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Linking environment to ecology in arid land consumers : two case studies

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LINKING ENVIRONMENT TO ECOLOGY IN ARID LAND CONSUMERS: TWO CASE STUDIES

by

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B.S., Biology, New Mexico State University, 2004

DISSERTATION

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ABSTRACT

The physical environment has a profound influence over many aspects of animal ecology, such as governing the pace and timing of phenology and the patterns of activity across space and time. In extreme habitats such as deserts, the most important components of the physical environment are precipitation and temperature. Not only do these vary temporally and spatially, but this variation may influence the life history and ecology of species. My research examines how variance in temperature and precipitation influences the ecology of two species of desert dwelling consumers (desert woodrats and desert tortoises) over differing temporal scales. In Chapter 1, I describe how temperature constrains the daily activity of desert woodrats for much of the year. In the spring and summer, woodrats significantly reduce nocturnal activity outside of the den as environmental temperature increases. Indeed, during the warm summer months, nightly activity does not begin until temperature drops below the physiological lethal limit. This relationship is dependent upon gender and body mass. In Chapter 2, I estimate the tissue carbon incorporation rates and diet-to-tissue discrimination in desert tortoises. Characterizing these relationships in consumers is a critical part of being able to more accurately study how animal diet varies through the use of stable isotope analyses.

However, our current understanding of the tissue isotope dynamics in terrestrial ectotherms such as tortoises is poorly developed. In Chapter 3, I examine how precipitation shapes the nutritional ecology of desert tortoises on a seasonal basis across a precipitation gradient. Due to differences in digestibility and nutritional content, foraging on C_3 vs. C_4/CAM plant resources has important fitness repercussions for the consumer. I use the variance in growth ring carbon isotope ratios to estimate the dietary integration of C_3 and C_4/CAM plant resources across tortoise populations. Individual tortoise dietary breadth, as estimated by carbon isotopic variances, becomes more generalized as local rainfall decreases. I find that juvenile desert tortoises have a more narrow dietary breadth relative to adults, and adult male tortoises have less specialized diets compared to adult female tortoises.

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Introduction

The term ‘ecology’ was originally coined in 1866 by the biologist Ernst Haeckel to define the study of the relationships organisms have with their environment, or the “economy of nature” (Stauffer 1957). The term ‘environment’ refers to a biotic component, including all of the species that an organism interacts with, as well as an abiotic component, or the physical environment. The physical environment encompasses everything that can be thought of as being a physical or chemical variable, like temperature or humidity. Altogether these factors play an important role in characterizing the fundamental niche of an organism. Ecological interactions such as predation further refine where the animal is actually found, a concept called the realized niche (Hutchinson 1978; Tracy and Christian 1986). An organism’s niche is dynamic, and is subject to constraints involving the environment. Precipitation and temperature are the most commonly modeled physical variables that contribute to the determination of how an animal integrates itself in its surroundings. In this work I use stable isotope analyses and estimates of activity to examine how the ecology of two species of desert consumers, desert woodrats and desert tortoises, changes across gradients of temperature and moisture.

In Chapter 1, I document how desert woodrat activity is influenced on a daily basis by ambient air temperatures. Desert woodrats, *Neotoma lepida*, are small herbivorous murid rodents that occur widely throughout the desert southwest. Despite this, they are relatively recent occupants of desert environments, and indeed are not especially ‘good’ desert animals having few adaptations for water conservation or effective heat dissipation. Indeed these animals have their evolutionary origins in relatively equitable regions of coastal California (Patton et al. 2008). Daily high air

temperatures are above the upper lethal temperatures for desert woodrats for weeks at a time at our study site in Death Valley, CA, USA, and may not drop below the lethal temperature until well after sunset. I find that the amount of time that desert woodrats spend active outside of the thermally buffered den declines significantly as ambient air temperatures increase. My results suggest that woodrat activity is tightly constrained during the hot summer months, and that animals face a tradeoff between remaining in the thermal safety of the den *vs*. emerging to obtain resources and being exposed to potentially lethal air temperatures.

In chapter two I explore the carbon tissue isotope dynamics in desert tortoises. Because consumers are isotopically coupled to their diet, stable isotope analyses are a commonly used set of methods employed to better understand diverse aspects of animal ecology, from patterns of migration to tracing the flow of energy across a landscape. However, our confidence in interpreting consumer tissue stable isotope data are only as reliable as the available laboratory validation studies allow, and certain groups of organisms, such as terrestrial ectotherms, are under-represented in these validation experiments (Dalerum and Angerbjorn 2005; Warne et al. 2010). In Chapter 2, I estimate the carbon incorporation rates and diet-to-tissue discrimination in several tissues from desert tortoises undergoing a diet switch. Data such as these provide a more robust framework for interpreting the stable isotope data from similar consumers in a field setting.

In the third chapter I examine how desert tortoise dietary breadth changes across a precipitation gradient. To do this, I use growth ring series from individual tortoises spanning up to three decades. C_3 and C_4/CAM plants have non-overlapping carbon

isotope ratios. The tissue carbon isotope ratios in consumers reflect those of integrated food resources. Consequently, I use the variance in carbon isotope ratios to trace the flow of these plant resource groups in desert tortoises, and to estimate dietary breadth across populations. In this way I use growth ring sequences from individual tortoises as a 'walking' dietary chronology. I find that in hotter and drier regions, individual tortoise dietary specialization is relatively low, while in more mesic environments desert tortoises shift to a more specialized feeding strategy. Additionally, there are important differences in the plant resource groups integrated and the degree of dietary specialization between tortoise age classes (juvenile *vs*. adult) and between males *vs*. females.

Each of these chapters is a discrete body of work, but they are complementary in their goals of providing a better understanding of how animal species are linked to their environment. Through gauging the ecological responses of animals to a spectrum of moisture and temperature gradients, we can begin to better appreciate the potential ecological impacts that global climate change may usher into the future.

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Chapter 1: The influence of the thermal environment on activity patterns of the desert woodrat (*Neotoma lepida)*

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Abstract

Environmental temperature influences the ecology and life history of animals. In habitats near the thermal range boundary, fluctuations in temperature may influence the ability of species to persist. Desert woodrats *(Neotoma lepida*) occupy one of the hottest and most extreme environments in the Western hemisphere, Death Valley, California, despite limited adaptations for water conservation or efficient heat dissipation. Moreover, woodrats have a relatively low tolerance for high temperature. Thus, we hypothesized temperature might influence both the timing and duration of activity. To test this idea, we attached iButton sensors to 56 animals over a two year period and recorded activity. Each sensor was set at 5 or 15 minute intervals and stored ~2,000 records before retrieval. As expected, we found a strong relationship between ambient temperature and the onset and duration of activity, influenced by both body size and gender. Woodrats did not emerge until ambient temperature fell below 42°C. As daily high temperatures increased, both sexes had fewer nightly activity bouts of shorter duration. Our results suggest woodrat activity is tightly constrained during the climatically intense summer months; animals face a tradeoff between remaining in the thermal safety of the den *vs.* emerging to obtain resources.

Introduction

Deserts are arguably one of the most extreme and seemingly inhospitable terrestrial environments on Earth. Shaded air temperatures in excess of 45°C are routine, and surface temperatures may exceed 85°C (Hunt et al. 1966). Yet these regions are populated by their own distinctive biota, whose ecologies are largely shaped by their thermal environments. Animals living in deserts face numerous problems that are all ultimately related to the need to maintain homeostasis in a harsh environment. These problems can be especially severe for mammals who typically maintain core temperatures that are significantly cooler than ambient temperature and who may not have abundant access to water for evaporative cooling. Satisfying the basic/constant requirements for food and water are also difficult when primary productivity is unpredictable and sporadic, as is common in deserts. Although species in deserts are necessarily adapted to the harsh environment, nonetheless, at times they may be close to their thermal limits. For example, unusually severe heat waves recently contributed to massive bird and fruit bat die-offs in western and southeastern Australia (Serventy 1971; Wolf 2000; Welbergen et al. 2008; McKechnie and Wolf 2010). Hence, warming temperatures in deserts over the next decades may influence animals in deserts more than in other habitats. An important area study is how desert animals will fare in the face of anthropogenic warming and drying trends.

Woodrats (*Neotoma*) are a genus of medium-bodied herbivorous, murid rodents. Several species are found in the Southwestern deserts, including the desert woodrat, *Neotoma lepida*. This is somewhat surprising because woodrats have a relatively low tolerance for high temperatures (Brown 1968; Smith et al. 1995; Smith and Betancourt

1998). Moreover, they lack the physiological adaptations common in other desert dwelling mammals, such as the ability to produce highly concentrated urine, a counter current water recovery system in the nasal cavity, or aestivation during unfavorable conditions (Schmidt-Nielsen and Schmidt-Nielsen 1950; MacMillen 1964; Tracy and Walsberg 2002). Indeed, the name "desert woodrat" is a bit of a misnomer; the species originated in coastal California and only expanded into the Mojave Desert during the late Pleistocene (Patton et al. 2008).

Woodrats are able to survive in desert habitats because of their ability to construct houses. All species build complex structures of locally gathered debris that can reach several meters in height and diameter. These houses or dens typically contain several entrances and multiple food and nest chambers (Stones and Hayward 1968). They are important thermal refugia, ameliorating air temperature by as much as 3 - 9°C (Vorhies 1945; Lee 1963; Brown 1968). Dens also provide significant protection against predators (Lee 1963; Brown 1968; Stones and Hayward 1968; Cameron and Rainey 1972; Smith 1995).

Here we describe the influence of temperature on the movements and daily activity patterns of the desert woodrat, *N. lepida* in Death Valley, California, USA. This is one of the hottest and driest environments in the Western hemisphere and one that poses significant challenges to animals. Because of the extreme topography, Death Valley lies in a severe rain-shadow, which coupled with below sea-level elevation, contribute to a hyper-arid climate. Maximum daily temperatures regularly exceed 50°C during the summer; much higher than the upper lethal temperature for *N. lepida* of ca. 41°C, although different investigators have found this figure to vary slightly in either

direction (38 - 40°C; Lee 1963; 41.4°C; Brown and Lee 1969; 43°C; Nelson and Yousef 1979). Moreover, temperature is projected to increase between 2.0 – 5.0°C within the next 100 years (Cayan et al. 2009). Because woodrats are highly sensitive to high temperatures (Smith et al. 1995; Smith and Betancourt 1998; Smith et al. 1998), we suspected that the thermal environment might constrain the duration and onset of foraging and other activities. Woodrats are highly sedentary and display high site fidelity. Indeed, in this habitat, most animals limit nightly activity to the confines of the mesquite hosting the home den (*personal observation* of radio-collared individuals). While the den ameliorates environmental temperature to some degree, woodrats must leave their dens to forage and to search for mates. Thus, temperature may directly impact fitness by modulating activity levels. Here, we address the following questions: 1) *Is the onset of foraging and other activities influenced by ambient temperature?* 2) *Is the duration of activity constrained by temperature?* 3) *Do gender and body mass influence the onset and duration of activity?*

Materials and Methods

Study Area

Our study site lies on a ca. 10 ha plot of scattered honey mesquite, *Prosopsis glandulosa*, about 2 km northwest of the Furnace Creek Ranger Station at Furnace Creek, Death Valley (N 36° 27.7°0 W 116° 52.00°) at an elevation of -77 m (Fig. 1). The site is situated between the base of a large alluvial fan extending from the Funeral Mountains to the east, and the salt pan occupying ca. 520 km^2 on the valley floor (Hunt et al. 1966). It is in this area of relatively shallow subsurface water that *P. glandulosa* grows, often in continuous linear formations, tracking available ground water (Fig. 1B). Temperatures

here are among the hottest in the world and nightfall brings little relief (Roof and Callagan 2003). The intense heat is coupled with irregular annual precipitation that averages 4.8 cm/yr. There is a complete lack of an herbaceous understory or other vegetation, tying the desert woodrat, and its need for succulent vegetation, solely to the honey mesquite. *N. lepida* build their dens at the bases of the mesquite at this site.

