Examination of the State-Dependency and Consequences of Foraging in a Low-Energy System, the Gila Monster, *Heloderma Suspectum*

by

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ABSTRACT

Foraging has complex effects on whole-organism homeostasis, and there is considerable evidence that foraging behavior is influenced by both environmental factors (e.g., food availability, predation risk) and the physiological condition of an organism. The optimization of foraging behavior to balance costs and benefits is termed state-dependent foraging (SDF) while behavior that seeks to protect assets of fitness is termed the asset protection principle (APP). A majority of studies examining SDF have focused on the role that energy balance has on the foraging of organisms with high metabolism and high energy demands ("high-energy systems" such as endotherms). In contrast, limited work has examined whether species with low energy use ("low-energy systems" such as vertebrate ectotherms) use an SDF strategy. Additionally, there is a paucity of evidence demonstrating how physiological and environmental factors other than energy balance influence foraging behavior (e.g. hydration state and free-standing water availability). Given these gaps in our understanding of SDF behavior and the APP, I examined the state-dependency and consequences of foraging in a low-energy system occupying a resource-limited environment - the Gila monster (*Heloderma suspectum*, Cope 1869).

In contrast to what has been observed in a wide variety of taxa, I found that Gila monsters do not use a SDF strategy to manage their energy reserves and that Gila monsters do not defend their energetic assets. However, hydration state and free-standing water availability do affect foraging behavior of Gila monsters. Additionally, as Gila monsters become increasingly dehydrated, they reduce activity to defend hydration state. The SDF behavior of Gila monsters appears to be largely driven by the fact that Gila monsters

must separately satisfy energy and water demands with food and free-standing water, respectively, in conjunction with the timescale within which Gila monsters balance their energy and water budgets (supra-annually versus annually, respectively). Given these findings, the impact of anticipated changes in temperature and rainfall patterns in the Sonoran Desert are most likely going to pose their greatest risks to Gila monsters through the direct and indirect effects on water balance.

DEDICATION

I dedicate my dissertation work to the support of my many friends and my family. Thank you to my loving mother, Cyndy Wright, for all the words of encouragement, love, and support. To my brother Cory Wright, who has always pushed me to be my best and to his wife, Cathy Wright, for her continued support. To my niece Madeline Wright, whom I one day hope becomes a scientist! And especially to my grandparents, Louis and Elizabeth Headley, who always taught me to do what I loved and work hard every day.

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CHAPTER 1

INTRODUCTION

Organisms rely on a suite of behavioral and physiological strategies to cope with spatial and temporal variation in resources (i.e., energy and water). However, the strategy used to address one need may negatively impact another, so an organism must coordinate its behavioral and physiological responses to best accommodate all of its needs (Stahlschmidt et al., 2011). Foraging is a critical behavior that can have complex effects on organism homeostasis, with the potential to both positively and negatively affect vital needs (e.g., energy and water balances). Therefore, foraging requires an organism to integrate information regarding environmental conditions and internal physiological condition to optimize foraging behavior (Charnov, 1976). There has been considerable research investigating factors that influence foraging behavior, and studies have established that food supply, time allocation, and predation risk are important factors driving foraging behavior (e.g., Brown 1988, 1992, 1999; Abrams, 1991; McNamara and Houston, 1994; Olsson et al, 2002). However, recent studies show that other factors including gut fullness, energy reserves, and free-standing water availability can also impact foraging behavior (Kotler et al., 1998; Metcalfe et al., 1998; Burrows et al., 2000; Aubret and Bonnet, 2005; Hochman and Kotler, 2006; Kotler et al., 2010). The optimization of foraging behavior to balance costs and benefits is termed state-dependent foraging (SDF, Nonacs, 2001), and this leads to the protection of assets that aid in survival and reproduction (asset protection principle, APP, Clark, 1994).

The vast majority of studies examining SDF focuses on animals with high metabolism and high energy demands (“high-energy systems”, endotherms such as small mammals), with little work examining species with low energy use (“low-energy systems” such as ectotherms, but see Aubret and Bonnet, 2005; Aubret et al., 2007). Terrestrial vertebrate ectotherms, in particular infrequently feeding species, are typically in negative energy balance, balance energy budgets over longer periods of time, and rely extensively on energy reserves to cope with extended periods without eating (e.g., Bonnet et al., 1999). While high-energy systems must balance their energy budgets over shorter time periods (e.g., hours to days) and thus must continually balance risk and rewards associated with foraging, infrequently feeding ectotherms may not practice a SDF strategy with regards to energetic state. Low-energy systems simply may be able to tolerate not foraging during challenging or “risky” times (e.g., elevated predation risk or elevated ambient temperatures), as they are unlikely to experience lethal or non-lethal consequences of aphagia over such short periods of time. By studying SDF in terrestrial, low-energy vertebrate systems, we can better understand which factors favor the use of SDF and the APP.

Although energy balance is crucial for survival and fitness, so too is water balance, and water and energy budgets must be paid using discrete currencies. Failure to maintain hydration state can have lethal and non-lethal effects. Non-lethal effects of dehydration include hyperthermia, reduced meal consumption (e.g., Watts, 1999; Maloiy et al., 2008), and reduced activity (e.g., Davis and DeNardo, 2009). For numerous organisms, meal consumption serves as a source of both energy and water intake, and many organisms maintain water balance exclusively, or nearly so, through meal consumption and metabolic water production (e.g., Minnich and Shoemaker, 1970; Karasov, 1983; Cooper, 1985; Nagy and Gruchacz, 1994; Degen et al., 1997; Williams et al., 2001; Ostrowski et al., 2002). However, other organisms require free-standing water to maintain hydration state (e.g., Schmidt-Nielsen et al., 1956; Beaupre, 1996; Lillywhite et al., 2012; Appendix A, Wright et al., 2013). Thus, factors such as hydration state, availability of drinking water, and the extent to which organisms rely on various sources of water may guide SDF behavior. Despite the critical importance of maintaining water balance, the majority of studies examining SDF consider only the energetic state of the study organism as the "physiological condition" of the animal, and a very limited number of studies have examined how free-standing water availability impacts high-energy systems (but see Kotler et al., 1998; Hochman and Kotler, 2006; Shrader et al., 2008). If we are to truly understand how organisms co-manage various physiological needs, we must broaden our understanding of SDF behavior beyond the influence of energy balance to include factors involved in water balance.

Understanding the interaction between physiological condition and foraging behavior has become increasingly important in lieu of recent and anticipated anthropogenically-induced climate change. Over the past 50 years, the Earth's mean air temperature has risen 0.13°C per decade, precipitation patterns have changed dramatically, and the frequency and intensity of heat waves have increased (Tebaldi et al., 2006; IPCC, 2007). Climate models now predict (globally) approximately 0.20°C per decade increases in temperature, an increase in the frequency and duration of heat wave events, and continued shifts in precipitation patterns (Meehl and Tebaldi, 2004; Tebaldi et al., 2006; IPCC, 2007). Rapid climate change may pose significant challenges to organisms, and an inability to adjust to altered environmental conditions can result in fragmented populations, reduced species distributions, and loss of biodiversity. Indeed, a large emphasis of climate change research has been placed on examining how it will impact organism behavior and physiology, particularly with respect to how changing temperature will impact the energy budgets. Elevated temperature can result in hyperthermia and perhaps death (McKechnie and Wolf, 2010); however, increased air temperature can also have less severe but important non-lethal impacts on organisms by altering performance, elevating metabolic rate or increasing water loss (Dillon et al., 2010; McKechnie and Wolf, 2010). Elevated ambient temperatures may limit the foraging time for organisms as temperatures exceed their critical thermal maxima for a greater portion of the day. A reduction in foraging may, in turn, strain energy budgets, particularly when energy budgets are already strained due to other physiologically costly processes, such as reproduction (Sinervo et al., 2010). Coupled with anticipated reductions in plant biomass (Breshears et al., 2005; Allen et al., 2010), this altered foraging effort may result in local species die-offs due to an inability to maintain energy balance.

However, the impacts of climate change are not limited to effects on energy balance. Reduced precipitation strains water budgets by not only limiting free-standing water, but also reducing dietary water through a reduction in primary productivity (Breshears et al., 2005; Allen et al., 2010) and water content of meals. Furthermore, increases in ambient temperature, independent of any changes in precipitation, can contribution to water imbalance through increased evaporative water loss rates (McKechnie and Wolf, 2010). Clearly, reduced precipitation and increased temperature will likely have additive impacts on water balance of organisms, particularly those in water-limited environments (e.g., deserts). Therefore, it is imperative that our understanding of foraging decisions includes the reciprocal feedback between physiology and behavior as it relates to energy and water balance. Thus, for my dissertation, I investigated factors that influenced foraging behavior and the consequences of meal acquisition in a low-energy, vertebrate ectotherm inhabiting a resource limited environment.

Gila monsters, *Heloderma suspectum* (Cope 1869) are a long-lived (20+ years), medium-sized lizard whose range predominantly lies within the Sonoran Desert, which has considerable seasonal variation in temperature and rainfall, including a lengthy hot, dry season and a monsoonal pulse occurring in late July through August (Beck, 2005). Gila monsters tolerate limited food availability, no free-standing water, and thermal challenges during the hot, dry season (mid-May through mid-July, Beck, 2005). They exclusively consume the contents of vertebrate nests (i.e., eggs and nestlings), and their prey is widely distributed and temporally variable in availability (Beck, 2005). Thus, Gila monsters invest a significant amount of time and potentially energy into foraging and rely heavily on fat reserves to cope with extended periods in negative energy balance. Although Gila monsters are considered active foragers, they occupy sub-surface refugia 70 – 90% of the time (Beck, 1999; Beck, 2005; Davis and DeNardo, 2009, 2010). To cope with seasonal variation in free-standing water, Gila monsters use their urinary bladder as a reservoir to buffer changes in plasma osmolality during the dry season, allowing them to survive over 80 days without drinking water (Davis and DeNardo, 2007). However, once they deplete their hydric reserves, Gila monsters experience significant elevations in plasma osmolality (> 360 mOsm*kg^{-1}, Davis and DeNardo, 2009, 2010). Increased plasma osmolality has been shown to result in a concomitant reduction in surface activity (< 10% surface active), which has been suggested as a behavioral response to reduce further water loss (Davis and DeNardo, 2009). At the onset of the monsoon rainfall, Gila monster plasma osmolality returns to normosmotic levels within 24 to 48 hours of a single binge drinking event (Davis and DeNardo, 2007).

Although there is substantial information on the physiological ecology of Gila monsters with regards to water balance and their basic life history, there is a paucity of information on the responses they exhibit to temporal and spatial variability in food availability and the interaction between various physiological parameters and foraging decisions, including the consequences of meal consumption. As such, my dissertation takes a comprehensive look at foraging in Gila monsters by evaluating how energy state and hydric state influence foraging as well as the energetic and hydric consequences of foraging and meal acquisition. In Chapter 2 I determined the thermal sensitivity of standard metabolic rate (SMR) as well as the acclimation of SMR to extended exposure to cold temperatures, which is typical for the overwintering period when Gila monsters do not eat and are inactive. I then assessed the implications associated with energy use under anticipated warming scenarios. To determine whether Gila monsters use a SDF strategy to co-manage energy and water balance, in Chapter 3 I performed a field-based, food-supplementation study on free-ranging Gila monsters, measuring seasonal changes in body condition (energetic and hydration state) and foraging behavior (via surface activity). In Chapter 4 I examined whether natural correlations between resource states and surface activity of Gila monsters reflect a SDF strategy similar to that seen with the manipulative experiments where the population was more dichotomous in terms of resource state. Finally, to assess the hydric consequences of meal consumption, in Appendix A, I examined how meal consumption influenced the rate of dehydration of Gila monsters (Appendix A, Wright et al., 2013).

CHAPTER 2

EXAMINING COLD-ACCLIMATION METABOLIC RESPONSE AND THERMAL SENSITIVITY OF STANDARD METABOLIC RATE

Introduction

Winter often represents a physiologically challenging period when food is scarce and environmental temperatures limit performance, thus impacting activity and energy balance. To cope, many organisms reduce activity and body temperature during the winter to maximize the duration of energy stores. In fact, many organisms are capable of suppressing their metabolic rate beyond what thermal sensitivity curves of metabolic rates would predict (i.e., metabolic depression), thus resulting in considerable energetic savings (for examples see Christian et al., 1999; de Souza et al., 2004; Heldmaier et al, 2004; Jackson and Ultsch, 2010). Similarly, aestivating organisms can also show metabolic depression during summer months when environmental conditions are problematic (e.g., drought). There is considerable literature on the existence and extent of metabolic suppression during hibernation in mammals or either overwintering or aestivating anurans, chelonians, and invertebrates (for reviews, albeit not comprehensive, see Guppy and Withers, 1999; Geiser, 2004). However, there is limited information regarding acclimation or metabolic depression in overwintering squamates (but see Christian et al., 1999; Guppy and Withers, 1999; de Souza et al., 2004).

Gila monsters are medium-sized (adult body mass = 350 to 700 g, snout-to-vent length = 275 to 335 mm), long-lived (> 20 years), venomous lizards that primarily inhabit the Sonoran Desert of Arizona and Mexico (Bogert and Martin del Campo, 1956; Beck, 2005), where there is considerable seasonal variation in air temperature (range = -5.73°C to 49.50°C, D.F. DeNardo, personal communications), rainfall, and food availability (Beck, 2005). The primary active season of Gila monsters is from April through August, when vertebrates, on whose nest contents they feed, are breeding. For the remaining seven months of the year, Gila monsters typically do not feed and rely solely on energy stores. To conserve energy, activity is greatly reduced during months when food is not available, and this typically includes a period of complete inactivity during the coolest months (December through February, Beck, 2005; Davis and DeNardo, 2009, 2010).

Since the majority of Gila monsters' time is spent inactive in refugia, energy consumption is highly dependent on SMR, and, since SMR is highly dependent upon temperature, body temperature likely has considerable effect on energy expenditures in this species. Despite extensive reliance on refuge occupation to avoid undesirable air temperatures, Gila monsters experience a wide range of body temperatures, typically ranging from 25°C to 32°C during the active season and 11°C to 21°C during the quiescent overwintering period (Fig. 1). Therefore, knowledge of the extent of the thermal sensitivity of SMR is vital to understanding energy budgets in this species, as is the value of any metabolic depression during the overwintering period.

Climate models predict (globally) an increase in air temperature of approximately 0.2°C per decade (IPCC, 2007), and this complicates our understanding of energy balance in ectotherms. For the American Southwest, climate models project a 1- 4°C increase in air temperature by the year 2100 (IPCC, 2007). Although many overwintering ectotherms, including Gila monsters, occupy refugia and are thus buffered from air temperature, anticipated increases in air temperatures associated with climate change are likely to increase refugia temperatures (Bai et al., 2013). Increased refugia temperatures will potentially increase energy expenditure during overwintering. This, in turn, would reduce survival time. If we are to better understand the consequences of elevated temperatures on ectotherm energetics, it is important that we have a better understanding of the thermal sensitivity of SMR across a broad range of body temperatures as well as the extent and significance of metabolic depression during overwintering. Thus, I assessed the thermal sensitivity of Gila monster SMR across an ecologically relevant range of body temperatures, and I examined the extent and ecological significance of metabolic depression associated with extended exposure to cold temperatures.

Results

Experiment 1 – Thermal sensitivity of SMR

Linear Mixed-Model Output

This linear mixed-model revealed there was a significant effect of temperature on oxygen consumption (mixed-model analysis of variance, ANOVA: $F_{1,29} = 175.14$, $P < 0.0001$, Fig. 2). The best model for examining this relationship was model 5 (Table 1 and 2). Model 5 was selected over model 6 because the additional parameter added to model 6 did not reduce the second order Akaike information criterion (AICc), which is a small sample version of Akaike's information criterion (AIC) (Anderson et al., 2001), beyond the generally accepted required difference in difference in AICc values (Δ_{AICc}) of at least 2 when compared to model 5, which has fewer parameters.

Experiment 2 – Acclimation of SMR to Cold Temperatures

There was a significant effect of time on the oxygen consumption of yearling Gila monsters (repeated measures analysis of variance, rmANOVA: $F_{19,76} = 7.558$, $P << 0.0001$, Fig. 3) across the entire experiment. Tukey's HSD post-hoc analysis revealed that there were no differences in oxygen consumption of Gila monsters across all time points at 30°C, regardless of whether they occurred prior to or following cold exposure. Generally, the oxygen consumption of yearling Gila monsters when exposed to 16°C was significantly lower than both the pre and post 30°C cold exposures. However, there were two important observations. First, the metabolic rate of animals 24 hours after being exposed to 16°C was not significantly different from their metabolic rate at 30°C prior to and following cold exposure. Additionally, the metabolic rate of yearling Gila monsters on the first day at 30°C after being exposed to 16°C was not significantly different from any values of metabolic rate calculated at 16°C.

Discussion

Thermal sensitivity of SMR

As expected, the SMR of Gila monsters increases with increasing temperature (Figs 2, 4). The SMR values collected in experiment 1 match well with SMR values collected previously from helodermatids at two temperatures (15°C and 25°C, Beck and Lowe, 1994; Fig. 4). Additionally, my data confirm previous assertions that Gila monster SMR is relatively low for a squamate (lizards and snakes) of its size (Fig. 5, Beck and Lowe, 1994). My observed Gila monster SMR was 16% lower than that predicted for lizards and 20% lower than that predicted for snakes based on a log-log relationship between

SMR and body mass. When comparing the mass-adjusted metabolic rate (ml O_2 *hr^{-1}*$g^{-0.69}$) of lizards in the family Varanidae (both Varanidae and Helodermatidae are within the infraorder Anguimorpha, Wiens et al., 2012), which are similarly active foragers, Gila monster SMR is 43% lower than that of varanids. However, when comparing the mass-adjusted metabolic rate of lizards from the family Anguidae, which are sister taxa to Helodermatids, the mass-adjusted SMR of Gila monsters is 35% higher.

The temperature coefficient of metabolism (Q_{10}) from 20°C to 30°C, a temperature range that includes the majority of body temperatures of Gila monsters during their active season (Fig. 1), was 3.46. This value is similar to, but somewhat higher than, the Q_{10} of 3.0 found by Beck and Lowe (1994) for helodermatid lizards from 15°C to 25°C. The difference in Q_{10} between the two studies is likely attributable to the non-linear relationship between temperature and metabolic rate. A polynomial relationship (model 6) provided the lowest AICc value, but was not selected for the results of experiment 1, because the reduction in AICc value was not sufficient to justify the greater number of parameters (the addition of a temperature squared component, Table 1). However, when the data from experiment 1 are combined with those from experiment 2 and those from Beck and Lowe (1994), a polynomial equation better reflects the relationship between temperature and metabolic rate (Fig. 4). In fact, extrapolating the linear relationship from experiment 1 would not effectively predict the results from experiment 2 or the 15°C value from Beck and Lowe (1994), and such extrapolation would be unwarranted, as it would result in negative values for metabolic rate below 17.8°C (Fig. 4). Using the polynomial equation in Fig. 4 better predicts the cumulative data. In fact, the polynomial equation explains much of the measured difference in Q_{10} between the two studies (predicted Q_{10} values for 15-25°C and 20-30°C are 2.8 and 3.7, respectively). This analysis clearly demonstrates the importance of collecting data on the thermal sensitivity of metabolic rate across the entire range of temperatures experienced by the individual in order to avoid problems associated with extrapolation. Additionally, the Q_{10} values from 20°C to 30°C of Gila monsters reported here and by Beck and Lowe (1994) fall within or near the range of values calculated from metabolic rates determined at 20°C and 30°C for a variety of lizards (1.89 – 3.21, Andrews and Pough, 1985; Beck and Lowe, 1994) and snakes (1.57 – 3.39, Andrews and Pough, 1985; McCue and Lillywhite, 2002; Greene et al., 2013).

Acclimation of SMR to Cold Temperatures

In this study I demonstrate that Gila monsters experience a significant reduction in metabolic rate during extended exposure to 16°C air temperature. Metabolic rate was 3.16 ml $O_2 * hr^{-1}$ when measured 24 hrs after exposure to 16°C. Continued exposure to 16°C further reduced metabolic rate to 0.91 ml $O_2 * hr^{-1}$ at four days and as low as 0.59 ml $O_2 * hr^{-1}$ at 23 days post-exposure. These values, respectively, are 71% and 81% lower than the initial 24 hour post-cold exposure values. When I compared the acclimated 16°C metabolic rate to the Gila monster SMR at 30°C (which approximates preferred body temperature), the metabolic depression is even more substantial. At 30°C, SMR of Gila monsters was 7.09 ml $O_2 * hr^{-1}$, which is more than 15 times greater than SMR when cold-acclimated to 16°C.

To compare the extent of Gila monster metabolic depression to other vertebrate taxa, I calculated metabolic depression as the ratio of metabolic rate during overwintering or aestivation to either SMR (for ectotherms at their preferred body temperature) or resting metabolic rate (for mammals). Gila monster metabolic depression (0.11) is near the lower limit of the responses exhibited by amphibians (range 0.09 to 0.84) and reptiles (range 0.19 to 0.77), but is near the upper limit of values reported for hibernating mammals (range 0.012 to 0.15; Fig. 6).

The acclimation response of Gila monster SMR to cold temperatures is rapid compared to what has previously been observed in other hibernating or aestivating species. For example, Kayes et al. (2009) observed that aestivating striped burrowing frogs, *Cyclorana alboguttata*, took 5 weeks before metabolic rates reached low steady states that were 82% lower than their non-acclimated metabolic rate at the same temperature. Common frogs, *Rana temporaria*, submerged in 3°C water gradually reduced their metabolic rate 62% over 90 days (Donohoe et al., 1998). While the proportional reduction in metabolic rate of these two frogs is similar to what I found in Gila monsters, the time required for acclimation was considerably less in Gila monsters. Although I did not examine the physiological mechanisms that could explain the rapid metabolic suppression I observed, a number of possible explanations exist, including reduction of organ mass and tissue-specific oxygen consumption (Secor, 2005; Kayes et al., 2009; Naya et al., 2009) as well as reduction of hydrolytic activity of enzymes in the digestive tract (Naya et al, 2009). Given that Gila monsters can undergo rapid up-and-down regulation of digestive tissue in response to feeding (Christel et al., 2007), these mechanisms may be plausible. Clearly, the rapidity and magnitude of metabolic depression in response to cold temperature in Gila monsters deserve further study.

Ecological Significance

The thermal sensitivity of SMR for Gila monsters and, more generally, ectotherms, is particularly important when considering the impact of changes in mean surface temperature globally and regionally. From 1906 to 2005, the global mean surface temperatures have increased by 0.74 °C, and warming rates over the past 50 years have doubled from 0.07 °C per decade to 0.13 °C per decade. Further, global surface air temperatures are projected to increase 1.8-4.0°C by 2100 (Meehl, et al., 2007; IPCC, 2007).

Previous studies examining the impact of changes in air temperature have shown that elevated temperatures will predictably increase the energy expenditure for ectotherms (Dillon et al., 2010). However, even during the active season, Gila monsters spend a majority of their time occupying refugia (e.g., burrows, Beck, 1990; Davis and DeNardo, 2009, 2010) and generally limit their surface activity to time periods when temperatures are 20°C to 32.5°C (Fig. 1). During overwintering, when Gila monsters are inactive within refugia, their body temperature is relatively constant with a slight, steady decrease as winter progresses (typically shifting from 17°C to 12°C as the winter progresses, D. F. DeNardo, unpublished data). This extended period of low body temperature helps conserve fat reserves during a period when there is no food available.

Projected changes in mean surface air temperatures would entail an increase in refugia temperatures (Zheng et al., 1993; Bai et al., 2013). Gila monsters could respond to increased refugia temperatures by either tolerating the higher temperatures, preferentially selecting burrows with cooler temperatures (e.g., white-throated wood rat, *Neotoma albigula*, middens), or by digging deeper burrows, the latter of which may be difficult, as caliche in the Sonoran Desert soil can limit burrow depth. If Gila monsters were to experience increased body temperatures during the overwintering period, this would result in higher metabolic rate and therefore increased energy expenditure during this time. I calculated the potential mean fat loss (g) by free-ranging Gila monsters under four different climate scenarios. For current conditions, I used hourly body temperature of free-ranging Gila monsters during the 2010-2011 overwintering period (Chapter 3), and I converted these body temperatures to energy consumption using two different polynomial curves modeling thermal sensitivity of SMR (one created using the non-acclimated SMR at 16°C and one created using the acclimated SMR at 16°C). To calculate the effect of projected warming scenarios on energy expenditure of overwintering Gila monsters, I repeated this approach, adding 1°C, 3°C, and 5°C to each of the body temperatures from 2010 to 2011. These increases in temperature cover the range of projected increases determined by climate models for the American Southwest (Christensen et al., 2007; Gutzler and Robbins, 2010).

Under current overwintering conditions, cold-acclimated Gila monsters oxidize 3.24 g of fat, whereas that number would increase to 5.87 g of fat if there were no acclimation. The biological significance (in terms of energy savings) of acclimating versus not acclimating is relatively large, as the acclimated animals oxidize 45% less fat than non-acclimated animals. When we compare the energy saved relative to the fat oxidized during the active season (assuming pure lipid oxidation), the energy savings is still considerably large. Active, free-ranging Gila monsters oxidize an average of 9.52 g and 11.00 g of fat during the dry and monsoon components of the active season, respectively (C. D. Wright, unpublished data, Chapter 3), and the energy saved via cold acclimation during winter equates to 28% and 24% of the total fat oxidized in the dry and monsoon season, respectively.

As expected, an increase in the body temperature of Gila monsters under the three projected climate scenarios results in a subsequent increase in energy expenditure. Overwintering, cold-acclimated Gila monsters are projected to consume 3.66, 4.92, and 6.76 g of fat under the +1, +3, and +5°C warming scenarios, respectively, while non-acclimated, overwintering Gila monsters are projected to burn 5.86, 6.39, and 7.64 g of fat under the +1, +3, and +5°C warming scenarios, respectively (Fig. 7). Additionally, as body temperatures increase under the three projected climate scenarios, the energetic costs projected for cold-acclimated and non-acclimated animals converge, likely because of the convergence of the two polynomial curves (acclimated and non-acclimated). Regardless, given the fact that Gila monster survival is based on frugal energy consumption that is balanced on an annual to supra-annual scale (Chapter 4, Fig. 13A) because of seasonal food availability, projected warming scenarios may compromise overall energy balance across multiple seasons and threaten species distribution and possibly even persistence.

Materials and Methods

Experiment 1 - Thermal Sensitivity of SMR

To determine the temperature dependence of SMR of Gila monsters, I measured SMR at six ecologically relevant temperatures. For this experiment, I used six captive, adult (3 males, 3 females) Gila monsters (mean initial mass = 484 g, range = 396 to 624 g) obtained from the Arizona Game and Fish Department (AZGFD) and held under wildlife holding license SP598954. All animal use was conducted under Arizona State University's (ASU) Institutional Animal Care and Use Committee (IACUC) protocol 12-1244R. Between experimental trials, animals were maintained in a room set at 25.5 ± 0.5°C on a 13:11 light:dark cycle. Animals were housed in individual solid-walled, opaque containers with an expanded metal top and a sub-surface heating element at one end, which enabled the animals to behaviorally thermoregulate. The Gila monsters were fed meals of dead mice approximately biweekly, and provided water *ad libitum*.

