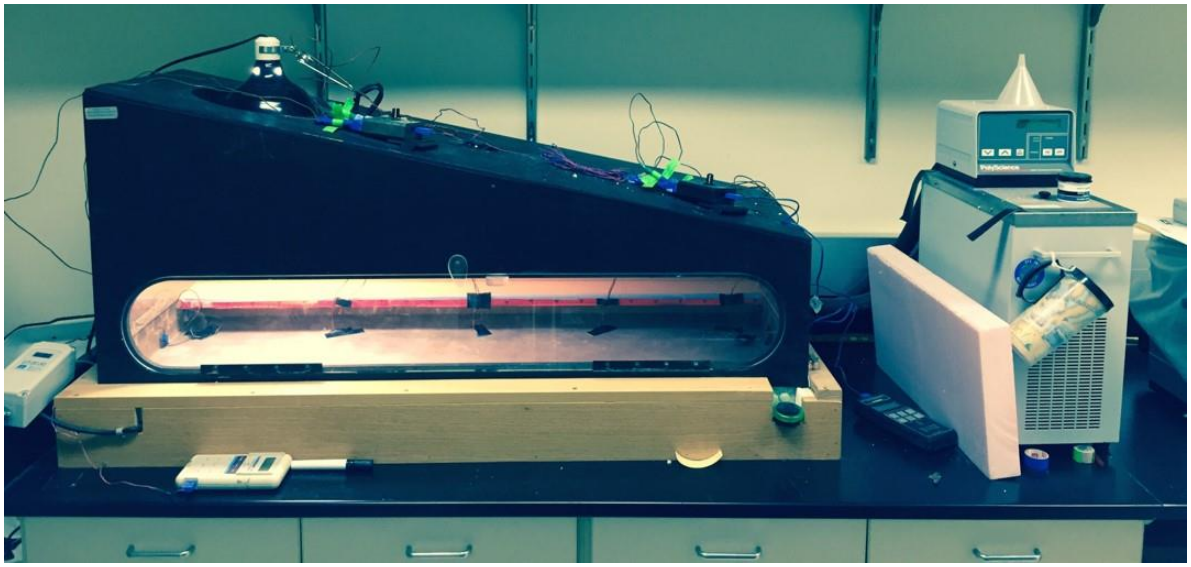


Figure 10. Graphical representations of hypotheses about thermoregulation behavior of *Sceloporus occidentalis*. Graphs in A represent the H_{T0} (*i.e.* populations at all sites can effectively thermoregulate to the known, narrow range of field-active body temperature of *S. occidentalis*), Graphs in B represent H_{T1} (*i.e.* challenging conditions force lizards to thermoregulate to the edges of the acceptable field-active body temperature range of *S. occidentalis*). Graphs in C represent H_{T2} (*i.e.* conditions at each site prohibit lizards from being able to maintain body temperatures within the field-active body temperature range of *S. occidentalis*). Slash lines indicate the mean T_b value.

Photo 8. The terraria in which lizards were house while in lab. Each terrarium contained a small refugium, water bowl, and heating rock, and were heated by a heating lamp suspended above the terrarium.