Trapping Protocol

We live-trapped *Neotoma lepida* on a monthly basis from 2003 to 2008. We permanently placed Sherman live-traps in each of 40 individually marked mesquite clumps, or complexes (Fig. 1B). The site was visited monthly and trapped for 3 consecutive nights for a total of ~500 trap nights/month, or 19,500 trap nights over the duration of the study. Traps were baited with apple and during the cooler months both oatmeal and insulating polyester batting were included. Upon initial capture, we individually marked animals with numbered fingerling tags clipped onto each ear (National Band and Tag Co., model #1005-1). At each subsequent capture, we recorded mass, sex, reproductive status, and assessed body condition. We worked under supervision of the Animal Care and Use Committee of the University of New Mexico, and followed appropriate guidelines as outlined by Gannon et al. 2007. (Protocol # 08UNM073-TR-100489).

Because of high coyote activity, our traps were wired to branches located 2 – 4 m up in the mesquite canopy, instead of on the ground. An additional modification to our trapping protocol was necessary because of the extreme temperature regimes. During May – September traps were not set until 2220h because of the high night time temperatures. Even with this modification, traps had to be checked and processed within

an hour as rats began dying from hyperthermia if forced to remain outside the den for longer periods. In contrast, nightly lows often dip below 0°C during the winter months, and despite the addition of polyester batting, hypothermia was a problem, especially with the smaller females. Accordingly, during the winter months, traps were closed by 0000h.

Temperature Recording

We used miniature temperature data-loggers (Thermochron iButtons, model DS1921G, Dallas Semiconductor, 3.3 g) mounted on collars to study the movements of free-ranging woodrats. Because we are interested in activity patterns, we concentrated on changes in temperature rather than the actual ambient temperature. As the thermal environment outside the den was so much warmer than inside, we were able to determine where animals were by the temperatures recorded on the iButtons. We set the iButtons to record at 15 minute intervals. This provided 2,048 consecutive readings, or ~ 22 days per usage interval. A subset of animals (N = 3) wore collars set to intervals of five minutes. The small size of this data-logger combined with its high-capacity memory allowed us to passively record activity patterns of naturally behaving animals over long periods of time.

In both 2006 and 2007, we outfitted 56 rats (32 males, 24 females) with iButtons. These were mounted on circular foam platforms using metal snaps, and placed around the neck of a rat using a cable tie. The cable ties were threaded through a small piece of foam, and a metal snap base was epoxied to this. The opposite end of the snap was glued to the iButton itself, which allowed rapid retrieval and exchange. The total package weighed between 6 – 8 g, and did not exceed 10% of animal mass. Upon recapture of the animal, data were downloaded via a reader connected to a computer interface and a new collar installed.

Temperature Records

We used two sources to characterize the temperature for the site, which differed in the degree of spatial averaging and duration. First, we empirically recorded temperatures at a variety of heights within the mesquite canopy. The iButtons were placed at 0.3 m and 2.0 m within several mesquite trees. These heights are biologically relevant because they represent the height of woodrat dens and the foraging trails of the rats, respectively. Second, we obtained a 100 year Death Valley weather dataset compiled by Roof and Callaghan (2003). From these data we characterized the historical record of daily maximum/minimum temperatures for the site compared with those from the individual rat collars and the available ambient temperature records (Fig. 2).

Data Analysis

Over the course of the study we collected > 44,000 individual woodrat activity readings, which complicated the processing of data. To make these data more tractable, we assigned each day to a relative measure of temperature intensity. Daily high temperature is robustly related to nocturnal temperature ($r = 0.91$; $p = 0.000$, $df = 363$). We extracted daily high temperatures, and binned them into four measures of thermal stress based on biologically relevant benchmarks: low, medium, high, and severe, representing temperatures below the thermoneutral zone (< 31°C), within the thermoneutral zone (31°C to 35°C), above the thermoneutral zone (>35°C < 41°C), and above upper lethal temperature (>= 41°C), respectively (Lee 1963; Brown and Lee 1969; Nelson and Yousef 1979). That these benchmarks derived from the literature were relevant for the woodrats in Death Valley was evident in the trap mortality records;

animals died if they were forced to spend greater than one hour in temperatures of > 40°C.

We divided data between nocturnal and diurnal periods and defined the onset of activity as a demarcated, abrupt deviation from baseline diurnal den temperature. Because postural adjustments or movements within the den could be reflected in rat temperature profiles, we used the mean standard deviation for an individual rat's recorded diurnal temperature chronology as the cutoff for separating outside activity from activity within the den. If the difference between two consecutive temperatures was greater or equal to the mean diurnal standard deviation for that given rat, we gave that record a value of 15 minutes (derived from the period of time between two data points). We summed every rat's activity on a nightly basis to estimate time spent outside of the den. We recorded the number of nightly activity bouts for a rat as the total number of episodes where there was at least 15 minutes of activity.

Statistical Analyses

To study how temperature constrains onset and duration of activity and to examine the influence of body mass and gender on these measures, we fit linear mixed effects models with sex, body condition, and daily high temperature category as fixed factors. Because we had many repeated measurements on a limited number of individuals for largely non-overlapping periods of time, the utility of tests such as repeated measures ANOVA is limited. To account for pseudo-replication, we treat rat identity as a random factor in all of our mixed effects models. This allowed us to examine how the factors of interest (i.e., temperature) influenced woodrats, while accounting for the between rat variance. We set significance at $\alpha = 0.05$, and used SPSS

19, SigmaPlot 8.0, and Microsoft Excel for all analyses and figures. All means are followed by the standard errors (SE) reported with the marginal means from the mixed effects models.

Results

Adult body mass of woodrats at Furnace Creek demonstrates a clear annual cycle (Fig. 3). The median body mass of the population declines sharply in the summer months. These changes are not the result of decreases of body mass of individual animals, but rather, reflect higher mortality of larger individuals (Smith et al. in prep.).

Onset of activity

We retrieved 34 of 99 (21 of 32 individuals) iButtons affixed to males, and six of 41 (7 of 24 individuals) on female rats. After accounting for equipment malfunction, usable data consisted of 468 woodrat nights from 14 individuals (10 males and 4 females) with over 44,000 individual readings. The mean monthly ambient temperature at the initiation of nightly woodrat activity closely tracks mean monthly daily high temperatures. During the summer months of June through August where mean daily maximum temperature is consistently > 45°C, woodrats did not begin nightly foraging activities until ambient temperatures were below 42°C (Fig. 4). This is a significant threshold as it represents the empirically derived upper lethal temperature (Lee 1963; Nelson and Yousef 1979). We also observed significant differences in timing of nocturnal emergence between male and female woodrats; females initiated nightly movements considerably later than males (mean female emergence post-sunset: 95.3 ± 11.3 min *vs.* mean male emergence post-sunset: 59.5 ± 6.7 min; $F_{(1,9.6)} = 7.0$; $P = 0.025$).

Body size is an important determinant of the onset of nightly activity. Larger rats were active less than smaller rats; each ~1g increase in body mass led to an activity reduction by 2.5 minutes (ß = -2.5 ± 0.6; $F_{(1,263)} = 18.9$; P = 0.000). Moreover, this difference was exacerbated in males; larger individuals emerged over 1 min/g later (min post-sunset) than did smaller ones (ß = 1.2 ± 0.4; $F_{(1,22.4)} = 8.4$; P = 0.008). For example, males with body mass of 1 SD greater than the population mean (160 g; mean male mass = 136.0 ± 23.4 g; n = 207) emerged later in the evening (72.0 ± 9.0 min *vs.* 49.0 ± 7.0 min) and were less active overall (246.0 ± 15.0 min *vs.* 311.0 ± 22.0 min) than those smaller than 160 g.

Duration of activity

Woodrats are nocturnal animals. Because of differences in photoperiod, the duration of potential activity time varies seasonally. The decrease in activity observed with increasing daily temperature persisted even after accounting for the proportion of available nocturnal hours utilized ($F_{(3,454.2)} = 13.8$; P = 0.000; Table 1). The difference in activity following 'low' temperature and 'severe' temperature days was striking (40.1 ± 5.4% *vs.* 23.9 ± 5.2%). As might be expected, time outside the den was intermediate following 'medium' and 'high' temperature days (31.4 ± 5.5% and 29.4 ± 5.4%, respectively). Interestingly, at each temperature level animals in good condition were active 6.5 ± 2.3% longer than those in poor condition (34.4 ± 5.3% *vs.* 27.9 ± 5.3%, respectively; $F_{(1,429.9)} = 7.7$; P = 0.006). It also appeared that individuals occupying larger mesquite complexes engaged in higher levels of activity (ß = 0.04 ± 0.01; $F_{(1,17.3)} = 8.4$; P = 0.01).

As daily high temperatures increased over the summer, all woodrats showed a precipitous decline in nightly activity (Table 1), but at any specific temperature, males were active for significantly longer than females. On average, this was 133.3 ± 58.4 minutes per night ($F_{(1,15.9)} = 5.2$; P = 0.036; mean female activity: 124.0 ± 49.8 min *vs.* mean male activity: 257.3± 29.2 min; $F_{(1,15.9)} = 5.2$; P = 0.036). This trend was not monotonic, however, and activity closely mapped the actual diurnal temperature. For example, following 'low' temperature days, woodrats were almost 80% more active than after 'severe' temperature days (251.4 ± 29.1 minutes *vs.* 142.4 ± 28.4 minutes; Tukey's LSD; P = 0.000). While there was a trend for nocturnal activity to be higher after 'medium' temperature relative to 'high' temperature days (190.7 ± 31.2 minutes *vs.* 177.9 ± 30.3 minutes), this was not significant (Tukey's LSD; P = 0.44).

As might be expected, activity patterns changed during the winter/spring reproductive season and were different for males and females. Male rats were consistently active for about half the night time hours (41–52%) during the months of January through July, when corrected for changing day length (Fig. 5). When females were no longer receptive, however, there was a significant decline in male activity. In September and October, for example, woodrats were active for only 10-12.0 % of the night time hours (Fig. 5). Activity increased markedly just prior to when females once again became sexually receptive (e.g., in December 366 min; 43.0 ± 5.0%).