During experimental trials, the Gila monsters were at rest and in a post-absorptive, normosmotic state. I used flow-through respirohygrometry to measure the rate of O_2 consumption and the water content of the air during trials at 20.0, 22.5, 25.0, 27.5, 30.0, 32.5 ± 0.1°C. This temperature range represents approximately 95% of the body temperatures that free-ranging Gila monsters experience in the wild during the active season (April until November, Fig. 1). During trials, Gila monsters were housed individually in dual-ported, cylindrical, enameled steel metabolic chambers (volume = 7.0 L, height = 13.5 cm, diameter = 20.0 cm) which were placed inside an environmental chamber in complete darkness, thus mimicking inactivity in a refuge in the wild.

Chambers were opaque on the bottom and sides, but the tops were transparent to enable periodic observation. The windows on the chamber tops were covered during trials to minimize activity. All chambers had grated platforms made of minimally hygroscopic material, so that any excrement would fall through the grating and away from the animals. Any trials during which excrement was produced were repeated, since the excrement would affect the hygrometric measurements. Animals were not provided food or water during the trials and were allowed to adjust to the respirometry chambers for approximately 12 hours prior to recording any data, as Gila monsters can spend an extended period of time exploring new environments. Trials were completed within 24 hours of an animal's exposure to the trial temperature to minimize acclimation to that temperature.

Up to six animals, each in its own metabolic chamber, were subjected to a trial at the same time. Outdoor air was compressed and then delivered to the metabolic chambers through a supply system that passed the air through a desiccant (Drierite, W.A. Hammond Drierite Co. LTD., Xenia, OH, USA) before the air line was bifurcated, sending part of the air through a mass flow controller (MFC) (UNIT Instruments, Yorba Linda, CA, USA) and the rest through a 20 L capacity rotameter. The MFC delivered air at 300 ± 1 $ml*min^{-1}$ to the metabolic chamber that was currently being sampled, while the rotameter distributed air equally (300 $ml*min^{-1}$ each) though a manifold and then to the remaining animal chambers as well as an empty chamber (the latter being used to determine baseline values for the supply air). I used an array of two-way solenoid valves controlled by a datalogger (23X micrologger, Campbell Scientific, Logan, UT, USA) to sequentially deliver supply air from the MFC to each of the chambers for 180 minutes.

Effluent from the metabolic chambers flowed into separate spill tubes. A peristaltic pump pulled air through an array of one-way solenoid valves to sequentially sub-sample from each spill tube. Both the two-way and one-way solenoids were controlled so that the chamber being sub-sampled at a given time was the one that was receiving its supply air from the MFC. The peristaltic pump delivered the subsampled air to a hygrometer (RH-300 water vapor analyzer, Sable Systems International, Las Vegas, NV, USA), through a Drierite column, and then through an oxygen analyzer (FC-1B oxygen analyzer, Sable Systems International, Las Vegas, NV, USA) that was calibrated with outside air prior to each use. The 99% equilibration period for my experimental chambers was approximately 117 minutes (Lasiewski et al., 1966), so the 180 minute trial duration safely ensured the collection of a minimum of 30 minutes of stable data for water content and oxygen consumption. Minimally hygroscopic tubing (Bev-a-line) was used to plumb the entire system.

Environmental chamber air temperature, MFC flux, the status of each solenoid valve, as well as the effluent's dew point, percent oxygen content, and barometric pressure were continually monitored and recorded every minute by a 23X datalogger. Environmental chamber air temperature was monitored using two type-T thermocouples placed on the upper and lower levels of the environmental chamber, which had a small fan running at all times to prevent stratification. The two temperature readings were averaged to determine the temperature of the environmental chamber.

Oxygen consumption ($ml*min^{-1}$) was determined using equation 10.2 from Lighton (2008):

$$V_{O2} = FR_i*(F_{iO2} - F'_{eO2}) / [1 - F'_{eO2}*(1 - RQ)] \quad (1),$$

Where V_{O2} is the rate of oxygen consumption, FR_i is the incurrent mass flow rate scrubbed of water vapor, F_{iO2} is the fractional concentration of the incurrent oxygen, F'_{eO2} is the fractional concentration of the excurrent oxygen scrubbed of water vapor, and RQ is the respiratory quotient, or V_{CO2}/V_{O2}. Given the animals were at rest and in a post-absorptive state, I assumed animals were catabolizing a ratio of 20:75:5 percent proteins to fats to carbohydrates (representing a post-absorptive carnivore), and calculated that an RQ of 0.71 would be most appropriate (Gessaman and Nagy, 1988).

Experiment 2 - Acclimation of SMR to Cold Temperatures

I determined whether the SMR of Gila monsters acclimates to the cool inactive period by evaluating SMR throughout a trial during which they were maintained at their approximate preferred body temperature (30°C) over an 8-day period, then abruptly shifted (within 1 hr) to a temperature typical of body temperature during the majority of the over-wintering period (16°C) for 35 days, and finally abruptly returned to 30°C for a final 20 days. For this experiment, I used eight captive, yearling Gila monsters (mean mass = 138 g, range = 114 to 165 g) acquired from a private breeder and held under

AZGFD wildlife holding license SP577864. At the time of the trials, the animals were in post-absorptive, normosmotic states. Animals were not fed throughout the trial, but received water after each temperature treatment and needed in order to maintain hydration state, which was confirmed by measuring body mass weekly throughout the experiment. This experiment was conducted in accordance with ASU IACUC protocol 09-1044R.

Due to the relatively small size and thus low metabolic rate of yearling Gila monsters, I determined SMR by measuring oxygen consumption using closed-system respirometry. Each animal was individually housed in a plastic, air-tight chamber, and all chambers were placed in an environmental chamber that tightly regulated air temperature (±0.5°C). T-port valves secured with silicone sealant were installed on opposite sides of each container to enable delivery and sampling of air and to maintain a seal during trials. During non-sampling periods, room air was pumped through a series of coils within the environmental chamber (to equilibrate supply air to chamber temperature) and then through a manifold that split the air supply to reach all animal chambers.

The temperature of the environmental chamber was regulated using a datalogger (21X micrologger, Cambell Scientific, Logan, Utah, USA) and monitored using two miniature temperature loggers (iButton model DS1922L, Maxim, Thermochron, Dallas, TX, USA) placed inside the environmental chamber, one high and one low within the chamber, which was equipped with a small fan operating at all times to prevent stratification. For each temperature treatment, oxygen consumption was assessed within 24 hours of initial exposure to the temperature and then repeatedly measured over the duration of that trial (five times for the initial 8-day 30°C treatment, nine times during the 35-day 16°C treatment, and six times during the 20-day final 30°C treatment).

Oxygen consumption was determined by collecting an initial air sample from each animal chamber, sealing each container for a pre-determined duration, and then collecting a final air sample from each chamber. The initial air sample was collected by connecting a 140 ml syringe to the effluent port, opening the port, and then withdrawing air into the syringe. After collection of the final air sample, both ports were opened, and the supply air was reconnected to the supply port. The durations that the chambers were sealed were based on pilot trials to identify sufficient oxygen suppression (approximately 2.0%) for each temperature.

Oxygen concentration was determined for each air sample using an oxygen analyzer (S-3A/I Oxygen Analyzer, AEI Technologies, Pittsburgh, PA, USA). Samples were passed through a Drierite column and then into the analyzer at a rate of 50 $ml*min^{-1}$ using a syringe pump (model KDS230, KD Scientific INC., Holliston, MA, USA). Prior to each use, the analyzer was calibrated using a syringe containing outside air processed through the analyzer as described for the trial samples.

Oxygen consumption ($ml*min^{-1}$) was determined using the following equation:

$$[O_{2initial} - O_{2final}] \times V / T \quad (2),$$

Where *V* is the functional volume of the container (i.e., container volume minus animal volume, assuming a density of 1.0 g*cm^{-1}, which is similar to the density of 0.98 g*cm^{-1} suggested by Lighton (2008) when animal volume is unknown) and *T* is the time between sampling points in minutes.

Meta-analysis of Gila monster SMR

I combined the data from both of my experiments with those from Beck and Lowe (1994). Doing so allowed me to (1) determine whether SMR was consistent among experiments, and, if so, (2) determine the temperature-SMR relationship across a wider range of temperatures than those used in experiment 1. To adjust for differences in body mass across the various experiments, I calculated the allometrically scaled metabolic rates for my data (ml O_2 *hr^{-1}*g$^{-0.69}$, Beck and Lowe, 1994). I then examined the relationship between temperature and the allometrically scaled metabolic rate by performing regression analyses on the data.

Statistical Analysis

Experiment 1 – Thermal sensitivity of SMR

I analyzed the effects of temperatures on oxygen consumption (ml O_2*hr^{-1}) using a linear, mixed-model approach. Individual animals were included as a random factor. Following Zuur et al. (2009), I began by fitting a linear mixed model with all possible main effects, interactions, and random error fitted by restricted maximum likelihood (REML) using the "nlme" library (Pinheiro et al., 2011) of the R statistical package (version 2.13.1; R Development Core Team, 2011). I compared this model to a generalized least squares model with all possible main effects and interactions fitted with REML, but with random error excluded. I then refitted the linear mixed model with all possible main effects, interactions, and random error, and I progressively simplified the model by dropping the highest-order terms. These models were refitted using maximum likelihood. The best model was selected using AICc analysis (Anderson et al., 2001). AICc tables were calculated using the “AICcmodavg” library of the R statistical package.

Experiment 2 – Acclimation of SMR to Cold Temperatures

To determine whether and to what extent Gila monsters acclimate to cold temperatures and to assess the impact of cold exposure on SMR when re-exposed to 30°C, I examined the effect of time on the oxygen consumption (ml O_2*hr^{-1}) using a rmANOVA. The rmANOVA was completed using the “stats” library of the R statistical package.

Tables

Table 1: Model selection used to describe the relationship between SMR and temperature in Gila monsters using corrected Akaike information criterion (AICc). K = number of parameters in the function, w_{iAICc} = Akaike weight, Δ_{AICc} = difference in AICc values compared to "best" model, LL = log likelihood of each model. The model in boldface was selected for the analysis.

Model #	Description of Model	K	AICc	Δ_{AICc}	w_{iAICc}	LL
1 (Full model)	Y ~ Mass*Temperature + Temperature^2	7	201.94	4.11	0.05	-91.97
2	Y ~ Mass*Temperature	6	202.36	4.53	0.04	-93.73
3	Y ~ Temperature +Mass	5	200.75	2.92	0.10	-94.38
4	Y ~ Mass	4	258.23	60.40	0.00	-124.47
5	Y ~ Temperature	4	198.08	0.25	0.38	-94.39
6	Y ~ Temperature + Temperature^2	5	197.83	0.00	0.43	-92.91

Table 2: Statistical results for linear mixed-effects model fitted by restricted maximum likelihood.

Model #	Description of Model	AIC	Log Likelihood
5	Y ~ Temperature	197.67	-94.84

Figure Legends

Figure 1: Histogram depicting the frequency of occurrence of body temperatures during the overwintering period (hatched bars, n = 21,598) and active season (open bars, n = 43,325) for free-ranging Gila monsters. Data were collected as part of another study over the course of a single overwintering period and active season.

Figure 2: Mean oxygen consumption of adult Gila monsters across the range of temperatures that is most typical for free-ranging Gila monsters during their active season. The data are fitted with a linear mixed-effects model. Error bars indicate ± 1 standard error of the mean (s.e.m.).

Figure 3: The effect of temperature exposure duration on mean oxygen consumption for yearling Gila monsters. Brackets along the X-axis represent the temperature at which the animals were maintained during the days that fall within those brackets. Chamber temperature was changed on Day 9 and Day 46. Trial temperatures were chosen because 30°C approximates selected body temperature during the active season, while 16°C is the most common body temperature during overwintering. Error bars indicate ± 1 s.e.m.

Figure 4: Thermal sensitivity of allometrically mass-adjusted oxygen consumption from experiments 1 and 2 as well as from previously published work (Beck and Lowe, 1994). The light grey text and dashed line present a linear regression based solely on data from experiment 1, while the black text and solid line present a polynomial regression for all data. For experiment 2 data at 16°C, the upper point represents oxygen consumption prior to acclimation, while the lower point represents the mean of the post-acclimation values. Mass-adjusted consumption rates (using the allometric equation determined by Beck and Lowe, 1994) were used because of the considerable variation in animal size among studies. Error bars indicate ± 1 s.e.m.

Figure 5: Log-log relationship of the standard metabolic rate (SMR) at 25°C to body mass of individual adult Gila monsters from experiment 1 (n = 6, open circles) compared with adults of other lizard species (n = 33, closed triangles), snake species (n = 44, open squares), and previously collected data on the SMR of helodermatid lizards at 25°C from Beck and Lowe (1994; crosses). Linear log-log regressions for other adult lizards (solid line), adult snakes (dashed line), and helodermatids (dotted line) are plotted. All animals were measured under standard conditions (data from Andrews and Pough, 1985; Beck and Lowe, 1994, McCue and Lillywhite, 2002; and Greene et al., 2013). Data for the SMR of other adult lizards as well as helodermatids at 25°C were taken from Beck and Lowe (table 1 and Fig. 1, 1994) while data for the SMR of snakes at 25°C were either taken directly from Andrews and Pough, (1985), McCue and Lillywhite

(2002), and Greene et al. (2013) or, if SMR was measured at any temperatures other than 25°C, those SMR values were adjusted using either species-specific Q_{10} values or an average Q_{10} of 2.4 (average was calculated from reported and calculated values of Q_{10} of other species of snakes).

Figure 6: Comparison across taxa of the ratio of the depressed metabolic rate (DMR) during overwintering or aestivation against either SMR or resting metabolic rate (RMR). Open squares represent the reported or calculated ratios (DMR / SMR) for aestivating amphibians and reptiles, while open triangles represent calculated ratios (DMR / SMR or RMR) for hibernating amphibians and reptiles. All mammals for which data are reported here are hibernators. The closed circles represents the ratio of DMR to SMR for Gila monsters, using values of 0.79 ml O_2*hr^{-1} (mean SMR after acclimation to 16°C) and 7.09 ml O_2*hr^{-1} (mean SMR at 30°C), respectively.

Figure 7: Projected mean mass of fat utilized by free-ranging Gila monsters calculated using two different thermal sensitivity polynomial curves based on the polynomial regression presented in Figure 4 under different climate scenarios. The "current" climate scenario represents the calculated amount of fat burned given the actual body temperatures during the overwintering period (December 1st through February 28th) for the 2010 – 2011 season. I then calculated the mass of fat that would have been burned if the animal's body temperatures during the 2010 – 2011 overwintering period increased by 1°C, 3°C, and 5°C. This range of values is based on the projected increased air temperatures from 2010 – 2100 predicted for the American Southwest in the IPCC's 4th annual report (Christensen et al., 2007). The clear bars, designated "acclimated", were calculated using a polynomial regression similar to that presented in Figure 4, but excluding the non-acclimated value for 16°C ($y = 0.1948 – 0.0245x + 0.0009044x^2$). The black bars, designated "not-acclimated", were calculated using a similar approach but with the acclimated value at 16°C excluded ($y = 0.3785 – 0.03776x + 0.001141x^2$).

Figures

Figure 1

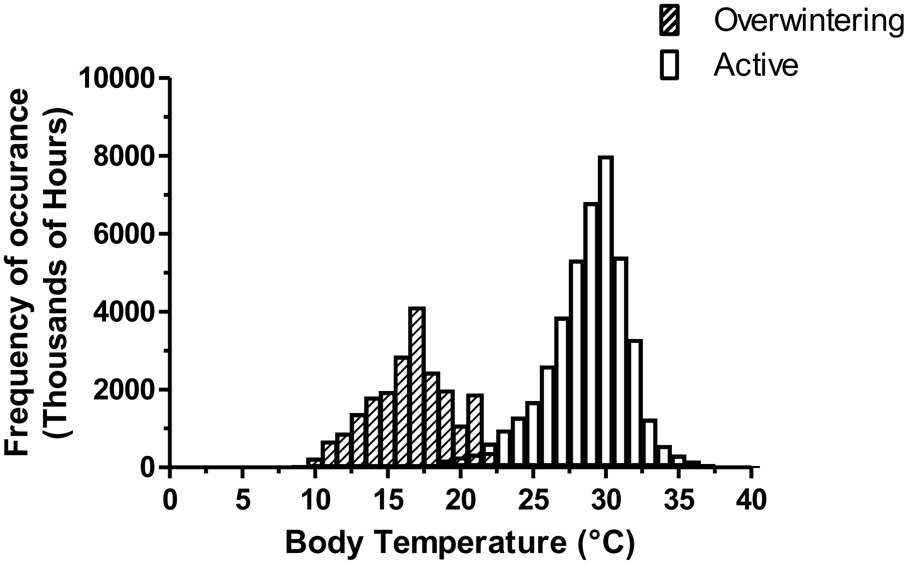


Figure 2

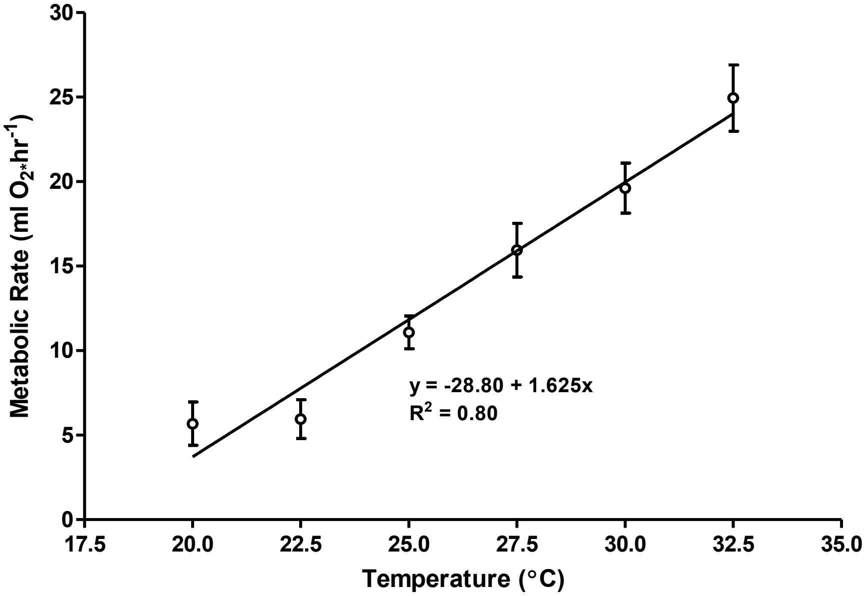


Figure 3

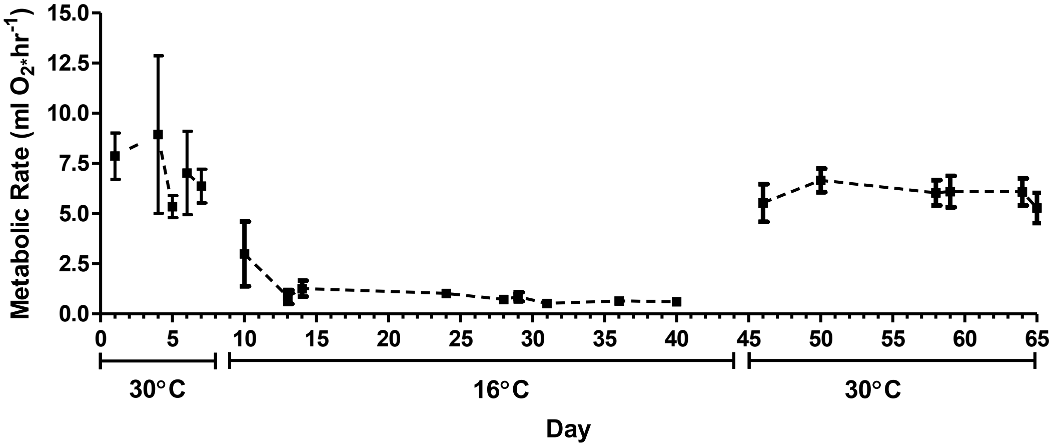


Figure 4

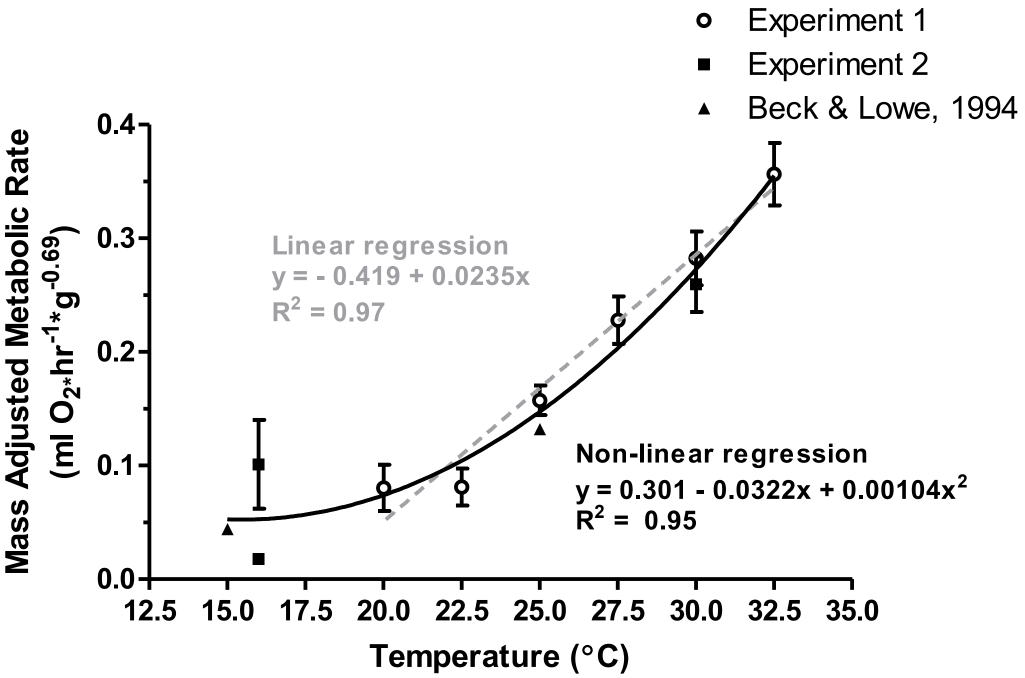


Figure 5

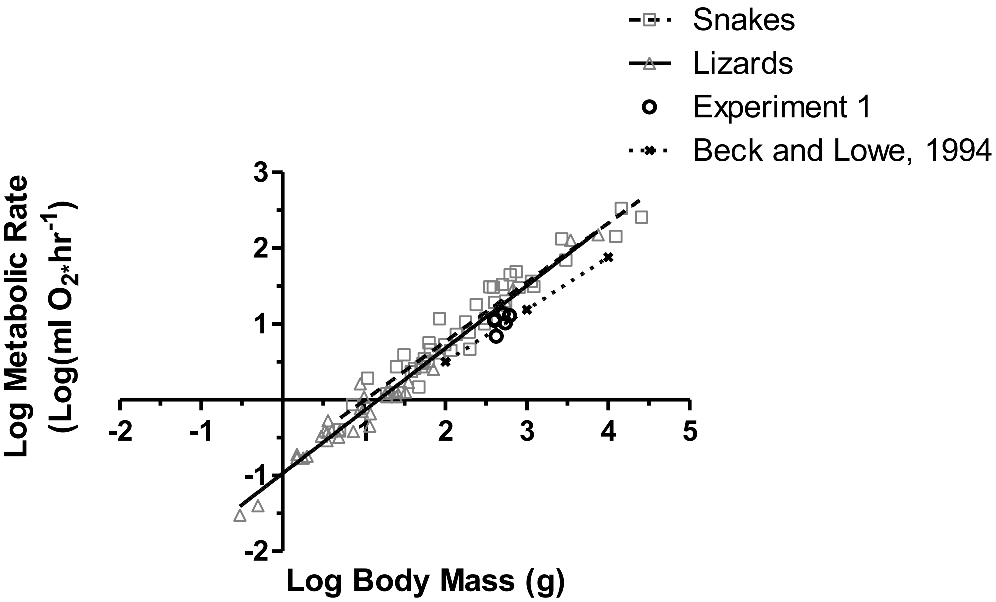


Figure 6

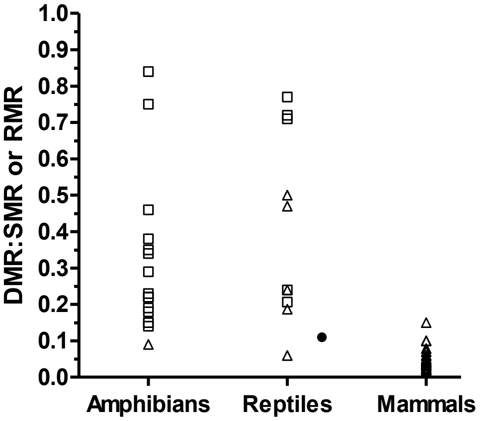
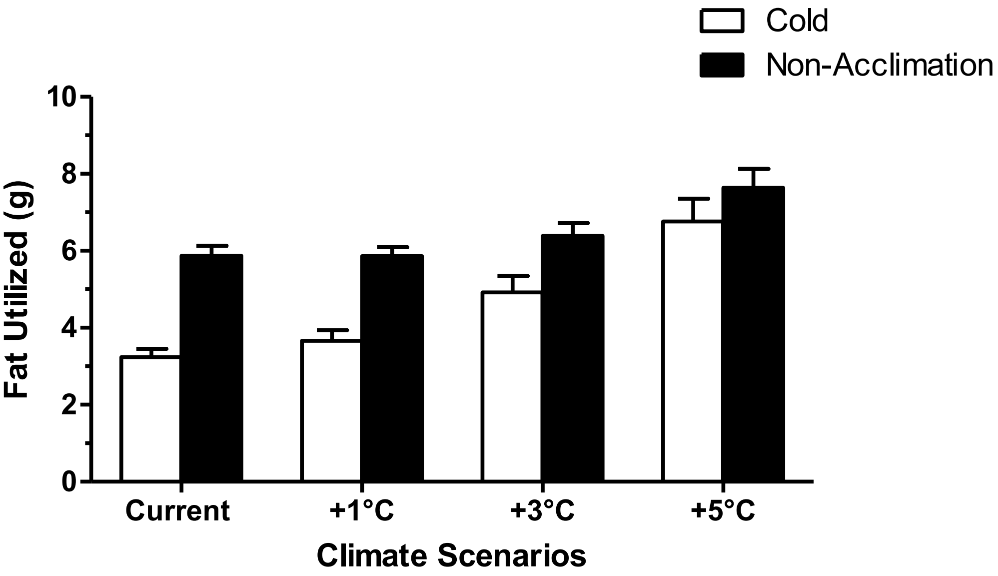


Figure 7


Cold
Non-Acclimation
Fat Utilized (g)
10
8
6
4
2
0
Current
+1°C
+3°C
+5°C
Climate Scenarios

CHAPTER 3

DO LOW ENERGY ORGANISMS PRACTICE STATE-DEPENDENT FORAGING STRATEGIES? INSIGHT FROM A SUPPLEMENTAL FEEDING STUDY

Introduction

Survival requires organisms to use a suite of activities to address various physiological needs including energy balance and water balance. The timing and duration of each activity is influenced by the interaction between environmental conditions and the physiological state of the organism. For example, foraging theory recognizes that organisms integrate information pertaining to environmental conditions and physiological state to optimize foraging behavior (Charnov, 1976). Although it is well established that food supply and predation risk are primary environmental drivers of foraging patterns in animals (Brown 1988, 1992, 1999; Abrams, 1991; McNamara and Houston, 1994; Olsson et al, 2002), energy demands and other physiological and ecological factors can be equally important in determining foraging activity (e.g., gut fullness, energy reserves, or free-standing water availability, Burrows and Hughes, 1991; Kotler et al., 1998; Aubret and Bonnet, 2005; Hochman and Kotler, 2006; Kotler et al., 2010). Foraging models recognize that organisms seek to maximize benefits associated with foraging (i.e., energy and or water intake) while minimizing costs (time allocation, predation risk, physiological costs of activities) (Clark, 1994; Nonacs, 2001), and this foraging strategy is termed SDF (Nonacs, 2001). Additionally, the APP (Clark, 1994) predicts that organisms will adjust their foraging strategies such that assets that are important for survival and fitness (e.g., energy or hydric reserves) are protected.

