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Thermal selection in *Sceloporus occidentalis* during exercise recovery

A Thesis Presented

by

Morgan Ann Halley

To the Keck Science Department

Of Claremont McKenna, Pitzer, and Scripps Colleges

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Senior Thesis in Biology

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Abstract

Ectotherms regulate body temperature (T_b) primarily through behavioral interactions with their environment. Consequently, ambient temperature (T_a) significantly affects both their activity levels and metabolic processes. These animals have limited aerobic scopes and must rely on anaerobic metabolism to support intense activity; lactate production and glycogen depletion are two consequences of anaerobic metabolism that must be dealt with during exercise recovery. It has been suggested that, in many ectothermic species, T_b may affect the rate of lactate clearance and glycogen restoration during recovery from intense exercise. I investigated thermoregulatory behavior in Western Fence Lizards (*Sceloporus occidentalis*) to determine preferred T_b during exercise recovery. I hypothesized that animals would select T_a 's that result in higher T_b 's during the recovery period on the basis that biochemical processes occur at faster rates at high temperatures, which may facilitate a more rapid reduction of lactate and replenishment of glycogen stores. However, I found that control animals maintained a constant T_b of 33°C, while exercised animals cooled T_b to 30°C 20 minutes after exercising, and eventually warmed to 32°C by the 2 hour mark. Animals were found to be able to cool T_b by a maximum of 0.175°C/min given the available temperatures, which allowed for rapid cooling of 3-4° in less than 20 minutes in exercised animals. My results suggest a benefit of lowered T_b to facilitate exercise recovery in these animals. Behavioral hypothermia in *S. occidentalis* may be caused by physiological triggers associated with intense activity such as hypoxia, hypercapnia, or elevated blood lactate concentration. However, these speculations must be confirmed by further research.

Introduction

Animals of the *Lacertilia* suborder of reptiles, known as lizards, are ectotherms; they are organisms whose body temperatures (T_b) are determined largely by their environmental temperature. As with endotherms, who internally regulate T_b using the heat byproduct of their metabolic processes, ectotherms must sustain exercise by generating adenosine-5'-triphosphate (ATP; Randall *et al.* 2002). To do this, glucose that is stored in the animal's muscle and liver cells as glycogen is broken down to pyruvate during glycolysis. This process converts NAD^+ to $NADH$. However, there is a finite amount of NAD^+ in the cell. Therefore, to regenerate NAD^+ , $NADH$ must be oxidized; this is normally done during oxidative phosphorylation during aerobic metabolism, which endotherms generally rely more heavily on than ectotherms during activity (Randall *et al.* 2002).

Intense activity in ectotherms is primarily supported anaerobically. This is due to the limits of their aerobic scope. Resting metabolic rates of amphibians and reptiles are 10-20% of those of comparably sized birds and mammals (Bennett and Dawson 1976; Feder 1976; Figure 1). Sustained locomotor activity causes this rate to increase by a factor of their basal metabolic rate (BMR) for both ectotherms and endotherms. For endotherms, the absolute magnitude of this difference is enormous due to their high initial RMR. Birds have been found to increase BMR by factors up to 17.5, 12, and 8 for flying, running, and swimming, respectively, but generally most values fall between 5 and 10 (Brackenbury 1984, Gessaman and Nagy 1988, Kooyman and Ponganis 1994). For passerine birds, the magnitude of this difference in oxygen consumption is around $4.8 \text{ ml O}_2 \cdot (\text{g}\cdot\text{h})^{-1}$ BMR to about $15.6 \text{ ml O}_2 \cdot (\text{g}\cdot\text{h})^{-1}$ at summit metabolism (Dutenhoffer and Swanson 1996). The aerobic scope of ectotherms scales by a similar factor range; most lizard species are able to increase aerobic

scope by factors between 6 and 7.5 (Gleeson 1981). However, the absolute magnitude of this difference is much smaller. Gleeson (1981) found that the monitor lizard *Varanus salvator* increases oxygen consumption rates from $0.144 \text{ ml O}_2 \cdot (\text{g-h})^{-1}$ to $0.84 \text{ ml O}_2 \cdot (\text{g-h})^{-1}$. The physiology of this metabolic difference between endotherms and ectotherms is complex but due to several main factors. Blood oxygen capacity in ectotherms, for instance, is 25-50% lower than that of endotherms. Reduced oxygen capacity in the blood of these animals is due to less capillary surface area (Dessauer 1970, Pough 1969), as well as a greater distance from cell to capillary (Altman and Dittmer 1971), both of which hamper the diffusion of gases. Despite these limitations, ectotherms are able to support most of their daily activity aerobically. In *Sceloporus jarrovi*, for example, it was found that lactate concentration in free ranging lizards did not increase during routine activity (Pough and Andrews 1985). However, pushing animals to sustain activity beyond their aerobic scope forces them to obtain energy via anaerobic metabolism; this occurs at a much lower activity level for ectotherms than for endotherms (Gatten *et al.* 1992).