While male activity patterns represent both foraging and searching for mates that of females is largely centered on the search for resources. Females exhibited a steady decline in nighttime activity from a high of 34.0 ± 5.0% (283 min) in January, to a low of 5.0 ± 2.0% (32 min) in April. As temperatures warm, females became active for a larger

proportion of the night (Fig. 5). We have no data for July – August, but note that female rats paralleled the September lull in activity for males (10 ± 9.0%), as well as the marked activity increase in December (47.0 ± 4.0%).

Woodrats made significantly fewer trips outside the den as daily high temperatures rose ($F_{(3,437.4)} = 8.2$; P = 0.000; Table 1). Indeed, under 'severe' conditions, the number of trips outside the den was cut almost in half (Table 1). There was no significant difference between males and females. Larger woodrats engaged in fewer activity bouts overall (ß = -0.06 ± 0.03; $F_{(1,459.5)} = 5.2$; P = 0.024) and animals inhabiting larger mesquite 'complexes' initiated more activity bouts (ß = 0.04 ± 0.01; $F_{(1,53.0)} = 34.9$; P = 0.000). The decrease in activity with rising temperature was quite consistent; woodrats instigated 0.1 fewer activity bouts for each one degree increase in daily ambient temperature (ß = -0.08 ± 0.03; $F_{(3,437.4)} = 8.2$; P = 0.000).

Not only did woodrats reduce the number of activity episodes as temperatures increased, but male and female rats spent less time active on a given bout of movement as temperatures increased (34.5 ± 3.1 *vs.* 22.2 ± 2.6 minutes after 'low' and 'severe' temperature days, respectively; Tukey's LSD; P = 0.000; Table 1). The mean duration of an activity bout following daily temperature exceeding the upper critical temperature was significantly less than that following days within the thermoneutral zone (Tukey's LSD; P = 0.045). This pattern was moderated by animal condition; woodrats in good condition spent on average 4.4 ± 1.9 minutes more per activity bout than those in poor shape ($F_{(1,254.6)} = 5.2$; P = 0.024). Again, animals living in larger mesquite 'complexes' invested more time in each activity bout (ß = 0.02 ± 0.001; $F_{(1,15.8)} = 9.9$; P = 0.006), and

less time per activity bout as seasonal night length increased (ß = -0.04 ± 0.01; $F_{(1,353.8)}$ = 9.9; P = 0.002).

Discussion

Our results clearly demonstrate that environmental temperature influences the activity patterns of woodrats at Furnace Creek, and moreover, that the magnitude of the effect depends on gender and body mass. As temperature increased, animals have less time to spend for the essential activities of mating and foraging. Higher temperature leads not only to a reduction of nightly activity bouts, but also limits the duration of each.

Temperatures of over 50°C can occur anytime during the spring and summer months. Indeed, between April and October the mean daily maximum temperature at Furnace Creek, Death Valley never drops below 35°C. Yet, numerous studies have demonstrated that the maximum temperature in the thermoneutral zone of *N. lepida* is 35°C, and the upper lethal temperature is around 41°C (Lee 1963; Brown and Lee 1969; Nelson and Yousef 1979).

The ability of woodrats to survive in an environment that has temperatures consistently over lethal is entirely due to the den and the availability of succulent mesquite. However, woodrats are unable to store sufficient forage within the den to meet water demands during the warm summer months; clipped mesquite loses virtually all of its water content within five hours (J. Martin, personal communication, 2011). Thus, animals are forced to leave the thermal neutrality of their dens to obtain required resources even under the most extreme of conditions. Regardless, woodrats did not commence nightly activities until ambient temperatures were ≤ 41°C, which is at the

empirically derived upper lethal temperature (Lee 1963; Brown and Lee 1969; Nelson and Yousef 1979; Fig. 3).

Woodrat body mass and gender are important determinants of how ambient temperature constrains the initiation and extent of activity outside of the den. Male woodrats at this site are always active longer than females (Fig. 5; Table 1), and larger animals are active less and emerge later than smaller individuals. We suggest that this may be due in part to the demanding energetic requirements sustained by reproducing females. The energetic demands of reproduction are especially high in the lactating female, and one strategy that can be employed to minimize energy costs is to minimize locomotor activities (Sorensen et al. 2005*a*). Indeed various rodent species limit activity during peak energy requirements associated with reproduction (Slonaker 1925; Richards 1966; Randolph et al. 1977; Wade and Schneider 1992). Additionally, five years of trapping data suggest that female woodrats at this site are under more intense thermal selection of body mass following peak summer temperatures, a phenomenon probably intertwined with the intense demands of reproduction (Fig. 3; Smith et al. in prep.), which may compel them to remain within the temperature-buffered den for longer periods of time. Male woodrats are not as constrained by such high costs of reproduction enabling them to invest in behaviors such as mate searching and scent marking while occupying larger home ranges (Cranford 1977; Vaughan and Schwartz 1980; Conditt and Ribble 1997; Henke and Smith 2000), but see MacMillen (1964) and Bleich and Schwartz (1975). Indeed, individual males in this population are more likely than females to engage in long movements culminating in the switching of occupied mesquite trees (H. Lease, personal communication, 2012).

In addition to body mass, body condition and home mesquite complex size were important factors influencing the onset and duration of activity. Lethal temperature scales inversely with body size in *Neotoma* (Smith et al. 1995; Smith and Charnov 2001), thus it is not surprising that larger woodrats were forced to emerge from their dens later, and engaged in less outside activity. For each one gram increase in body mass, there was a two and a half minute reduction in nocturnal activity. Large males (>160g) were on average active 65 minutes less each night and emerged 23 minutes later, relative to small males (<160g). Further, at a specific temperature, rats in better body condition were active for 40.7 ± 12.7 minutes longer per night and 5.1 ± 2.0 minutes per activity bout than those in poor body condition. Rats in poor condition generally had patchy, dull pelage, and a thin and bony overall feel to them. Rats in this state may minimize the proportion of their time devoted to intra-specific interactions and/or require longer periods of time within the thermally buffered den to maintain homeostasis, which results in reduced activity.

The size of mesquite trees occupied by woodrats was also an important determinant of activity. Individuals living in larger home complexes were active for 0.02 ± 0.007 minutes per activity bout, initiated 0.04 ± 0.006 more activity bouts, and were 0.2 ± 0.08 minutes more active per night for each meter increase in home complex area (213 – 1,216 m^2). Larger complexes presumably offer better predator protection (pers. obs.), and probably have better thermal buffering capacity in the form of older, more established den sites as well as greater canopy coverage to buffer against daily high temperatures. Also, larger complexes simply hold more dens. Animals living within 'good' complexes such as these probably invest more time making territorial rounds of

their home territory and may encounter and engage in more rat-rat interactions as a result of the larger area of mesquite occupied.

Our results suggest the warming climate over the next decades will likely influence the fitness of desert woodrats in Furnace Creek, Death Valley. Annual temperatures in California are projected to increase up to 5°C over the next century (Cayan et al. 2009). Mean maximum July temperatures (the hottest month of the year) have already risen almost 1.0°C over the last 2 decades (Roof and Callaghan 2003). Indeed, the mean maximum temperature for every day in July has increased over the past 20 years, relative to the almost 100 year mean maximum temperatures (Roof and Callaghan 2003).

Warmer temperatures led to decreased time spent outside of the den for both males and females. The reduction in activity we found (Table 1) shortens drastically the time available for meeting energy and water requirements. For example, field and laboratory studies suggest that a typical 125 g adult woodrat requires 161 kJ/day and 5.0 g H_2O/day just to maintain body mass (Lee 1963; Karasov 1989; Schmidt-Nielsen 1997). Desert woodrats have dry matter feeding intake rates of 0.16 g min^{-1} and 2.89 g $meal^{-1}$ kg^{-1} (Sorensen et al. 2005*b*) and an apparent energy digestibility of ca. 60% (Karasov 1982; Karasov 1989; Smith 1995). Given that a "severe" temperature day resulted in a reduction of activity by 49 minutes and 2 activity bouts (Table 1), this corresponds to a potential loss of opportunity to gain 86.3 kJ of energy and 6.8 g of water (for a loss of 49 minutes; Table 2). This is a substantial amount, representing 54% of the energy and 136% of the total daily water requirements of a 125 g animal (minus losses in feces and urine, and not taking into account metabolic water production). Furthermore, our

calculation ignores the potential loss of water and energy required for maintaining homeostasis in the face of foraging in extreme temperature. Clearly, even warmer temperatures would more drastically influence this physiological balance. At some point, the tradeoffs must be great enough that remaining in the den despite a lack of water or forage is preferable. Larger animals are even more susceptible to the negative effects of high temperatures, and likely face stiffer tradeoffs. Indeed, we found a significant difference in the body mass of animal trap moralities in the summer *vs.* winter months. While small individuals were more likely to die in traps during the winter, this pattern was reversed in the summer months (104.8 ± 4.4 g *vs.* 121.7 ± 6.4 g, respectively; df= 22; t-test; P = 0.04). Despite our hourly checks of traps, these mortalities represented animals forced to remain away from the thermal safety of their dens for longer than was physiologically possible given the temperature extremes. Additionally, our monthly trapping data clearly indicates a seasonal pattern of mass with temperature (Fig. 3).

Water in particular, may be the driving force behind the continuation of foraging despite extreme temperatures. Not only are woodrats in Death Valley entirely dependent on freshly clipped mesquite to meet their water requirements (J. Martin, personal communication, 2011), but they have relatively high water requirements. For example, at temperatures up to 50°C Nelson and Yousef (1979) recorded evaporative water losses over 5.0 mg H_2O/mL O_2 in desert woodrats. These values are considerably higher than those of other rodent species (Schmidt-Nielsen and Schmidt-Nielsen 1950; Hudson 1962; Tucker 1965; Breyen et al. 1973; Bradley et al. 1975). On multiple occasions at our study site, we observed heat-stressed *N. lepida*, profusely coating the anterior portions of their body with saliva. This undoubtedly helps alleviate heat loads, but presumably

produces a severe water deficit detrimental to fitness. Any factor, such as increasing ambient temperature, which constrains the time spent outside of the den makes homeostasis more difficult to achieve. Interestingly, larger animals should be better able to withstand water deprivation because of their higher mass (Peters 1983; Calder 1984); this may help explain why animals are so large on this site (Smith and Charnov 2001).

A major emphasis of much of modern science is to understand how organisms will respond to the warmer temperatures, drier conditions, and more frequent extreme weather predicted over coming decades. Woodrats at Furnace Creek are already operating at close to their thermal limits. Higher summer temperatures may further restrict activity outside of the den, thus compromising the survivorship and fecundity of animals on the site. For example, woodrat activity is reduced to 115 minutes at 55°C and 96 minutes at 60°C, scarcely accommodating the time required to intake the minimal amount of mesquite necessary to meet energetic requirements (Fig. 6). Indeed, our computations suggest that this unique population of rodents may be unable to persist under the most pessimistic of climate change scenarios.

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Table 1.1. The effect of daily maximum temperature on desert woodrat (*Neotoma lepida*) activity the following night.