Although, there are a number of studies linking foraging decisions and physiological state, a vast majority of the work focuses on animals with high metabolic rates and thus high energy demands (i.e., “high-energy systems”, e.g., Brown et al., 1992; Olsson et al., 2002; Hahn et al., 2005; Kotler et al., 2010). There is also a fair amount of work that examines invertebrates and aquatic ectotherms (e.g., Burrows and Hughes, 1991; Metcalfe et al., 1998; Burrows et al., 2000; Koh and Li, 2003; Wojdak, 2009). However, little work has examined SDF in terrestrial vertebrate ectotherms, where energy demands are low and energy budgets balanced over extended periods of time (but see Aubret and Bonnet, 2005; Aubret et al., 2007). Studying SDF in vertebrate ectotherms, particularly those that feed infrequently will provide valuable insight into whether current theories regarding SDF are broadly applicable or are restricted to certain physiological or ecological conditions as well as the extent to which mechanisms driving SDF are conserved across species. Therefore, I examined the interaction between physiological state and foraging decisions in a low energy vertebrate ectotherm occupying a highly resource-limited environment.

The Gila monster is an excellent study organism for examining the applicability of SDF to low energy systems. The Gila monster is a relatively large lizard whose distribution is predominantly limited to the Sonoran Desert. Consequently Gila monsters must endure limited food availability, no free-standing water, and considerable thermal challenges during a relatively lengthy hot, dry season (mid-May through mid-July, Beck, 2005). They exclusively feed on the contents of vertebrate nests, a resource that is limited both temporally and spatially (Beck, 2005). To cope with temporal variation in food availability, Gila monsters tolerate extended periods of negative energy balance and, during those times, rely extensively on fat reserves. Because their prey is widely distributed spatially, Gila monsters dedicate a substantial amount of effort to foraging and are considered active foragers (Beck, 2005), however their energetic cost of locomotion is low in comparison to other squamate reptiles (John-Alder et al., 1983). To endure lengthy hot, dry periods, Gila monsters use their urinary bladder as a water reservoir (Davis and DeNardo, 2007) and, upon depletion of the reservoir, tolerate considerable increases in plasma osmolality (>360 $mOsm*kg^{-1}$, Davis and DeNardo, 2009, 2010). Furthermore, increased plasma osmolality leads to a reduction in surface activity, presumably to reduce water loss (Davis and DeNardo, 2009). During challenging environmental conditions, Gila monsters may spend 95% of their time in refugia (Beck, 1990; Beck, 2005; Davis and DeNardo, 2009, 2010). At the onset of the first summer rains, Gila monsters will binge drink free water, which rapidly returns them to a normosmotic state (Davis and DeNardo, 2007). With this return to normal hydration, surface activity greatly increases (Davis and DeNardo, 2009, 2010). Interestingly, despite the high water content of their meals (~70%), meal consumption provides little hydric benefit to Gila monsters (Appendix A, Wright et al., 2013). As such, energy and water represent discrete currencies that come from distinct sources.

Although there is considerable information on how hydric state and free-standing water availability influences Gila monster activity, there is a paucity of information on behavioral responses used to cope with temporal and spatial variability in food availability as well as the influence that energy and hydric states have on foraging decision. Thus, I performed a food-supplementation study on free-ranging Gila monsters to determine how meal consumption influences their physiology and behavior. I hypothesized Gila monsters use SDF strategies to co-manage their energetic and hydration states; however, because they must satisfy energy and water budgets with discrete currencies, their foraging behavior will be driven primarily by the most proximal need based on their physiological condition. I predicted that animals in our feeding supplementation, when compared to sham-manipulated animals, would experience an improved body condition, no difference in hydration state, reduced activity, and, as a consequence of reducing activity, reduced energy expenditure. To elucidate the mechanisms driving the SDF behavior, I also examined the seasonal energy budgets of Gila monsters. Specifically I calculated the energy expenditure associated with refuge occupation, surface activity, and the net energetic cost of foraging. I hypothesized that because Gila monsters occupy refugia for extended periods of time and because of their low cost of locomotion, a majority of the energy budget of Gila monsters is attributed to time Gila monsters spend at rest rather than surface active. Because of this, I further predicted that energy state will have less of an influence on foraging strategies of Gila monsters than hydric state.

Results

Surface Activity Estimates

The best model to examine the impact of season and treatment on the proportion of surface activity was the following model: proportion of time surface active ~ season + treatment with animal ID as a random factor and weighted variances for season to account for heterogeneity in the spread of the residuals for season (Table 3 and 4). This model revealed there was a significant effect of season on the proportion of time surface active (mixed-model ANOVA: $F_{2,20} = 84.82$, $P < 0.0001$, Fig. 8A). However, treatment did not have a significant effect on surface activity (mixed-model ANOVA: $F_{1,12} = 3.57$, $P = 0.0833$, Fig. 8A). Post-hoc analysis indicated that surface activity was significantly lower during the dry season when compared to either the spring or monsoon seasons; however activity during the spring and monsoon season was statistically the same.

Energy Expenditure per Day

The best model to examine the impact of season and treatment on the energy expenditure per day was the following model: energy expenditure per day ~ season with animal ID as a random factor and weighted variances for treatment to account for heterogeneity in the spread of the residuals for treatment (Table 5 and 6). This model revealed there was a significant effect of season on energy expenditure (mixed-model ANOVA: $F_{2,18} = 13.70$, $P > 0.0001$, Fig. 8B); however, as treatment was dropped from the model, treatment and the interaction between treatment and season did not have a significant effect on energy expenditure. Post-hoc analysis indicated that energy expenditure per day was significantly higher in the spring than either the dry or monsoon seasons. Additionally, Gila monsters expended the same amount of energy per day during the dry season as they did during the monsoon season.

Condition – Change in Tail Volume

The best model to examine the impact of season and treatment on the change in tail volume was the following model: change in tail volume ~ treatment with animal ID as a random factor (Table 7 and 8). This model revealed there was a significant effect of treatment on change in tail volume (mixed-model ANOVA: $F_{1,14} = 8.60$, $P = 0.0109$, Fig. 8C). Post-hoc analysis indicated that fed animals gained more tail volume (energy reserves) than sham manipulated animals.

Total Body Water

The best model to examine the impact of season and treatment on the proportion of body water in Gila monsters was the following model: proportion of body water ~ 1 with animal ID as a random factor and weighted variances for season to account for heterogeneity in the spread of the residuals for season (Table 9 and 10). This model revealed there was no effect of season, treatment, or a season*treatment interaction (Fig. 8D).

Hydration State

The best model to examine the impact of season and treatment on the osmolality of Gila monsters was the following model: plasma osmolality ~ sample period with animal ID as a random factor and weighted variances for sample period to account for heterogeneity in the spread of the residuals for osmolality between samplings (Table 11 and 12). This model revealed there was a significant effect of sampling period on plasma osmolality (mixed-model ANOVA: $F_{5,61} = 22.12$, $P < 0.0001$, Fig. 8E). The initial spring plasma osmolality of Gila monsters was significantly lower than the plasma osmolality taken from all other sampling periods, excluding the final sample taken in the monsoon. Additionally, plasma osmolality was significantly higher in both sampling periods in the dry season when compared to both the initial and final samples taken in the monsoon. Finally, the final sample collected in the spring was significantly higher than the final sample taken in the monsoon period.

Energy Expended Performing Different Activities

The best model to examine the impact of season, behavior, and treatment on energy expenditure of resting and active Gila monsters during the dry and monsoon seasons was the following model: energy expenditure ~ season*activity + treatment with animal ID as a random factor and weighted variances for behavior to account for heterogeneity in the spread of the residuals for energy expenditure (Table 13 and 14). This model revealed there was a significant interaction between season and behavior on the energy expended by Gila monsters in the two treatment groups, as well as significant main effects of season and behavior (mixed-model ANOVA – season*behavior: $F_{1,29} = 12.91$, $P = 0.0012$, season: $F_{1,29} = 5.44$, $P = 0.0268$, activity: $F_{1,29} = 28.03$, $P < 0.0001$, Fig. 9). Treatment did not significantly affect energy expenditure. Post-hoc analysis showed that Gila monsters expended significantly less energy associated with surface activity compared to refuge use during the dry season. Additionally, energy expenditure associated with inactivity was greater in the dry season than the wet season. Finally, Gila monsters expended more energy in the wet season while inactive than in the dry season when surface active.

Cost of Foraging

The best model to examine the impact of season and treatment on the non-transformed energetic cost of foraging for Gila monsters was the following model: energetic cost of foraging ~ season with animal ID as a random factor and weighted variances for treatment to account for heterogeneity in the spread of the residuals (Table 15 and 16). This model revealed there was not a significant main effect of season (mixed-model ANOVA – season: $F_{1,8} = 4.29$, $P = 0.072$, Fig. 10). Similarly, there was no significant effects of treatment or the season*treatment interaction on the energetic cost of foraging for Gila monsters. Post-hoc power analysis did reveal that, given the effect size observed between seasons, than an alpha of 0.05 provided sufficient power (> 0.8) to detect a significant difference amongst seasons.

Discussion

Food supplementation of Gila monsters led to an increase in energy stores but did not reduce surface activity or alter energy expenditures (resting, active, or total). Accordingly, the net cost of activity (which we equate to the cost of foraging, E_{CoF}) was not significantly different between fed and unfed animals. Hydration state was also unaffected by treatment and both groups exhibited seasonal changes in hydration state similar to those previously reported for free-ranging Gila monsters (Davis and DeNardo, 2009). I also compared the average field metabolic rate (FMR) value of sham-manipulated (CON) animals to the FMR of other reptiles (reported in Nagy et al., 1999) and found that Gila monster FMR is relatively quite low, much like other xeric species from the American Southwest (Fig. 11).

The Role of Energetic and Hydric Currencies in Satisfying Physiological Demands

Although food supplementation significantly improved the energy state of free-ranging Gila monsters, augmenting food intake failed to result in an improved hydration state for wild Gila monsters. Previous work examining the impact of water supplementation of free-ranging Gila monsters demonstrated that water augmentation significantly improved hydration state and resulted in increased surface activity compared to control animals. Water-supplemented animals also experienced a significant increase in tail volume relative to control animals, particularly during the dry season, which was attributed to increased foraging efforts because animals were no longer hydrically limited (Davis and DeNardo, 2009). Additional work has shown that meal consumption fails to improve the hydration state of Gila monsters regardless of their initial hydration state (Appendix A, Wright et al., 2013). Collectively, these results indicate that Gila monsters must rely on discrete currencies to separately satisfy energy and water budgets, relying on food consumption and free-standing water to meet energy and water demands, respectively. The complete reliance on two separate sources of energy and water income is uncommon for xeric reptiles and other xeric species (for examples see Thompson et al., 1997; Znari and Nagy, 1997; Nagy and Gruchacz, 1994; Ostrowski et al., 2002). Although a vast majority of SDF studies consider energetic state, no studies to my knowledge have taken into account how preformed dietary water nor hydration state affect behavioral decisions. Studies that do examine how the availability of oral free-standing water affects animal foraging did not consider the physiological condition of the organism (e.g., hydration state) (Kotler et al., 1998; Hochman and Kotler, 2006; Shrader et al., 2008). My results clearly show that hydration state can be an equally potent driver of activity, as both treatment groups significantly reduce activity when dehydrated during the dry season, and as such, future SDF analyses should consider the effect of energy and water balance as well as how meal consumption impacts both energy and water balance. The decoupling of sources of energy and water provides a unique opportunity to examine how energy intake and water availability differentially influence SDF in Gila monsters. As Gila monsters must satisfy energy and water demands via separate resources, they provide an excellent study system for examining the differential effects of increased food and water availability and or intake on foraging behavior in animals.

Energetic State, SDF, the APP, and Possible Mechanisms Driving Foraging Behavior

My results suggest that, in regards to energy balance and expenditure, Gila monsters do not use a SDF strategy and do not defend their energy assets, as fed animals did not exhibit changes in surface activity that were significantly different to that of sham animals. Sham and fed animals were similarly active, but the sham animals experienced either a loss or minimal gain in energy reserves while fed animals experienced an increase in energy reserves across the active season (Fig. 8A,C). These results do not align with the predictions set forth by the APP and SDF theory (Clark 1994; Nonacs, 2001) and thus indicate that, unlike other species, Gila monsters do not defend energy reserves. For example, Godfrey and Bryant (2000) found that reducing reserves increased the rate of foraging and thus energy expenditure in European robins, *Erithacus rubecula*. Aubret and Bonnet (2005) found that during sloughing events when eye opacity was greatest, well fed tiger snakes, *Notechis scutatus,* often refused to eat while less-fed snakes with lower body reserves consumed meals regardless of their eye opacity.

Although SDF has been observed in a wide variety of high-energy systems, SDF theory may not be broadly applicable to both high- and low-energy systems, likely because of different energetic demands and timeframes within which these different taxa must balance energy budgets. Gila monsters likely balance their energy budgets on either an annual or supra-annual basis (Chapter 4, Fig. 13A). High-energy systems must balance their energy budget on a shorter time scale and thus must frequently weigh costs and benefits associated with foraging. However, because they have lower metabolic demands, low-energy, infrequently feeding animals may simply be able to wait out extended periods without food availability or periods during which predation risk is high rather than optimize foraging behavior based on physiological condition, risk, and the marginal value of energy (Nonacs, 2001). However, my study is one of a select few examining the SDF strategies of low-energy, vertebrate ectotherms, and in reality our collective understanding of the foraging behavior of low-energy, infrequently-feeding vertebrate systems is still extremely limited. Thus future work should continue to examine the applicability of SDF theory and the APP in a wider array of low-energy systems.

One possible explanation for Gila monsters not using SDF to manage energy balance is that Gila monsters, because they are venomous, likely have a low predation risk which might be similar regardless of whether they are occupying a refuge (where they are dug up by large predators) or being surface active (where they may better defend themselves by more easily positioning their mouth towards the predator). Numerous studies have shown that predation risk is a potent driver of foraging behavior and both risk and energy state can interact to impact SDF strategies. Olsson et al. (2002) found that European starlings, *Sturnus vulgaris*, had higher giving-up density (GUD, with a high GUD reflecting reduced foraging effort) in patches with higher predation risk and in patches with more food. Additionally, starlings from high-quality habitats, and thus in better overall energy balance, foraged less intensely relative to birds from lower-quality habitats, lowering their predation risk because the marginal value of energy was perceived to be lower for birds from high-quality habitats. Kotler et al. (2010) found that Allenby's gerbils, *Gerbillus andersoni allenbyi*, increased vigilance and reduced foraging effort early in the lunar cycle when predation risk was highest. Thus for Gila monsters, unlike the aforementioned examples, the likely low overall predation risk couple with the risk of predation being comparatively equal regardless of their behavioral decision and their reliance on extensive energetic reserves may result in Gila monsters not using a SDF strategy to manage energy balance.

The energetic cost of activity may be another possible explanation as to why I observed no difference in the surface activity between food-supplemented (FED) and CON animals. Although my animals were inactive for extended periods of time (> 90% of the time during the dry season; > 70% of the time during the monsoon season, Fig. 8A), the energetic cost of foraging represented a significant, yet statistically equivalent cost for animals in both treatment groups. E_{CoF} comprises a significant percentage of their total seasonal energy budget during the dry and monsoon seasons for both sham-manipulated and fed animals (sham, dry season = 30%; sham, monsoon season = 33%; fed, dry season = 24%; fed, monsoon season = 34%, Fig. 3). Work examining the impact of E_{CoF} in other species has shown that the E_{CoF} can drive SDF behavior. Grubb and Greenwald (1982) found that when the energetic cost of foraging at two patches differed for house sparrows', *Passer domesticus*, but predation risk was the same, sparrows foraged at the less energetically costly patch. Additionally, when predation risk varied (but the energetic cost did not), sparrows foraged at the patches with less predation risk. Although the E_{CoF} comprised a large percentage of the total metabolism for Gila monsters in both treatment groups, as was likely the case with predation risk, E_{CoF} was equivalent across groups. A similar E_{CoF} between treatment groups may also help explain why I did not see a significant difference in activity levels between FED and CON animals; however, future studies should explicitly examine the impact of the cost of foraging on the SDF of Gila monsters and other low-energy systems so that we can better understand the extent to which foraging behavior of low-energy systems is driven by the energetic costs of foraging.

Hydration State and SDF

Although my results indicate that Gila monsters do not use a SDF strategy with regards to energy state, Gila monsters do defend their hydration state. My study, as well as previous studies, reveals that Gila monsters significantly reduced activity during the dry season when plasma osmolality is highest (Fig. 8A,E; Davis and DeNardo, 2009, 2010). The dry season is a considerable challenge to water balance because there is no free-standing water and dietary water in ingested meals does not significantly improve hydration state (Appendix A, Wright et al., 2013). Gila monsters supplemented with water during the dry season are normosmotic and have elevated surface activity relative to control lizards (Davis and DeNardo, 2009), providing further evidence that reduced activity during the dry season is a result of Gila monsters defending their hydric state. While my energy supplementation increased energy reserves (as measured in tail volume), it did not alter dry season foraging, suggesting that energy defense is not an intended benefit of reduced dry season activity. Although Gila monsters use a SDF strategy, the decoupling of hydric benefits from energy intake forces Gila monsters to protect hydric assets depending on their physiological condition and the availability of free-standing water.

Previous work examining the impact of water availability on the SDF behavior of organisms, albeit limited, has shown that free-standing water availability differentially impacts foraging strategies of organisms. Kotler et al. (1998) measured the GUD of the Australian raven, *Corvus coronoides*, and the sandy inland mouse, *Pseudomys hermannsburgensis*, in the presence or absence of adjacent drinking water. Ravens had a GUD that was 50% less in water-supplemented patches when compared to patches without free-standing water, indicating that ravens foraged more intensely when water was available. Conversely, the GUDs of sandy inland mice were unaffected by the presence of water. Shrader et al. (2008) found that when water was available, domesticated goats living in a semi-arid region had lower GUDs relative to patches where water was unavailable. Finally, Hochman and Kotler (2006) also found that when free-standing water was available, the Nubian ibex, *Capra nubiana*, foraged more intensely at a patch and thus had lower GUDs. One explanation for the variation in GUD in response to water availability may be the degree to which various sources of water (free-standing, dietary, and or metabolic water) contribute to hydration state. Although to my knowledge there is no information on the water budgets of sandy inland mice, numerous other rodent species are capable of satisfying their water balance through dietary and metabolic water (Nagy and Gruchacz, 1994). Conversely, although metabolic and dietary water can significantly contribute to their water balance, larger vertebrates must still periodically drink free-standing water in order to satisfy their water budgets (e.g., Schmidt-Nielsen et al., 1956; Ostrowski et al., 2002). As such, free-standing water availability may not have improved the overall quality of the patch for the sandy inland mice, while free-standing water was complementary to the other aforementioned species. Although free-standing water availability can differentially impact foraging behavior, hydration state can also be a potent driver of foraging, as I observed in my study and has been previously observed with Gila monsters (Davis and DeNardo, 2009). Work has shown that animals reduce meal consumption with increasing levels of dehydration (for examples, see Watts, 1999, Maloiy et al., 2008). As such, the foraging strategies of organisms may be impacted not just by the presence or absence of free-standing water, but also the degree to which various sources of water satisfy the water balance of a given organism as well as that organism's hydration state. As organisms become increasingly dehydrated, the value of a given patch of resources increases when water is present. Therefore, future studies examining the SDF behavior of organisms should incorporate measurements of the hydration state of a foraging animal as well as examine how various sources of water contribute to whole-organism water balance.

Ecological Implications

For many species, including xeric-dwelling species, increased ambient temperatures and reduction in rainfall events will likely become the norm as projected climate scenarios pan out. By the year 2100, models predict that the American Southwest will experience an approximately 1-4°C increase in temperature and as much as a 10-20% decrease in precipitation (IPCC, 2007). Such changes will likely challenge energy balance of desert organisms through the cumulative effects of elevated metabolic rate (Dillon et al., 2010), reduced foraging time (Sinervo et al., 2010), and reduced primary productivity (Breshears et al., 2005; Allen et al., 2010). While receiving less attention, anticipated climate change will similarly impact water balance. For small desert birds, the expected climate scenario may challenge survival because of its effect on water balance (McKechnie and Wolf, 2010). Similarly, the cumulative data for Gila monsters suggests that water, not energy, balance represents the most acute physiological demand and therefore drives activity decisions. Therefore, although examining the impact of rising temperatures on energy expenditure, foraging, and primary productivity is important, water availability and expenditures must be included in this suite of assessments in order to best predict the impact that climate change will have on desert organisms.

Materials and Methods

Study Site and Weather Conditions

The study was conducted over a single Gila monster active season (April through September, 2010) at a 3 km^2 long-term study site located in the Arizona Upland subdivision of the Sonoran Desert in Pinal County, Arizona (32°, 36'N, 111°, 07'W; 800-1,000 m elevation). The active season for Gila monsters in the Sonoran Desert is marked by three seasons which are categorized based on temperature and rainfall. The spring season (March to May) which is a cool, dry period; the dry season (May to late July) which has elevated air temperatures and no rainfall; and the monsoon season (late July to September) which is marked by elevated air temperatures with intermittent periods of heavy rainfall.

Throughout the study I recorded all rainfall events ($\geq$ 2 mm) and hourly shaded air temperature (T_{air}; ± 0.2°C) using automated loggers (rain gauge model RG3-M and StowAway Tidbit temperature logger, Onset Computer, Bourne, MA, USA) placed at a central location at the site. From the raw data from these loggers, I calculated total seasonal precipitation and daily maximum and minimum T_{air}.

Sample Sizes and Radiotelemetry

This research was conducted in accordance with ASU's IACUC under protocol 09-1044R and under the AZGFD scientific collecting permit SP577864. I captured 15 (7 male and, 8 female) adult Gila monsters at or near the beginning of the active season (late March through early April). Upon capture, each Gila monster was transported to ASU where I intracoelomically implanted both a radiotransmitter (13.0 g model SI-2, Holohil Systems, Carp, Ontario, CA) and a temperature logger (programmed to record temperature (±0.5°C) hourly for the duration of the study, Thermochron iButton model DS1922L, Maxim, Dallas, USA) into its cavity using previously described methods (Davis and DeNardo, 2009). After surgery but before recovery from anesthesia, I collected several measurements of body condition (see description below). Each animal was returned to its capture site within 72 hours of the initial capture. Gila monsters were then tracked weekly and, at the beginning of each month, captured to collect measurements of body condition and provide either the meal supplementation or sham procedure.

Meal Supplementation

I randomly assigned males and females separately to either a FED or CON treatment group. 3 males and 4 females were placed in the FED group while 4 males and 4 females were placed in the CON group. During the first 7-10 days of each month, all Gila monsters were captured to evaluate body condition and hydration state (see below) and then provided their treatment just prior to release. Animals in the FED group were fed two previously frozen but thawed mice (total mass = 60.0 ± 0.1 g). This meal represented approximately two-thirds of the average monthly caloric demand of free-ranging Gila monsters (D. F. DeNardo, personal communications). Large hemostats were used to place the meal into the back of the oral cavity of FED lizards and to administer the sham treatment (inserting the hemostats into the oral cavity without a meal) to the CON lizards. After feeding, I monitored each lizard for approximately 15 minutes to ensure that the animal did not regurgitate. The animal was then released at its point of capture. Although I could not control possible consumption of natural food resources, by giving the FED Gila monsters approximately two-thirds of their monthly caloric demand, I substantially augmented food intake but did not remove the need to forage to maintain energy balance.

Body Condition and Hydration State

During the initial, monthly, and final captures of each animal, I evaluated body condition and hydration state. I measured body mass (± 1 g) using either an electronic scale in the lab (Acculab, GS-2001, Edgewood, NY, USA) or a 1,000 g capacity spring scale (Pesola AG, Baar, CH). As body mass can be highly variable between samples in Gila monsters because of feeding, drinking, reproduction, and defecation, I also evaluated body condition by measuring tail volume since Gila monsters store fat in their tails (Bogert and Martin del Campo, 1956; Beck, 2005). I dipped the lizard's tail, up to the vent, into a 250 ml graduated cylinder filled with water. I then measured the volume (± 1 mL) of water displaced by the lizard's tail by refilling the 250 ml graduated cylinder from a second graduated cylinder (Davis and DeNardo, 2009).

To assess hydration state, I collected 0.25 ml blood from the caudal vein to measure plasma osmolality ($mOsm*kg^{-1}$) and total body water (the latter is described below). Samples were placed in screw-top vials and stored in a cooler until they were taken to the laboratory. In the lab, plasma was separated from whole blood by centrifugation. Approximately half of the plasma sample was flame-sealed within glass microcapillary tubes and then stored at 4°C for isotope analysis at a later date (see below). The remaining plasma was stored in sealed microcentrifuge tubes at -80°C until plasma osmolality was measured in triplicate using a vapor pressure osmometer (model 5500, Wescor, Inc., Logan, UT, USA) as previously described (Davis and DeNardo, 2007; Appendix A, Wright et al., 2013).

Field Metabolic Rate and Total Body Water Estimates

I assessed seasonal FMR ($kJ*day^{-1}$) and total body water content (mL) of each animal using the doubly labeled water technique (Lifson and McClintock 1966; Nagy, 1983). I assessed FMR over a period of approximately 30 days during each of the three seasons (spring: beginning of April to the beginning of May; dry, hot period: beginning of June to the beginning of July: monsoon: beginning of August to the beginning of September).