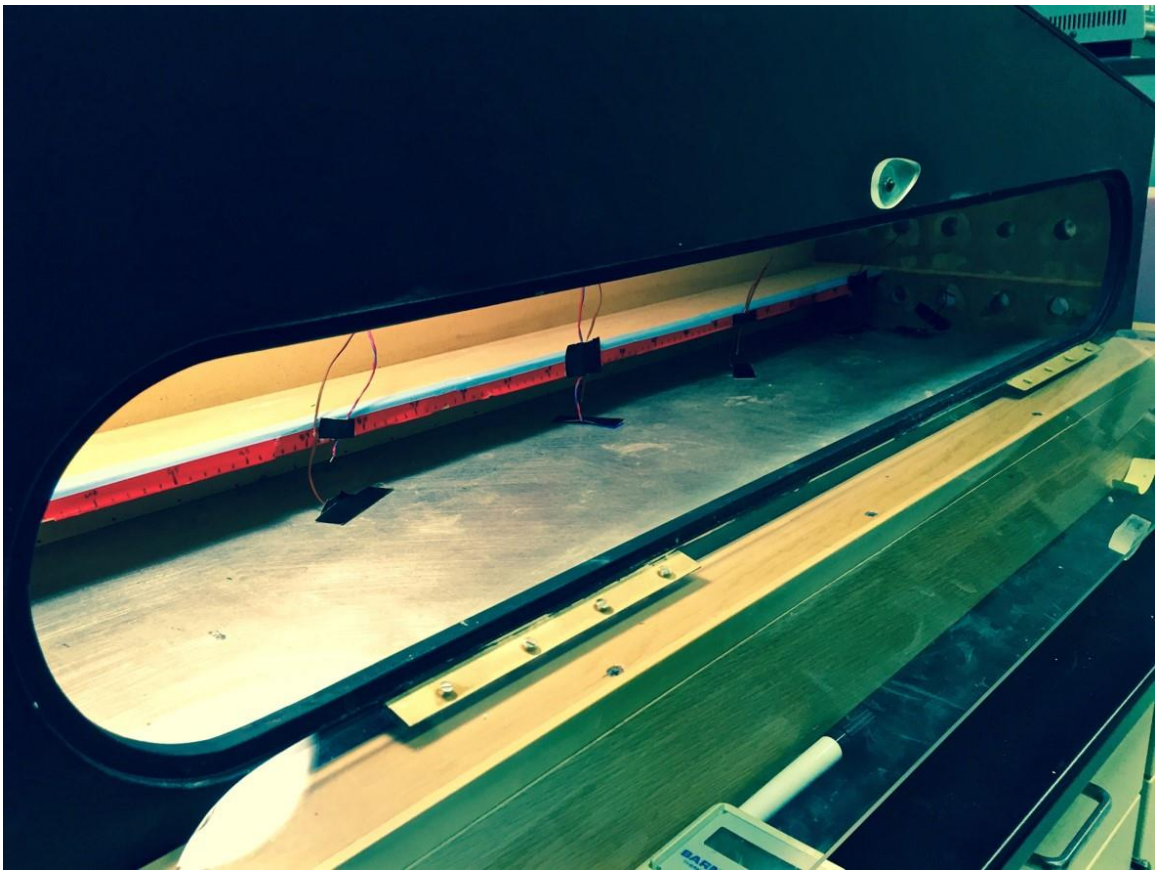


Photo 9-10. Pictured are (from top to bottom) 1) the thermal gradient (center), including the ethylene glycol cooling pump (right) and heating coil device (left), and 2) the interior of the thermal gradient.