Activity parameter	**Low < TNZ (< 31°C)**	**Medium TNZ (31 – 35°C)**	**High >TNZ < ULT (>35C < 41°C)**	**Severe >ULT (>= 41°C)**
Activity (min)	251.4 ± 29.1[a]	190.7 ± 31.2[b]	177.9 ± 30.3[b]	142.4 ± 28.4[c]
% night active	40.1 ± 5.4[a]	31.4 ± 5.5[b]	29.4 ± 5.4[b]	23.9 ± 5.2[c]
Mean activity bout duration (min)	34.5 ± 3.1[a]	27.1 ± 3.0[b]	25.3 ± 2.8[b,c]	22.2 ± 2.6[c]
# activity bouts	11.3 ± 3.9[a]	9.1 ± 3.9[b]	8.3 ± 3.9[b]	6.8 ± 3.9[c]

Note: Different letters represent significant differences between temperature categories (Tukey's LSD; $P < 0.05$). Marginal mean values are presented with model standard errors. Daily high temperatures are binned into low, medium, high, and severe categories based on the empirically determined thermoneutral zone (TNZ) and upper lethal temperatures (ULT) for *N. lepida*.

Table 1.2. Hypothetical lost opportunities for energy and water intake associated with reductions in desert woodrat (*Neotoma lepida*) activity.

Activity reduction	**Mesquite leaf energy (kJ/g dry matter)**	**Mesquite leaf water (% water by mass)**	**Intake per unit time (g dry matter/min)**	**Intake per meal (g dry matter/meal)**	**Energy loss (kJ)**	**Water loss (g)**
49 minutes	18.35	46.3	0.16	*	86.3	6.8
2 activity bouts	18.35	46.3	*	0.36	8.0	1.3

Note: We assume that a single activity bout equates to one meal. Intake rate data are drawn from Sorenson, Heward, and Dearing (2005*b*) captive *N. lepida* of 125 g. Mesquite leaf energy content from Baptista and Launchbaugh (2001), and leaf water content is J. Martin (personal communication, 2011).

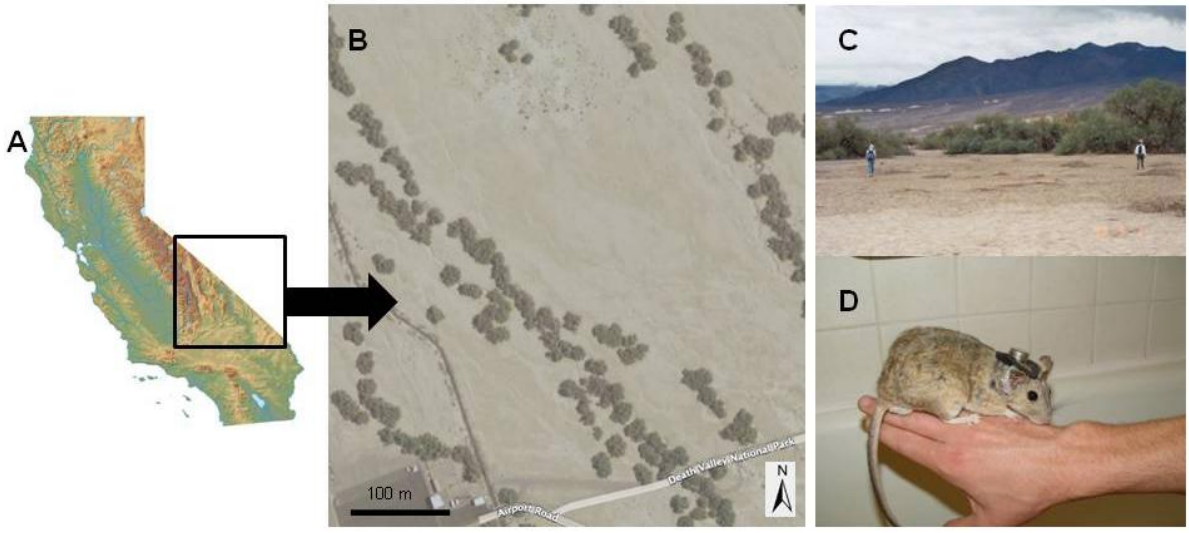

Figure 1.1. Furnace Creek, Death Valley, California *Neotoma lepida* study site. A) Approximate location of Death Valley, California. B) Aerial image of our trapping location, showing individual mesquite clumps where trapping grids are located, and the barren nature of the intervening landscape. C) Ground view of several trapped mesquite 'site complexes.' D) *N. lepida* equipped with iButton.

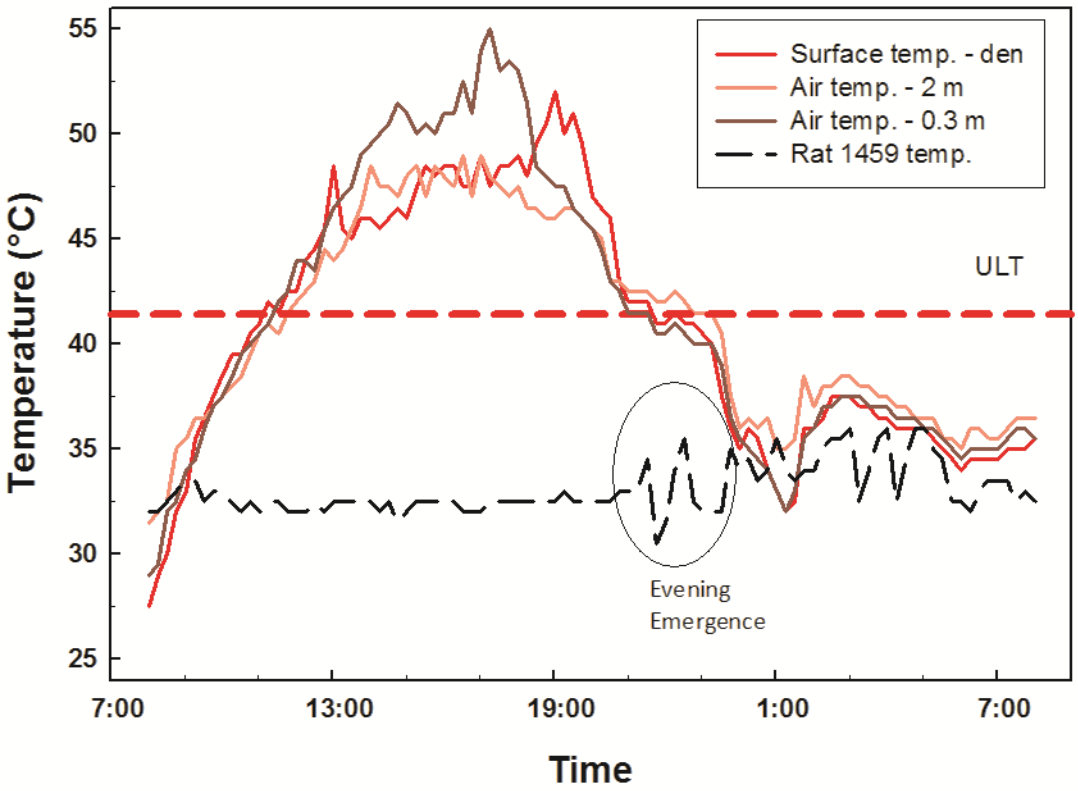

Figure 1.2. Twenty-four hour tracing of ambient and woodrat iButton temperature. iButton temperature chronologies illustrating temperature spikes indicative of activity outside of the thermal stability of the den, for one *N. lepida* over a one day period in June 2007, relative to iButton traces recorded at variable heights within a mesquite complex on our study site. (30 cm = iButton placed on a wooden stake 30 cm above a den, 2 m = iButton placed on a wooden stake 2 m above the ground in the mesquite canopy, and den = iButton placed on the surface of the same woodrat den.) Upper lethal temperature (ULT) is given as 41.4°C.

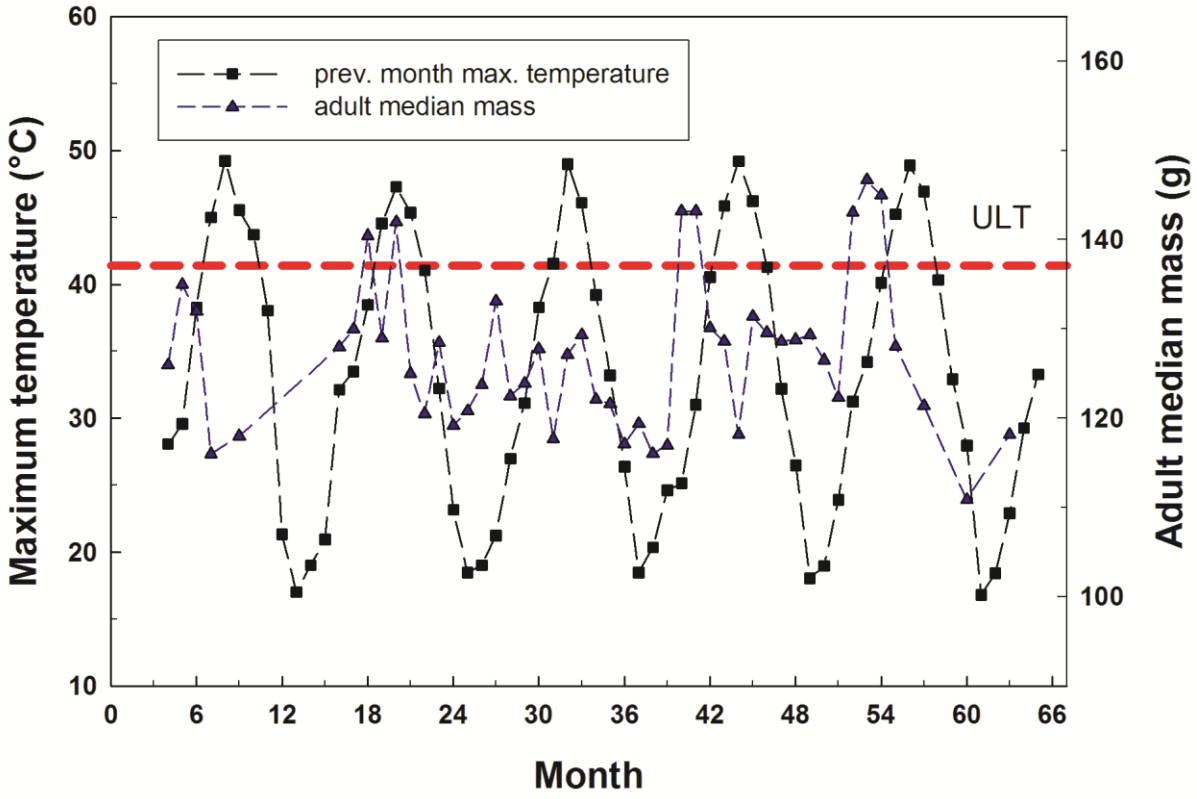

Figure 1.3. Monthly adult median woodrat body mass plotted against mean maximum monthly daily temperatures for the previous month. Upper lethal temperature (ULT) is given as 41.4°C.