The previously described flame-sealed plasma samples from the months representing the beginning of a season were used to determine background isotopic composition of the animal. For these months, following the collection of the blood sample and body condition metrics but prior to administering the FED or CON treatment, I injected each animal with 0.15 mL of doubly labeled water at a 1 to 4 ratio of deuterium ($[^2H_2]^{16}O$) to oxygen-18 ($^1H_2[^{18}O]$). Twelve to 24 hrs post-injection we collected a second blood sample (0.15 mL) to determine post-injection isotopic enrichment in the animal. Pilot trials determined that 12 to 24 hours was necessary for the isotope to equilibrate in the body water (C. D. Wright, unpublished).

Isotopic water samples were extracted from plasma samples by cryogenic vacuum distillation (Ehleringer, 1989). Samples were then processed using a Laser Water Isotope Analyzer (DLT-100 Liquid Water Isotope Analyzer, Los Gatos Research, Mountain View, CA, USA) to determine deuterium to hydrogen-1 and oxygen-18 to oxygen-17 isotopic ratios. Standard waters were calibrated against the international standards SLAP and VSMOW and run with samples to provide corrections. Isotope ratios were expressed using the delta notation (δ) in parts per thousand (‰) as:

$$\delta X = (R_{\text{sample}}/R_{\text{standard}} - 1)\times 1000 \quad (3)$$

Where R_{sample} and R_{standard} are the molar ratios of $^{2}H/^{1}H$ or $^{18}O/^{17}O$ of the sample and reference, respectively. Samples were referenced against international standards VSMOW.

Total body water was calculated from the dilution of deuterium in the equilibrated post-injection blood samples. I calculated rates of CO_2 production by using the changes in isotopic enrichments between the post-injection blood samples at the beginning of a season's sampling period and the sample taken at the beginning of the next month (Lifson and McClintock 1966; Nagy 1980, 1983; Speakman, 1997). I averaged the two body masses taken at the time of the blood samplings and assumed that the mass-specific water pools were stable during the experimental period. I used an energy equivalence of 27.8 $kJ*L^{-1}$ CO_2 to convert the CO_2 production into energy expenditure and thus used a respiratory quotient of 0.71.

Surface Activity Estimates

At the end of the study, I removed the implanted transmitter and temperature loggers from each animal, and used the hourly body temperature data along with the shaded air temperature data to estimate surface activity of each animal using previously described Temperature-Based Activity Estimation (TBAE, Davis et al., 2008). TBAE is 96% accurate at predicting whether a Gila monster was above or below ground for a given hour (Davis et al., 2008). From these data, I calculated the proportion of time each Gila monster was surface active during each season. The hourly body temperatures were also used in calculating the energetic costs of refuge occupation and of foraging (described below).

Cost of Foraging

Using TBAE as described above, I determined the timing and duration of activity bouts and refuge occupations during the entire hot, dry season and monsoon season. I limited my analysis to only the dry and monsoon seasons, because these two seasons represent the extremes of environmental challenges and, during these seasons, surface activity of Gila monsters is predominantly for foraging. In contrast, during the spring, Gila monsters are also surface active to bask, and, for males, to search for mates (Beck, 2005). For each individual refuge occupation, I also calculated the mean body temperature.

Using the body temperature data, FMR data, TBAE estimated periods of refuge occupation and surface activity, and thermal sensitivity of metabolic rate data from another study (Chapter 2), I estimated several energy expenditures for each of the two seasons:

1) E_{total}, which is the total energy expenditure based on the doubly labeled water technique (described above). Additionally, for the FED group, I also subtracted from E_{total} the energy invested into the digestion of the meal provided during that sample period. Christel et al. (2007) found that the energetic cost of digesting a meal (termed specific dynamic action, or SDA, Secor, 2009) for a rodent meal that was 10% of a Gila monster's body mass was 60 kJ (Christel et al., 2007), while the SDA for a rodent meal

that was 15% of a Gila monster's body mass was 71 kJ (S. Secor, personal communications). The meals I provided were 14.5 ± 0.5% of the body mass of food-supplemented animals, so I subtracted 71 kJ from the initial E_{total} of FED animals.

2) E_{rest}, which is the total energetic expenditure during refuge use. E_{rest} was calculated by summing the estimated energy expended during each hour of refuge occupation. I estimated hourly energy expenditure by first converting the body temperature during each hour of refuge occupation to an estimate of hourly metabolic rate (ml O_2*hr^{-1}) using a non-linear regression of standard metabolic rate and temperature (Chapter 2). Hourly metabolic rate was then converted to energy expended (kJ) by multiplying the hourly metabolic rate by the animal's body mass raised to the 0.69 power (Beck and Lowe, 1994), and by 19.62 kJ*ml O_2^{-1} (I assumed the animals were catabolizing a ratio of 20:75:5 percent proteins to fats to carbohydrates, which is typical of a post-absorptive carnivore, Gessaman and Nagy, 1988).

3) E_{active}, which is the energy expenditure during activity. E_{active} was estimated by subtracting E_{rest} from E_{total}.

4) E_{min}, which estimates the energy that a Gila monster would have expended if it stayed in its refuge rather than becoming surface active. E_{min} was calculated much like E_{rest} except that I used the average temperature of the refuge occupation prior to a given bout of surface activity and the duration of a given surface activity event.

5) $E_{CoF,}$ where E_{CoF} equals E_{active} minus E_{min} (i.e., the additional energy expended by becoming surface active).

Statistical Analysis

Given that Gila monsters are secretive lizards and spend up to 95% of the time in refugia (Beck 1990; Beck, 2005; Davis and DeNardo 2010), I was unable to capture and collect data from animals during some sampling periods. To compensate for unbalanced data sets, I used a linear mixed-model approach to analyze my data. I analyzed the effects of season (spring, dry, and monsoon), treatment (fed versus sham-manipulation), and the season-treatment interactions on surface activity, energy expenditure per day, change in tail volume, total body water, plasma osmolality, and the cost of foraging. Additionally, I examined the effects of behavior (resting versus surface active), season, treatment, and their interactions on energy expenditure during the dry and monsoon seasons to determine differences between active and resting energy expenditure. Individual animals were included as a random factor. Following Zuur et al. (2009), I started out by fitting a linear mixed model with all possible main effects, interactions, and random error fitted by REML using the ‘‘nlme’ ’library (Pinheiro et al., 2011) of the R statistical package (version 2.13.1; R Development Core Team, 2011) and compared this model to a generalized least squares model with all possible main effects and interactions fitted with REML, but excluding random error. I then refitted the linear mixed model with all possible main effects, interactions, and random error, and I progressively simplified the model by dropping the highest-order terms. These models were refitted using maximum likelihood. Terms that were dropped from a given model did not significantly affect the response variable. The best model was selected using AICc which is a small sample version of AIC (Anderson et al., 2001). AICc tables were calculated using the "AICcmodavg" library of the R statistical package. The optimal model was selected as the model with the smallest AICc value (Zuur et al., 2009). The optimal model was then refitted with REML and I performed an ANOVA on said model to generate significance terms. Additionally, when significant main effects and or interactions were observed, I performed post-hoc analyses to determine differences between levels of a factor. I used the package "multcomp" and "lsmeans" to determine differences across factors for main effects and interactions, respectively.

Tables

Table 3: Model selection used to describe the relationship between surface activity, season, and treatment in Gila monsters using corrected Akaike information criterion (AICc). K = number of parameters in the function, w_{iAICc} = Akaike weight, Δ_{AICc} = difference in AICc values compared to “best” model, LL = log likelihood of each model. The model in boldface was selected for the analysis.

Model #	Description of Model	K	AICc	Δ_{AICc}	w_{iAICc}	LL
1 (Full model)	Y ~ season*treatment	10	-100.15	7.26	0.01	64.48
2	**Y ~ season + treatment**	**8**	**-107.42**	**0.00**	**0.52**	**64.37**
3	Y ~ season	7	-107.16	0.25	0.46	62.58
4	Y ~ treatment	6	-68.95	38.10	0.00	40.66
5	Y ~ 1, 1 = no interaction or main effect	5	-68.95	38.47	0.00	41.92

Table 4: Statistical results for linear mixed-effects model fitted by REML.

Model #	Description of Model	AIC	Log Likelihood
2	Y ~ season + treatment	-85.45	50.73

Table 5: Model selection used to describe the relationship between energy expenditure per day and season and treatment in Gila monsters using corrected Akaike information criterion (AICc). K = number of parameters in the function, w_{iAICc} = Akaike weight, Δ_{AICc} = difference in AICc values compared to “best” model, LL = log likelihood of each model. The model in boldface was selected for the analysis.

Model #	Description of Model	K	AICc	Δ_{AICc}	w_{iAICc}	LL
1 (Full model)	Y ~ season*treatment	9	188.09	8.99	0.01	-81.30
2	Y ~ season + treatment	7	182.30	3.19	0.17	-81.99
3	**Y ~ season**	**6**	**179.10**	**0.00**	**0.82**	**-82.00**
4	Y ~ treatment	5	191.42	12.31	0.00	-89.64
5	Y ~ 1, 1 = no interaction or main effect	4	188.66	9.56	0.01	-89.64

Table 6: Statistical results for linear mixed-effects model fitted by REML.

Model #	Description of Model	AIC	Log Likelihood
3	Y ~ season	172.16	-80.08

Table 7: Model selection used to describe the relationship between change in tail volume, season, and treatment in Gila monsters using corrected Akaike information criterion (AICc). K = number of parameters in the function, w_{iAICc} = Akaike weight, Δ_{AICc} = difference in AICc values compared to "best" model, LL = log likelihood of each model. The model in boldface was selected for the analysis.

Model #	Description of Model	K	AICc	Δ_{AICc}	w_{iAICc}	LL
1 (Full model)	Y ~ season*treatment	8	50.32	5.67	0.05	-14.54
2	Y ~ season + treatment	6	49.81	4.87	0.07	-17.40
3	Y ~ season	5	53.91	8.96	0.01	-20.92
4	**Y ~ treatment**	**4**	**44.94**	**0.00**	**0.80**	**-17.81**
5	Y ~ 1, 1 = no interaction or main effect	3	49.74	4.79	0.07	-21.48

Table 8: Statistical results for linear mixed-effects model fitted by REML.

Model #	Description of Model	AIC	Log Likelihood
4	Y ~ treatment	49.20	-20.60

Table 9: Model selection used to describe the relationship between the proportion of body water in Gila monsters and season and treatment using corrected Akaike information criterion (AICc). K = number of parameters in the function, w_{iAICc} = Akaike weight, Δ_{AICc} = difference in AICc values compared to "best" model, LL = log likelihood of each model. The model in boldface was selected for the analysis.

Model #	Description of Model	K	AICc	Δ_{AICc}	w_{iAICc}	LL
1 (Full model)	Y ~ season*treatment	10	-119.47	5.32	0.03	73.81
2	Y ~ season + treatment	8	-121.66	3.13	0.10	71.31
3	**Y ~ season**	**7**	**124.79**	**0.00**	**0.48**	**71.26**
4	Y ~ treatment	6	-121.20	3.59	0.08	67.96
5	Y ~ 1, 1 = no interaction or main effect	5	-123.96	0.83	0.31	67.92

Table 10: Statistical results for linear mixed-effects model fitted by REML.

Model #	Description of Model	AIC	Log Likelihood
4	Y ~ season	-106.87	60.43

Table 11: Model selection used to describe the relationship between the plasma osmolality of Gila monsters and season and treatment using corrected Akaike information criterion (AICc). K = number of parameters in the function, w_{iAICc} = Akaike weight, Δ_{AICc} = difference in AICc values compared to "best" model, LL = log likelihood of each model. The model in boldface was selected for the analysis.

Model #	Description of Model	K	AICc	Δ_{AICc}	w_{iAICc}	LL
1 (Full model)	Y ~ month*treatment	19	704.88	15.55	0.00	-327.31
2	Y ~ month + treatment	14	691.76	2.43	0.23	-328.75
3	**Y ~ month**	**13**	**689.33**	**0.00**	**0.77**	**-328.99**
4	Y ~ treatment	9	733.50	44.17	0.00	-356.50
5	Y ~ 1, 1 = no interaction or main effect	8	732.01	42.68	0.00	-357.02

Table 12: Statistical results for linear mixed-effects model fitted by REML.

Model #	Description of Model	AIC	Log Likelihood
4	Y ~ month	657.40	-315.70

Table 13: Model selection used to describe the relationship between energy expended performing different activities and season, behavior, and treatment in Gila monsters using corrected Akaike information criterion (AICc). K = number of parameters in the function, w_{iAICc} = Akaike weight, Δ_{AICc} = difference in AICc values compared to "best" model, LL = log likelihood of each model. The model in boldface was selected for the analysis. We did not perform a compressive model analysis (dropping all terms) because our analysis indicated that season*behavior interaction could not be dropped from the model.

Model #	Description of Model	K	AICc	Δ_{AICc}	w_{iAICc}	LL
1 (Full model)	Y ~ season*treatment*behavior	11	520.78	5.52	0.02	-245.51
2	Y ~ season*treatment* + season*behavior + behavior*treatment	10	517.72	2.47	0.11	-245.72

3	Y ~ season*treatment* + season*behavior	9	516.23	0.98	0.24	-246.61
4	Y ~ season*treatment + treatment*behavior	9	527.05	11.80	0.00	-252.02
5	Y ~ season*activity + treatment* behavior	9	516.58	1.32	0.20	-246.79
6	Y ~ season*treatment + behavior	8	524.33	9.08	0.00	-252.22
7	**Y ~ season*behavior + treatment**	**8**	**515.25**	**0.00**	**0.39**	**-247.68**
8	Y ~ treatment*behavior + season	8	526.10	10.84	0.00	-253.10
9	Y ~ season + behavior + treatment	7	523.55	8.29	0.01	-253.30
10	Y ~ season + behavior	6	522.43	7.17	0.01	-254.14
12	Y ~ season + treatment	6	529.53	14.27	0.00	-257.69
13	Y ~ behavior + treatment	6	524.20	8.95	0.00	-255.02
14	Y ~ season	5	528.72	13.47	0.00	-258.61
15	Y ~ treatment	5	531.26	16.01	0.00	-259.88
16	Y ~ behavior	5	522.35	7.10	0.01	-255.43
17	Y ~ 1	4	529.67	14.41	0.00	-260.35

Table 14: Statistical results for linear mixed-effects model fitted by REML.

Model #	Description of Model	AIC	Log Likelihood
7	Y ~ season*behavior + treatment	473.88	-228.94

Table 15: Model selection used to describe the relationship between the cost of foraging and season and treatment in Gila monsters using corrected Akaike information criterion (AICc). K = number of parameters in the function, w_{iAICc} = Akaike weight, Δ_{AICc} = difference in AICc values compared to "best" model, LL = log likelihood of each model. The model in boldface was selected for the analysis.

Model #	Description of Model	K	AICc	Δ_{AICc}	w_{iAICc}	LL
1 (Full model)	Y ~ season*treatment	7	276.60	7.88	0.01	-127.57
2	Y ~ season + treatment	6	272.44	3.72	0.07	-127.60
3	**Y ~ season**	**5**	**268.72**	**0.00**	**0.47**	**-127.60**

4	Y ~ treatment	5	272.45	3.73	0.07	-129.46
5	Y ~ 1, 1 = no interaction or main effect	4	269.18	0.46	0.37	-129.48

Table 16: Statistical results for linear mixed-effects model fitted by REML.

Model #	Description of Model	AIC	Log Likelihood
3	Y ~ season	250.59	-120.29

Figure Legends

Figure 8: Seasonality of A) surface activity, B) energy expenditure, C) change in tail volume, D) proportion of body mass that is water, and E) plasma osmolality. For osmolality, there were two point samples, an initial and final, taken during each season. Hatched bars represent animals in the sham treatment group and filled bars are animals in the fed treatment group. Letters represent significant differences across sampling time points. "*" represents an overall treatment effect. Error bars are ± 1*s.e.m.

Figure 9: Energy expenditures of fed and sham manipulated Gila monsters at rest (filled bars) and during activity (unfilled bars). The hatched bars represent E_{min}, or the energy that a Gila monster would have expended if it stayed in its refuge rather than becoming surface active. The % values inside the unfilled bars represent the percent of total metabolism that is accounted for by the cost of foraging. "*" represents significant differences across time points or between treatment groups within a given season. Error bars are ± 1*s.e.m.

Figure 10: The energetic cost of foraging of free-ranging Gila monsters during the dry and monsoon seasons. Hatched bars represent animals in the sham treatment group and filled bars are animals in the fed treatment group. Error bars are ± 1*s.e.m.

Figure 11: Regression of the log mass versus log field metabolic rate (FMR) for data reported for "all reptiles" from Nagy et al., 1999 (small grey "x"s) and from Gila monsters (this study, enlarged filled triangle). The solid line and dashed lines represents the best fit line and the 95% confidence intervals, respectively, for the Nagy (1999) data. Reptiles that fall well below the 95% confidence interval are identified.

Figures

Figure 8

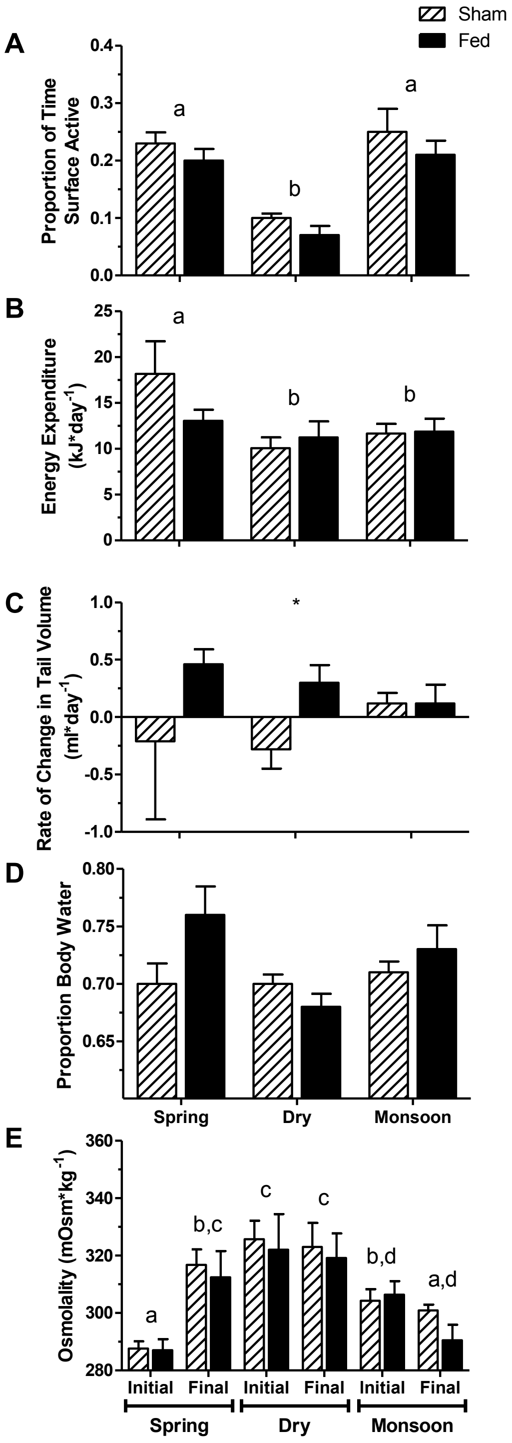


Figure 9

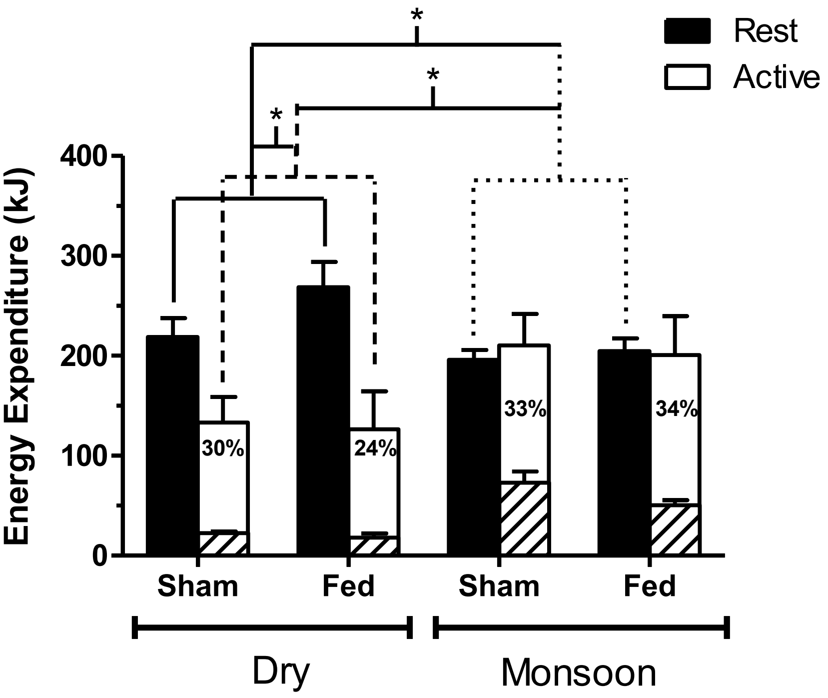


Figure 10

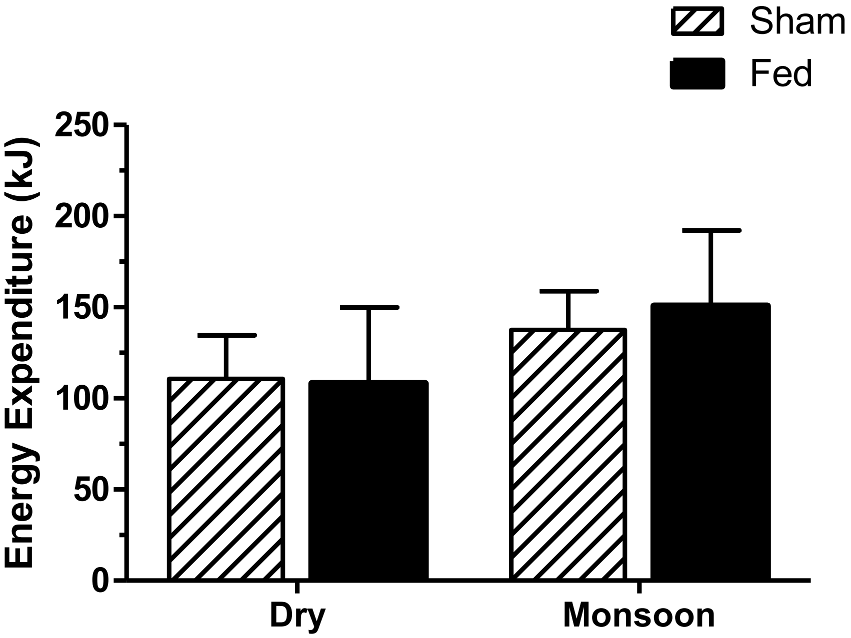
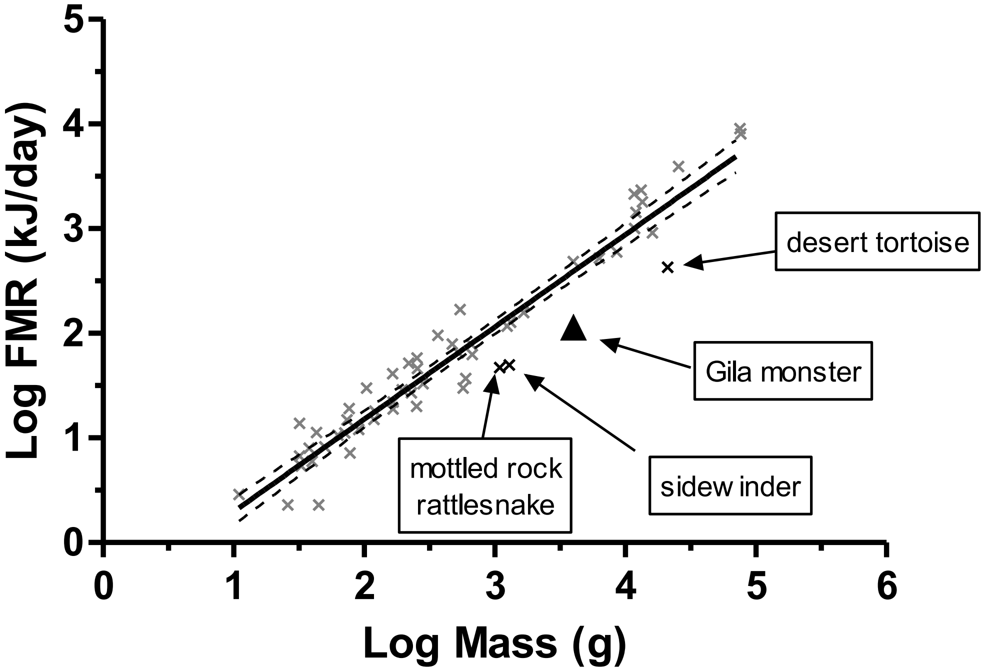


Figure 11



Log FMR (kJ/day)
Log Mass (g)
0
1
2
3
4
5
6
desert tortoise
Gila monster
mottled rock rattlesnake
sidew inder

CHAPTER 4

THE IMPACT OF INDIVIDUAL VARIATION IN PHYSIOLOGICAL STATE ON FORAGING BEHAVIOR ACROSS MULTIPLE TIME SCALES: IMPLICATIONS FOR FUTURE STUDIES EXAMINING STATE-DEPENDENT FORAGING

Introduction

Foraging is a critical behavior that, if successful, satisfies the energetic and, in some species, hydric needs of an organism. Foraging decisions integrate information regarding environmental conditions and physiological state and foraging theory recognizes that organisms seek to optimize foraging behavior (Charnov, 1976). It is well established that food supply and predation risk are important factors influencing foraging patterns in animals (i.e., Brown 1988, 1992, 1999; Abrams, 1991; McNamara and Houston, 1994; Olsson et al., 2002). However, other physiological and ecological factors can be just as critical in influencing foraging activity (e.g., energy demands, energy reserves, gut fullness, or free-standing water availability, Burrows and Hughes, 1991; Kotler et al., 1998, 2010; Aubret and Bonnet, 2005; Hochman and Kotler, 2006; Aubret et al., 2007). Research on the foraging behavior of organisms now recognizes that organisms optimize their behavior such that benefits associated with foraging are maximized while potential costs and risk are mitigated. Thusly, assets that are critical for survival and fitness are protected. These strategies are often referred to as SDF (Nonacs, 2001) and the APP, Clark, 1994).

Although there is a large body of research examining SDF, our understanding of how physiological condition impacts the foraging strategies of a broad array of taxa is still limited. For example, most SDF research has focused on animals with high metabolic rates and thus high energy demands (i.e., high-energy systems such as birds and mammals) and little is known regarding whether low metabolism, infrequently feeding terrestrial vertebrates (i.e., low-energy systems as exemplified by many squamates reptiles) use SDF strategies (but see Aubret and Bonnet, 2005; Aubret et al., 2007). Additionally, the vast majority of studies examining SDF consider only the energetic state of the forager while only a limited number of studies have examined how water availability impacts foraging behavior (e.g., Kotler et al., 1998; Hochman and Kotler, 2006; Shrader et al., 2008). Even for studies considering how free-standing water availability impacts the foraging strategies of organisms, to date none have explicitly examined how the organism's hydration state impacts foraging decisions and whether variation among species may be attributable to the relative importance of the various water sources (free-standing, dietary, and metabolic). Such information may explain why free-standing water availability differentially affects foraging behavior across species (e.g., Kotler et al., 1998; Hochman and Kotler, 2006; Shrader et al., 2008).