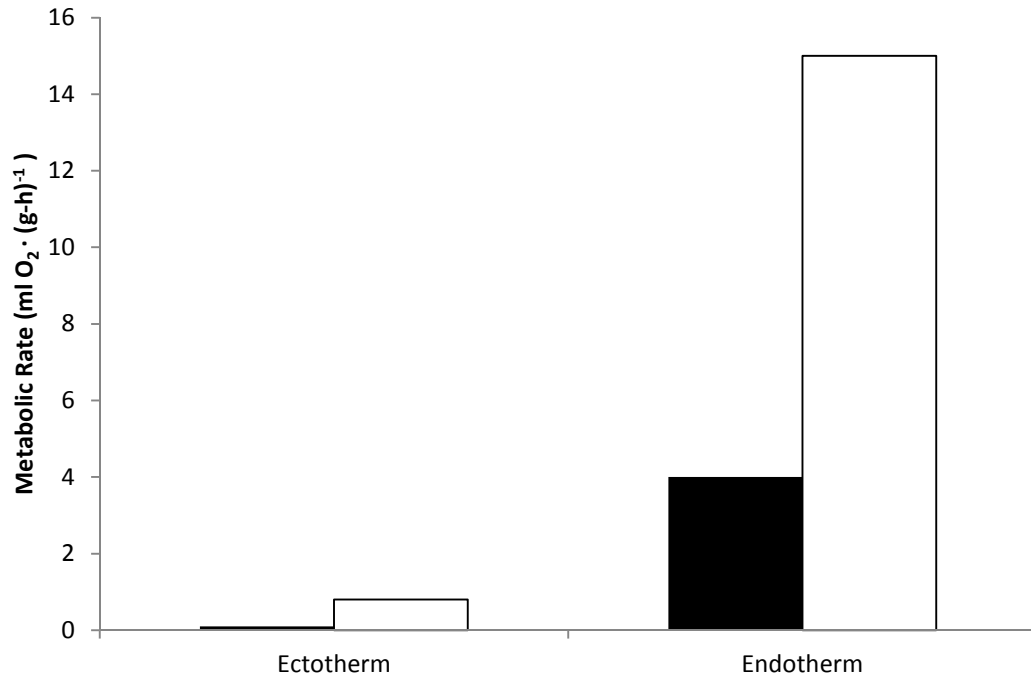


Figure 1. Aerobic scope difference between endotherms and ectotherms (■ = BMR, □ = summit metabolism).

Reptiles have demonstrated tremendous anaerobic capacity and have evolved methods to compensate for low blood oxygen capacity; many are able to deliver most, if not all, bound oxygen to tissues. Oxygen is released from hemoglobin at a higher partial pressure in lizard blood than it is in the blood of birds and mammals of similar mass (Pough 1977a, 1977b). Levels of oxygen in the venous blood of some reptiles may even fall to as low as 1ml/100ml blood or less (White 1959, Tucker 1966), whereas mammals are much less tolerant of low venous oxygen tensions; O₂ content for mammals does not fall below 5ml/100ml blood (Altman and Dittmer 1971). It has even been shown that reptiles can endure complete de-oxygenation of their blood, so long as circulation is maintained (Belkin 1968). The ability to cope with low oxygen levels has been demonstrated in a variety of

snakes, lizards, turtles and crocodiles, which survived from 30min -17h in a nitrogen atmosphere (Belkin 1963).

The sprawling gait of lizards may necessitate increased reliance on anaerobic metabolism during intense activity. Every footstep twists the thorax and causes one side of the lungs to be compressed while the other expands; relaxed breathing, in which both lungs are able to expand simultaneously, is prevented during this movement (Carrier 1987; Carrier 1991). Wang *et al.* (1997) found that increasing treadmill running speed from 0.28 to 1.1 m · s⁻¹ caused *Iguana iguana* to decrease oxygen consumption from 10 ml · (kg · min)⁻¹ to 6.7 ml · (kg · min)⁻¹. This observation in the laboratory may be substantiated with the everyday observation that many lizards and amphibians run in spurts; the pauses supposedly allow them to breathe (Carrier 1987). Conversely, locomotion in mammals does not twist the thorax to the same extent, allowing most mammals to take one breath per stride (Carrier 1987; Carrier 1991). As their ability to inhale oxygen is limited, lizards are found to rely more heavily on anaerobic metabolism during exercise; small reptiles, for instance, convert glycogen to lactate anaerobically for 60-90% of their total metabolic energy during burst activity (Bennett and Licht 1972; Bennett and Gleeson 1976; Gratz and Hutchison 1977). It has been shown that *S. occidentalis* utilize anaerobic energy production for 67-72% of total energy used in 2 minutes of activity (Bennett and Gleeson 1976, Gleeson 1979). During anaerobic mechanism, NADH donates electrons to pyruvate during glycolysis to regenerate NAD⁺ and maintain the glycolytic pathway. This process, known as lactic acid fermentation, generates lactic acid byproduct, which can have various physiological consequences (Figure 2).

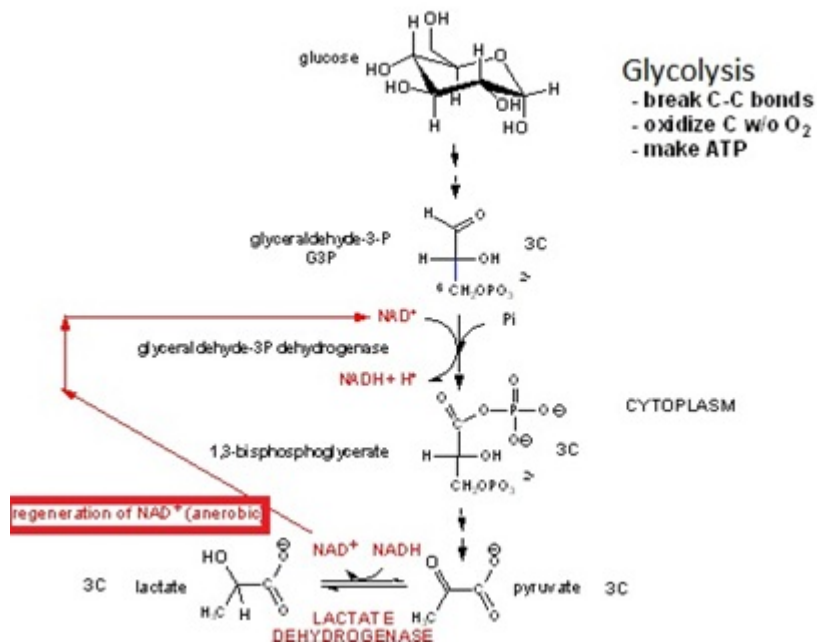


Figure 2. Anaerobic metabolism in regeneration of NAD^+ from NADH during glycolysis to make adenosine-5'-triphosphate (ATP). (Modified from <http://employees.csbsju.edu/hjakubowski/classes/ch331/oxphos/catabolismoverview.gif>).