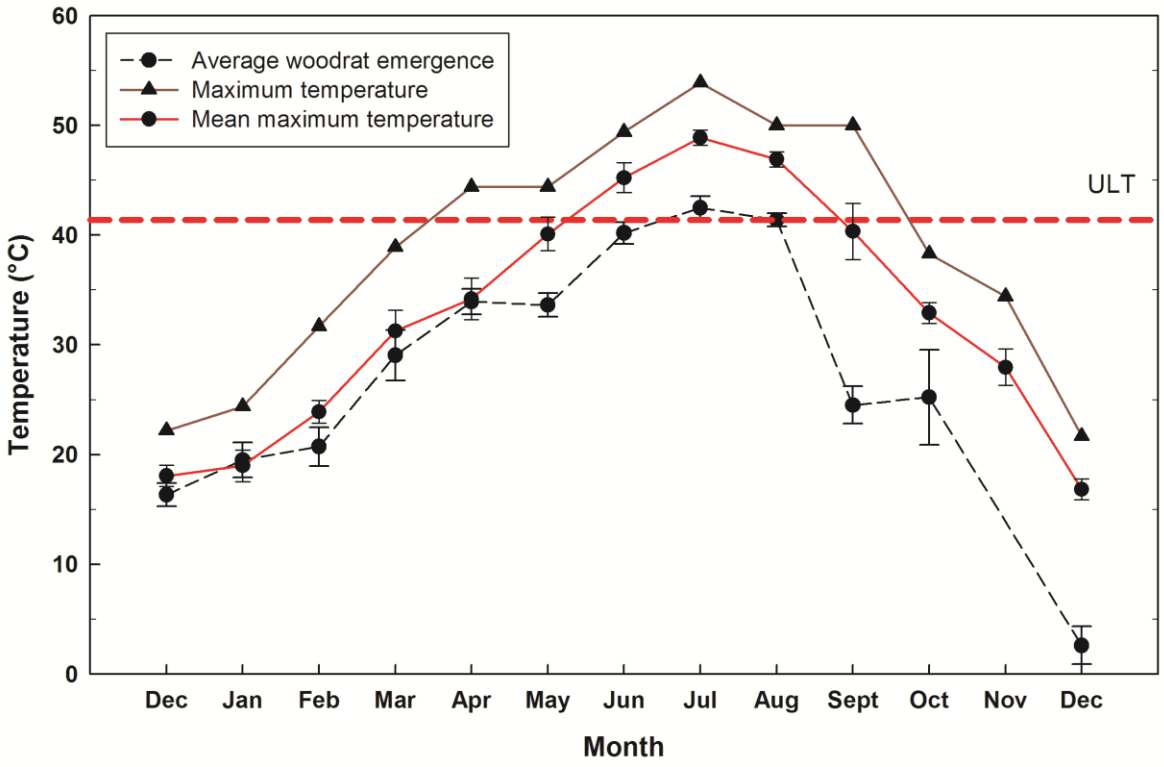

Figure 1.4. Mean ambient temperature upon initiation of nightly activity for woodrats relative to monthly mean daily maximum temperature. (Error bars represent 95% confidence intervals.) N = 468 woodrat nights; 10 males and 4 females. The dashed reference line is the upper lethal temperature (ULT; 41.4°C) for *N. lepida*.

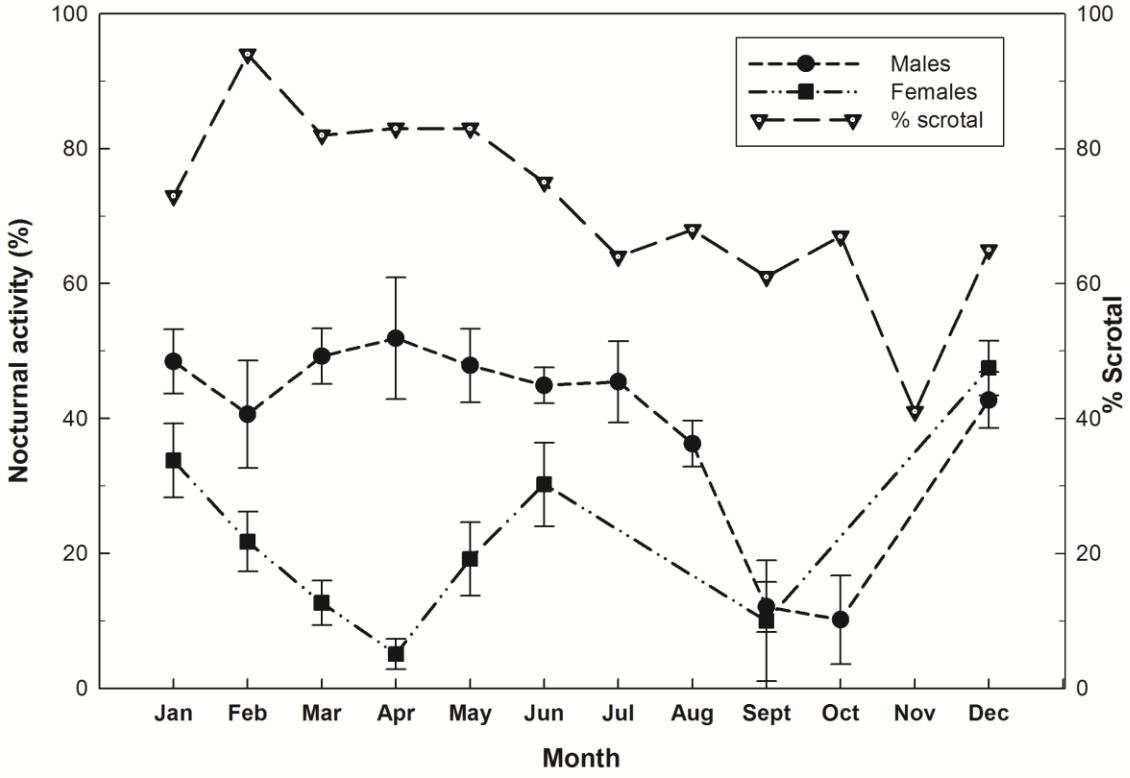

Figure 1.5. Woodrat activity as a percent of available nocturnal hours used relative to time of year, sex, and for males, an index of sexual activity, the percent of reproductive males (any male noted as being scrotal or partially scrotal for a given trapping period) on a monthly basis. (We have no November activity data for males, and no October – November activity data for females.) N = 468 woodrat nights; 10 males and 4 females.

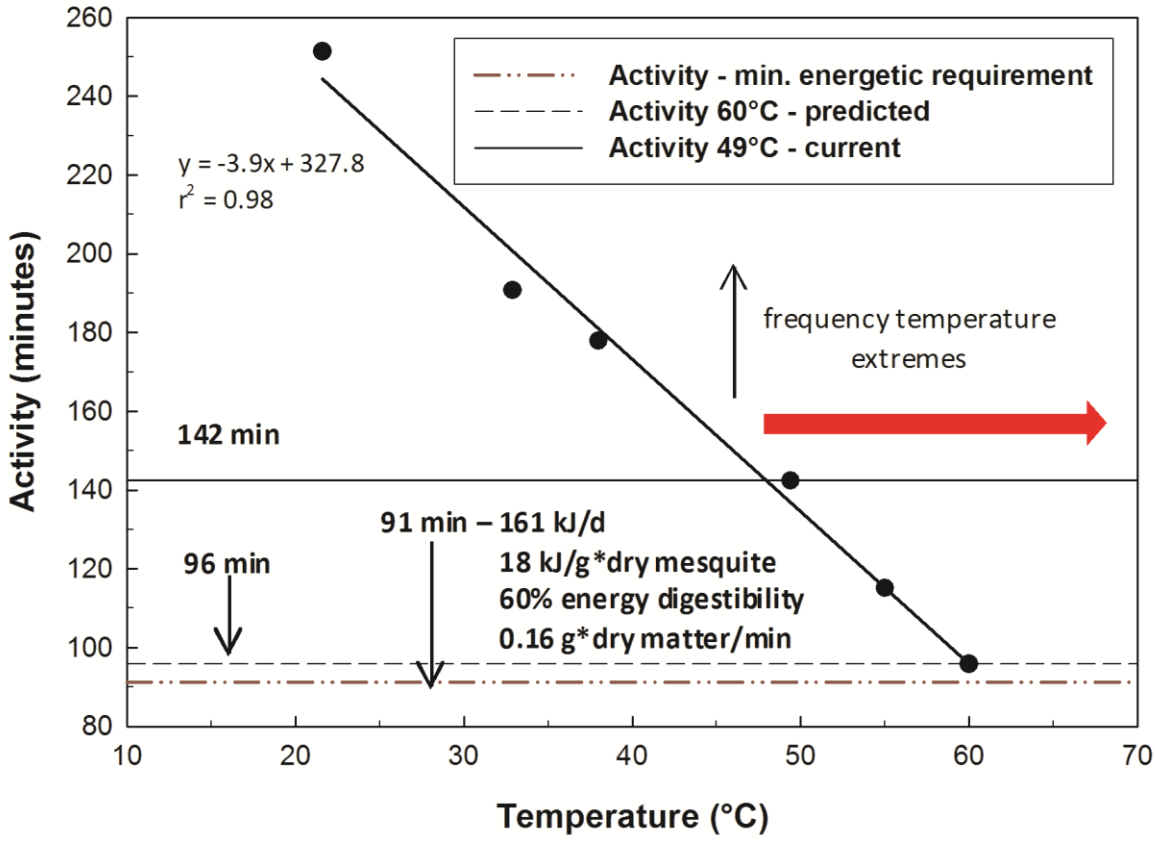

Figure 1.6. Predicted reductions in *N. lepida* activity under scenarios of increased temperatures, given the observed relationship between ambient temperature and activity. Minimal energetic requirements for a 125 g woodrat are 161 kJ, which necessitates 91 minutes of feeding given published intake rates and apparent energy digestibility.

Chapter 2: Tissue carbon incorporation rates and diet-to-tissue discrimination in ectotherms – tortoises are really slow

This chapter has appeared in similar form as: Murray, I.W. and B.O. Wolf. 2012. Tissue carbon incorporation rates and diet-to-tissue discrimination in ectotherms – tortoises are really slow. Physiol Biochem Zool 85:96-105.

Abstract

Understanding carbon incorporation rates and diet-to-tissue discrimination ($\Delta^{13}C_{tissue\text{-}diet}$) in animals is necessary to interpret stable isotope data collected from animals in the field. Our current understanding of the carbon dynamics in terrestrial ectotherms such as snakes, lizards and turtles is poorly developed. Here we use a diet switch experiment to estimate carbon incorporation rates and diet-to-tissue discrimination factors in growing desert tortoises (*Gopherus agassizii*). Average carbon retention times for red blood cells (RBCs) and plasma were 126.7 ± 40.3 and 32.9 ± 14.5 days, respectively. Tissue carbon incorporation rates were affected by both growth and metabolism with growth accounting for 50% of the carbon turnover in RBCs and 13% of carbon turnover in plasma. At equilibrium, scute keratin (0.8 ± 0.1‰) and plasma (1.0 ± 0.2‰) showed enriched discrimination values ($\Delta^{13}C$) compared to the test diet, but RBC $\Delta^{13}C$ values were indistinguishable from diet (0.2 ± 0.3‰). We also found that new keratin continued to contribute significant material to previously grown keratin rings on the tortoise's shell. Changes in the $\delta^{13}C$ of previously laid down growth rings indicated that the old rings closest to the region of new growth received about 73% of the carbon from the current diet; these data suggest that the interpretation of dietary history using growth rings must recognize that each ring may represent the weighted average of the

diet over several seasons. These results continue to highlight the importance of laboratory experiments in interpreting isotopic data derived from field studies.