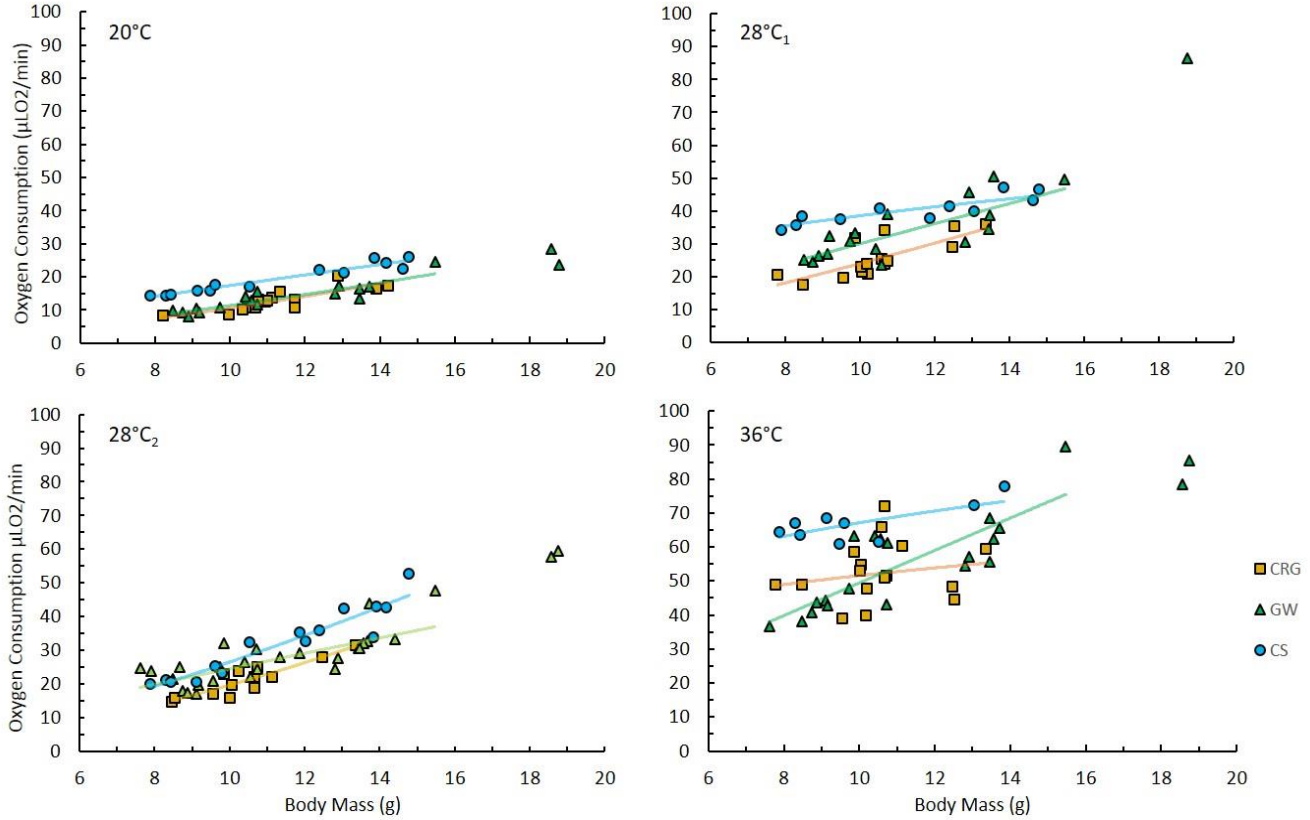


Figure 11. RMR (metabolism of fed, resting lizards, measured as microliters of oxygen per minute) of *Sceloporus occidentalis* from all three sites, plotted with respect to body mass (grams) for each of three body temperatures (20°C 28°C and 36°C). The SMR (metabolism of post-absorptive, resting lizards) was also measured at 28ºC (28°C_2). Power trendlines were used to show the relationship between body mass and whole-animal rate of oxygen consumption for lizards at each site. Y-axes were standardized for easier comparison of rates of oxygen consumption among body temperatures.

APPENDIX B — EQUIPMENT PHOTOS AND SPECIFICATIONS

Thermocron iButtons, Model DS1921G (Maxim Integrated)

Power Requirements:

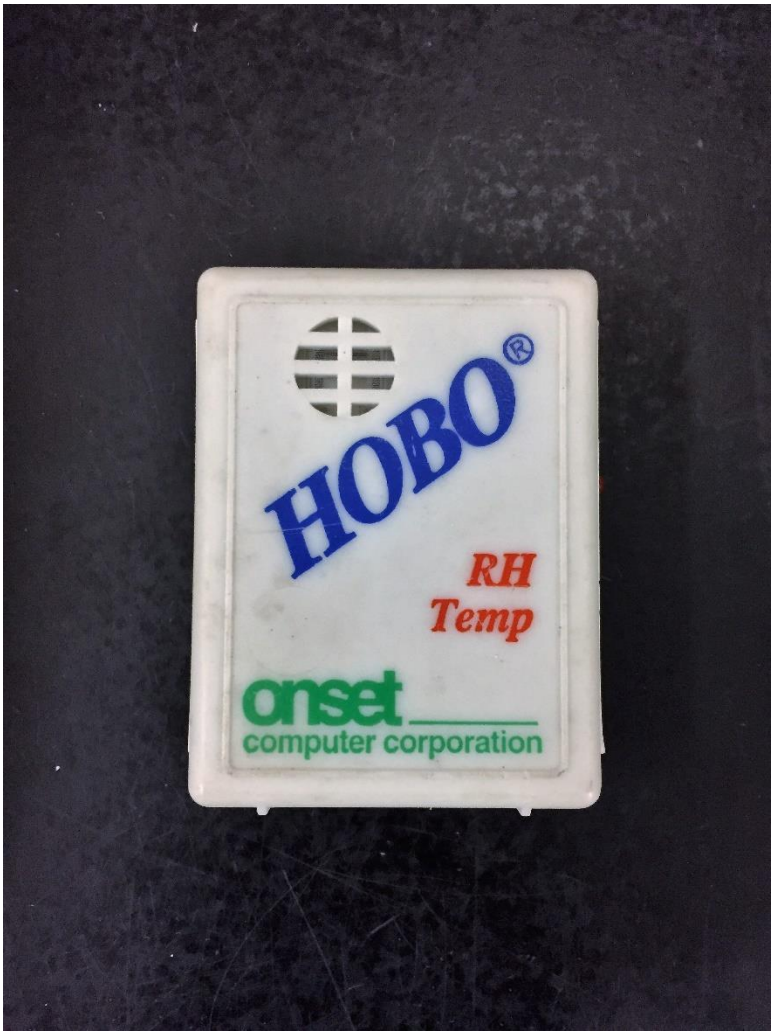
- Internal battery provided. Battery life, depending on usage rate, is approximately 8-10 years.

Accuracy and Resolution:

- Accurate to within 1°C when measuring from -30 to 70°C; resolution of 0.5°C.

Measurement Range:

- Time Interval of Measurements: 1-255 minutes.
- Operative temperature range: -40 to 85°C.



HOBO Temperature and Relative Humidity Logger, H8 Family (Onset Computer Corporation)

Dimensions (W x D x H) and Weight:

- 4.8 x 2 x 6 cm (1.9 x 0.8 x 2.4 in.); approximately 28 g (1 oz.).

Power Requirements:

- 1 CR-2032 (lithium) user-replaceable battery

Accuracy and Resolution:

- Temperature: Accuracy better than within 2°C; resolution of 1°C.
- Relative Humidity (RH): Accuracy better than within 5% RH; resolution of 1% RH.

Measurement Range:

- Temperature: -20°C to 70°C.
- RH: 25 to 95% RH, over the operating temperature range of 5°C to 50°C.

FoxBox Field Gas Analysis System (Sable Systems International, Inc.)

Dimensions (W x D x H) and Weight:

- 28 x 25 x 18 cm (11 x 10 x 7 in.); 15 kg (7 lbs.)

Power Requirements:

- 12-24 volts of direct current.

Accuracy and Resolution:

- Oxygen: Accuracy better than 0.2% of full scale (1 — 100%); resolution 0.001%.
- Carbon Dioxide: Accuracy better than 1% of calibrated span; resolution to 1 ppm/0.0001%.
- Barometric Pressure: Accuracy better than 0.1% of full scale; resolution 0.001 kPa 0 -10%.