SDF research has greatly benefitted from the use of manipulative, field-based experiments that help to identify factors that influence foraging decisions. However, it is equally critical to examine long-term (e.g., multi-year) data sets of unmanipulated organisms to ascertain whether manipulative experiments effectively predict responses to natural variation in environmental and physiological conditions.

Studying long-term variation in the foraging activity of natural animals may become increasingly important in lieu of observed and anticipated climate change. From 1956 to 2005, Earth's air temperature has risen 0.13°C per decade (doubling the trend from 1906 to 1956; IPCC, 2007). Moreover, precipitation patterns have changed substantially (e.g., reduced precipitation in subtropical/arid regions), and the number of heat waves has become more frequent (IPCC, 2007). Climate models now predict (globally) future increases of approximately 0.2°C per decade, an increase in the frequency and duration of heat wave events, and continued shifts in precipitation patterns, (Meehl and Tebaldi, 2004; Tebaldi et al., 2006; IPCC, 2007). A large body of work has shown that there are behavioral and physiological consequences associated with observed and anticipated climate change. Temperature, in particular, has received a great deal of interest with regards to its impacts on the energetics of ectotherms, as metabolism is a non-linear function of body temperature. Additionally, elevated temperatures coupled with more variable precipitation may lead to a reduction in net primary productivity (Breshears et al., 2005; Allen et al., 2010) and, therefore, reduced food availability for organisms. Elevated body temperatures are predicted to increase metabolic rates (Dillon et al., 2010) as well as reduce foraging time (Sinervo et al., 2010), which could potentially strain energy budgets and lead to local species extinction (Sinervo et al., 2010). However, even within ectotherms, there may be variation in the extent to which elevated metabolic rates and reduced foraging time will impact energy balance and thus fitness and survivorship. Low-energy squamates reptiles, for example, can balance their energy budgets over long periods. For example, the aspic viper, *Vipera aspis*, exhibits changes in maternal body condition that cycle annually to supraannually based on the timing of reproduction and parturition (Bonnet et al., 1999). As such, the effect of acute elevations in temperature (e.g., heat waves) on the energy budgets of ectotherms may be minimal. However, the additive effect of long-term elevated ambient temperatures and reduced rainfall may prevent even low-energy systems from maintaining energy balance.

Although elevations in ambient temperature may have important long-term energetic consequences, they may have a more potent acute influence on water balance. Intense heat waves, which are predicted to become more frequent, are expected to have a dramatic effect on acute survivorship of small desert birds through their impact on water balance (McKechnie and Wolf, 2010). Even if non-lethal, negative impacts of climate change on water balance can have broad implications on an organism including indirect effects on energy balance as dehydration can reduce meal consumption (Watts, 1999; Maloiy et al., 2008) and foraging activity (Davis and DeNardo, 2009). Clearly, expected temperature and precipitation changes will not act distinctly on energy and water balance, respectively, but, instead, will interact to jointly affect both physiological processes.

Broadening our understanding of the interaction between the physiological state (e.g., energetic and hydration states) and foraging strategies in a wide array of taxa across different time scales (e.g., seasonal versus multi-year) will provide a more extensive understanding of how anticipated changes in temperature and rainfall patterns may affect both energy and water balance of organisms. As such, I investigated the extent to which individual variation in the physiological state of free-ranging, low-energy ectotherms in a resource limited environment affected surface activity, and thus foraging behavior, across multiple time scales (seasonal and multi annual).

The Gila monster is an excellent study organism for examining how interannual variation in physiological condition affects foraging behavior within the context of climate change and SDF. Gila monsters are a long-lived (20+ years), medium-sized lizard whose primary range lies within the Sonoran Desert (Beck, 2005). As such, Gila monsters cope with limited food availability, no free-standing water, and considerable thermal challenges during the hot, dry season (mid-May through mid-July, Beck, 2005). They are also vertebrate-nest specialists, feeding exclusively on the contents of vertebrate nests, a resource that is both temporally and spatially limited (Beck, 2005). To cope with temporal variation in food availability, Gila monsters tolerate extended periods of negative energy balance and rely extensively on fat reserves. Because their prey is widely distributed spatially, Gila monsters invest a significant amount of time and energy foraging (Beck, 2005); however they occupy sub-surface refugia 70 to 90% of the time (Beck, 1990; Beck, 2005; Davis and DeNardo, 2009). Unlike most species Gila monsters utilize a water reservoir, the urinary bladder, to endure lengthy hot, dry periods (Davis and DeNardo, 2007). Once Gila monsters deplete their hydric reserves, they experience significant increases in plasma osmolality (>360 $mOsm*kg^{-1}$, Davis and DeNardo, 2009, 2010). Increased plasma osmolality results in a concomitant reduction in surface activity, which is thought to reduce water loss (Davis and DeNardo, 2009). At the onset of the first summer rains, Gila monsters binge drink free-standing water, returning their osmolality to a normosmotic state within 24 to 48 hours (Davis and DeNardo, 2007). Interestingly, Gila monsters are incapable of improving their hydric state via meal consumption (Appendix A, Wright et al., 2013). Thus, Gila monsters maintain energetic and water balance using discrete currencies from two difference sources, food and free-standing water (Appendix A, Wright et al., 2013). Manipulative studies providing supplemental water or food have demonstrated that activity is driven by hydration state rather than energy reserves and meal consumption (Davis and DeNardo 2009; Chapter 3).

In this study, I sought to determine whether previous experimental results (Chapter 3) accurately reflect the relationship between physiological state and activity based on natural individual variation in physiological state. Based on previous results, I predicted that natural hydric condition would be correlated with surface activity (an indicator of foraging activity), but surface activity will not correlate with energy reserves.

Results

Surface Activity and Tail Volume

The best model to examine the impact of tail volume, season, and year on surface activity was the following model: proportion of time surface active ~ season + year with animal ID as a random factor and weighted variances for season to account for heterogeneity in the spread of the residuals for season (Table 17 and 18). This model revealed there was a significant effect of season and year on the proportion of time Gila monsters were surface active (mixed-model ANOVA: season – $F_{1,26} = 291.33$, $P < 0.0001$; year – $F_{2,26} = 12.00$, $P << 0.0001$, Figs 12A, 13C). As this model dropped tail volume from the analysis, I also used this model to examine the difference in surface activity across season and year.

Thus, there was no significant effect of tail volume on surface activity. Post-hoc analysis indicated that surface activity was significantly lower during the dry season when compared to the monsoon season and animals were significantly more active in 2010 compared to 2005 and 2006 active season; however there was no difference in activity between 2005 and 2006.

Surface Activity and Osmolality

The best model to examine the impact of osmolality, season, and year on surface activity was the following model: proportion of time surface active ~ season + year*osmolality with animal ID as a random factor and weighted variances for season to account for heterogeneity in the spread of the residuals for season (Table 19 and 20). This model revealed there was a significant effect of season, year, and osmolality on the proportion of time surface active (mixed-model ANOVA: season – $F_{1,25} = 260.38$, $P < 0.0001$; year - $F_{2,25} = 4.40$, $P = 0.0231$; osmolality – $F_{1,25} = 8.51$, $P = 0.0074$, Fig. 12B). Additionally, there was a significant interaction between year and osmolality (mixed-model ANOVA: $F_{2,25} = 3.50$, $P = 0.0456$, Fig. 12B). Post-hoc analysis indicated that surface activity was significantly lower during the dry compared to the monsoon season; additionally, surface activity was significantly lower during the 2005 season compared to the 2010 season, while there was no difference in activity between 2006 and either 2005 or 2010.

Tail Volume across Seasons and Years

The best model to examine the impact of season and year on tail volume was the following model: change in tail volume ~ season with animal ID as a random factor

(Table 21 and 22). This model revealed there was no effect of year, season, or a year*season interaction on the change in tail volume for Gila monsters (mixed-model ANOVA: season – $F_{1,28} = 2.75$, $P = 0.1083$, Fig. 13A).

Plasma Osmolality across Seasons and Years

The best model to examine the impact of season and year on plasma osmolality was the following model: plasma osmolality ~ month*year with animal ID as a random factor and weighted variances to account for heterogeneity in the spread of the residuals across months (Table 23 and 24). This model revealed there was a significant interaction between season*year (mixed-model ANOVA: season*year – $F_{1,25} = 10.16$, $P = 0.006$, Fig. 13B). Post-hoc analyses revealed that the plasma osmolality of Gila monsters in the dry season of 2006 was significantly higher than the plasma osmolality of Gila monsters in the monsoon season of the 2005, 2006, and 2010 field season. Additionally, the plasma osmolality of Gila monsters in the dry season of 2005 was significantly lower than the plasma osmolality of Gila monsters in the dry season of 2006. Additionally, the plasma osmolality of Gila monsters in the dry season of 2010 was significantly higher than the plasma osmolality of Gila monsters in the 2005 dry season or the monsoon season of 2006 and 2010.

Discussion

Broader Contributions to SDF Theory

My results demonstrate that the foraging behavior of Gila monsters is insensitive to changes in energy balance (Fig. 12A,B). The lack of an effect of energy reserves on surface activity is likely because Gila monsters balance their energy budgets annually to supraannually, as Fig. 13A shows that Gila monster tail volume is not significantly different across seasons or years. My results align with previous findings (Chapter 3) and demonstrate that Gila monsters do not use a SDF strategy based on energy reserves. These results based on natural variation are in agreement with previous results from manipulative experiments that show, despite enhancing energy reserves, food supplementation has no effect on foraging activity (Chapter 3).

Although there was no relationship between energy reserves and surface activity, surface activity is sensitive to changes in hydration state, particularly as Gila monsters become moderately and severely dehydrated (Fig. 12B). The impact of hydration state on surface activity is likely due to the fact that, unlike energy balance, Gila monsters balance their hydric budgets within a single active season (Fig. 13B). My results here are in agreement with previous studies (Chapter 3), which found that both food-supplemented and sham-manipulated Gila monsters experienced a significant increase in plasma osmolality as well as a concomitant reduction in surface activity during the dry season. Additionally, water supplementation enhanced both plasma osmolality and surface activity of Gila monsters during the dry season (Davis and DeNardo, 2009). Collectively these results suggest that Gila monsters use a SDF strategy with regards to hydration state influencing surface activity. However, the findings by Davis and DeNardo (2009) also suggest that Gila monsters do not defend hydration state during the dry season, as defense of hydric reserves would imply that, regardless of hydration state, Gila monsters would reduce their surface activity when free-standing water is unavailable. Although my results do not agree with the findings by Davis and DeNardo (2009), my work broadens our understanding of the role of hydration state in driving the surface activity of Gila monsters. My results show that wild, free-ranging Gila monsters during the dry season do defend their hydration state, as Fig. 1B shows that despite similar plasma osmolalities in the dry season and monsoon season; Gila monsters in the dry season have significantly reduced surface activity relative to animals in the monsoon season.

The lack of sensitivity of foraging behavior to changes in energy reserves for Gila monsters does not mirror what has been regularly observed in a number of taxa (Burrows and Hughes, 1991; Brown et al., 1992; Metcalfe et al., 1998; Burrows et al., 2000; Olsson et al., 2002; Koh and Li, 2003; Hahn et al., 2005; Wojdak, 2009; Kotler et al., 2010). Additionally, my results do not match those from the only other low-energy, infrequently feeding vertebrate ectotherm, the tiger snake, *Notechis scutatus*. For tiger snakes, energetic state influences foraging behavior (Aubret and Bonnet, 2005; Aubret et al., 2007).

Energy reserves may not significantly impact the foraging behavior of Gila monsters because they have an extremely low standard metabolic rate, even relative to other squamate reptiles (Beck and Lowe, 1994; Chapter 2, Fig. 5) and their seasonal energy expenditure is significantly lower than other squamates based on their body size (Chapter 3, Fig. 11). Their low energy expenditure, in conjunction with the fact that Gila monsters rely on extensive fat reserves that do not significantly fluctuate annually or supraannually (Fig. 13A), suggests that Gila monsters have ample opportunities to balance their energy budgets throughout a single active season. The extended timescale with which Gila monsters balance their energy budgets may thus reduce the overall impact that biological factors such as lipid reserve levels and predation risk have on the foraging behavior of Gila monsters, unlike in other species where energy demands may have to be balanced over shorter periods of time. However, given the paucity of information regarding the applicability of SDF and the APP to low-energy, infrequently feeding systems, there is a need for more studies examining the interaction between physiological conditions and foraging behavior in other low-energy, infrequently feeding systems.

Previous studies, albeit limited in number, have revealed variable results regarding the presence of water on SDF (Kotler et al., 1998; Hochman and Kotler, 2006; Shrader et al., 2008). Furthermore, none of the studies considered the extent to which free-standing water contributes to water balance or how variation in hydration affects foraging strategy. All of these studies supplemented free-standing without considering the relative importance of water source (free-standing, dietary, metabolic) to the species' water balance. This alone could explain the variable results. Species that rely on free-standing water, whether exclusively or in part may be particularly responsive to water availability and hydration state. Given that Gila monsters are atypical in their ability to maintain an internal water reservoir (Davis and DeNardo, 2007), I would expect that activity of other species that are reliant on free-standing water would be even more sensitive than Gila monsters to hydric condition. Clearly, if we are to better understand the broad applicability of foraging theories and the relative importance of energy and water to foraging decisions, it is crucial that we consider the timescales within which organisms balance their energy and water budgets (which are influenced by rates of expenditures and storage capabilities), the relative importance of the different sources of water, and the environmental limitation of resources.

Implications Associated with Global Climate Change

Recently, a large body of work has focused on examining the effect that anticipated changes in climate will have on the physiology and behavior of organisms. In particular, a great deal of effort has focused on examining how increasing temperature will affect the energy balance of organisms, particularly for small vertebrate ectotherms (which serve as an excellent model for examining the impact of elevated temperatures on energy balance). Continued elevation of ambient temperature have been shown to alter sex-ratios of hatchling reptiles and result in lethally high incubation temperatures for developing turtle offspring (Hawkes et al., 2007; Schwanz and Janzen, 2008). Elevated ambient temperatures are also predicted to constrain energy budgets of ectotherms by reducing foraging time (Sinervo et al., 2010), elevating metabolic rate (Dillon et al., 2010), and reducing food availability through reduced primary productivity (Breshears et al., 2005; Allen et al., 2010). Energetic constraints could results in mortality and ultimately lead to local extinction events if climate change follows current projected scenarios (Sinervo et al., 2010). However, such outcomes for ectotherms require long-term impacts, since these species have relatively low metabolic rates and many are capable of maintaining substantial energy stores. Therefore, an increased frequency and intensity of heat waves will likely have limited effect on the overall energy balance of ectotherms that have lengthy energy budget timescales. Additionally, even long-term chronic changes in temperature may have limited impact on energy balance of these species if they simply shift the seasonality of peak energy acquisition (e.g., to earlier in the spring).

Although less considered to date, a more immediate threat to survival may be the impact on water balance from the anticipated reduction and increased variability in precipitation events coupled with extended droughts and more frequent heat waves (Meehl and Tebaldi, 2004; Tebaldi et al., 2006; IPCC, 2007). Water availability greatly influences the ability of organisms to cope with elevated temperatures. For example, small desert birds are inactive during the midday summer heat and thus do not have access to water during this period. Anticipated elevations in maximum daily temperatures will elevate midday evaporative water loss rates beyond sustainable levels and likely jeopardize survival, particularly of smaller species (McKechnie and Wolf, 2010). Such dramatic effects on water balance can be realized as a result of a single extremely hot afternoon, which is not the case for energy balance.

The consequences of climate change on water balance may be exacerbated in organisms that rely on free-standing water. Even the Gila monster, which possesses an abnormally large internal water reservoir, emphasizes water balance in making activity decisions under current climate conditions. Expected climate change is likely to reduce free-standing water availability in deserts, and thus further emphasize the importance of prioritizing water balance. Whether this will be the case for other species, especially those that are similarly reliant on free-standing water but lack a physiological buffer to environmental water shortages, requires further studies that integrate a thorough assessment of the temporal aspects of energy and water balances with expected changes in both temperature and precipitation.

Materials and Methods

Study Site and Weather Conditions

The study was conducted over three Gila monster active season (April through September, 2005, 2006, and 2010) at a previously described 3 km^2 site in the Arizona Upland subdivision of the Sonoran Desert in Pinal County, Arizona (32°, 36’N, 111°, 07’W, 800 –1,000 m elevation) (Davis and DeNardo, 2009).

During each field season, rainfall ($\geq$ 2 mm) was measured using an automated rainfall gauge (model RG3-M, Onset Computer, Bourne, MA, USA) left continuously in the field, and T_{air} (±0.2°C) recorded hourly using an automated temperature logger (StowAway Tidbit, Onset Computer, Bourne, MA, USA) placed at a central location at the site. Direct solar radiation on the temperature data logger was reduced by shading the logger in an uncapped PVC tube hanging vertically from a tree branch approximately 1 m above the ground. For the 2005 season, problems with the rainfall gauge required the use of rainfall data from the nearest National Climate Data Center, Picacho Arizona, climate station (32°39’N, 111°24’W, 557 M; COOP 026513) (Davis and DeNardo, 2009). Comparison of data from this climate station and my field site during the 2006 and 2010 seasons demonstrated that the two sites had similar rainfall patterns.

Sample Sizes and Radiotelemetry

All research was conducted in accordance with ASU's IACUC (protocol 01-671R for 2005 and 2006, protocol 09-1044R for 2010) and under AZGFD scientific collecting permits (SP683420, SP739769 and SP577864 for 2005, 2006, and 2010, respectively). I included 30 (2005 – 5M : 6F; 2006 – 6M : 6F; 2010 – 4M : 3F) adult Gila monsters in my analysis. All Gila monsters were collected at or near the beginning of the active season (late March through early April) and, upon capture, each animal was transported to ASU where a radiotransmitter (13.0 g model SI-2, Holohil Systems, Carp, Ontario, CA) and a temperature data logger (Thermochron iButton, model DS1922L, Maxim, Dallas, USA) were intracoelomically implanted using a technique similar to that used in previous studies (Taylor et al., 2004). Throughout the active season (beginning of April through the beginning of September), animals were tracked weekly while body condition and hydration state were assessed monthly.

Body Condition and Hydration State

Measurements of body condition and hydration state were collected during the first 7 to 10 days of each month. Processing of each animal was mostly done at the site of capture, but also done in the lab if the animal was being returned to the lab for surgery at the beginning and end of the study. I measured body mass using a 1,000 g capacity spring scale (Pesola AG, Baar, CH). As body mass can be highly variable between samples in Gila monsters because of feeding, drinking, reproduction, and defecation, body condition was also inferred by measuring tail volume (± 1 mL), since Gila monsters store fat caudally (Bogert and Martin del Campo, 1956; Beck, 2005). Tail volume was measured using water displacement from a graduated cylinder.

Hydration state was assessed using plasma osmolality (mOsm*kg^{-1}). 0.15 – 0.20 mL of blood was collected from the caudal vein using a heparanized 1 mL syringe. Samples were stored in screw-top vials and stored in a cooler until they were taken back the laboratory. Plasma was then separated from whole blood by centrifugation and stored in sealed containers at -80°C until the samples were analyzed in triplicate using a vapor pressure osmometer (model 5500, Wescor, Inc., Logan, UT, USA) using a previously described calibration and analysis procedure (Davis and DeNardo, 2007; Appendix A, Wright et al., 2013).

Surface Activity Estimates

Hourly temperature data from the surgically implanted data loggers were used to estimate the surface activity of each animal. At the end of the active season, all lizards were brought to ASU to have their temperature loggers surgically removed and downloaded. Surface activity was determined using TBAE which compares body temperature (T_b) and T_{air} data (Davis et al., 2008). TBAE is 96% accurate at predicting Gila monster surface activity and refuge occupation during each hour (Davis et al., 2008). Using this technique, I calculated seasonal proportions of hours that animals were surface active (number of hours on surface/total hours). During the hot, dry season and monsoon Gila monster surface activity is almost exclusively attributable to foraging.

Statistical Analysis

To compensate for unbalanced data sets, I used a linear mixed-effects model approach to determine the effects season (dry and monsoon), year (2005, 2006, and 2010), and either plasma osmolality or tail volume have on surface activity. Following Zuur et al. (2009), I started out by fitting a linear mixed model with all possible main effects, interactions, and random error fitted by REML using the ‘‘nlme’ ’library (Pinheiro et al., 2011) of the R statistical package (version 2.13.1; R Development Core Team, 2011). I then compared this model to a generalized least squares model with all possible main effects and interactions fitted with REML, but excluding random error. I refitted the linear mixed model with all possible main effects, interactions, and random error, and I progressively simplified the model by dropping the highest-order terms. These models were refitted using maximum likelihood. Terms that were dropped from a given model did not significantly affect the response variable. The best model was selected using AICc, which is a small sample version of AIC (Anderson et al., 2001). AICc tables were calculated using the “AICcmodavg” library of the R statistical package. The optimal model was selected as the model with the smallest AICc value (Zuur et al., 2009). The optimal model was then refitted with REML and I performed an ANOVA on said model to generate significance terms. Additionally, when significant main effects and or interactions were observed, I performed post-hoc analyses to determine differences between levels of a factor. I used the package “multcomp” and “lsmeans” to determine differences across factors for main effects and interactions, respectively.

Tables

Table 17: Model selection used to describe the relationship between surface activity and tail volume, season, and year for free-ranging Gila monsters using corrected Akaike information criterion (AICc). K = number of parameters in the function, w_{iAICc} = Akaike weight, Δ_{AICc} = difference in AICc values compared to "best" model, LL = log likelihood of each model. The model in boldface was selected for the analysis.

Model #	Description of Model	K	AICc	Δ_{AICc}	w_{iAICc}	LL
1 (Full model)	Act ~ Month*Year*TV	15	-163.99	19.96	0.00	104.06
2	Act ~ Month*Year + Month*TV + Year*TV	13	-167.21	16.75	0.00	101.66
3	Act ~ Month*Year + Month*TV	11	-172.98	10.98	0.00	100.96
4	Act ~ Month*Year + Year*TV	12	-170.76	13.20	0.00	101.60
5	Act ~ Month*TV + Year*TV	11	-173.02	10.94	0.00	100.98
6	Act ~ Month*Year + TV	10	-176.28	7.67	0.02	100.96
7	Act ~ Year + Month*TV	9	-178.50	5.46	0.05	100.50
8	Act ~ Month + Year*TV	10	-176.09	7.87	0.01	100.86
9	Act ~ Month + Year + TV	8	-181.47	2.48	0.21	100.49
10	Act ~ Month + TV	6	-166.86	17.10	0.00	90.41
11	Act ~ Year + TV	7	-116.84	67.12	0.00	66.75
12	**Act ~ Month + Year**	**7**	**-183.96**	**0.00**	**0.71**	**100.31**
13	Act ~ TV	5	-107.61	76.34	0.00	59.49
14	Act ~ Year	6	-119.49	64.47	0.00	66.72
15	Act ~ Month	5	-168.74	15.21	0.00	90.05
16	Act ~ 1	4	-109.89	74.07	0.00	59.39

Table 18: Statistical results for linear mixed-effects model fitted by REML for the best fitting model from table 1.

Model #	Description of Model	AIC	Log Likelihood
12	Act ~ Month + Year	-155.69	84.85

Table 19: Model selection used to describe the relationship between surface activity and plasma osmolality, season, and year for free-ranging Gila monsters using corrected Akaike information criterion (AICc). K = number of parameters in the function, w_{iAICc} = Akaike weight, Δ_{AICc} = difference in AICc values compared to "best" model, LL = log likelihood of each model. The model in boldface was selected for the analysis.

Model #	Description of Model	K	AICc	Δ_{AICc}	w_{iAICc}	LL
1 (Full model)	Act ~ Month*Year* Osm	15	-178.99	16.71	0.00	111.16
2	Act ~ Month*Year + Month* Osm + Year*TV	13	-185.94	9.76	0.00	110.76
3	Act ~ Month*Year + Month*Osm	11	-185.70	10.01	0.00	107.15
4	Act ~ Month*Year + Year*Osm	12	-189.08	6.63	0.02	110.54
5	Act ~ Month*Osm + Year*Osm	11	-192.90	2.80	0.12	110.75
6	Act ~ Month*Year + Osm	10	-188.73	6.97	0.02	107.05
7	Act ~ Year + Month*Osm	9	191.47	4.23	0.06	106.88
8	**Act ~ Month + Year*Osm**	**10**	**-195.70**	**0.00**	**0.50**	**110.53**
9	Act ~ Month + Year + Osm	8	-194.30	1.40	0.25	106.82
10	Act ~ Month + Osm	6	-175.72	19.99	0.00	94.76
11	Act ~ Year + Osm	7	-109.94	85.76	0.00	61.10
12	Act ~ Month + Year	7	-190.26	5.44	0.03	103.40
13	Act ~ Osm	5	-105.65	90.05	0.00	58.48
14	Act ~ Year	6	-103.25	92.45	0.00	58.56
15	Act ~ Month	5	-177.90	17.80	0.00	94.60
16	Act ~ 1	4	-113.35	82.36	0.00	61.10

Table 20: Statistical results for linear mixed-effects model fitted by REML for the best fitting model from table 3.

Model #	Description of Model	AIC	Log Likelihood
8	Act ~ Month + Year*Osm	-126.12	73.06

Table 21: Model selection used to describe the relationship between tail volume, season, and year in Gila monsters using corrected Akaike information criterion (AICc). K = number of parameters in the function, w_{iAICc} = Akaike weight, Δ_{AICc} = difference in AICc values compared to “best” model, LL = log likelihood of each model. The model in boldface was selected for the analysis.

Model #	Description of Model	K	AICc	Δ_{AICc}	w_{iAICc}	LL
1 (Full model)	TV ~ Year*Month	8	396.80	5.03	0.03	-188.64
2	TV ~ Year + Month	6	392.94	1.18	0.19	-189.49
3	TV ~ Year	5	393.02	1.26	0.18	-190.83
4	**TV ~ Month**	**4**	**391.76**	**0.00**	**0.34**	**-191.44**
5	TV ~ 1	3	392.18	0.42	0.27	-192.83

Table 22: Statistical results for linear mixed-effects model fitted by REML.

Model #	Description of Model	AIC	Log Likelihood
4	TV ~ Month	383.68	-187.84

Table 23: Model selection used to describe the relationship between plasma osmolality, season, and year in Gila monsters using corrected Akaike information criterion (AICc). K = number of parameters in the function, w_{iAICc} = Akaike weight, Δ_{AICc} = difference in AICc values compared to “best” model, LL = log likelihood of each model. The model in boldface was selected for the analysis.