Introduction

Stable isotopes of carbon ($\delta^{13}C$) are routinely used as a tracer for estimating the movement of energy through consumers and their different tissue pools (DeNiro and Epstein 1978; Hobson and Clark 1992; Michener and Schell 1994). The robust interpretation of these data depends upon an understanding of the tissue carbon dynamics (i.e. carbon incorporation rates and discrimination factors) in the consumers of interest. The current literature focuses on terrestrial endotherms (birds and mammals) and fishes, with many fewer studies describing these processes in reptiles and amphibians (Dalerum and Angerbjorn 2005; Warne et al. 2010). Differences in the physiology, thermal biology and habitats used by amphibians and reptiles preclude simply transferring what we know about carbon dynamics of other vertebrates to these groups (Zug 1993; Pough et al. 2004). As a consequence, there are significant gaps in our knowledge of tissue carbon dynamics for many taxa, and these data are needed to provide confidence limits on stable isotope data obtained from field studies (Gannes et al. 1997; Martínez del Rio et al. 2009; Warne et al. 2010). Recent studies of tissue carbon dynamics provide a starting point for understanding how the carbon turnover dynamics of reptiles differ from other vertebrates. Warne et al. (2010) has reported on these processes in lizards, Fisk et al. (2009) on juvenile snakes and Reich et al. (2008) and Seminoff et al. (2006, 2007) on aquatic turtles, but to our knowledge there is no published information available describing these processes in any terrestrial turtle species. Tortoises are expected to differ from other terrestrial ectotherms, such as lizards, because they are long-lived and attain relatively

large sizes compared to most lizards. Here, we present tissue carbon retention times and discrimination factors from a diet-switch experiment in growing juvenile desert tortoises, *Gopherus agassizii*. Desert tortoises are herbivorous reptiles occupying arid regions of the southwestern United States and Mexico characterized by plant communities with carbon isotope values of -32.3‰ to -12.0‰ VPDB (n = 94 Sonoran desert plant species; Murray and Wolf unpublished data). Many past studies have used organisms at or near their adult size, where the only driver of carbon incorporation rates is catabolic tissue turnover. However, we argue that it is important to examine the influence of growth and catabolism because both of these processes are biologically relevant to our understanding of stable isotope tissue kinetics in ecological systems.

Methods

We obtained seven captive-bred hatchling desert tortoises while they were hibernating during their first winter after hatching. Tortoises were maintained indoors in a stock tank (Rubbermaid® model # 4243) in the University of New Mexico Biology Department. The vertebral scutes of individual tortoises were marked with unique numbers using a permanent marker. Animals lived on a substrate of gravel, and simulated solar radiation/heat was provided by 100 watt heat lamps and ZooMed® UVB 10.0 fluorescent bulbs to maintain a diurnal temperature gradient within the enclosure that ranged from 29 to 39°C (mean diurnal body temperature; 32.3 ± 0.6°C). A 14h/10h light/dark photoperiod was maintained and animals were kept at normal activity temperatures and fed throughout the year. Tortoises were fed and watered daily. Tortoises were weighed (Ohaus model V31XH2 ± 0.1 g) and measured (straight carapace

length ± 1.0 mm) every 30 days. The project was approved by the university's institutional animal use and care committee (UNM-IACUC #10-100471-MCC). Tortoises were fed diets with unknown isotopic values for up to 2 months after hatching and before their first hibernation (when we acquired them). We warmed the tortoises up in the spring (approximately 6 months after hatching) and fed them for 307 days on a diet of Zoomed grassland tortoise chow® ($\delta^{13}C$ = -25.0 ± 0.1‰ VPDB). We then switched tortoises to a diet of Mazuri tortoise chow® ($\delta^{13}C$ = -21.9 ± 0.2‰) for 371 days. These two commercial diets differ in their nutritional composition (i.e. 9% vs. 15% protein for Zoomed and Mazuri diets, respectively) which may contribute to different patterns of carbon isotope dynamics.

Blood tissue dynamics experiment

We analyzed the isotopic composition of red blood cells (RBCs), plasma solutes, and scute keratin. We sampled blood from between two to seven tortoises on days 0, 2, 6, 10, 20, 40, 70, 99, 181, 282, 293, 321, and 371. Blood was drawn with a 27 gauge needle and syringe from the dorsal cervical sinus, transferred to a hematocrit tube and centrifuged (relative centripetal force = 14,800 x g for 2 – 3 min) down into plasma and RBC components. Tortoises became noticeably more difficult to extract blood from as the experiment progressed, possibly due to the accumulation of scar tissue. We followed blood preparation and analysis methods detailed in Warne et al. (2010).

Scute growth ring experiment

Turtles generally grow via the addition of successive growth rings visible on keratinized scutes overlain on the bony shell (Cagle 1946; Wilson et al. 2003). Throughout the study, the desert tortoises grew and added between two to four rings

during the initial 307 days on the baseline diet, and between two and five rings during the 371 days on the new test diet. On day 0, the start of the diet switch and day 371, we sampled scute keratin from all tortoises using non-overlapping regions of the same scute for both sampling periods. Using a razor saw (Revell #88-6964) and No. 21 scalpel we cut and lifted a 15 mm wide strip of keratin bisecting the growth rings on the 2nd left pleural scute. We sampled only the scute and did not cut into the bony carapace. (Tortoises were not harmed during this procedure, and the scute eventually regenerates.) Each strip was scrubbed, washed in a 2:1 chloroform/methanol solution to extract any superficial lipids, and dried before being cut to separate individual growth rings with a razor blade under a dissecting scope. Keratin samples from individual growth rings were cut into 0.4 – 0.7 mg pieces and loaded into tin capsules (Costech 3x5mm #041074) for $\delta^{13}C$ analysis. Some rings were too wide to analyze as a single sample; in these cases, the ring was divided horizontally, and representative parts from each section were loaded into separate tins. Each of these ring parts was then averaged to acquire one $\delta^{13}C$ value per ring.

We measured the $\delta^{13}C$ values of tissue samples using a continuous flow isotope ratio mass spectrometer (Thermo-Finnigan IRMS Delta Plus) connected to a Costech ECS 4010 Elemental Analyzer in the UNM Earth and Planetary Sciences Mass Spectrometry lab. The precision of these measurements was ± 0.1‰ SD based on repeated measurements of internal lab standards. All sample runs included regularly spaced lab standards (soy $\delta^{13}C$ = -27.2‰ VPDB) throughout the run that were calibrated against international standards used to correct tissue sample raw values. All values are reported using delta notation (δ) in parts per thousand (‰) as $\delta X = (R_{sample}/R_{standard} - 1)$ *

1000. The R_{sample} and $R_{standard}$ represent the ratio of heavy to light isotopes ($^{13}C/^{12}C$) for the sample and standard.

Statistical Analyses

Animal tissue isotopic incorporation rates may be best estimated using one-compartment or two-compartment models. One-compartment models assume that stable isotope ratios from ingested foods mix in one compartment or pool and get replaced at constant rates by isotope ratios of newly eaten food items. In two-compartment models, the overall incorporation rates are integrated over multiple compartments in a given tissue with stable isotope ratios turning over, or being replaced, at different rates (Cerling et al. 2007; Kurle 2009). Some data support the existence of multiple carbon compartments operating within an animal (McCue 2007; Podlesak and McWilliams 2007; McCue 2011; McCue et al. 2011), but determining the kinetics of various carbon pools in animals that utilize a microbial gut fauna for nutrient assimilation, such as desert tortoises, may be less straight forward (Nieto and Lobley 1999). The reaction–progress variable method (RPV) developed by Cerling et al. (2007) is a valuable tool used to evaluate whether a one- or two-compartment model best fits carbon incorporation rates in a given tissue. In the RPV method, a diet switch experiment is modeled as the fractional approach to equilibrium:

$$(\delta^t - \delta^{eq})/(\delta^{eq} - \delta^{init}) = (1 - F) \qquad (1)$$

here δ^t is the isotope value at time t, δ^{eq} is the isotopic equilibrium value, and δ^{init} equals the initial isotope value. The RPV method treats a turnover experiment as the fractional approach to equilibrium, leading to values of F = 0 at day 0, and F = 1 at equilibrium. Incorporation rates can then be displayed as the fraction of change at time t, between 0

and 1, in essence normalizing them. The RPV results can be log transformed (ln(1 – F)) and graphed versus time, which allows the fractional approach to equilibrium to be represented as a straight line. An intercept of < 0 means that multiple pools are contributing to the isotopic value of the tissue, while an intercept > 0 means that there is a delay before the material from the new diet is incorporated into the tissue. A plotted intercept of 0 means that a single pool contributes 100% to isotope exchange. Visual inspection of ln(1 – F) over time initially determines whether or not one or multiple compartments are influencing isotope incorporation. We then used the approach of Martínez del Rio and Anderson-Sprecher (2008) to quantitatively select the best-fit model. We estimated isotope incorporation rates with non-linear regression in SigmaPlot 8.0®. If the one compartment model was deemed most robust, we used the equation:

$$\delta_t = \delta_{eq} - (\delta_{eq} - \delta_{init})\, e^{(-T/\,tau)} \qquad (2)$$

if the two compartment model was most robust, we used the equation:

$$\delta_t = \delta_{eq} - (\delta_{eq} - \delta_{init})[pe^{(-T/\,tau_1)} + (1\text{-}p)e^{(-T/\,tau_2)}] \qquad (3)$$

In both cases, δ_{eq}, δ_{init}, and δ_t are equilibrium, initial, and time 't' isotope ratios, T is time in days, tau (τ) is carbon residence time, or the mean length of time that a carbon atom is retained in a particular tissue pool, and p is the fractional contribution of each compartment to the two compartment model. We follow Martínez del Rio and Anderson-Sprecher (2008) and Warne et al. (2010), and use τ to present isotope incorporation rates. Other investigators report tissue element half-lives ($t_{1/2} = \tau\ln(2)$) derived from fractional rates of incorporation ($\lambda = 1/\tau$) (Hobson and Clark 1992, Carleton and Martínez del Rio 2005, Cerling et al. 2007). We used Akaike's information criteria corrected for small sample sizes (AICc) to test the goodness of fit of the one or two-compartment models for

each tissue. Here the AICc increases as a function of the number of model parameters. In short, the model exhibiting the lowest AICc is the preferred model. Thus, the one compartment model is supported if $AICc_1$ is smaller than $AICc_2$, and the two compartment model is supported if the reverse is true (Burnham and Anderson 2002 & Martínez del Rio and Anderson-Sprecher 2008).