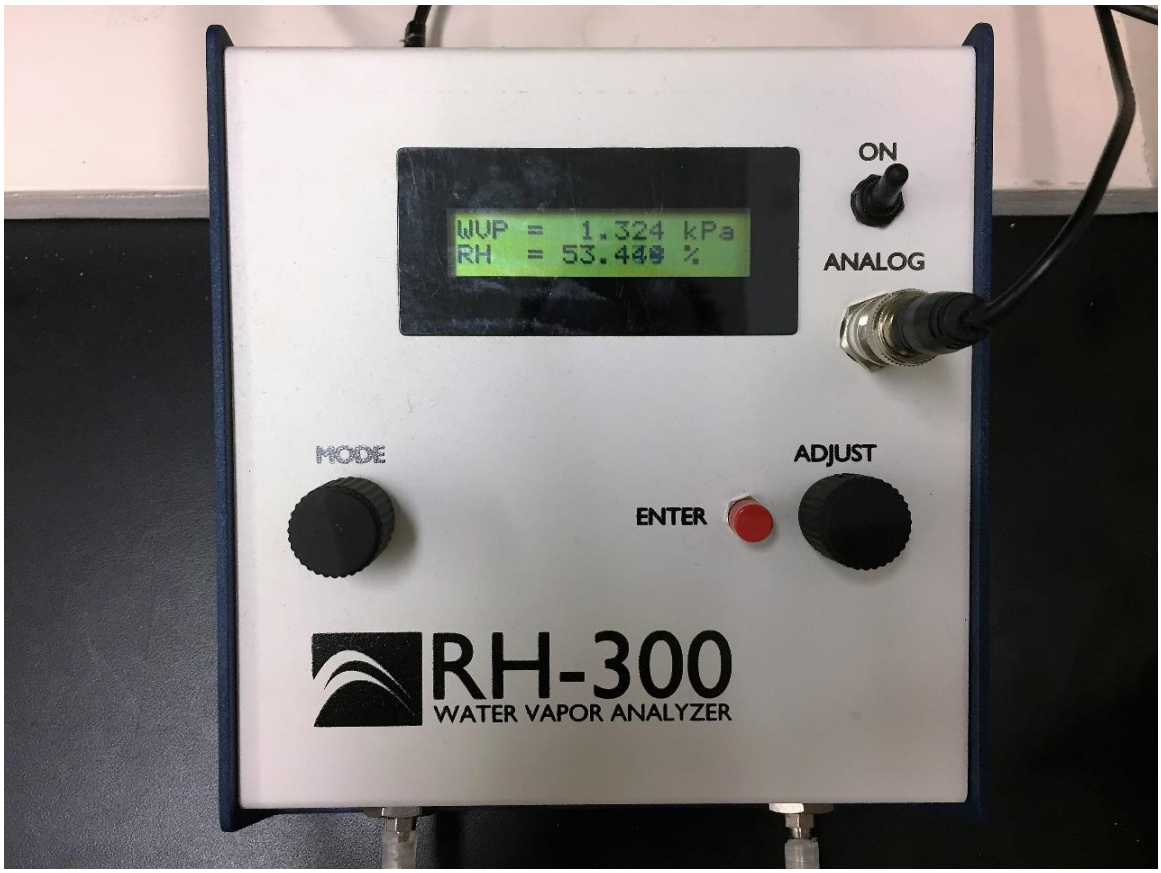
Measurement range:

- Oxygen: 0 — 100% of air sample, with best accuracy occurring above 1%.
- Carbon Dioxide: 0-5% of air sample.
- Barometric Pressure: 30-110 kilopascals.

Flow Range:

- 20 — 1,500 milliliters per minute. Measured and controlled by linearized mass flow meter, with accuracy within 2% of the reading.

**Note: The data readouts on the equipment in this appendix are examples of the user interface of the devices and are not related to my research.*



RH-300 Water Vapor Analyzer (Sable Systems International, Inc.)

Dimensions (W x D x H) and Weight:

- 15.2 x 15.2 x 10.2 cm (6 x 6 x 4 in.); 1.4 kg (3 lbs.)

Power Requirements:

- 12-24 volts of direct current.

Accuracy and Resolution:

- Relative Humidity (RH): Accuracy better than 1% RH from 0 — 95%, and better than 2% from 0 to 100% RH; Resolution 0.001 RH%.
- Water Vapor Density (WVD): Resolution up to 0.0001 $\mu g\ ml^{-1}$.
- Water Vapor Pressure (WVP): Resolution of 0.01 Pa (to 1000 Pa); 1 Pa from 1000 — 20,000 Pa.