Due to their heavy reliance on anaerobic metabolism during exercise, recovery from activity for these animals involves reducing built up lactic acid and restoring muscle glycogen and body oxygen stores as quickly as possible to avoid predation and other threats. It has been shown that *S. occidentalis* are quite efficient at removing lactate and restoring glycogen, with lactate concentrations returning to resting rates after 90 minutes following exercise, and glycogen replenished within 150 minutes for fasted lizards (Gleeson 1982). Oxygen consumption rates were also found to return to resting rates after 66 minutes. Lab rats, however, were found to replace only 50% of depleted muscle glycogen during the first 4

hours following exercise (Gleeson 1982). Many endotherms, in fact, take several hours to restore muscle glycogen and restoration is often incomplete until a dietary source of carbohydrates is ingested (Bergström *et al.* 1967; Pernow and Saltin 1971; Brooks *et al.* 1973; Gaesser and Brooks 1980). However, dietary supplement of carbohydrate during 48 hours of recovery caused supercompensation of glycogen stores in *S. occidentalis* muscle (compared with normal resting muscle glycogen stores, +66% in muscle and +800% in liver; Gleeson 1982). It was instead found that the exponential decay of lactate concentration corresponded to the exponential increase of muscle glycogen during recovery for *S. occidentalis*, indicating that there is an inter-conversion between the two (Gleeson 1982).

Lizards have been shown to reduce lactic acid by converting as much as 50% of it back to glycogen (Gleeson 1996). This process is similar to that of the mammalian Cori cycle, in which lactate is converted to glucose in the liver, but with a key difference: for lizards, gluconeogenesis occurs directly within the muscles, which allows glycogen stores to be replenished at a much faster rate (Hitchcox 2009). Gleeson and Dalessio (1990) observed that glycogen depletion corresponded with lactate increase in *Dipsosaurus dorsalis* during exhaustive exercise, and that lactate concentration declined with complete resynthesis of glycogen in red muscle tissue during a 2 hour exercise recovery period. This pattern of glycogen resynthesis is absent in endotherms, who convert lactic acid back into pyruvic acid and into the citric acid cycle where it is oxidized; this occurs for up to 90% of the lactate generated during exercise in mammals (Gleeson 1996). Because of their low metabolic rates, however, lactate oxidation would occur very slowly for ectotherms. Indeed, reptiles and amphibians oxidize less than 20% of lactate generated during exercise (Gleeson 1996).

The cost of gluconeogenesis during exercise recovery is substantial in ectotherms. Post-exercise excess oxygen consumption (EPOC) is a measure of the metabolic cost of exercise recovery. In *D. dorsalis*, lactate removal via gluconeogenesis has high aerobic costs, which account for 37% of EPOC (Hancock and Gleeson 2008). The process of converting each glucose unit costs 6-7 ATP, which is quite expensive relative to the 2-3 ATP that are yielded during glycolysis to generate lactate, and the cost is borne entirely during exercise recovery. In order to cover this cost, some lactate is oxidized; in *D. dorsalis*, 16% of lactate was oxidized within 2 hours of exercising (Gleeson and Dalessio 1989). Other associated costs during exercise recovery are phosphocreatine repletion (32%-50% EPOC), ATP repletion (8%–13% EPOC), elevated ventilatory activity (2% EPOC), elevated cardiac activity (2% EPOC), and oxygen store resaturation (1% EPOC; Hancock and Gleeson 2008; Gleeson 2002). The rate at which these processes occur may be affected by T_b .

Ambient temperature (T_a) is important both during activity and recovery from activity for ectotherms, and thermoregulatory behavior may have implications for how quickly and efficiently they are able to recover from exercise. This study investigated thermoregulatory behavior and T_b selection through environmental interactions in *S. occidentalis* during exercise recovery. It was expected that lizards would choose to be at an T_a that results in higher T_b as biochemical processes occur at faster rates at high temperatures, and may potentially help remove lactic acid, regenerate muscle glycogen, and restore other processes to pre-exercise levels at a faster rate during exercise recovery (Randall *et al.* 2002).

Materials and Methods

Animals

Sceloporus occidentalis were collected between the months of June and August, 2012, from various sites around the Pitzer College campus in Claremont, CA under California Division of Fish and Game collecting permit 11273. In accordance with our permit, which stipulated that no breeding females be caught, only 20 male lizards, which ranged from 3 - 16g, were captured for this study. After capture, animals were housed in terraria in the W.M. Keck Science Center constant temperature room at 27° C, where they had access to a photothermal gradient between 6am and 8pm. Air temperatures in the terraria ranged from 27-34° C, while surfaces in direct light heated up to temperatures between 40-50° C. A sandy substrate was used, and PVC pipes that ran the length of each terrarium, as well as oak leaves, rocks, and wood pieces, were available as shelter. To allow animals closer access to heat lamps, wooden ramps were placed in each terrarium at about a 45° tilt. Lizards were misted with water every day, and 4-5 waxmoth larvae or crickets were provided on a feeding schedule that allowed animals to be fasted for 48 hours prior to a trial, so that digestion did not affect metabolism or T_b preference (Table 1).

Table 1. Sample of experiment schedule denoting trial regimen and feeding regimen.