There are two processes (growth and catabolism) that potentially contribute to the fractional rate of incorporation of material into the tissues of an animal. The models presented thus far only account for incorporation of materials into tissues due to catabolism (c) (Hesslein et al. 1993) and thus fail to account for the second potential source of incorporated material associated with an animal's growth. Our tortoises were hatchlings at the start of the experiment and grew rapidly over the entire course of the experimental treatment and thus growth (k) must be considered in our models of incorporation. Thus if we substitute λ for $1/\tau$, equation (2) becomes:

$$\delta_t = \delta_{eq} - (\delta_{eq} - \delta_{init})\, e^{-(k + c)T} \qquad (4)$$

We can then use an exponential model ($y = ae^{(kt)}$) to estimate the fractional growth rate (k in $g^* day^{-1}$) of juvenile desert tortoises in SigmaPlot 8.0®. Since $\lambda = k + c$, our estimates of carbon incorporation rates (i.e. $\lambda = 1/\tau$) and fractional growth rates (k) allow us to quantitatively parse out the contributions of catabolism and growth to overall tissue turnover. Thus, if λ and k are indistinguishable, then growth can be assumed to be the only determinant of isotopic incorporation after the diet switch. If λ is higher than k, the difference is the influence of tissue catabolism.

Diet-to-tissue discrimination ($\Delta^{13}C_{tissue\text{-}diet}$) values were reported as the difference between tortoise tissue $\delta^{13}C$ values at equilibrium and $\delta^{13}C$ of the diet. We used t-tests to

determine whether or not there were differences between tissue and diet $\delta^{13}C$ values. We used paired t-tests to examine $\delta^{13}C$ values in growth rings sampled on day 0, with the same rings re-sampled on day 371. All $\delta^{13}C$ estimates are given as mean ± SE‰ VPDB.

Results

At the start of the diet switch, desert tortoises had mean plasma and RBC $\delta^{13}C$ values of -23.4 ± 0.4‰ and -24.2 ± 0.2‰, respectively, after feeding on an exclusive diet of Zoomed grassland tortoise diet® for 10 months (-25.0 ± 0.1‰). Five tortoises had three growth rings at the start of the diet switch (day 0), one had two, and one had four growth rings. Samples obtained from these rings had mean $\delta^{13}C$ values of -22.3 ± 0.5, -24.0 ± 1.8, -24.3 ± 0.6, -24.6 ± 0.2, and -24.7 for rings 0 – 4 (here we use ring 0 for the neonatal scute, and ring 4 is the most distally grown annulus). All of the tortoises avidly fed on the Mazuri tortoise diet® (-21.9± 0.2‰) starting at day 0, and running through day 371. Tortoises grew rapidly, and this growth was well described by an exponential function ($r^2 = 0.80$) with a fractional growth rate (k) of 0.004 ± 0.0002 $g*day^{-1}$ (Figure 1). When the RPV was applied to our results the linearized output for plasma and RBC supported the use of a two-compartment model, as evidenced by the negative intercepts with confidence intervals that did not overlap the origin (range = -0.70 to -0.14; Figure 2). The negative intercepts evident for plasma and RBC using the RPV method supported selection of the two-compartment model, but the one-compartment model was supported by the AICc comparisons due to the smaller value of $AICc_1$ relative to $AICc_2$ (plasma: $AICc_1 = 9.9$; $AICc_2 = 15.2$; RBC: $AICc_1 = 13.4$; $AICc_2 = 17.9$). Although model goodness of fit comparisons were equivocal (plasma: one-compartment adjusted $r^2 = 0.60$; two-compartment adjusted $r^2 = 0.58$; RBC: one-compartment adjusted r^2 =

0.78; two-compartment adjusted $r^2 = 0.81$), our selection of the less parameterized one-compartment model for plasma and RBC was informed by the model AICc comparisons (Burnham and Anderson 2002).

Mean (± SE) carbon retention time (τ) in tortoise plasma was 32.9 ± 14.5 days and 126.7 ± 40.3 days in RBC (plasma = $-20.9 + 2.5e^{-T/32.9}$; RBC = $-21.7 + 2.5e^{-T/126.7}$). We do not present carbon retention times for scute keratin because we did not sample this tissue on a continuous schedule.

The estimated values of carbon incorporation rates (λ) for plasma (0.03) and RBC (0.008) are both higher than we would expect if growth ($k = 0.004$ g*day^{-1}) alone is the determined rates of tissue carbon incorporation. Thus, catabolic tissue turnover played a key role in carbon incorporation of both plasma and RBCs and growth contributed significantly to RBC carbon incorporation rates (50 ± 16%), but minimally to that of plasma (13 ± 6%).

Desert tortoise plasma diet-to-tissue discrimination ($\Delta^{13}C_{tissue\text{-}diet}$) was 1.0 ± 0.2‰, which was significantly enriched over that of diet (-21.9 ± 0.0‰; one-sample t-test, $t = 5.52$, $P < 0.05$). However, tortoise RBC $\Delta^{13}C$ (0.2 ± 0.3‰) was not significantly different from zero (one-sample t-test, $t = 0.85$, $P > 0.05$). Using keratin sampled from each of the tortoises' most recently accrued annulus after the diet switch (mean $\delta^{13}C$ = -21.1 ± 0.1‰) we observed a $\Delta^{13}C$ for scute keratin of 0.8 ± 0.1‰, which is significantly enriched over diet (one-sample t-test, $t = 6.6$, $P < 0.003$). Desert tortoise plasma (1.6 ± 0.4‰) and scute keratin (0.6 ± 0.3‰) $\Delta^{13}C_{tissue\text{-}diet}$ values after ten months on the pre-switch, Zoomed diet (-25.0 ± 0.1‰) were indistinguishable from those measured after the diet switch to the Mazuri diet (plasma; one-sample t-test, $t = 1.62$, $P > 0.05$; keratin; one-

sample t-test, $t = -0.5$, $P > 0.05$). However, the tortoise RBC $\Delta^{13}C_{tissue\text{-}diet}$ while eating the more depleted pre-switch Zoomed diet (0.8 ± 0.2‰) was significantly greater than the value measured in animals maintained on the Mazuri diet (one-sample t-test, $t = 3.04$, $P < 0.05$).

The $\delta^{13}C$ values for individual growth rings already extant at the start of the diet switch, and sampled pre- and post-diet switch, reflected that of the new diet, in accordance to their proximity to the zone of new ring addition at the distal most edge of the scute. This regular change in the carbon isotope ratios of pre-existing growth rings after a switch to an isotopically distinct diet can be thought of as 'carbon creep.' Ring 0, 1, and 2 all showed non-significant trends towards enrichment (creep towards the new diet) when measured on day 371 relative to day 0 (0.9 ± 0.6‰, 0.4 ± 1.8‰, and 1.6 ± 0.8‰ for rings 0 through 2, respectively). Ring 3 was significantly enriched by 2.2 ± 0.6‰ on day 371 compared to day 0 (paired t-test, $t = -3.7$, $P < 0.01$). Only one tortoise had 4 rings at the start of the diet switch, and this ring was enriched 3.4‰ by day 371 (Figure 3).

Discussion

We report on the carbon isotope incorporation and diet-to-tissue discrimination (Δ) in multiple tissues from juvenile tortoises feeding on isotopically distinct diets, and explore the influence of growth on incorporation rates in different tissue pools. We also provide a valuable 'calibration' of how to interpret the stable isotope chronologies evident in discretely deposited growth ring chronologies on tortoise scutes. Our survey of the literature suggests that these are the first measurements of diet-to-tissue discrimination and carbon incorporation rates reported in terrestrial turtles. Desert

tortoise plasma and RBC $\delta^{13}C$ values achieved equilibrium with that of diet, but had variable carbon incorporation rates and $\Delta^{13}C_{tissue\text{-}diet}$ values. Use of the RPV method supported the need for two-compartment models to best characterize incorporation rates in plasma and RBC, but AICc comparisons strongly suggested that a one-compartment model best suited the data. Blood plasma had significantly faster incorporation rates relative to RBCs, and despite the exponential growth shown by all tortoises, catabolism remained an important determinant of incorporation rates, particularly in blood plasma. The carbon isotope ratios in previously deposited growth rings are significantly influenced by current dietary isotope ratios, particularly for those rings closest to actively growing scute edges; an indicator of new tissue addition over multiple seasons after a growth ring is initially deposited. Our study parallels that of Warne et al. (2010), but it is important to note that while lizards and tortoises are both terrestrial ectotherms, differences in diet, body size, growth patterns, and longevity among these groups are factors that importantly influence tissue carbon dynamics. *Gopherus* tortoises are obligate herbivores that grow rapidly for their first 20 years and can live for 50 years or more (Germano 1992; 1994), whereas the *Sceloporus* lizards in Warne et al. (2010) are insectivores that mature in about one year, and live no longer than four years (Vinegar 1975; Jones and Ballinger 1987). In the following discussion we underscore the similarities and differences between tortoises, and other reptiles, we put our results into a broader framework based on the literature, and illustrate how this study broadens our understanding of stable isotope dynamics in terrestrial ectotherms in general, and chelonians in particular.

Carbon incorporation rates

The current literature suggests that carbon incorporation rates increase with decreasing body size, an idea supported by the observation of successively declining isotopic incorporation rates in larger species of birds and lizards (Carleton and Martínez del Rio 2005; Warne et al. 2010), and in line with the metabolic theory of ecology describing the dependence of ecological and physiological processes on metabolic rate and body size (Brown et al. 2004). Carleton and Martínez del Rio (2005), have postulated that rates of carbon incorporation are heavily dependent upon tissue-specific protein turnover rates, which tend to be significantly higher as metabolic rate increases. For example, endothermic animals such as mammals and birds, which have total metabolic rates that are seven to ten times those of ectotherms, have carbon incorporation rates that are seven to twenty times faster than those in similarly sized lizards (Warne et al. 2010). Despite distinct ecological differences and evolutionary trajectories in the tortoise lineage, our data are similar to those reported for other reptiles of comparable size. The half-life ($t_{1/2} = \tau \ln 2$) for carbon in desert tortoise plasma was 23 days, which is similar to the data on plasma half-life in reptiles of similar size (i.e., 14 days for hatchling loggerhead sea turtles) (Reich et al. 2008), but four to seven times slower than birds (three days) and mammals (six days) of the same size (Hobson and Clark 1993; Kurle 2009).