Measurement range:

- RH: 0-100%
- WVD: 0-10 $\mu g\ ml^{-1}$
- WVP: 0 to 20,000 Pa

*Photo showing the FoxBox and RH-300 connected via an analog cable. A temperature probe (measuring air temperature in the Percival temperature cabinet) is connected to the FoxBox via another analog cable. The FoxBox then sends RH, $\%CO_2$, $\%O_2$, barometric pressure, and temperature data to the lab computer via a serial cable. The serial data is read on the lab computer using a serial daemon program before being recorded by Expedata software (Sable Systems International, Inc.).

Biological Incubator, I-35 Series (Percival Scientific, Inc.)

Exterior Dimensions (W x D x H):

- 87.6 x 80 x 198.1 cm (34.5 x 31.5 x 78 in.)

Power Requirements:

- 115/1/60 volts.

Interior Space

- 0.71 m^3 (25 ft^3)

Temperature Range and Accuracy:

- -18 — 60°C; Accuracy within 0.5°C.

Sample Chamber (Scientific Technical Services, WWU)

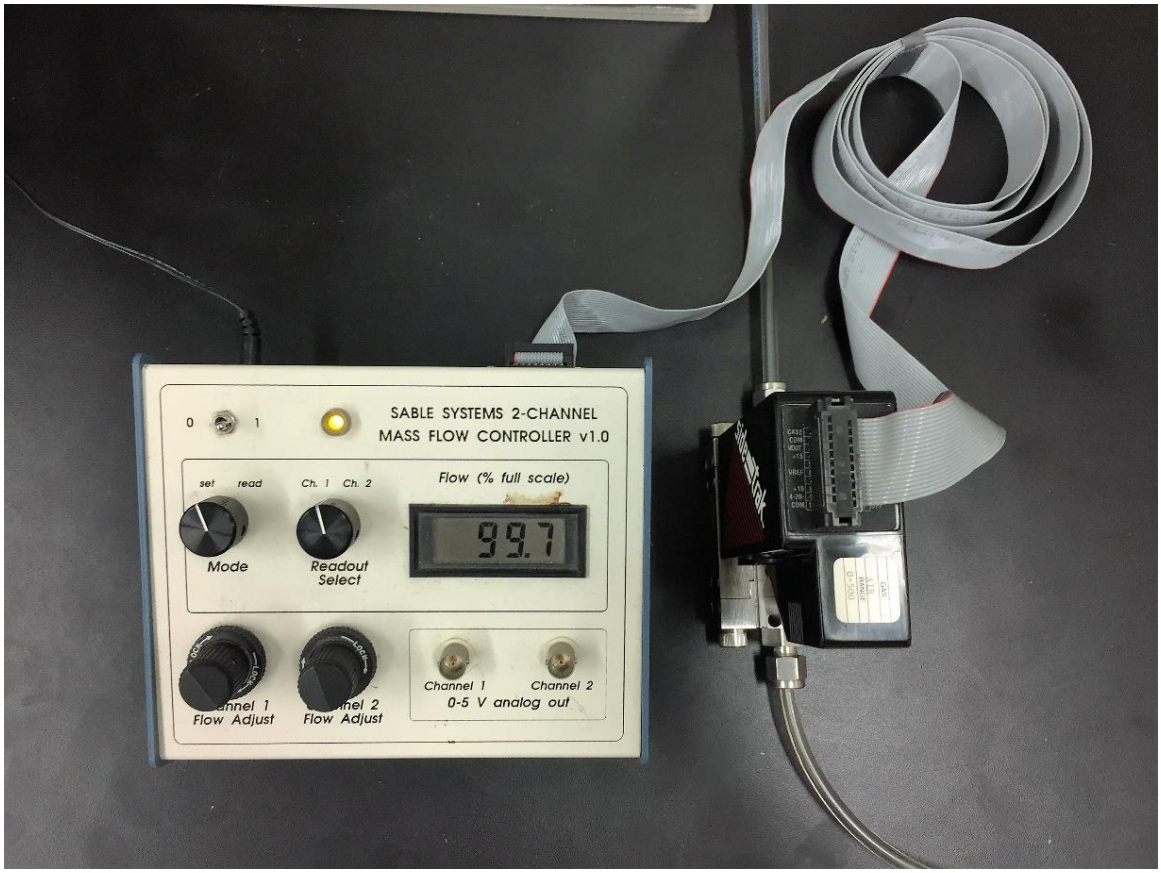
Dimensions (Length by Diameter):

- 16 x 4.5 cm

Volume:

- 180 ml without rubber stoppers inserted; 170 ml with rubber stoppers inserted.