06/10/2012		Feed 5-8 +2 following treatment	5pm -> incubate 1-4	#1 Treat 5-8 +2, TG	
06/11/2012	Feed 1-4 following treatment	9am feed 5-8 +2, remove food at 12pm		#2 Treat 1-4, TG	
06/12/2012	9am feed 1,3,4, remove food at 12pm			5pm -> incubate 5-8 +2	
06/13/2012		Feed 5-8 +2 following treatment	5pm -> incubate 1,3,4	#2 Treat 5-8 +2, TG	
06/14/2012	Feed 1,3,4 following treatment	9am feed 5-8 +2, remove food at 12pm		#3 Treat 1,3,4, TG	
06/15/2012	9am feed 1-4, remove food at 12pm			5pm -> incubate 5-8 +2	
06/16/2012		Feed 5-8 +2 following treatment	5pm -> incubate 1,3,4	#3 Treat 5-8 +2, TG	
06/17/2012	Feed 1,3,4 following treatment	9am feed 5-8 +2, remove food at 12pm		#4 Treat 1,3,4, TG	
06/18/2012	9am feed 1,3,4			5pm -> incubate 5-8 +2	Release 1,3,4
06/19/2012		Feed 5-8 +2 following treatment		#4 Treat 5-8 +2, TG	
06/20/2012		9am feed 5-8			Release 5-8 +2, Catch 9-16

Trial Equipment Design

A thermal gradient was also kept in the constant temperature room at 27° C. It consisted of eight lanes of insulated track. Underneath each lane were heating coils, which increased in density at one end to provide a range of temperatures, from 27° C to 47° C along the length of each lane. This range approximated daytime temperatures to which the lizards might have access at this time of year (Adolph 1990). Inside each lane of the gradient, a

sandy substrate and a wooden cover for shelter were provided. Plastic covers over each lane prevented animals from escaping during trials.

Thermal Selection Experiment

Before trials, lizards (n=16) were incubated at 34° C overnight, because this is the optimal T_b for sprinting of this species (Adolph 1987). The morning after incubation, lizards were run on a treadmill. A 1.5'x2' plexiglass frame was constructed to rest on the treadmill and prevent animals from escaping while exercising. Animals were run at 0.447 meters per second; *S. occidentalis* can sprint above speeds of 0.894 meters per second, but it was suspected that exercising them at greater speeds would rapidly lead to exhaustion (Sinervo *et al.* 1991). To sustain exercise, lizards were prodded by hand. Each animal participated in four different exercise treatments over an 11 day period, with 72 hours of rest between treatments. The treatments included a control (which did not involve exercise, but took the animals through all other trial steps including handling) and 30, 60, and 90 second exercise trials. Ninety seconds was chosen as the upper limit for treatment duration, as preliminary tests of the lizards' stamina showed that few could sustain exercise beyond this time; virtually all became exhausted and lost their righting response.

The sequence of each animal's treatment was randomized using a random number generator to minimize any effect of treatment order. After exercise, all animals were placed at the cool end of the thermal gradient to minimize T_b bias due to initial placement. Once in the thermal gradient, each animal's T_b was measured at 20, 40, 60, and 120 minute intervals. Skin temperature measurements were made using a FLIR[®] E30 thermal imaging camera, taking thermal images at points between the animal's dorsal anterior limbs, mid dorsum, and

dorsal posterior limbs (Figure 3). These measurements were found to be comparable to cloacal temperature readings; prior to beginning data collection, cloacal readings and thermal images were taken of the mid dorsum for a small sample of lizards and were found to be correlated ($t = 1.55$, $df = 10$, $p = 0.152$). The accuracy of infrared thermometers for measuring T_b in amphibians and small lizards has been verified (Rowley *et al.* 2007, Hare *et al.* 2007). This method was also chosen to decrease handling of the animals, which has the potential to initiate a stress response and elevate plasma corticosterone, which could affect the results of this study (Moore *et al.* 1991). Elevated levels of corticosterone have been shown to significantly alter thermoregulatory behavior in geckos; *Hoplodactylus maculatus* was found to exhibit heat-seaking behaviors and select for higher T_b in a thermal gradient when injected with corticosterone compared with placebo animals (Preest and Cree 2008).

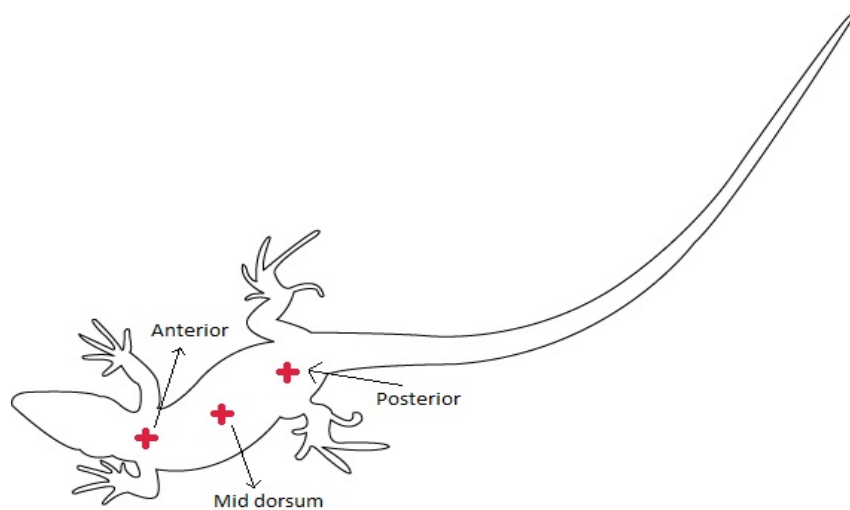


Figure 3. Thermal imaging point locations on *S. occidentalis* during exercise recovery. (Modified from <http://www.stencil-workshop.com/Stencil/Animal/Lizard/lizard-2.gif>)

Body temperature cooling experiment

A follow-up study was carried out to examine the T_b changes in cooling animals. In the previous study, an initial temperature reading was not taken immediately following placement in the thermal gradient. This left a gap in observed T_b changes from 0 to 20 minutes in the thermal gradient. The object of this study was to measure the maximum rate at which T_b could cool, from 34° C at incubation to 27° C, the lowest available temperature in the thermal gradient. The thermal gradient was turned off and the constant temperature room set at 27° C in order to ensure an even temperature of 27° C in the area lizards were to be placed in. Dividers were placed in the lanes to confine the lizards to a smaller area with a length of 19cm. Lizards ($n = 4$) were again incubated at 34° C overnight, and placed in the modified lanes of the thermal gradient the next morning. Thermal images were taken every minute for 20 minutes at mid dorsum points to observe the rate of skin temperature cooling.