Model #	Description of Model	K	AICc	Δ_{AICc}	w_{iAICc}	LL
1 (Full model)	**Osm ~ Year*Month**	**9**	**433.11**	**0.00**	**0.94**	**-205.36**
2	Osm ~ Year + Month	7	444.43	11.33	0.00	-213.91
3	Osm ~ Year	6	445.48	12.38	0.00	-215.79
4	Osm ~ Month	5	439.73	6.63	0.03	-214.20
5	Osm ~ 1	4	441.16	8.05	0.02	-216.14

Table 24: Statistical results for linear mixed-effects model fitted by REML.

Model #	Description of Model	AIC	Log Likelihood
1	Osm ~ Year*Month	398.57	-190.29

Figure Legends

Figure 12: Relationship between surface activity and A) tail volume and B) plasma osmolality across seasons and years. Unfilled symbols represent values from the dry season while filled symbols represent values from the monsoon season. Squares, triangles, and diamonds represent the 2005, 2006, and 2010 field seasons, respectively. The solid lines represent a linear regression that combines all data.

Figure 13: Plots depicting how A) tail volume, B) plasma osmolality, and C) surface activity vary between seasons and among years. Unfilled columns represent the dry season; filled columns represent the monsoon season. Letters represent significant differences between seasons and or a season*year interaction, while an “*” indicates a significant difference between years. Error bars are ± 1 s.e.m..

Figures

Figure 12

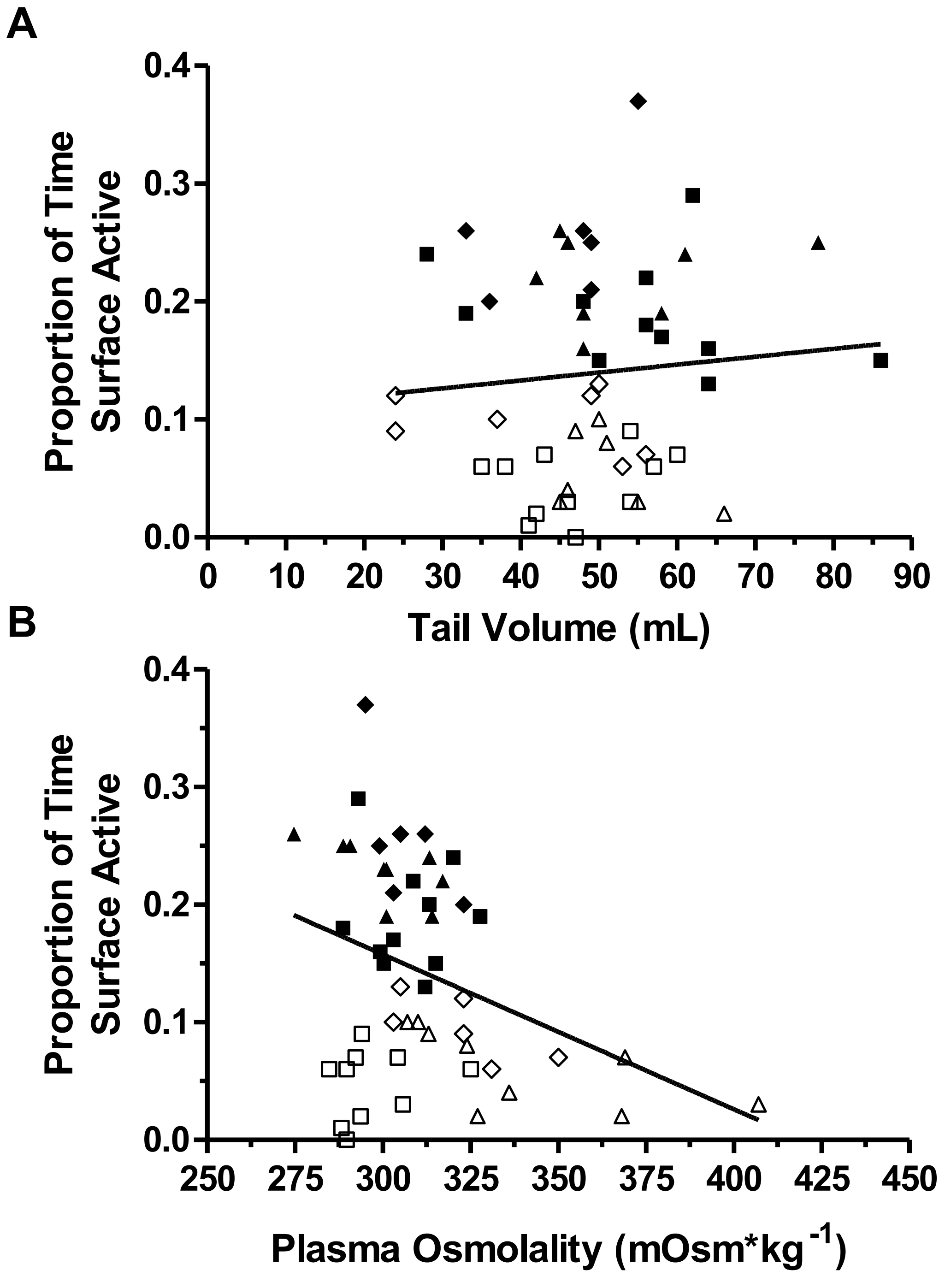
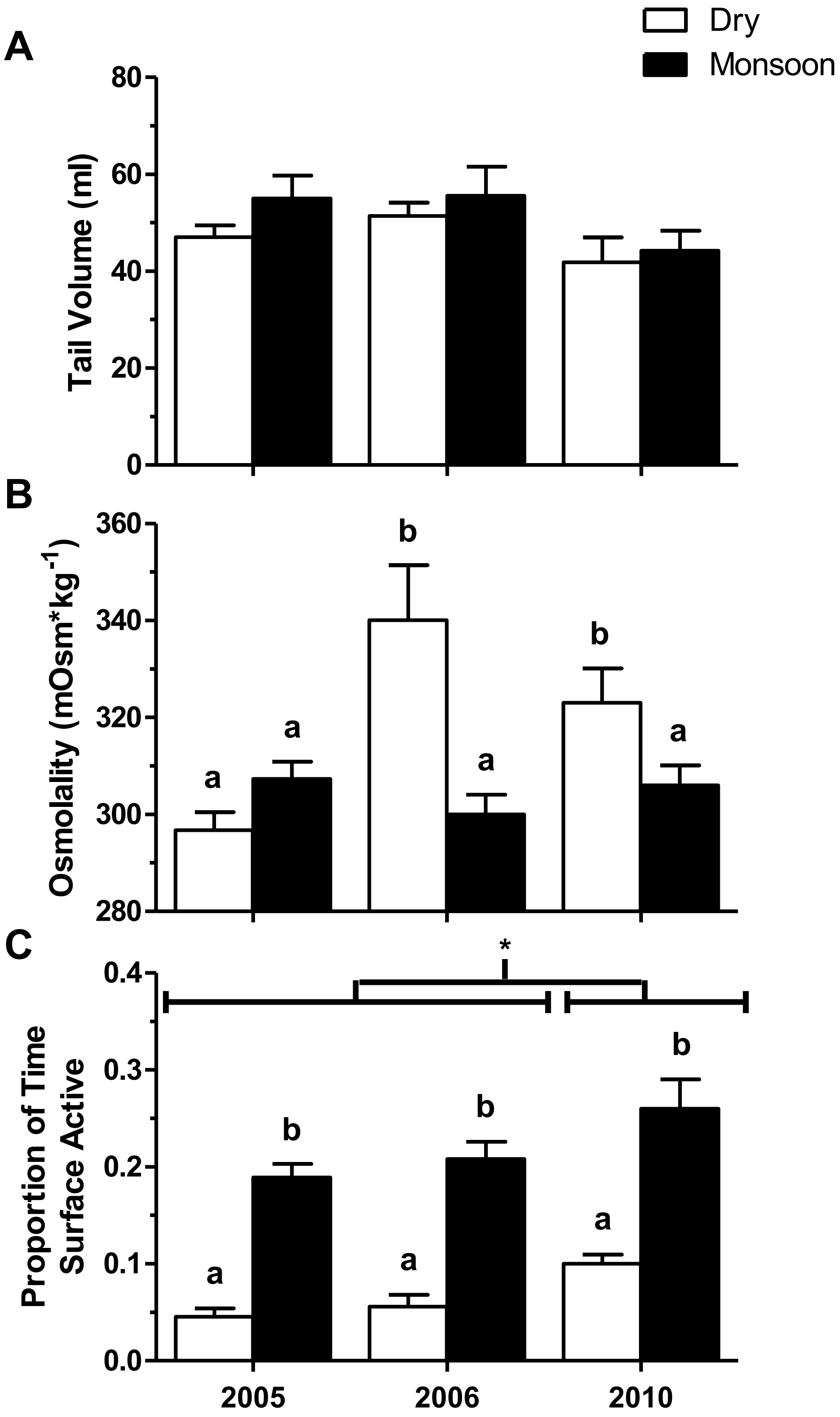


Figure 13



Dry
Monsoon
A
80
60
40
20
0
Tail Volume (ml)
B
360
340
320
300
280
Osmolality (mOsm*kg^{-1})
a
a
b
a
b
a
C
*
0.4
0.3
0.2
0.1
0.0
Proportion of Time Surface Active
a
b
a
b
a
b
2005
2006
2010

CHAPTER 5

CONCLUSIONS

My work revealed that Gila monsters do not use an SDF strategy to manage their energy reserves nor do they defend their energetic assets (Chapters 3 and 4). Food supplementation of free-ranging Gila monsters enhanced energy reserves, but did not affect surface activity, which serves as an indicator of time foraging, particularly during the dry and monsoon seasons (Chapter 3, Fig. 8A,C). Similarly, I found no correlation between surface activity and energy stores among unmanipulated, free-ranging Gila monsters (Chapter 4, Fig. 12A).

These findings contrast with what has been observed in a wide variety of taxa, that energy reserves and energetic state significantly affect foraging behavior (Godfrey and Bryant, 2000; Olsson et al., 2002; Kotler et al., 2010; Wojdak, 2009). This is also true for the only other terrestrial ectotherm studied the tiger snake (*Notechis scutatus*, Aubret and Bonnet, 2005; Aubret et al., 2007). Ours is the first study demonstrating that energy state does not influence foraging behavior, and thus questions the universality of SDF theory. Energetic state may not be a potent driver of foraging behavior for Gila monsters because they balance their energy budgets supraannually (Chapter 4, Fig. 12A) rather than over shorter periods of time (e.g., seasonally, daily) and because they have relatively low energy expenditure, even for a squamate reptile (Beck and Lowe, 1994; Chapter 2, Fig. 5). Coupled with the use of extensive energy reserves, Gila monsters simply may not have to use a SDF strategy to manage energy reserves and thus can avoid risk-inducing behaviors such as exposure to predation or undesirable abiotic conditions. However, given our considerable lack of knowledge regarding SDF behavior in low-energy vertebrate ectotherms, my findings highlight the importance of the need for more studies examining the extent to which other low-energy systems practice energy-based SDF strategies.

Although Gila monsters do not use SDF to manage energy reserves, they do use an SDF strategy to manage hydration state and, in accordance with the APP, defend their hydric assets as they become moderately and significantly dehydrated (Chapter 3 and 4). In Chapter 3, both food-supplemented and sham-manipulated animals experienced an increase in plasma osmolality as the hot, dry season progressed (Fig. 8E), similar to previous studies (Davis and DeNardo, 2009, 2010). As animals in both treatment groups became increasingly dehydrated in the dry season, they reduced their surface activity (Chapter 3, Fig. 8A). However, after the onset of the monsoon, plasma osmolality returned to normosmic levels and surface activity significantly increased in both treatment groups. Davis and DeNardo (2009) found that water-supplemented free-ranging Gila monsters were significantly more active than sham-manipulated animals during the dry season, and, as a consequence of increased foraging effort, gained energy reserves throughout the active season. Furthermore, I found a negative correlation between plasma osmolality and surface activity in unmanipulated free-ranging Gila monsters (Chapter 4, Fig. 12B). Collectively, these results demonstrate that Gila monsters use an SDF strategy to manage their hydration state. Additionally, the fact that Gila monsters exhibit reduced surface activity during the dry season even when plasma osmolality is similar to that during the monsoon season (Chapter 4, Fig. 12B) strongly suggests that Gila monsters defend their hydric reserves in accordance with the APP.

While limited in number, the few previous SDF studies examining the effect of water availability on foraging behavior have shown that the presence of free-standing water affects foraging behavior (Kotler et al., 1998; Hochman and Kotler, 2006; Shrader et al., 2008). However, the effect varied among species. For Gila monsters, foraging behavior is driven by the their hydration state (which reflects the availability of free-standing water), the fact that they balance their water budgets annually (Chapter 3, Fig. 8E; Chapter 4, Fig. 13B), and because they satisfy energetic and hydric demands using two distinct sources of income, food and free-standing water, as meal consumption does not improve their hydric state (Appendix A, Wright et al., 2013). Thus, Gila monsters defend hydration state during the dry season in order to minimize water loss prior to the onset of the monsoon when they can correct their water balance by drinking (Davis and DeNardo, 2009, 2010; Chapters 3 and 4). Unlike my research, none of the previous studies examining the effect of water availability on foraging considered the extent to which free-standing water contributes to either water balance or how variation in hydration state may have affected foraging strategies. Without considering the relative importance of various water sources (free-standing, dietary, metabolic), it is difficult to know the extent to which free-standing water influences water balance, and thus whether to expect foraging behavior to be affected by the presence of free-standing water. Additionally, as dehydration has been shown to reduce meal consumption (for examples, see Watts, 1999; Maloiy et al., 2008), without first identifying the hydration state of an organism, it is difficult to make predictions regarding the influence that the presence of water should have on foraging behavior. Given my findings and the limitations of previous studies, it is clear that SDF studies, especially those in water-limited environments, need to incorporate measurements of hydration state, water availability, and water budgets in order to more fully understand driving forces behind foraging decisions.

My work has shown that, for Gila monsters, foraging behavior can be differentially affected by energy reserves, hydric reserves, and free-standing water availability under current climate scenarios. However, in the face of rapid, anticipated changes in temperature and rainfall patterns, the long-term survivorship of Gila monsters may be at risk. Although projected changes in temperature and rainfall may strain the energy budgets of some organisms, energy balance may not be the greatest short-term challenge faced by low-energy ectotherms that are often in negative energy balance and rely on lipid reserves to cope with variation in food availability. However, long-term energy balance may be threatened by continued reductions in rainfall and increases in ambient temperatures. For Gila monsters, climate-induced changes in energy expenditure and prey availability have a limited effect on their ability to balance their energy budgets within a single active season. However, the additive effect of elevated ambient temperatures and reduced rainfall may threaten their ability to maintain long-term energy balance, thus threatening survivorship. For low-energy organisms, elevated ambient temperatures may be less important to survivorship than water availability. The effect of reduced and more variable precipitation coupled with more intense and frequent heat waves may threaten short-term survivorship of organisms by straining water balance (McKechnie and Wolf, 2010). Unlike many species, Gila monsters rely on their urinary bladder to buffer changes in hydration state (Davis and DeNardo, 2007) and occupy sub-surface thermal refugia for extended periods (Beck, 1990; Beck, 2005; Davis and DeNardo, 2009, 2010; Chapters 3, Fig. 8A; Chapter 4, Fig. 12C). Nevertheless, they become increasingly dehydrated during the dry season (Davis and DeNardo, 2009, 2010; Chapter 3, Fig. 8E; Chapter 4, Fig. 13B). Although Gila monsters can tolerate extended periods without rainfall, the non-lethal and lethal impacts of elevated temperatures and reduced rainfall may be more dramatic for those species that lack internal water reservoirs and cannot escape challenging thermal conditions. Additionally, sources of dietary water may become less reliable due to reduced, more variable precipitation and or drought-induced reductions in plant biomass (Breshears et al., 2005; Allen et al., 2010), challenging the ability of a number of organisms to maintain water balance. Given potential lethal and non-lethal consequences of anticipated changes in climatic patters on organismal water balance, it is crucial that we not only investigate how changes in temperature affect energy balance, but also how reductions in rainfall and more intense and frequent heat waves alter water balance. As foraging and meal acquisition can affect both energy and water balance, by investigating the interaction between foraging strategies and body condition, we can better predict and potentially mitigate the impacts of continued climate change.

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APPENDIX A

MEAL CONSUMPTION IS INEFFECTIVE AT MAINTAINING OR CORRECTING WATER BALANCE IN A DESERT LIZARD, *HELODERMA SUSPECTUM*

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RESEARCH ARTICLE

Meal consumption is ineffective at maintaining or correcting water balance in a desert lizard, *Heloderma suspectum*

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SUMMARY

Many xeric organisms maintain water balance by relying on dietary and metabolic water rather than free water, even when free water may be available. For such organisms, hydric state may influence foraging decisions, since meal consumption is meeting both energy and water demands. To understand foraging decisions it is vital to understand the role of dietary water in maintaining water balance. We investigated whether meal consumption was sufficient to maintain water balance in captive Gila monsters (*Heloderma suspectum*) at varying levels of dehydration. Gila monsters could not maintain water balance over long time scales through meal consumption alone. Animals fed a single meal took no longer to dehydrate than controls when both groups were deprived of free water. Additionally, meal consumption imparts an acute short-term hydric cost regardless of hydration state. Meal consumption typically resulted in a significant elevation in osmolality at 6 h post-feeding, and plasma osmolality never fell below pre-feeding levels despite high water content (~70%) of meals. These results failed to support our hypothesis that dietary water is valuable to Gila monsters during seasonal drought. When considered in conjunction with previous research, these results demonstrate that Gila monsters, unlike many xeric species, are heavily reliant on seasonal rainfall and the resulting free-standing water to maintain water balance.

Key words: dehydration, dietary water, water balance, reptile.

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INTRODUCTION

Energy and water balances are both crucial to organism survival, yet the degree to which energy and water represent discrete currencies varies among species. In the most dichotomous situation, food consumption provides the organism with energy, while drinking fulfils water requirements. However, because free-standing water can be both temporally and spatially limited in the environment, dietary water (i.e. the water present in the consumed meal) can make up a considerable portion of an organism's water intake. In fact, some species can heavily or fully rely on dietary water for maintaining water balance (Karasov, 1983; Golightly and Ohmart, 1984; Cooper, 1985; Nagy and Medica, 1986; Green et al., 1991; Nagy et al., 1991; Zhi-long et al., 1992; Nagy and Gruchacz, 1994; Znari and Nagy, 1997; Ostrowski et al., 2002).

Reliance on dietary water may have a considerable influence on foraging decisions (Kotler et al., 1998). Many xeric species will shift their diet to items with greater water content as forage dries up or as free water becomes increasingly limited (e.g. Karasov, 1983; Golightly and Ohmart, 1984; Nagy and Gruchacz, 1994). In addition to its influence on diet preference, dietary water can also influence activity. If dietary and metabolically produced water completely satisfies water requirements, as is the case for a variety of desert rodent species (Nagy and Gruchacz, 1994; Degen et al., 1997), then those organisms can continue to remain active even when free water is unavailable. However, if meal consumption does not enable an organism to maintain water balance, the organism may dehydrate, which can lead to a reduction in activity to minimize water loss. The desert tortoise (*Gopherus agassizii*), a xeric species that inhabits the American Southwest, relies on dietary water as an important contributor to water balance (Nagy and Medica, 1986; Peterson, 1996a; Peterson, 1996b; Henen et al., 1998). However, despite having food available during extended periods of drought, a desert tortoise experiences a dramatic increase in its plasma osmolality, reduces activity, and relies on fluid stored in its urinary bladder to survive. Despite being well adapted to their xeric lifestyle, desert tortoises are heavily reliant upon seasonal rainfall for survival (Nagy and Medica, 1986; Peterson, 1996a; Peterson, 1996b; Henen et al., 1998). Given the existing variation among species, understanding the extent to which a meal influences water balance can be important in determining the vulnerability of organisms to hydric limitations of their environment.

The role of dietary water in water balance has predominantly been investigated in herbivorous species, where different forage can have dramatically different water and protein content (the latter being critical due to protein catabolism, leading to the need to eliminate large amounts of nitrogenous wastes). In contrast, carnivorous diets tend to be more consistent in both water and protein content. While this consistency reduces the value of diet shifts, it does not address whether dietary water is a significant contributor to water balance in carnivorous species. Most prey consists of ~70% water, so meal consumption could contribute substantially to water balance.

To date, the vast majority of work on dietary water contributions to water balance has relied on indirect field assessments that use injections of isotopically labeled water (e.g. Karasov, 1983; Cooper, 1985; Green et al., 1991; Nagy et al., 1991; Zhi-long et al., 1992; Nagy and Gruchacz, 1994; Znari and Nagy, 1997; Ostrowski et al., 2002) or measurements of stable isotopes in body water (e.g. Wolf and Martinez del Rio, 2000). While these studies provide considerable information on a larger scale, the specifics related to the intake of individual meals cannot be evaluated. Thus such work would benefit from complementary studies that make more direct assessments under tightly controlled conditions. Therefore, we conducted a set of laboratory experiments on an infrequently feeding carnivore to evaluate the short-term impact of meal consumption on plasma osmolality and to determine whether consumption of a meal can maintain the hydration state over extended periods of time.

The Gila monster (*Heloderma suspectum* Cope 1869) is an ideal species to examine the impact of meal consumption on hydration state. It is the largest lizard in North America and inhabits the xeric American Southwest where summer temperatures frequently exceed 40°C and free water can be unavailable for 2–3 months (Beck, 2005). To endure lengthy dry periods, Gila monsters use their urinary bladder as a water reservoir (Davis and DeNardo, 2007) and, upon depletion of the reservoir, tolerate considerable increases in plasma osmolality [$>$360 mosmol kg^{-1} (Davis and DeNardo, 2009; Davis and DeNardo, 2010)]. Furthermore, increased plasma osmolality leads to a reduction in surface activity, presumably to reduce evaporative water loss (Davis and DeNardo, 2009). At the onset of the first summer rains, Gila monsters will binge drink free water, rapidly returning their osmolality to a normosmotic state (Davis and DeNardo, 2007).

Although extensive work has examined the role of free water in the physiological ecology of Gila monsters, no work has examined how meal consumption may affect hydration state, especially during periods of extreme water limitation. Given the importance that dietary water plays in the water budgets of other animals, and that Gila monsters have a specialized diet of vertebrate nestlings and eggs (Beck, 2005) that contain ~70% water, we hypothesized that dietary water is a valuable supplemental water resource to Gila monsters during seasonal drought. Accordingly, we determined the impact of dietary water on rates of dehydration and rehydration in Gila monsters. In our first experiment, we examined to what extent dehydration rate is altered by a single meal and the effectiveness of different meal types in rehydrating Gila monsters. We predicted that the rate of dehydration in water-deprived Gila monsters would be significantly slower in animals provided with a meal compared with those given no food. Additionally, when animals are in an extremely dehydrated state, we predicted that meal consumption would considerably improve hydration state, but, unlike a single drinking event, would not fully rehydrate Gila monsters. In our second experiment, we examined the acute (first 48 h) hydric implications of consuming different meals at various stages of hydration (normosmotic, moderate dehydration and extreme dehydration) and determined how multiple meals influence the time it take Gila monsters to reach an extreme dehydration state. We predicted that ingestion of a meal results in an acute water cost at all hydration states, and that such negative effects would be more substantial in rodent meals because of the more complex requirements for digestion. Additionally, we predicted that egg meals would significantly extend the time to reach extreme dehydration relative to rodent meals.

MATERIALS AND METHODS

Study animals and experimental housing

For both experiments, we used 12 long-term captive, adult (experiment 1: mean initial mass 530 g, range 415–639 g; experiment 2: mean initial mass 469 g, range 405–690 g) Gila monsters obtained from the Arizona Game and Fish Department and held under holding licence SP577864. Additionally, all experiments were conducted in accordance with Arizona State University's Institutional Animal Care and Use Committee under protocol 09-1044R. Animals were housed in individual opaque containers (length, 34 cm; width, 21.5 cm; depth, 13.4 cm) with screen lids to allow for exposure to the environmental chamber conditions and to permit visual observation. As the environmental chamber had multiple levels, animal cages were rotated within the experimental chamber once or twice per week.

During the experiments, Gila monsters were housed in an environmental chamber at 30.0±0.2°C that received affluent air with a dew point of 3.5±1.3°C. These values approximate the preferred body temperature of the species (Beck, 2005) and the ambient humidity during the hot, dry season in the Sonoran Desert (authors' personal observations). Air temperature was maintained using a feedback design where a datalogger (21X micrologger, Campbell Scientific, Logan, UT, USA) received input from a thermocouple placed within the chamber and, based on this input, provided variable power to a heating element (iQ FlexHeat, CaloriQue LLC, West Wareham, MA, USA) within the chamber. A small fan placed adjacent to the heating element ran continuously to circulate the air within the chamber.

To achieve the desired dew point, room air was bubbled serially through two 1 liter humidifying bottles, the first being at room temperature (~25°C) and the second heated to ensure that exiting air was completely saturated when it cooled back to room temperature. Air then flowed serially through two 1 liter condensation bottles (both at room temperature, ~25°C) to remove excess moisture. This humidified air then flowed through a mass flow controller (UNIT Instruments, Yorba Linda, CA, USA) and into a small refrigerator set to attain the desired dew point. The air then exited the refrigerator and warmed to room temperature before flowing into the environmental chamber (mean flow rate, 1789±1 ml min^{-1}). The entire air flow system was plumbed with minimally hygroscopic Bev-a-line tubing.

Output of supply air flow rate from the mass flow controller and dew point of the environmental chamber from the hygrometer were recorded by the datalogger every minute. A small pump drew air from the environmental chamber to a flow-through hygrometer (RH-100, Sables Systems, Logan, UT, USA). Additionally, the dew point of the supply air was monitored daily using a bypass system connected to the flow-through hygrometer, enabling us to monitor the supply air dew point while minimizing disturbances to both the environmental chamber conditions and the animals. The supply air flow rate and the dew point of the supply air were adjusted as needed throughout the duration of both experiments (although both were quite stable throughout the duration of both experiments and rarely needed adjustment).

Experiment 1

Dehydration component – effect of a single meal on dehydration rate

The goal of the dehydration component of experiment 1 was to examine whether ingesting a single meal affected the rate of dehydration in captive, free water-deprived adult Gila monsters. We used a single meal since Gila monsters are infrequent binge feeders and thus might only ingest a single meal over the course of the hot, dry season.