*Photo showing the sample chambers in the Percival cabinet while connected to the respiration system. The wooden rack holding the chambers was designed and constructed by Scientific Technical Services at Western Washington University.



Mass Flow Meter/Controller Electronics (2-Channel) v1.0 (Sable Systems International, Inc.)

Power Requirements:

- 11-15.5 volts of direct current.

Accuracy and Resolution:

- Within 1% of the requested mass flow unit output.

Side-Trak Mass Flow Meter, 840 Series (Sierra Instruments, Inc.)

Weight:

- 2.00 lbs. (.91 kg)

Power Requirements:

- 15 volts of direct current.

Accuracy:

- Within 1% of the full scale including linearity over 15-25°C and 10-60 psia.

Flow Range:

- 0-10 standard cubic centimeters per minute, to 0-15 standard liters per minute.

Air Pump (Get the Model) (Gast Manufacturing, Inc.)

Dimensions (W x D x H) and Weight:

- 13.6 x 19.9 x 19.5 cm (5.35 x 7.88 x 7.6 in.); 14.5 lbs. (6.58 kg).

Power Requirements:

- 115 volts of AC

Max Flow:

- 45.3 dm^3/min (1.6 ft^3/min)

APPENDIX C — FOXBOX/EXPEDATA USER GUIDE

NOTE: ExpeData should be setup after the respirometry system is already turned on and fully operational, as I've written the follow instructions using the assumption that the system will already be communicating with the lab computer. For instructions regarding the setup of the FoxBox and RH300, please refer to the set-up guide provided by Sable Systems International.

DATA COLLECTION:

1. First, you must open the "Serial Daemon" program. Click on the Windows icon at the bottom left of the screen, and type "Serial Daemon" into the search bar to find the program.

2. Once the program is opened, you'll notice a dropdown menu that initially reads "9600N981". Click on that menu, and select "115200N81" from the drop-down options. Then click "Monitor Instrument". If everything is working properly, a string of data will populate the text bar. You will leave the Serial Daemon program running until you are done with data collection.

3. Now open the desktop icon for ExpeData v.1.9.8 (the user agreement may pop up; just click the box for "I've read and understand…" and then "accept").

4. Select "Acquire" from the toolbar at the top of the window, and then select "Setup Data Acquisition…" from the dropdown menu.

5. When the window entitled "Connect to Data Acquisition System(s)" appears, just click "OK".

6. When the "Acquisition Parameters" window appears, click "Setup…", and then select "Load setup…" from the dropdown menu.

7. From the "EXPEDATA Load Setup" window, select the "FoxBox Setup File.stp" and then click "Open". Upon hitting "Open", a window should appear that reads "The setup file matches the acquisition system(s) currently active. Setup file accepted & OK for use". Click "OK".

8. To ensure that the system is working correctly, click "Monitor" on the "Acquisitions Parameters" window. A smaller window entitled "Channel Monitor" should appear with six channels showing values. The values are:

    - BP – Barometric Pressure
    - O2 – Percent Oxygen
    - FR – Flow Rate
    - CO2 – Percent Carbon Dioxide
    - WVP – Water Vapor Pressure
    - Temp – Temperature

9. Confirm that all six channels are reading correctly by comparing the values in the "Channel Monitor" window with those being shown on the interfaces of the FoxBox, RH-300, and the Percival cabinet. Some minor differences between these values can be expected, as ExpeData is reading the information from the respirometry

system on a one-second delay; however, the values should still be very similar. Once it has been confirmed that the values in ExpeData are correct, click "OK". Then select the green check mark on the "Acquisition Parameters" window.