Data Analysis

T_b data for the thermal selection experiment were compared using a two-way repeated measures analysis of variance (ANOVA) in SPSS 19. This analysis tested for the effects of exercise treatment and time elapsed after exercising on T_b selection.

Results

Temperature selection experiment

There were significant effects of exercise treatment and time following exercise on dorsal anterior, mid dorsum, and dorsal posterior body temperature in *S. occidentalis* (Figures 4, 5, and 6). Control animals had a relatively constant T_b , which averaged around 33°C throughout the exercise recovery period. Animals that were exercised initially cooled their T_b 's to an average of about 30°C, and then warmed to an average of about 32°C by the 2 hour mark.

A Mauchly's test indicated that sphericity could not be assumed for exercise ($df = 5$, $p = 0.013$) or for time ($df = 9$, $p < 0.001$) so a Greenhouse-Geisser correction (Epsilon = 0.566) was used for dorsal anterior skin temperature data analysis. Exercise treatment and time were found to have significant effects on dorsal anterior body temperature of *S. occidentalis* ($p = 0.005$ and $p < 0.001$, respectively; Figure 4). Control animals showed little variation in dorsal anterior body temperature throughout exercise recovery and T_b fluctuated between 32.2°C and 32.7°C. Exercised animals showed more variation in dorsal anterior body temperature. Lizards exercised for 30 seconds averaged 29.2°C at 20 minutes after exercising and 32.2°C at 120 minutes after exercising. Lizards exercised for 60 seconds averaged 29°C at 20 minutes after exercising and 32.1°C at 120 minutes after exercising. Lizards exercised for 90 seconds averaged 28.4°C at 20 minutes after exercising and 32.5°C at 120 minutes after exercising. It is also important to note that a significant interactive effect was found between time and exercise ($p = 0.006$), which indicates that different treatment groups may have different thermoregulatory behavior at any given time during exercise recovery.

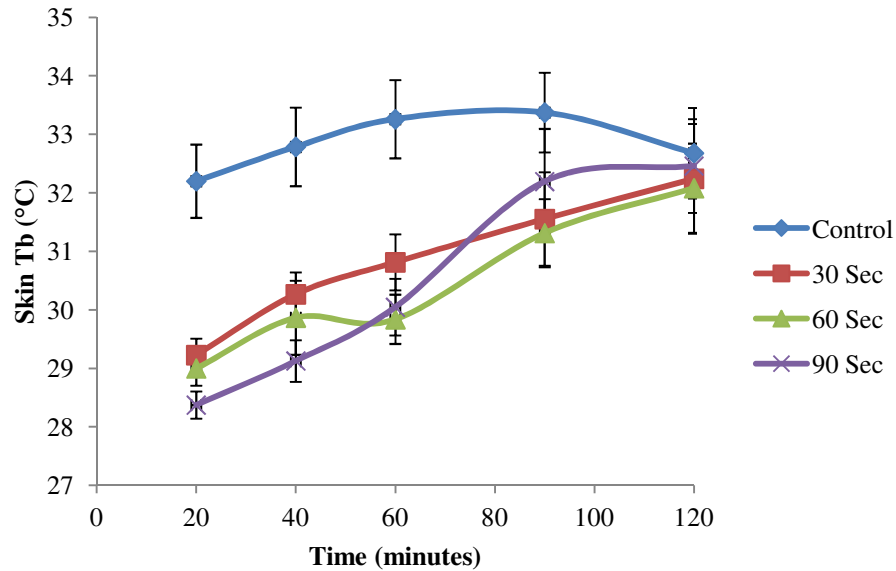


Figure 4. Effect of exercise treatment and time after exercise on *S. occidentalis* dorsal anterior skin temperature (mean \pm standard error, n = 16)

A Greenhouse-Geisser correction (Epsilon = 0.562) was also used for mid dorsum body temperature data analysis because a Mauchly's test indicated sphericity could not be assumed for time (df = 5, $p < 0.001$) or for exercise ($p = 0.012$). Exercise treatment and time were found to have significant effects on mid dorsum body temperature in *S. occidentalis* ($p = 0.006$ and $p < 0.001$, respectively; Figure 5). Control animals showed little variation in mid dorsum body temperature throughout exercise recovery and T_b fluctuated between 32.1°C and 32.6°C. Exercised animals showed more variation in mid dorsum body temperature. Lizards exercised for 30 seconds averaged 29.2°C at 20 minutes after exercising and 32.2°C at 120 minutes after exercising. Lizards exercised for 60 seconds averaged 29°C at 20 minutes after exercising and 32°C at 120 minutes after exercising. Lizards exercised for 90 seconds averaged 28.4°C at 20 minutes after exercising and 32.4°C at 120 minutes after exercising. A significant interactive effect between exercise and time was also found ($p =$

0.007), which indicates animals may exhibit different thermoregulatory at any given time after exercising depending on their exercise treatment.