For a minimum of 14 days prior to the start of the experiment, Gila monsters were maintained with *ad libitum* water but without food to ensure that animals were normosmotic (~290–300 mosmol kg^{-1}) and post-absorptive. As Gila monsters use water stored in their urinary bladders to buffer changes in plasma osmolality (Davis and DeNardo, 2007), each Gila monster had its urinary bladder drained *via* trans-urethral bladder catheterization (for details, see Davis and DeNardo, 2007) just prior to beginning the experiment. Ultrasonography (Concept/MLV, Dynamic Imaging, Livingston, UK) was used to confirm that the urinary bladder was empty after catheterization. After catheterization, the animal was returned to its normal housing container, but without water. Twenty-four hours after trans-urethral bladder catheterization, an initial mass (g), tail volume (ml, serves as an estimate of energy stores), and a 0.1 ml blood sample (for plasma osmolality) were collected from each animal. Animals were then placed in their experimental containers in the environmental chamber without water. Body mass, tail volume and a blood sample were collected weekly throughout the experiment. Blood samples in both experiments were collected from the caudal vein using a heparinized 1 ml syringe.

As animals reached a moderately dehydrated state (~320–330 mosmol kg^{-1}), they were alternately assigned into one of two treatment groups: fed ('Fed') and non-fed ('Con') animals. Animals receiving a meal were fed two previously frozen but thawed juvenile rats (total mass 60.0±0.1 g). Sixty grams of thawed rat represents approximately two-thirds of the average monthly caloric demand of free-ranging Gila monsters (D.F.D., unpublished data). Following assignment into treatment groups, we continued to monitor plasma osmolality, mass and tail volume of animals weekly. However, to ensure the safety of the animals and to obtain a more precise estimate of days to dehydration, blood samples were collected more frequently as osmolality approached the upper limits of dehydration (>340 mosmol kg^{-1}). Once a lizard reached a plasma osmolality greater than 350 mosmol kg^{-1} [which approximates the near-maximum osmolality reached by free-ranging Gila monsters in the Sonoran Desert (Davis and DeNardo, 2009)], a final mass, tail volume, blood sample and ultrasound of bladder dimensions were collected, and the number of days to maximum dehydration was recorded. Animals then entered the rehydration experiment described below.

Rehydration component – effect of different meal types on rehydration

To address the degree to which different meal types rehydrate Gila monsters, the dehydrated animals from the first component of experiment 1 were alternately assigned to one of two rehydration treatments: animals were fed 60 g of either juvenile rat ('Rat') or blended chicken egg ('Egg'), excluding shell, immediately following the final blood sample of the dehydration component. Gila monsters prey nearly exclusively on the contents of vertebrate nests (Beck, 2005), so our treatments represent two ecologically relevant meals. Following feeding, the animals were returned to the environmental chamber and remained without free water. Given the high starting osmolality during this rehydration component, blood samples were collected prior to and 48 h after feeding. Once an animal reached either a normosmotic (<310 mosmol kg^{-1}) or extremely dehydrated (>350 mosmol kg^{-1}) state, the animal was removed from the study and provided *ad libitum* access to free water (Fig. 1A).

Experiment 2 – acute and long-term impact of multiple meals on hydration state

The goal of experiment 2 was to assess the acute hydration cost of digesting meals for adult Gila monsters at varying states of hydration, and examine the effect of consuming multiple meals on the rate of dehydration in these same animals. Similar to experiment 1, all animals were normosmotic, in a post-absorptive state, and had their urinary bladders drained via trans-urethral bladder catheterization prior to beginning experiment 2. As before, all animals were checked by ultrasound following catheterization to ensure that the urinary bladders were empty. Initial processing was completed 24 h after catheterization as described above.

The experimental design followed that of experiment 1 except for a change in the frequency and timing of meal treatments and post-feeding blood sampling (Fig. 1B). After baseline measurements were collected, animals were assigned to one of two treatments: Rats or Eggs meals. Each animal was scheduled to receive four supplementations while in the environmental chamber – a meal while in an normosmotic state (beginning of experiment), a meal when moderately dehydrated (320–330 mosmol kg^{-1}), oral free water supplementation (at a volume similar to the amount of dietary water in the meal, mean=42±2 ml) when extremely dehydrated (>350 mosmol kg^{-1}), and a meal when the animal returned to an extremely dehydrated state after free water supplementation. In addition to weekly processing of the animals, we collected blood samples 6, 24 and 48 h as well as 6 days (144 h) after each treatment (the 6-day sample was not collected after the third feeding). Mass and tail volume were collected at time 0, 48 h and 6 days post-treatment). Forty-eight hours after the third feeding, animals were provided with a bowl of water for 3 h, allowing them sufficient time to binge drink free water to satiation. Twenty-four hours post-binge drinking, a final blood sample and body mass were collected.

Determination of plasma osmolality

Plasma was separated from whole blood by centrifugation, and then plasma was stored in sealed containers at −80°C until the samples were analyzed. Weekly plasma samples were processed within 24 h of blood collection so that we could closely monitor hydration state. Frozen plasma samples were thawed and osmolality of samples was determined in triplicate using a vapor pressure osmometer (model 5500, Wescor, Logan, UT, USA). Before analyzing samples, the osmometer was calibrated using the three-step factory protocol using osmolality standards of 290 and 1000 mosmol kg^{-1}. To verify that the osmometer was consistent throughout the experiments, a sample of pooled plasma collected from well-hydrated captive adult Gila monsters (~290–300 mosmol kg^{-1}) was analyzed in triplicate after completing calibration procedures, as well as after every 20 triplicate samples. If the pooled plasma sample readings varied beyond the error range of the osmometer (±6 mosmol kg^{-1}), then the 290 standard was analyzed in triplicate as well. If the osmolality of both samples fell outside the error range of the osmometer, the osmometer head was cleaned, the osmometer was recalibrated, and pooled Gila monster plasma was re-run in triplicate prior to continuing analysis of samples, beginning with the 20 triplicate samples that fell between an accurate pooled sample reading and the pooled sample reading that varied beyond the osmometer error range.

Water content of meals

We determined the water content of both meal types (juvenile rat and shell-free chicken egg) using a sample size of five for each meal type. We also determined the water content of the two most commonly consumed prey of Gila monsters – desert cottontail rabbit (*Sylvilagus audubonii*) pups (*N*=1) and Gambel's quail (*Callipepla gambelii*) eggs (*N*=3). We could not use these natural preys for our experiments due to their limited availability, but we wanted to compare the water content of our experimental meals to that of the natural prey. Water content was determined by placing each item individually in a small, pre-weighed aluminum tray. Prey wet mass was calculated as initial total mass minus empty tray mass. The trays were then placed in a vacuum-sealed oven, and the prey dried

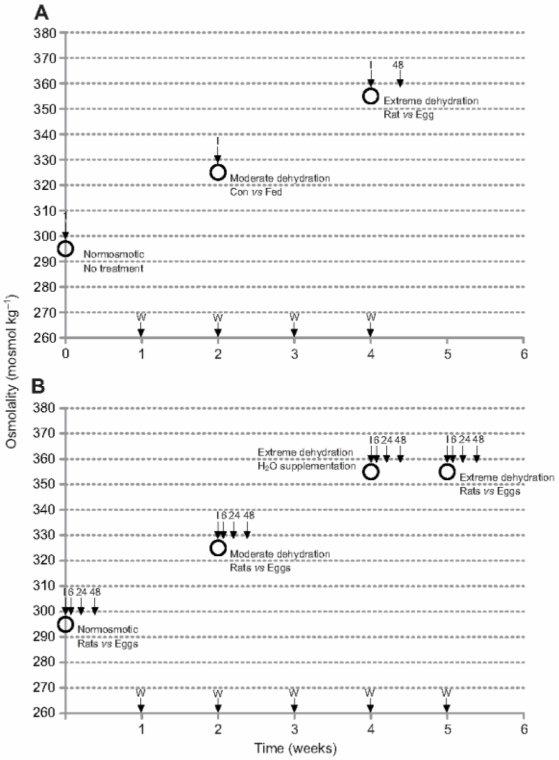

Fig. 1. Diagrammatic representation of the experimental designs for (A) experiment 1 and (B) experiment 2. Circles indicate the target hydration state for each manipulation; arrows and 'W' depict weekly processing of animals (see Materials and methods); arrows and 'I', '6', '24' and '48' represent sampling points prior to and at 6, 24 and 48 h after manipulation, respectively.

at 65°C until mass remained unchanged for at least 3 days. Prey dry mass was calculated as end total mass minus empty tray mass, and water content (%) was then calculated as (prey wet mass – prey dry mass)/(prey wet mass) × 100.

Data analysis

Experiment 1 – dehydration component

The effect of a single meal on dehydration time was assessed by comparing the number of days animals in the Con *versus* Fed treatment groups took to reach the extremely dehydrated state by using a non-parametric *t*-test (Mann–Whitney), since the assumptions of an unpaired Student's *t*-test and Welch's *t*-test were violated. Because the amount of time animals took to dehydrate varied, as did the final plasma osmolality, we also compared the rate of change in plasma osmolality of animals prior to and following treatment at the moderately dehydrated state using a repeated measures analysis of variance (rmANOVA).

Experiment 1 – rehydration component

To determine the effect of meal consumption on rehydration of extremely dehydrated Gila monsters, the mean plasma osmolality at times 0 and 48 h post-treatment were compared between Rat and Egg treatment groups using an rmANOVA.

Experiment 2

To determine the acute effect of meal consumption on hydration state, mean plasma osmolality at 0, 6 h, 24 h, 48 h and 6 days (144 h) were compared between treatments using an rmANOVA. This analysis was applied separately to the four manipulation events (feeding when normosmotic, second feeding when moderately dehydrated, free water supplementation when extremely dehydrated, third feeding when extremely dehydrated) plus the binge drinking event at the end of the experiment (comparing osmolality at time 0 and 24 h post-binge drinking). Snout–vent length (SVL) was initially used as a covariate in each analysis; however, because there was no significant effect of SVL in any of the aforementioned tests, the rmANOVAs were completed excluding SVL as a covariate.

The effect of multiple meals on dehydration time was assessed by comparing the number of days animals in the Rats *versus* Eggs treatment groups took to reach an extremely dehydrated state by using a non-parametric *t*-test (Mann–Whitney), since the assumptions of an unpaired Student's *t*-test and Welch's *t*-test were violated.

Water content of meals

The difference in the water content of our rat *versus* blended chicken egg meals was assessed by comparing the percent water content of each meal type using an unpaired Student's *t*-test, as the variances were equal and the data were normally distributed. The variances between the blended chicken egg *versus* fresh quail eggs were not equal; however, the data were normally distributed, so we compared the difference in water content between blended chicken egg *versus* fresh quail eggs by using a Welch's *t*-test. As our sample size for the juvenile desert cottontail rabbits was $N=1$, we did not use a statistical test to compare its water content with the water content of our rat meals.

RESULTS

Water content of meals

There was no difference in mean water content of juvenile rats and chicken eggs (rats 70.4±0.3%, eggs 74.5±0.2%, unpaired *t*-test: $P=0.374$). Similarly, we found no difference in water content of fresh quail eggs relative to chicken eggs (quail eggs 72.3±0.7%, Welch's *t*-test: $P=0.073$). Finally, although we lacked a sufficient sample size for statistical comparison (due to difficulties in obtaining samples), a single nestling desert cottontail rabbit had a water content of 76.5%.

Experiment 1 – dehydration component

There was no difference in the number of days it took the Con *versus* Fed treatment groups to reach an extreme dehydration state (Con 32.5±4.86 days, Fed 32.5±2.66 days, Mann–Whitney test: $P>0.20$, Fig. 2). Similarly, after we calculated the rate of change in osmolality prior to and following treatment at the moderately dehydrated state, we found that there was no effect of time or treatment on rate of change in osmolality, nor was there an interaction between time and treatment (rmANOVA time: $F_{1,10}=0.766$, $P=0.40$; rmANOVA treatment: $F_{1,10}=2.753$, $P=0.13$; rmANOVA time and treatment: $F_{1,10}=0.766$, $P=0.40$).

Experiment 1 – rehydration component

There was a significant effect of treatment on the osmolality of extremely dehydrated Gila monsters fed Egg or Rat (rmANOVA treatment: $F_{1,10}=9.37$, $P=0.012$, Fig. 3), and there was an interaction between time and treatment (rmANOVA time and treatment: $F_{1,10}=15.16$, $P=0.003$, Fig. 3). There was no effect of time on osmolality of extremely dehydrated Gila monsters after feeding (rmANOVA time: $F_{1,10}=4.558$, $P=0.059$, Fig. 3). Tukey–Kramer *post hoc* analysis revealed that: (1) there was no difference in starting osmolality (pre-feed) between either treatment group, (2) the Egg group did not experience a significant change in osmolality 48 h post-feeding, (3) animals in the Rat treatment had a significantly higher osmolality 48 h post-feeding relative to pre-feeding, and (4) animals in the Rat treatment had a significantly higher osmolality 48 h post-feeding relative to those in the Egg treatment (Fig. 3).

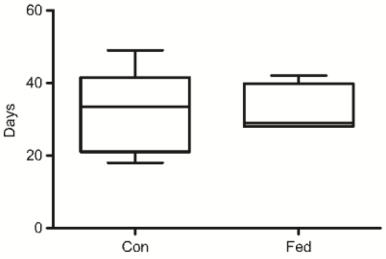

Fig. 2. The total number of days it took moderately dehydrated Gila monsters to reach an extreme dehydration state following a rat meal (Fed) or no meal (Con). Boxes represent quartiles and error bars 95% confidence intervals.

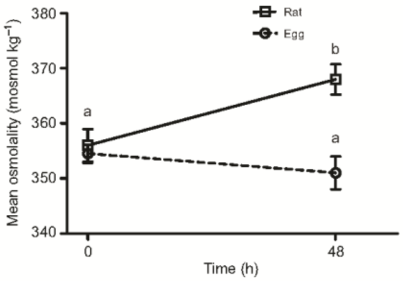

Fig. 3. Osmolality of extremely dehydrated animals before and 48 h after feeding either Egg or Rat. Rat-fed animals experienced a significant increase in osmolality while Egg-fed animals had no change in osmolality 48 h after feeding. Letters represent significant differences across sampling time points. Error bars are ±1 s.e.m.

Experiment 2

Both time and treatment had an effect on the osmolality of normosmotic Gila monsters (rmANOVA time: $F_{4,40}=23.555$, $P<0.001$; rmANOVA treatment: $F_{1,10}=9.414$, $P=0.012$, Fig. 4A). However, there was no interaction between time and treatment at this hydration state (rmANOVA time and treatment: $F_{4,40}=2.230$, $P=0.083$, Fig. 4A). *Post hoc* analysis revealed that 6 h post-feeding, the osmolality of normosmotic Gila monsters was elevated above baseline and remained elevated for the duration of the sampling period (6 days post-treatment). Additionally, animals in the Rats treatment had a significantly higher osmolality compared with Gila monsters in the Eggs treatment (Fig. 4A).

Time had a similar effect on osmolality of moderately dehydrated Gila monsters (rmANOVA time: $F_{4,32}=6.602$, $P=0.001$, Fig. 4B). However, there was no effect of treatment on osmolality at this hydration state, nor was there an interaction between time and treatment (rmANOVA treatment: $F_{1,8}=0.025$, $P=0.88$; rmANOVA time and treatment: $F_{4,32}=2.521$, $P=0.06$, Fig. 4B). *Post hoc* analysis showed that within 6 h of consuming a meal, plasma osmolality of moderately dehydrated animals in both treatment groups increased significantly above pre-feeding levels and remained at this elevated state for 24 h post-feeding before returning to pre-feeding levels 48 h and 6 days post-treatment (Fig. 4B).

As was the case with the previous two hydration states, time had an effect on osmolality of extremely dehydrated Gila monsters supplemented with an amount of free water similar to the amount of dietary water in their respective meal treatments (rmANOVA time: $F_{4,36}=100.129$, $P<0.001$, Fig. 5). As with the mildly dehydrated state, there was no effect of treatment on osmolality, nor was there an interaction between time and treatment, (rmANOVA treatment: $F_{1,9}=2.618$, $P=0.14$; rmANOVA time and treatment: $F_{4,36}=0.689$, $P=0.60$). *Post hoc* analysis revealed that within 6 h of free water supplementation, the plasma osmolality of extremely dehydrated Gila monsters in both treatment groups was significantly lower than

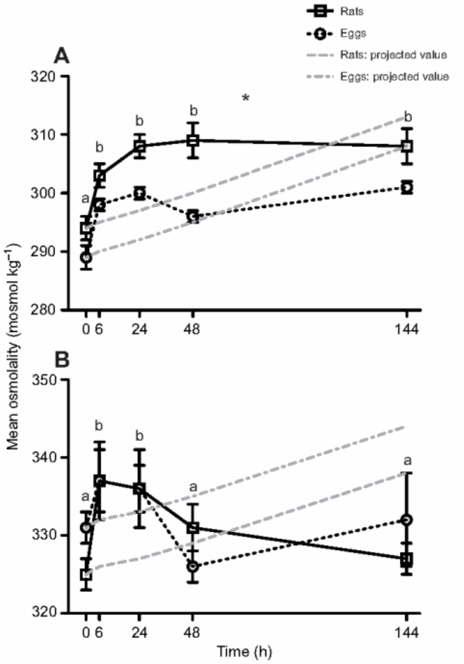

Fig. 4. Mean osmolality of (A) well-hydrated and (B) moderately dehydrated Gila monsters 0, 6, 24, 48 and 144 h (6 days) post-feeding with either Eggs or Rats. Light gray lines represent projected plasma osmolality values for animals in both treatment groups. These values were determined by using the rates of change in plasma osmolality determined in experiment 1 for Gila monsters in the Con treatment group. Letters represent significant differences across sampling time points. *Significant overall treatment effect. Error bars are ±1 s.e.m.

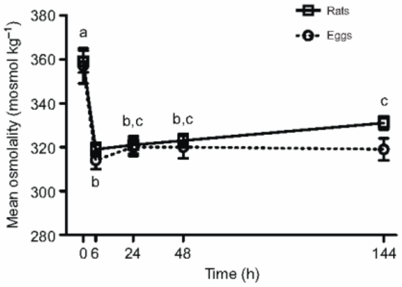

Fig. 5. Average osmolality of extremely dehydrated Gila monsters 0, 6, 24, 48 and 144 h (6 days) post-free water supplementation. There was a significant effect of time on osmolality, but there was no effect of treatment or an interaction between time and treatment. Letters represent significant differences across sampling time points. Error bars are ±1 s.e.m.

plasma osmolality prior to water supplementation, and it remained reduced throughout the sampling period (Fig. 5).

Four Gila monsters in the Eggs treatment and one in the Rats treatment exhibited clinical signs of dehydration (e.g. lethargy with reduced response to stimulation) before they reached the final meal treatment. Due to concerns for the well-being of the animals, we removed these animals from the study and provided them with water *ad libitum* for 3 h (binge drinking treatment). Due to this unanticipated response, our sample sizes for Eggs and Rats treatments at an extremely dehydrated state were unbalanced (N=2 for Eggs, N=5 for Rats) and too small to perform a valid statistical analysis.

Once animals either exhibited clinical signs of dehydration or had their final blood sample drawn while in the final feeding treatment, we examined the effect of a single binge drink on recovery from dehydration. We found that time and treatment independently had an effect on the osmolality of these extremely dehydrated Gila monsters (rmANOVA time: $F_{1,10}$=297.24, P<0.001; rmANOVA treatment: $F_{1,10}$=6.913, P=0.03, Fig. 6). There was no interaction between time and treatment (rmANOVA time and treatment: $F_{1,10}$=1.588, P=0.24) (Fig. 6). *Post hoc* analysis revealed that the osmolality of animals in both the Eggs and Rats groups had significantly decreased within 24 h of a binge drinking event (Fig. 6), similar to previous findings (Davis and DeNardo, 2007). Additionally, *post hoc* analysis revealed that the Rats group had a significantly higher mean osmolality relative to the Eggs group (Fig. 6). This result probably occurred because more animals in the Rats treatment group reached the extreme dehydration state for a second time without exhibiting clinical signs of dehydration, contrary to their Eggs treatment counterparts.

Overall, there was no difference in the number of days it took the Rats and Eggs treatment groups to reach an extreme dehydration state (Rats 41.4±2.5 days, Eggs 46.9±4.9 days, Mann–Whitney test: U=18.5, N_1=6, N_2=6, P>0.20, Fig. 7).

DISCUSSION

Meal consumption and water balance

The average time required for a Gila monster to reach an extreme state of dehydration was minimally affected by meal consumption (Fig. 2). Gila monsters given a single rodent meal took no longer to dehydrate (32.5 days) then did the unfed controls (32.5 days) or unfed Gila monsters with empty urinary bladders in a previous study (33.3 days) (Davis and DeNardo, 2007). Feeding multiple egg or rat meals to Gila monsters without access to free water did extend the time to dehydration (46.9 and 41.4 days, respectively). However, frequent meal consumption by Gila monsters is unlikely during the hot, dry season when few prey species are nesting. Thus from an ecological prospective, Gila monsters fall on the free water dependence side of the 'continuum' between free water independence and free water dependence (Gettinger, 1984).

Free water dependence is uncommon, particularly in xeric reptiles. Dietary and metabolically produced water completely satisfy the water requirements of the carnivorous heath monitor (*Varanus rosenbergi*) during the driest times of the year in southern Australia. Even during wetter portions of the year (i.e. spring and winter), these sources of water contribute 74 and 58%, respectively, of their water requirements (Green et al., 1991). Free-ranging, semiarid-dwelling goannas (*Varanus caudolineatus*) were also able to maintain water balance during the summer (in Western Australia)

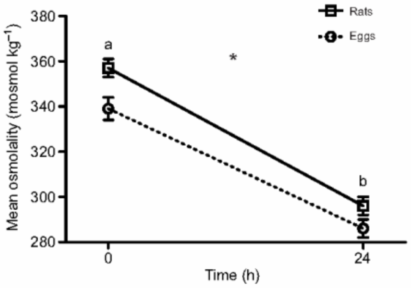

Fig. 6. Average osmolality of extremely dehydrated Gila monsters 0 and 24 h post-binge drinking (water provided *ad libitum* for 3 h). There was a significant effect of time and treatment; however, there was no interaction between time and treatment. *Overall treatment effect. Letters represent significant differences across sampling time points. Error bars are ±1 s.e.m.

despite little to no free water being available (Thompson et al., 1997). Free-living Bibron's agama (*Agama impalearis*), an insectivorous ambush predator that inhabits the arid regions of North Africa, satisfy their water demands entirely through dietary water and metabolic water production (Znari and Nagy, 1997). Numerous herbivorous, xeric-dwelling reptiles are also able to maintain water balance without drinking free water, e.g. the Namibian sand dune lizard *Angolosaurus skoogi* (Nagy et al., 1991) and the desert iguana *Dipsosaurus dorsalis* (Minnich and Shoemaker, 1970). Given their low water demands, many xeric reptiles are capable of capitalizing on available dietary water to balance their water budgets without drinking free water.

Even among xeric arthropods and endotherms, reliance on free-standing water is atypical. Cooper (Cooper, 1985) examined the water balance of two species of free-ranging tenebrionid beetles, the desert stink beetle (*Eleodes armata*) and the death feigning beetle (*Cryptoglossa verrucosa*), which are sympatric with the Gila monster. Both are capable of satisfying most of their water requirements *via* dietary and metabolic water (Cooper, 1985). At the extreme for high energy systems, a variety of desert rodent species, such as Merriam's kangaroo rats (*Dipodomys merriami*), are capable of satisfying their water requirements without drinking free-standing water (Nagy and Gruchacz, 1994; Degen et al., 1997). This independence from needing free-standing water to balance water budgets is also present among larger vertebrates. For example, Ostrowski et al. (Ostrowski et al., 2002) examined the water intake of free-living oryxes (*Oryx leucoryx*) during the summer in the Arabian Desert, finding that 14.4% of total daily water influx rate was metabolically produced water, while the remainder of their total water influx was obtained *via* the plants they consume. Like the oryx, kit foxes (*Vulpes macrotis*) obtain sufficient water to maintain water balance from both metabolic water and their prey (Golightly and Ohmart, 1984).

Although many species are capable of obtaining sufficient water from both cellular respiration and food, it appears that Gila monsters are precluded from using these strategies to maintain water balance. Having a high mass specific metabolic rate allows many species to capitalize on metabolic water production, which can significantly contribute to water balance. However, the contribution of this endogenously produced source of water to water balance is less significant in: (1) larger vertebrates, because of their low mass specific metabolic rates, and (2) low energy systems that generally have lower overall metabolic rates when compared with high energy systems of comparable size. Gila monsters are medium-sized lizards that have very low mass-specific metabolic rates and spend the majority of their time inactive in refugia, particularly during the hot, dry season when the proportion of time spent in the burrow can exceed 90% (Beck, 2005; Davis and DeNardo, 2010). Thus the energetic efficiency of Gila monsters dictates that metabolic water production can only provide a minimal contribution to both short- and long-term water balance.

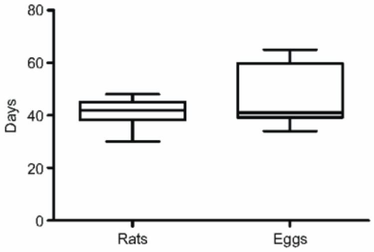

Fig. 7. Total number of days it took well-hydrated Gila monsters to reach an extreme dehydration state after two meals (Rats and Eggs), one at a well-hydrated and one at a moderately dehydrated state. Boxes represent quartiles and error bars 95% confidence intervals.

One key aspect of using dietary water to fulfil hydric needs is that animals often alter diet selection during dry periods. However, Gila monsters are nest specialists, and our data demonstrate little variation in water content among prey items as well as little variation in hydric benefits from consuming eggs *versus* juvenile rodents. These factors preclude Gila monsters from using a strategy many other species use to help maintain water balance.

However, Gila monsters are not alone in their inability to satisfy water requirements solely through dietary and endogenously produced sources. The xeric mottled rock rattlesnake (*Crotalus lepidus*) cannot fulfil water requirements *via* prey ingestion and metabolic water production alone, and is in negative water balance during the summer (Beaupre, 1996). Extensive work examining the physiological ecology of the desert tortoise, a species sympatric with the Gila monster, implies that green succulents are an important source of water for desert tortoises (Nagy and Medica, 1986; Peterson, 1996a; Henen et al., 1998). However, during the dry season, they consume dry plant matter that is osmotically stressful, requiring the tortoise to store excess ions and nitrogenous wastes in its bladder until they can be voided by drinking free-standing water that comes only with sufficient rainfall (Nagy and Medica, 1986; Peterson, 1996a; Peterson, 1996b; Henen et al., 1998). Recent evidence has also demonstrated that marine snakes, including sea kraits, sea snakes and file snakes, require fresh water to maintain water balance (Lillywhite and Ellis, 1994; Lillywhite et al., 2008; Lillywhite et al., 2012). Preliminary analysis of their water budgets indicates that dietary water is insufficient at correcting water balance (Lillywhite et al., 2008). Finally, the white-winged dove (*Zenaida asiatica*), another species sympatric with the Gila monster, relies heavily on saguaro cactus nectar and fruit throughout the summer, but they can also use free-standing water sources to maintain water balance as well (Wolf and Martinez del Rio, 2000).

Given their mobility, white-winged doves and many other birds can take advantage of free-standing water that may otherwise be inaccessible to less mobile species.