10. Once the "Recording: ExpeData" window is open, take a moment to find both the red arrow icon, the pause symbol icon, the disk icon, and the notepad icon (the notepad icon should be greyed out). The red arrow begins your data recording, the pause icon pauses it (of course), the disk icon saves your recording, and the notepad icon allows for you to place markers in your data set with labels that you write into the text box (for reference, the text box should read [MARKER NOTE TEXT] when you first see it).

11. Click the red arrow to start recording data (Reminder: you want to record a baseline value before you record any data involving an organism). Once you begin recording data, use the notepad icon to place a marker that you will label as "Baseline". When you are done recording your baseline value (about two minutes is enough time), click the pause button.

    NOTE: Do not save or exit the data collection window yet, as you will be recording both the organism's respiration data and a follow-up baseline measurement onto that same data file.

12. Next you will connect the tubing for the organism you wish to measure onto the respirometry pathway. Once done, repeat the previous steps used to record the baseline for recording the organism's respiration rate. This time, however, label your maker as "Organism 1", or whatever identifying label you've assigned to that individual.

13. Once you've finished recording the organism's respiration rate (usually 10 minutes is enough time), you will want to again record a baseline measurement.

14. When finished recording data, save the file using the disk icon. The first screen to appear is a note pad that allows for you to write information about the data file. Once you've written (or not written) your notes, click the green check button. Then, save the file to the desktop using a descriptive format, such as:

    "OrganismID_BodyMass_Date.exp"

DATA ANALYSIS:

1. Open desktop icon for ExpeData v.1.9.8 (you will not need Serial Daemon, so do not open it).

2. Click "File" and then select "Analyze Data Now!" from the dropdown menu.

3. In the "Automated Data Analysis" window, select "Choose File(s)". Select whichever data file you wish to analyze and then click "Open". Once you have selected a file, click "Run Macro" on the "Automated Data Analysis" window.

4. Left-click on the beginning of the graph and drag the mouse across the entire graph. This will result in a smaller window opening. In that smaller window, you will see a drop-down menu that

is displaying “BP”. Click on the menu and select “O2”. Doing so should change the figure from displaying BP to displaying O2. Now everything done henceforth is going to specifically be done to the percent oxygen data. Exit the smaller window.

5. Click “Transform” on the window displaying the graphed data. From the drop-down menu, select “General…” and enter this exact equation into the text box:

   O2*BP/(BP-WVP)

   This equation corrects for water vapor in the air sample that dilutes the oxygen value. Once entered, click “OK”.

6. Again select “Transform” from the task bar, and then select “Corrections”. From the dropdown list, select “Drift Correction…”. For the “Correct from” menu select “Start & End”, and for the “Scan for” menu select “Most level”. Also, click the box that reads “Span to this value: .2095” (make sure the value in the dropdown menu there is in fact .2095 before doing this). With your parameters set for your drift correction, click “Try It!” to apply the drift correction. Once the drift correction is complete, click “Done”.

7. Now the data is prepared for analysis. Select the data for the first baseline by left-clicking at the beginning of the recording and dragging to the end of the baseline measurement. The smaller pop-up window should only display the data you selected. Click “Mean”. Under “SELECTION A RESULTS” in the resulting pop-up window, find the calculated mean value. Record the mean into an Excel file.

8. Repeat this process to get the mean value of the second baseline measure and the organism respiration measure. Once you have these values, you can calculate the respiration rate of the organism (in $mLO_2\ min^{-1}$) using the following equation:

   Where “$BL1$” is the mean of the percent oxygen of the first baseline, “$BL2$” is the mean of the percent oxygen of the second baseline, “Org” is the mean of the percent oxygen of the organism’s oxygen consumption measurement, and “FR” is the flow rate ($mL\ min^{-1}$).

9. To get a mass-specific oxygen consumption value, divide the above calculated respiration rate value (which is in $mLO_2\ min^{-1}$) by the mass of the organism. The resulting value will be in units of ($mLO_2\ min^{-1}$ unit $mass^{-1}$).