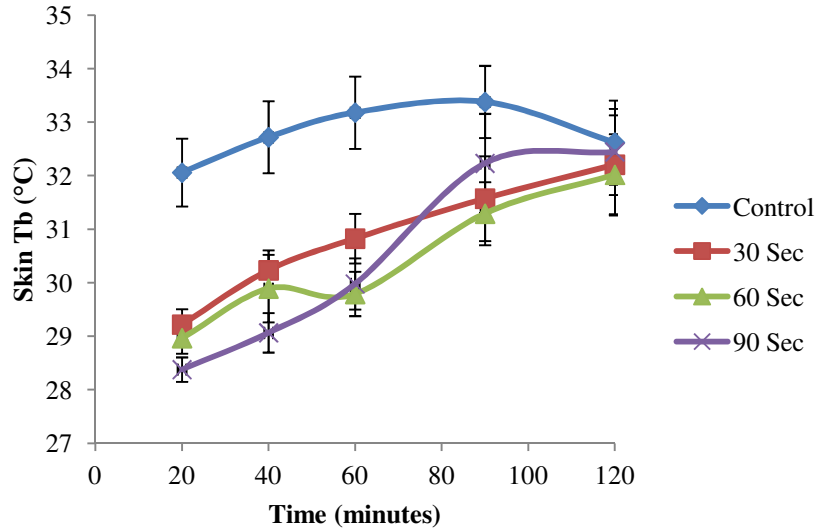


Figure 5. Effect of exercise treatment and time after exercise on mid dorsum skin temperature of *S. occidentalis* (mean \pm standard error, n = 16).

A Greenhouse-Geisser correction (Epsilon = 0.568) was again used for dorsal posterior body temperature data analysis as a Mauchly's test for indicated sphericity could not be assumed for exercise (df = 5, p = 0.014) or for time (p < 0.001). Exercise treatment and time were found to have significant effects on dorsal posterior body temperature in *S. occidentalis* (p = 0.006 and p < 0.001, respectively; Figure 6). Control animals showed little variation in dorsal anterior body temperature throughout exercise recovery and T_b fluctuated between 32°C and 32.6°C. Exercised animals showed more variation in dorsal anterior body temperature. Lizards exercised for 30 seconds averaged 29.2°C at 20 minutes after exercising and 32.2°C at 120 minutes after exercising. Lizards exercised for 60 seconds averaged 29°C at 20 minutes after exercising and 32°C at 120 minutes after exercising. Lizards exercised for 90 seconds averaged 28.4°C at 20 minutes after exercising and 32.5°C at 120 minutes after

exercising. A significant interactive effect between exercise and time was again found ($p = 0.012$), which indicates that, at any given time during exercise recovery, animals may exhibit different thermoregulatory behavior depending on their exercise treatment.

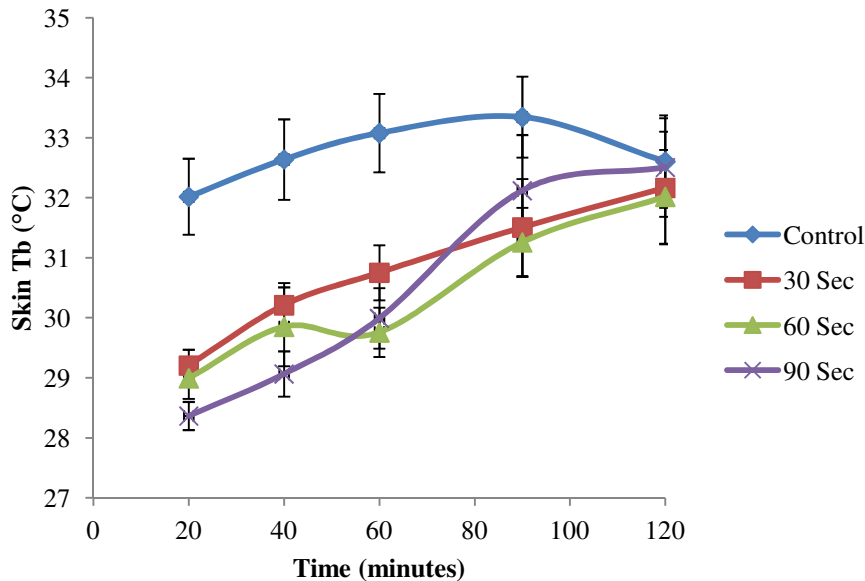


Figure 6. The effect of exercise treatment and time after exercise on posterior skin temperature of *S. occidentalis* exercise (mean \pm standard error, $n = 16$).

Body temperature cooling experiment

Both the average and individual temperatures for four lizards during the follow-up experiment declined over time at an average of $0.175\text{ }^{\circ}\text{C}/\text{minute}$ (Figures 7 and 8). There was some variation in this decline among the individual animals, possibly due to the relationship between surface area and volume, which correlates with body temperature change (Randall *et al.*, 2002). Lizards from this experiment group had an average body mass ($9.255 \pm 4.97\text{g}$, SD) similar to those from the previous group used in the thermal selection study ($9.68 \pm 3.10\text{g}$, SD).