The hydric cost of digestion

Our prediction that ingestion of a meal would result in a short-term water cost at all hydration states and that such negative effects would be more substantial in rodent meals was partially supported. At normosmotic and moderately dehydrated states, animals in both treatment groups exhibited a significant elevation in plasma osmolality at 6h post-feeding and it remained elevated for at least 24h. At 48h post-feeding, the osmolality of normosmotic animals remained elevated while the osmolality of moderately dehydrated animals returned to pre-feeding levels (Fig.4A,B). Additionally, consumption of egg or rodent at an extremely dehydrated state failed to improve hydration state within 48h post-feeding (Fig.3), thus failing to support our prediction that meal consumption would improve but not fully rehydrate extremely dehydrated Gila monsters. These results were similar regardless of meal type. Only when osmolality was >350 $\mathrm{mosmol\,kg^{-1}}$ did meal type affect the acute hydration response. Rodent-fed animals exhibited an osmolality that was significantly higher 48h post-feeding relative to baseline and when compared with the osmolality of animals 48h post-egg consumption (Fig.3). Additionally, the egg and rodent treatments failed to rehydrate Gila monsters to the extent of either a single binge drinking event (Davis and DeNardo, 2007) or to the extent that free water given in the amount equivalent to the dietary water did (Fig.5).

The fact that, when given in equal volumes, free water provides greater hydric benefit than dietary water, suggests a substantial water cost associated with meal digestion. Post-prandial energetic costs associated with digestion have been well documented in a wide variety of taxa (Secor, 2009), with some of the most dramatic responses occurring in infrequently feeding snakes such as the Burmese python (*Python molurus bivittatus*), which shows up to a 44-fold increase in metabolism during digestion compared with its standard metabolic rate (SMR) (Secor and Diamond, 1997). Although Gila monsters are infrequent binge feeders similar to Burmese pythons, they exhibit a more modest 4.0- to 4.9-fold increase in metabolic rate relative to SMR (Christel et al., 2007). Although the post-prandial metabolic response of many organisms, including the Gila monster, has been well documented, to our knowledge there are no studies quantifying the hydric cost of digestion.

In support of a significant hydric cost to digestion, Gila monsters, regardless of hydration state, showed an initial increase in plasma osmolality shortly after feeding. While osmolality decreased from this peak with time, it never went below pre-feeding levels (Fig.4A,B), as was seen when animals were given an equal volume of free water (Fig.5) or allowed a single binge drink (Fig.6). There are a number of possible mechanisms that might explain the post-prandial changes in plasma osmolality observed in Gila monsters. First, the rapid rise in plasma osmolality shortly after meal consumption might be driven primarily by the secretion of fluid into the lumen of the alimentary canal to aid in transport, digestion and absorption of nutrients *via* solvent drag. Additionally, evidence of post-prandial increases in: (1) the wet tissue mass of digestive and accessory organs and enterocyte volume (e.g. Starck and Beese, 2001; Cramp and Franklin, 2005; Lignot et al., 2005; Starck et al., 2007; Wood et al., 2007) and (2) blood flow to digestive organs (Starck and Wimmer, 2005), further demonstrate significant fluid investment into digestion. Hydric costs of digestion can also probably be attributed to the significant post-prandial increase in Gila monster metabolic rate (Christel et al., 2007), which would entail a concomitant increase in ventilatory water loss. Furthermore, hydric costs would be associated with eliminating meal-associated waste products in both the feces and urine. Clearly, given the void in our understanding of meal-associated hydric costs, further work is needed to quantify the relative importance of the various water-consuming aspects of digestion and how meal type might influence this balance.

One alternative explanation for increased plasma osmolality during digestion is the sudden increase in plasma constituents associated with nutrient absorption. However, while Gila monsters exhibit significant post-prandial increases in plasma glucose and triglyceride concentrations after feeding, these changes do not occur until 24h after meal consumption and remain elevated for at least 72h (Christel and DeNardo, 2007). This timeline does not reflect the pattern observed in plasma osmolality, where osmolality peaked at 6h post-feeding, and typically returned to near-baseline levels at 48h post-feeding (Fig.4A,B). Although the plasma osmolality of normosmotic animals remained elevated throughout our sampling period, the lack of any clear trends between our results and the temporal variation in plasma nutrient concentrations reported by Christel and DeNardo (Christel and DeNardo, 2007) indicates that the mechanisms proposed above are more likely to be driving the observed post-prandial changes in plasma osmolality.

Meal consumption, hydration state and state-dependent foraging strategies

Survival depends on an organism using a suite of activities to fulfil multiple physiological demands, especially energetic and hydric demands. When to use each activity and the extent to which an activity is used depends on the integration of information regarding environmental conditions as well as the individual's physiological condition. Foraging represents a major activity of most organisms and many studies have demonstrated that animals optimize foraging so as to maximize benefits and/or mitigate costs, and that these decisions are often driven by the internal state of the organism (Clark, 1994; Nonacs, 2001), which is termed state-dependent foraging (SDF) (Nonacs, 2001). Foraging-induced costs must be paid with discrete currencies (e.g. energy, water) and while energetic costs tend to receive the most attention in studies of SDF strategies, in arid environments water costs might be more important. This is especially true if food acquisition does not significantly benefit water balance, as is the case in our study. Some foods contain little water (e.g. dry vegetation) or may have high water cost associated with meal processing. Although water availability and the contribution of various sources of water (free, dietary and metabolic) to water balance can affect foraging behavior (Kotler et al., 1998), there is, to the best of our knowledge, no work examining the interplay between an organism's hydration state, the contribution of various sources of water, and the use of SDF strategies in organisms. Our work has shown that in some situations there can be little hydric benefit to meal consumption. Coupled with observations that free-ranging Gila monsters dramatically reduce surface activity when osmotically stressed during the hot, dry summer (Davis and DeNardo, 2009), these studies indicate that foraging behavior can be directly impacted by an organism's hydration state and that foraging activity can come at a significant hydric cost to the organism regardless of foraging success. Given the impact that food consumption can have on energy and water balance, future studies examining foraging behavior in organisms should be expanded to include an organism's hydration state and the hydric costs/benefits to foraging and meal acquisition. In doing so, we can build a more thorough understanding of the interplay between an organism's physiological condition and its foraging behavior.

LIST OF SYMBOLS AND ABBREVIATIONS

Con	treatment group in experiment 1 that did not receive a meal when moderately dehydrated
Egg	treatment group in experiment 1 that received a 60 g blended chicken egg meal when extremely dehydrated
Eggs	treatment group in experiment 2 that received a 60 g blended chicken egg meal or an amount of free water equivalent to that present in a 60 g blended chicken egg meal when normosmotic, moderately dehydrated and extremely dehydrated
Fed	treatment group in experiment 1 that did receive a 60 g rat meal when moderately dehydrated
Rat	treatment group in experiment 1 that received a 60 g rat meal when extremely dehydrated
Rats	treatment group in experiment 2 that received a 60 g rat meal or an amount of free water equivalent to that present in a 60 g rat meal when normosmotic, moderately dehydrated and extremely dehydrated
rmANOVA	repeated-measures analysis of variance
SDF	state-dependent foraging

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AUTHOR CONTRIBUTIONS

C.D.W. and D.F.D. made significant contributions to the conception, design and execution of the study, the interpretations of the findings, and the drafting and revising of the article. M.L.J. made significant contributions to the execution of the study.

COMPETING INTERESTS

No competing interests declared.

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APPENDIX B

STATEMENT OF PERMISSION FROM CO-AUTHORS TO USE THE FIRST AUTHORED PUBLICATION IN APPENDIX A AS A CHAPTER IN DISSERTATION

Per ASU's Graduate Education policy for documents containing separate studies or papers where the student submitting his or her dissertation is the first listed co-author, Dr. Dale F. DeNardo and Marin L. Jackson have provided explicit permission for me, Christian D. Wright, to use the following published article titled "Meal consumption is ineffective at maintaining or correcting water balance in a desert lizard, *Heloderma suspectum*" as an appendix in my dissertation titled "Examination of the state-dependency and consequences of foraging in a low-energy system, the Gila monster, *Heloderma suspectum*."

APPENDIX C

APPROVAL DOCUMENTATION FROM UNIVERSITY INSTITUTIONAL ANIMAL CARE AND USE COMMITTEE

Institutional Animal Care and Use Committee (IACUC)
Arizona State University

Tempe, Arizona 85287-1103
(480) 965-2179   FAX: (480) 965-7772

Animal Protocol Review

Protocol Number:	08-962R
Protocol Title:	Physiological Trade-Offs Associated with Life in a Xeric Environment
Principal Investigator:	Dale DeNardo
Date of Action:	11/15/2007

The animal protocol review was considered by the Committee and the following decisions were made:

☒ The original protocol was APPROVED as presented.
☐ The revised protocol was APPROVED as presented.
☐ The protocol was APPROVED with RESTRICTIONS or CHANGES as noted below. The project can only be pursued, subject to your acceptance of these restriction or changes. If you are not agreeable, contact the IACUC Chairperson immediately.
☐ The Committee requests CLARIFICATIONS or CHANGES in the protocol as described in the attached memorandum. The protocol will be reconsidered when these issues are clarified and the revised protocol is submitted.
☐ The protocol was approved, subject to the approval of a WAIVER of provisions of NIH policy as noted below. Waivers require written approval from the granting agencies.
☐ The protocol was DISAPPROVED for reasons outlined in the attached memorandum.
☐ The Committee requests you to contact ________________ to discuss this proposal.
☐ A copy of this correspondence has been sent to the Vice President for Research.
☐ Amendment was approved as presented.

RESTRICTIONS, CHANGES OR WAIVER REQUIREMENT:

Approved # of Animals: 242 Gila Monsters **Pain Level:** 60 - C; 182 - D

Approval Period: 11/15/2007 - 11/14/2010

Signature: Sheryl Tuxler ________________ Date: 11-21-07
for IACUC Chair or Designee

Original: Principal Investigator
cc: IACUC Office
IACUC Chair
ORSPA/SPS

Institutional Animal Care and Use Committee (IACUC)
Arizona State University

Tempe, Arizona 85287-1103
(480) 965-2179 FAX: (480) 965-7772

Animal Protocol Review

ASU Protocol Number: 08-962R
Protocol Title: Physiological Trade-Offs Associated with Life in a Xeric Environment
Principal Investigator: Dale DeNardo
Date of Action: 08/21/2008

The animal protocol review was considered by the Committee and the following decisions were made:

☐ The original protocol was APPROVED as presented.
☐ The revised protocol was APPROVED as presented.
☐ The protocol was APPROVED with RESTRICTIONS or CHANGES as noted below. The project can only be pursued, subject to your acceptance of these restriction or changes. If you are not agreeable, contact the IACUC Chairperson immediately.
☐ The Committee requests CLARIFICATIONS or CHANGES in the protocol as described in the attached memorandum. The protocol will be reconsidered when these issues are clarified and the revised protocol is submitted.
☐ The protocol was approved, subject to the approval of a WAIVER of provisions of NIH policy as noted below. Waivers require written approval from the granting agencies.
☐ The protocol was DISAPPROVED for reasons outlined in the attached memorandum.
☐ The Committee requests you to contact ______________ to discuss this proposal.
☐ A copy of this correspondence has been sent to the Vice President for Research.
☒ Amendment was approved to add two new participants to the protocol.

RESTRICTIONS, CHANGES OR WAIVER REQUIREMENT:

Approval Period: 11/15/2007 - 11/14/2010
Funded: AZ Game and Fish Dept.
Number: U07020
Title: Urban Influence of Gila Monster Ecology

Signature: ______________________ Date: 8/25/08
IACUC Chair or Designee

Original: Principal Investigator
cc: IACUC Office
IACUC Chair
ORSPA/SPS

Institutional Animal Care and Use Committee (IACUC)
Arizona State University

Tempe, Arizona 85287-1103
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Animal Protocol Review

ASU Protocol Number: 08-0962
Protocol Title: Physiological Trade-Offs Associated with Life in a Xeric Environment
Principal Investigator: Dale F Denardo
Date of Action: 10/23/2008

The animal protocol review was considered by the Committee and the following decisions were made:

☐ The original protocol was APPROVED as presented.
☐ The revised protocol was APPROVED as presented.
☐ The protocol was APPROVED with RESTRICTIONS or CHANGES as noted below. The project can only be pursued, subject to your acceptance of these restriction or changes. If you are not agreeable, contact the IACUC Chairperson immediately.
☐ The Committee requests CLARIFICATIONS or CHANGES in the protocol as described in the attached memorandum. The protocol will be reconsidered when these issues are clarified and the revised protocol is submitted.
☐ The protocol was approved, subject to the approval of a WAIVER of provisions of NIH policy as noted below. Waivers require written approval from the granting agencies.
☐ The protocol was DISAPPROVED for reasons outlined in the attached memorandum.
☐ The Committee requests you to contact ________________ to discuss this proposal.
☐ A copy of this correspondence has been sent to the Vice President for Research.
☒ Amendment was approved to change the vendor.

Approval Period: 11/15/2007 - 11/14/2010
Sponsor: Arizona Game and Fish Dept.
Number: U07020
Title: Urban Influence of Gila Monster Ecology

Signature: ________________________________ Date: 10/23/08
IACUC Chair or Designee

Original: Principal Investigator
cc: IACUC Office
IACUC Chair
ORSPA/SPS

Institutional Animal Care and Use Committee (IACUC)
Arizona State University

Tempe, Arizona 85287-1103
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Animal Protocol Review

ASU Protocol Number: 09-1044R
Protocol Title: Physiological Trade-Offs Associated with Life in a Xeric Environment
Principal Investigator: Dale DeNardo
Date of Action: 02/27/2009

The animal protocol review was considered by the Committee and the following decisions were made:

☒ The original protocol was APPROVED as presented.
☐ The revised protocol was APPROVED as presented.
☐ The protocol was APPROVED with RESTRICTIONS or CHANGES as noted below. The project can only be pursued, subject to your acceptance of these restriction or changes. If you are not agreeable, contact the IACUC Chairperson immediately.
☐ The Committee requests CLARIFICATIONS or CHANGES in the protocol as described in the attached memorandum. The protocol will be reconsidered when these issues are clarified and the revised protocol is submitted.
☐ The protocol was approved, subject to the approval of a WAIVER of provisions of NIH policy as noted below. Waivers require written approval from the granting agencies.
☐ The protocol was DISAPPROVED for reasons outlined in the attached memorandum.
☐ The Committee requests you to contact _______________ to discuss this proposal.
☐ A copy of this correspondence has been sent to the Vice President for Research.
☐ Amendment was approved as presented.

RESTRICTIONS, CHANGES OR WAIVER REQUIREMENT:

Approved # of Animals: 242 Gila Monsters, 90 Tortoise
Pain Level: 152 Gila Monsters D, all other animals C
Approval Period: 02/26/2009 - 02/25/2012
Funded: Arizona Game and Fish Department
Proposal Number: U07020
Title: Urban Influence of Gila Monster Ecology

Signature: ____________________________ Date: 2/27/09
IACUC Chair or Designee

Original: Principal Investigator
cc: IACUC Office
IACUC Chair
ORSPA/SPS

Institutional Animal Care and Use Committee (IACUC)
Office of Research Integrity and Assurance
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Animal Protocol Review

ASU Protocol Number: 09-1044R
Protocol Title: Physiological Trade-Offs Associated with Life in a Xeric
Principal Investigator: Dale DeNardo
Date of Action: 10/09/2009

The animal protocol review was considered by the Committee and the following decisions were made:

☐ The original protocol was APPROVED as presented.
☐ The revised protocol was APPROVED as presented.
☐ The protocol was APPROVED with RESTRICTIONS or CHANGES as noted below. The project can only be pursued, subject to your acceptance of these restriction or changes. If you are not agreeable, contact the IACUC Chairperson immediately.
☐ The Committee requests CLARIFICATIONS or CHANGES in the protocol as described in the attached memorandum. The protocol will be reconsidered when these issues are clarified and the revised protocol is submitted.
☐ The protocol was approved, subject to the approval of a WAIVER of provisions of NIH policy as noted below. Waivers require written approval from the granting agencies.
☐ The protocol was DISAPPROVED for reasons outlined in the attached memorandum.
☐ The Committee requests you to contact ________________ to discuss this proposal.
☐ A copy of this correspondence has been sent to the Vice President for Research.
☒ Amendment was administratively approved to perform pilot MRI evaluations of up to six Gila monsters.

RESTRICTIONS, CHANGES OR WAIVER REQUIREMENT:
Approved # of Animals: 242 Gila monsters, 90 Tortoise, 60 AZ Black Rattlesnakes
Pain Level: 152 Gila monsters D, all other animals C
Approval Period: 02/26/2009 – 02/25/2012
Sponsor: Arizona Game and Fish Department
Proposal Number: U07020
Title: Urban Influence of Gila monster Ecology

Signature: Sheryl Truxler ________________ Date: 10-9-09
for IACUC Chair or Designee

Original: Principal Investigator
cc: IACUC Office
IACUC Chair

Institutional Animal Care and Use Committee (IACUC)
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Animal Protocol Review

ASU Protocol Number: **09-1044R**
Protocol Title: **Physiological Trade-offs Associated with Life in a Xeric Environment**
Principal Investigator: **Dale DeNardo**
Date of Action: **02/26/2010**

The animal protocol review was considered by the Committee and the following decisions were made:

☐ The original protocol was APPROVED as presented.
☐ The revised protocol was APPROVED as presented.
☐ The protocol was APPROVED with RESTRICTIONS or CHANGES as noted below. The project can only be pursued, subject to your acceptance of these restriction or changes. If you are not agreeable, contact the IACUC Chairperson immediately.
☐ The Committee requests CLARIFICATIONS or CHANGES in the protocol as described in the attached memorandum. The protocol will be reconsidered when these issues are clarified and the revised protocol is submitted.
☐ The protocol was approved, subject to the approval of a WAIVER of provisions of NIH policy as noted below. Waivers require written approval from the granting agencies.
☐ The protocol was DISAPPROVED for reasons outlined in the attached memorandum.
☐ The Committee requests you to contact ________________ to discuss this proposal.
☐ A copy of this correspondence has been sent to the Vice President for Research.
☒ Amendment was approved by designated review to add a few additional but very minor activities.

RESTRICTIONS, CHANGES OR WAIVER REQUIREMENT:
Approved # of Animals: 242 Gila Monsters, 90 Tortoise
Pain Level: 152 Gila Monsters D, all other animals C **Species:**
Approval Period: 02/26/2009 - 02/25/2012
Funded: Arizona Game and Fish Department
Proposal Number: U07020
Title: Urban Influence of Gila Monster Ecology

Signature: ________________________________ Date: 2/26/10
IACUC Chair or Designee

Original: Principal Investigator
cc: IACUC Office
IACUC Chair

Institutional Animal Care and Use Committee (IACUC)
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Arizona State University
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Animal Protocol Review

ASU Protocol Number: **09-1044R**
Protocol Title: **Physiological Trade-Offs Associated with Life in a Xeric Environment**
Principal Investigator: **Dale DeNardo**
Date of Action: **02/09/2011**

The animal protocol review was considered by the Committee and the following decisions were made:

☐ The original protocol was APPROVED as presented.
☐ The revised protocol was APPROVED as presented.
☐ The protocol was APPROVED with RESTRICTIONS or CHANGES as noted below. The project can only be pursued, subject to your acceptance of these restriction or changes. If you are not agreeable, contact the IACUC Chairperson immediately.
☐ The Committee requests CLARIFICATIONS or CHANGES in the protocol as described in the attached memorandum. The protocol will be considered when these issues are clarified and the revised protocol is submitted.
☐ The protocol was approved, subject to the approval of a WAIVER of provisions of NIH policy as noted below. Waivers require written approval from the granting agencies.
☐ The protocol was DISAPPROVED for reasons outlined in the attached memorandum.
☐ The Committee requests you to contact ____________________ to discuss this proposal.
☐ A copy of this correspondence has been sent to the Vice President for Research.
☒ Amendment was approved by Designated Review to add new procedures and un-hatched quail eggs to the protocol.

RESTRICTIONS, CHANGES OR WAIVER REQUIREMENTS:

Total # of Animals:	**392**	**Pain Level:**	**D-152**	**Species:**	**Gila Monster**
			C-90		**Gila Monster**
			C-90		**Tortoise**
			C-90		**AZ Black Rattlesnake**

Sponsor: **AZ Game and Fish Department**
Proposal # **U07020**
Approval Period: **02/26/2009 – 02/25/2012**

Signature: ____________________ Date: 2/9/11
IACUC Chair/or Designee

Original: Principal Investigator
Cc: IACUC Office

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Animal Protocol Review

ASU Protocol Number: **12-1244R**
Protocol Title: **Physiological Trade-Offs Associated with Life in a Xeric Environment**
Principal Investigator: **Dale DeNardo**
Date of Action: **02/23/2012**

The animal protocol review was considered by the Committee and the following decisions were made:

☐ The original protocol was APPROVED as presented.
☒ The revised protocol was APPROVED as presented.
☐ The protocol was APPROVED with RESTRICTIONS or CHANGES as noted below. The project can only be pursued, subject to your acceptance of these restriction or changes. If you are not agreeable, contact the IACUC Chairperson immediately.
☐ The Committee requests CLARIFICATIONS or CHANGES in the protocol as described in the attached memorandum. The protocol will be considered when these issues are clarified and the revised protocol is submitted.
☐ The protocol was approved, subject to the approval of a WAIVER of provisions of NIH policy as noted below. Waivers require written approval from the granting agencies.
☐ The protocol was DISAPPROVED for reasons outlined in the attached memorandum.
☐ The Committee requests you to contact ____________________ to discuss this proposal.
☐ A copy of this correspondence has been sent to the Vice President for Research.
☐ Amendment was approved as presented.

Documentation of Level III Training will need to be provided to the IACUC office before the participant can perform procedures independently. For more information on Level III requirements see https://researchintegrity.asu.edu/training/animals/levelthree

Total # of Animals:	**260**	**Pain Level:**	**C-162, D-53** **E-45**	**Species:**	**Reptiles** **Mice**
Approval Period:	**02/23/2012 – 02/22/2015**				

Signature: C. Miller for D. Murphy Date: 2/24/12
IACUC Chair or Designee

Original: Principal Investigator
Cc: IACUC Office
IACUC Chair

Institutional Animal Care and Use Committee (IACUC)
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Animal Protocol Review

ASU Protocol Number:	**12-1244R Amendment #3**
Protocol Title:	**Physiological Trade-Offs Associated with Life in a Xeric Environment**
Principal Investigator:	**Dale DeNardo**
Date of Action:	**10/04/2012**

The animal protocol review was considered by the Committee and the following decisions were made:

Amendment was administratively approved to add Megan Murphy as additional personnel.

If you have not already done so, documentation of Level III Training (i.e., procedure-specific training) will need to be provided to the IACUC office before participants can perform procedures independently. For more information on Level III requirements see https://researchintegrity.asu.edu/training/animals/levelthree.

Total # of Animals:	**1,249**		
Species:	**Reptiles**	**Pain Level:**	**C-162; D-53**
Species:	**Mice**	**Pain Level:**	**E-45**
Protocol Approval Period:	**02/23/2012 – 02/22/2015**		

Sponsor:	**N/A**
ASU Proposal/Award #:	**N/A**
Title:	**N/A**

Signature: C. Miller　　　　Date: 10/4/12
IACUC Chair or Designee

Cc:　IACUC Office
IACUC Chair

Institutional Animal Care and Use Committee (IACUC)
Office of Research Integrity and Assurance
Arizona State University
660 South Mill Avenue, Suite 315
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Phone: (480) 965-4387 *FAX:* (480) 965-7772

Animal Protocol Review

ASU Protocol Number:	**12-1244R Amendment #4**
Protocol Title:	**Physiological Trade-Offs Associated with Life in a Xeric Environment**
Principal Investigator:	**Dale DeNardo**
Date of Action:	**10/10/2012**

The animal protocol review was considered by the Committee and the following decisions were made:

Amendment was administratively approved to add Courtney Holden as additional personnel.

If you have not already done so, documentation of Level III Training (i.e., procedure-specific training) will need to be provided to the IACUC office before participants can perform procedures independently. For more information on Level III requirements see https://researchintegrity.asu.edu/training/animals/levelthree.

Total # of Animals:	**260**		
Species:	**Mice**	**Pain Level:**	**E-45**
Species:	**Reptiles**	**Pain Level:**	**C-162; D-53**
Protocol Approval Period:	**02/23/2012 – 02/22/2015**		

Sponsor:	**N/A**
ASU Proposal/Award #:	**N/A**
Title:	**N/A**

Signature: C. Miller
IACUC Chair or Designee

Date: 10/10/12

Cc: IACUC Office
IACUC Chair

Institutional Animal Care and Use Committee (IACUC)
Office of Research Integrity and Assurance
Arizona State University
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Tempe, Arizona 85287-6111
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Animal Protocol Review

ASU Protocol Number:	**12-1244R Amendment #9**
Protocol Title:	**Physiological Trade-Offs Associated with Life in a Xeric Environment**
Principal Investigator:	**Dale DeNardo**
Date of Action:	**2/12/2013**

The animal protocol review was considered by the Committee and the following decisions were made:

Amendment was approved by Designated Review to add new procedures to the protocol.

If you have not already done so, documentation of Level III Training (i.e., procedure-specific training) will need to be provided to the IACUC office before participants can perform procedures independently. For more information on Level III requirements see https://researchintegrity.asu.edu/training/animals/levelthree.

Total # of Animals:	**260**		
Species:	**Reptiles**	**Pain Level:**	**C-162; D-53**
Species:	**Mice**	**Pain Level:**	**E-45**
Protocol Approval Period:	**02/23/2012 – 02/22/2015**		

Sponsor:	**N/A**
ASU Proposal/Award #:	**N/A**
Title:	**N/A**

Signature: C. Miller for D. Murphy Date: 2/15/13
IACUC Chair or Designee

Cc: IACUC Office
IACUC Chair

Institutional Animal Care and Use Committee (IACUC)
Office of Research Integrity and Assurance
Arizona State University
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Animal Protocol Review

ASU Protocol Number: **12-1244R Amendment #11**
Protocol Title: **Physiological Trade-Offs Associated with Life in a Xeric Environment**
Principal Investigator: **Dale DeNardo**
Date of Action: **4/18/2013**

The animal protocol review was considered by the Committee and the following decisions were made:

Amendment was administratively approved to add Guillaume Demare as additional personnel.

If you have not already done so, documentation of Level III Training (i.e., procedure-specific training) will need to be provided to the IACUC office before participants can perform procedures independently. For more information on Level III requirements see https://researchintegrity.asu.edu/training/animals/levelthree.

Total # of Animals:	**261**		
Species:	**Reptiles**	**Pain Level:**	**C-163; D-53**
Species:	**Mice**	**Pain Level:**	**E-45**
Protocol Approval Period:	**02/23/2012 – 02/22/2015**		

Sponsor: **N/A**
ASU Proposal/Award #: **N/A**
Title: **N/A**

Signature: C. Miller
IACUC Chair or Designee

Date: 4/23/13

Cc: IACUC Office
IACUC Chair