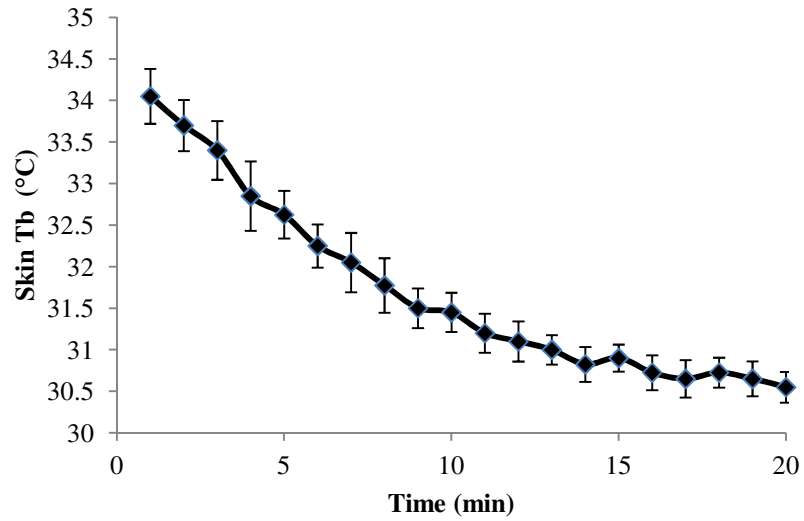


Figure 7. Average body temperature change over time from 34°C incubation to 27°C (n = 4).

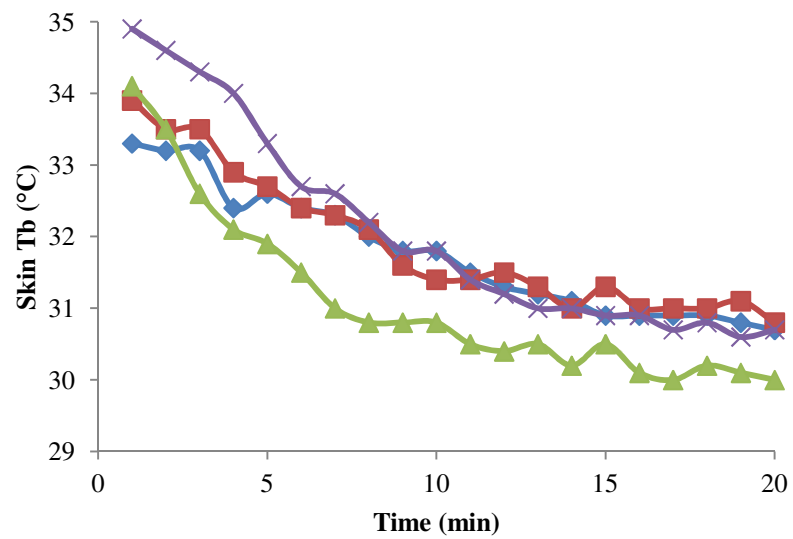


Figure 8. Individual body temperature change over time from 34°C incubation to 27°C (n = 4).

Discussion

Body temperature is known to have significant effects on both activity and recovery from activity in ectotherms. High T_b 's are associated with high levels of physical activity and many physiological processes (Randall *et al.* 2002). However, in some circumstances lizards are observed to move voluntarily to low temperature areas even when high temperatures are available to them (Regal 1967; Engbretson and Hutchison 1976). Low T_b has been shown to severely impede burst activity and sprint performance in *S. occidentalis* (Marsh and Bennett 1986). Animals are vulnerable to predation when they are slow-moving and burst activity is impaired; therefore, the benefits of behaviorally lowering T_b must be significant enough to outweigh this risk.

I found that *S. occidentalis* recovering from exercise select T_b 's lower than their preferred activity T_b . This effect, when compared with T_b of control animals, was found to be statistically significant. These results disproved my original hypothesis, that post-exercise lizards would select higher T_b 's in order to increase the rate of specific biochemical processes that would speed exercise recovery; gluconeogenesis and lactate reduction. For exercised animals, T_b was 4.8°C to 5.6°C lower than their preferred activity T_b of 34°C. This decrease in T_b occurred rapidly - within the first 20 minutes of placement in the thermal gradient. Declines in T_b following intense activity have been mirrored in other ectotherms; exhaustive exercise in the lizard *Anolis sagrei* caused animals to adjust their thermoregulatory behavior and lower T_b by about 5°C compared with control animals (Petersen *et al.* 2003). It is important to note that lizards exposed to stressors such as handling and induced exercise often raise their thermal set point (Cabanac and Gosselin, 1993; Morrow *et al.* 1993). Elevated levels of corticosterone, a stress hormone, have been found to alter

thermoregulatory behavior in the gecko *Hoplodactylus maculatus*, causing animals to select a higher T_b (Preest and Cree 2008). *S. occidentalis* was shown to behaviorally lower T_b during exercise recovery in this study; it is possible that the effects of exercise overrode the effects of stress caused by handling. In addition, although animals were placed at the cool end of the thermal gradient, it is unlikely this is the cause of lowered T_b because ectotherms have skin that is extremely sensitive to changes in ambient temperature (T_a); they would have been aware that warmer temperatures were available to them at the other end of the gradient (Hutchison and Dupré 1992). Additionally, control animals were placed at the cool end of the thermal gradient and did not exhibit T_b cooling, which indicates they quickly moved to warmer T_a 's and that exercised animals must have actively selected cooler T_a 's to reduce T_b .

Ectotherms commonly lower their energetic requirements by allowing T_b to fall (as do some endotherms – hummingbirds in torpor and hibernating ground squirrels; Calder 1994; Snapp and Heller 1981). There appear to be many signals that cause animals to behaviorally select environments that lower T_b during exercise recovery. Elevated blood lactate levels in ectotherms due to prolonged anaerobic metabolism during exercise may be one cause of blood acidosis, which has pronounced effects on thermoregulatory behavior in ectotherms. A marked effect was observed in hypoxic toads (*Bufo marinus*): after injection with sodium lactate, the animals decreased their mean T_b for several hours (Pörtner *et al.* 1994). Normoxic shore crabs (*Carcinus maenus*) were also found to select cooler microclimates when injected with a lactate solution (De Wachter *et al.* 1997). It was suggested that elevated lactate levels elicit a behavioral response in these animals to optimize energy balance and oxygen transport; lowering T_b causes a decrease in metabolic rate, which allows available oxygen and glucose stores to be replenished in tissues recovering from

exercise (Randall *et al.* 2002). De Wachter *et al.* (1997) noted that lactate injection also stimulated an increase in oxygen consumption rates in *C. maenus*, which would increase availability of oxygen to recovering tissues.

Other studies have shown that a significant decrease in arterial pH, not necessarily caused by high lactate concentration, is responsible for altered thermoregulatory patterns in ectotherms. Branco and Wood (1994) discovered that lowering the arterial pH of *Bufo marinus* to 7.1 by forcing animals to breathe air mixtures containing 8% CO₂ caused toads to select significantly cooler T_b than control animals. A study of the desert iguana *Dipsosaurus dorsalis* found that animals exhibited behavioral hypothermia when placed in a thermal gradient during exercise recovery (Wagner and Gleeson 1997). Further research to explain these findings demonstrated that hypercapnia-induced blood acidosis caused *D. dorsalis* to behaviorally select lower than preferred activity T_b during the exercise recovery period, indicating that hypercapnia may be a physiological trigger associated with behavioral hypothermia during exercise recovery (Wagner *et al.* 1999).

There is much evidence that acidosis alters thermoregulatory behavior in ectotherms. However, there are some discrepancies in responses to lactate-caused and hypercapnia-caused acidosis across species. Wagner *et al.* (1999) found that, while hypercapnia caused lizards to select lower T_b, laboratory-induced high blood lactate concentration did not. The effects of hypercapnia on thermoregulatory behavior, however, have been controversial in endotherms. A study of two rodent species *Proechimys yonenagae* and *Proechimys iheringi* found that only *P. yonenagae* exhibited a drop in T_b when exposed to air enriched with 5% or 10% CO₂ (Barros *et al.* 1998). It is possible that hypercapnia causes a non-regulated drop in T_b due to heat loss from hyperpnoea (increased depth of breathing) and vasodilation

(widening of blood vessels), rather than a regulated drop in T_b caused by altered thermoregulatory behavior (Lai *et al.* 1981). It is therefore plausible that other physiological signals aside from acidosis may be significant regulators of behavioral hypothermia.

Hypoxia in the absence of blood acidosis has been shown to cause ectotherms to select T_b 's that are cooler than their preferred activity temperature. The main benefits of this altered thermoregulatory behavior are clear: by lowering T_b , animals are able to reduce their metabolic rates and consequently reduce overall oxygen demands. The terrestrial toad, *Bufo marinus*, has been found to select low T_a under hypoxic conditions, which is physiologically advantageous. Toads at 15° C were found to have reduced metabolic rate, maintained arterial O_2 pressure, and increased arterial O_2 saturation, all of which allow O_2 to still be delivered maximally to tissues; animals at 25° C were found to have higher metabolic rates and reduced arterial O_2 pressure and saturation (Boutilier *et al.* 1987; Wood and Malvin 1991). Similar responses have been measured in reptiles; Hicks and Wood (1995) measured the T_b of four lizard species in a thermal gradient and found that subjecting them to hypoxic conditions caused them to select lower T_b than did normoxic conditions. In the lizard *Anolis sagrei*, hypoxic conditions (10% O_2) resulted in T_b depression of about 5°C caused by altered thermoregulatory behavior (Petersen *et al.* 2003). Furthermore, behaviors that cause low T_b following exhaustive exercise were eliminated in *A. sagrei* when animals were placed in a thermal gradient flooded with hyperoxic air (100% O_2), although blood lactate concentration and pH were not affected by this change (Petersen *et al.* 2003). Because exhaustive exercise causes elevated blood lactate concentration in lizards, this discovery suggests that hypoxia, rather than high blood lactate levels, is the main initiator for altered thermoregulatory behavior in these lizards. Exhaustive exercise has been shown to significantly decrease

arterial PO₂ in *Iguana iguana* and *Varanus exanthematicus* (by 20mmHg or more; Gleeson and Bennett 1982). Thus it is possible that hypoxia during exercise recovery is a common trigger for behavioral hypothermia in ectotherms.

Because of their sensitivity to T_a, climate change is predicted to particularly impact ectotherms. Ectotherms have been shown to have seasonal plasticity in preferred activity temperatures, which may provide a buffer against climate change and contribute to the animals' ability to adapt to a changing thermal environment (Gvozidik 2011). In *S. occidentalis*, it has been found that lizard populations at different geographic locations adapt to temperature differences between sites, and that seasonal variation may have different effects on the plasticity of thermal responses in these animals (McMillian 2010). However, studies of terrestrial insects have found that some specimens - those in tropic regions and high latitudes - are severely impacted by climate change; these studies have been compared with data from vertebrate taxa and used to conclude that the results are similar for terrestrial ectotherms (Deutch *et al.* 2008). Sensitivity to changing climate may be attributed to the aerobic scope of ectotherms; full oxygen delivery occurs only within the thermal optimum. Oxygen demand increases at high temperatures, and, at low temperatures, the aerobic capacity of mitochondria is limited; these factors lead to low levels of oxygen in the body fluids and a decrease in aerobic scope (Portner 2001). As a result, rising temperatures may lead to greater use of anaerobic metabolism in ectotherms; if this occurs during routine activity in addition to intense activity, this would result in more frequent and costly recovery periods. Shifts in mitochondrial densities and membrane adjustments that allow for higher rates of oxygen diffusion to tissues may aid in thermal tolerance (Portner 2001). However, it is evident that the physiological implications of climate change are significant for

ectotherms, and that many species may not be able to make the adjustments necessary for survival. The results of my experiment indicate that T_a is important during the recovery period for *S. occidentalis*; thus, if low temperatures are not available, recovery may be compromised. Further studies are needed to investigate the molecular basis for thermoregulatory behavior in these animals.

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