Desert tortoise RBC carbon $t_{1/2}$ (88 d) was greater than those values reported for other similarly-sized ectotherms (Here, we assume that reported whole blood values are similar to RBC values). For example, Bucheister and Latour (2010) found a half life of

23 days in flounder blood, while Reich et al. (2008) found values of 53 days in hatchling loggerhead turtle RBC. Reported values for mammalian and avian RBC and whole blood $t_{1/2}$ s are three to eight times faster than those reported here for tortoises (rat $t_{1/2}$ = 25 days; rat $t_{1/2}$ = 30 days; quail $t_{1/2}$ = 11.4 days; crow $t_{1/2}$ = 30 days) (MacAvoy et al. 2006; Hobson and Clark 1992; Hobson and Clark 1993; Kurle 2009). We suggest that tortoise plasma incorporation rates are likely similar to those of other ectotherms because plasma proteins are largely synthesized in the liver which is a metabolically active tissue, with high rates of turnover and similar function in most organisms (Haschemeyer and Smith 1979; Tieszen et al. 1983). RBCs, however, are in general long-lived, and unusually long-lived in turtles, circulating for 11 months (Krasilnikov 1971), > 500 days (Rodnan et al. 1957) or 600 - 800 days (Altland and Brace 1962). We propose that the relatively slow carbon incorporation rates seen in our study and by Reich et al. (2008) reflect the exceptionally long life cycle of chelonian RBCs. Because we studied rapidly growing juveniles maintained under constant, optimal conditions, our measured rates of carbon incorporation probably represent maximal rates for this species. McCue (2008) found rates of carbon turnover to be 6 to 7 times faster in rapidly growing sub-adult cockroaches, relative to adults, and Carleton and Martínez del Rio (2010) documented tissue carbon retention times 4 to 6 times longer in slow growing juvenile tilapia (*Oreochromis niloticus*) relative to more rapidly growing juveniles. Mature desert tortoises and/or tortoises experiencing seasonal temperature fluctuations are more likely to have slower incorporation rates than those seen here. If we remove the contribution of growth (k = 0.004) from our calculations of carbon retention times ($\lambda = k + c = 1/\tau$) for plasma and RBCs, we can estimate $t_{1/2}$s ($t_{1/2} = \tau \ln 2$) solely determined by tissue

catabolism in the non-growing animal. This simulation suggests that older tortoises would show plasma $t_{1/2}$ s of approximately 26 days (compared to 23 days in this study) and RBCs would have $t_{1/2}$ s of 173 days (compared to 88 days).

Relative contribution of growth and catabolism to carbon incorporation in tissues

Growth contributed 13% and 50% to the carbon incorporation rates of desert tortoise plasma and RBC, respectively. In rapidly growing animals whole body growth rates may obscure the characteristic catabolic turnover rates unique to different tissue pools in the body (Reich et al. 2008). Growth was a minor contributor to carbon incorporation in desert tortoise plasma, and our data were more similar to the ~ 10% contribution of growth to plasma found in adult lizards and mice (Warne et al. 2010; MacAvoy et al. 2005) relative to the 30% and 48% contribution of growth found in hatchling and juvenile loggerhead sea turtles (Reich et al. 2008). Our result is contrary to the expected result that quickly growing ectotherms (or endotherms) in the early stages of their life cycles will have growth rates that are major contributors to carbon incorporation rates. Indeed, some studies have shown that in larval fishes growth contributes 90% to carbon incorporation (Herzka and Holt 2000). The minor influence of growth observed in tortoise plasma incorporation rates is probably representative of the relatively rapid turnover rates of plasma solutes; that is, tissue catabolism is the primary determinant of plasma incorporation rate. Carleton and Martínez del Rio (2010) found that growth was a minor contributor to liver turnover in tilapia fish, and because liver and plasma proteins 'track' each other this suggests that this may be a general pattern. The observed contribution of growth to carbon turnover in RBC (50%) was almost identical to the 44%

that Reich et al. (2008) reported in juvenile loggerhead turtles and is nearer to values expected for young rapidly growing organisms relative to adult animals at or near their asymptotic masses (MacAvoy et al. 2005; Warne et al. 2010). Our results show that the high growth rates of juveniles did not mask the inherent catabolic turnover rates in these blood compartments even when growth was most rapid, although this result may be tissue-specific.

Diet-to-tissue Discrimination Factors

The carbon isotope ratios of diet and tissue often differ and can be described by a diet-to-tissue discrimination factor (Δ). These discrimination factors represent the sum of the biochemical processes that occur upon the incorporation of ingested nutrients, and may be tissue- and species-specific (Tieszen et al. 1983; Schoeller 1999; reviewed by Caut et al. 2009). Quantifying and understanding how these values vary among different tissues and taxa allow for rigorous interpretation of isotopic data that focuses on individual, population and community performance in the field. These 'off-sets' are an important part of any effort to isotopically characterize consumer diets from tissue samples. The factors that influence the isotopic spacing between diet and tissue are not well understood. Growth rate and temperature (for ectotherms) have the potential to impact stable isotope discrimination factors. For example in sea bass (*Dicentrarchus labrax*) and puffins (*Fratercula cirrhata*) individuals fed *ad libitum* (and growing more rapidly) had higher $\Delta^{13}C_{tissue\text{-}diet}$ values relative to individuals on restricted diets (Barnes et al. 2007; Williams et al. 2007), but Trueman et al. (2005) found no influence of growth rate on $\Delta^{13}C_{tissue\text{-}diet}$ values in salmon (*Salmo salar*). McCue (2008) found no impact of

food restriction or temperature on carbon isotope diet-tissue spacing in cockroaches, whereas Cherel et al. (2005) documented lower carbon isotope tissue-diet spacing in some tissues (plasma), but not others (RBCs) in fasting penguins (*Aptenodytes patagonicus*). However, Hobson et al. (1993) found no effect of limiting food intake on the $\Delta^{13}C_{tissue\text{-}diet}$ values for several tissues in quail (*Coturnix japonica*) and geese (*Chen rossii*). The tortoises in this study experienced no nutritional stress and grew rapidly (due to continual access to an optimal thermal gradient), which leads us to postulate that the resulting tissue carbon isotope discrimination factors may be higher than those seen in wild tortoises enduring episodic nutritional constraints and growing more slowly or not at all.

Additionally, the isotopic direction of a diet switch may affect reported isotopic discrimination factors. Some researchers have found that discrimination factors vary depending on the direction of the diet switch, such as when animals going from an enriched to a depleted diet (elimination), or a depleted to an enriched diet (uptake). However, the biochemical basis behind these observations are not well understood. This is an important observation, because it provides a more rigorous framework for the characterization and interpretation of tissue isotope data, and consequent linkage of animal resource use with distinct sources available in the environment. These studies have documented a greater offset between tissue and diet in elimination diets (Webb et al. 1998; Bearhop et al. 2002; Olive et al. 2003; Pilgrim 2005). In this study, desert tortoises were fed an uptake diet. Prior to the switch, tortoises were maintained on a constant diet for ten months, which was depleted, or an elimination diet. Samples taken on day 0 of the diet switch reflect ten months of an elimination diet. If we assume that plasma, RBC,

and scute were in equilibrium with the depleted diet after ten months, we can compare $\Delta^{13}C_{tissue\text{-}diet}$ values for tortoises on the uptake and elimination diets. Although tortoise RBCs were more enriched over diet when on an elimination diet, we found no difference between discrimination factors on the two diets for plasma and scute keratin, which is opposite to the trend observed in pygmy rattlesnakes (*Sistrurus miliarus*), marine worms (*Nereis virens*), great skuas (*Catharacta skua*) and locusts (*Locusta migratoria*) (Table 1).

Growth Rings

The growth rings, or annuli, on tortoise scutes are visible indentations on the carapace that mark the boundaries of discrete periods of growth (Cagle 1946; Carr 1952). Captive desert tortoises held under optimal conditions for growth may deposit multiple growth rings in a year (Jackson et al. 1976,1978; Tracy and Tracy 1995;), and our tortoises produced, from two to four rings during the 371 days after the diet switch. These rings are often thought of as being biologically inert once deposited, but detailed histological and radio-immunological studies by Alibardi (2005, 2006) and Alibardi and Toni (2006) have shown that metabolically active tissue underlies the previously deposited scute keratin and is incorporated into previously accrued growth rings. In short, a thin, living epidermal layer always underlies the non-living cornified scutes. When a new ring is formed, some of the keratinocytes (beta-cells) formed using resources available from current diet not only form a new keratinized growth ring at the distal edges of the growing scute, but also form a thin layer of corneous material that is compressed into the cornified matrix of previously laid down growth rings (Alibardi and

Toni 2006). This observation suggests important consequences for using the $\delta^{13}C$ measurements of growth rings to assess tortoise diets. If the $\delta^{13}C$ of the current diet differs from the $\delta^{13}C$ diet of the tortoise when the ring was originally grown, then the $\delta^{13}C$ of the old ring can actually change as additional rings are added on a new diet and some of this material is deposited under the older rings. As a consequence, some level of dilution, or carbon creep, reflective of current diet may be expected to influence the $\delta^{13}C$ of older rings more proximally located. We estimated the effects of carbon creep by measuring the $\delta^{13}C$ of all growth rings before the diet switch and then re-sampled these same rings along with new rings 371 days after the diet switch. Any new material deposited under old rings, thus had a $\delta^{13}C$ value that differed from that of the old ring. We observed that there was significant dilution of carbon isotope values in previously grown rings by the new diet. Not unexpectedly, the growth ring adjacent to the growing scute edge showed the most dilution with the new diet contributing 73% of the material to the older ring (ring 3). This relative contribution of new material decreased with progressively older growth rings (further from the growing scute edge) and accounted for 68% and 56% of the material in rings 2 and 1, respectively (Figure 3). This finding parallels the conclusions of Alibardi (2006) and Alibardi and Toni (2006) who found that a majority of radio-labeled histidine and proline appears in cells located at the growing edges of the scutes closest to the currently growing annulus, with a reduction in uptake further from the actively growing edges. This is an important process to consider when studying the stable isotope ratios in turtle growth rings because the stable isotope signal in a single annulus likely represents a cumulative record of diet over a period of time greater than that required for the deposition of that particular ring. We then conclude that

the often widely fluctuating carbon isotope ratios in sequential growth rings in wild tortoises (up to 5‰; Murray and Wolf, unpublished data) are in fact very conservative estimates of the magnitude of the actual changes in forage intake occurring during the periods of time encapsulated by individual growth rings.

Our data are a step forward in answering the call for more experimental data necessary to better understand stable isotope dynamics in various organisms (Gannes et al. 1997; Martínez del Rio et al. 2009). We add important contributions to the scarce dataset on terrestrial ectotherms, and provide the first data for tortoises. Data such as the reported $\Delta^{13}C_{tissue\text{-}diet}$ values and tissue carbon incorporation rates are vital for the stable isotope ecologist whose quest may be to characterize consumer nutrient fluxes in natural systems. To best interpret data like these, it is critical to know offsets between specific tissues and diet ($\Delta^{13}C_{tissue\text{-}diet}$) as well as the dietary window integrated by those tissues (carbon incorporation rates). In addition, we document and quantify the phenomena of currently growing annuli isotope ratios influencing the carbon values in inert, previously laid down growth rings (carbon creep). These data provide an important framework for interpreting the signal of past diet history locked within the keratin in tortoise growth ring chronologies.

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Table 2.1. Mean (± SE) $\delta^{13}C$ values at equilibrium and diet-to-tissue discrimination for *G. agassizii* tissues. Tissue $\delta^{13}C$ equilibrium estimates were derived from fitted models. $\Delta^{13}C_{tissue\text{-}diet}$ values found based on the model are compared with those in tortoise tissues in equilibrium with an isotopically distinct diet fed before the diet switch. RBC $\Delta^{13}C_{tissue\text{-}diet}$ values are significantly lower on the enriched uptake diet (Mazuri) relative to the depleted elimination pre-switch diet (Zoomed) (one-sample t-test, $t = 3.04$, $P < 0.05$). Plasma and scute keratin $\Delta^{13}C_{tissue\text{-}diet}$ values are not significantly different on the depleted and elimination diets.

Gopherus agassizii	Model $\delta^{13}C$ equilibrium	$\Delta^{13}C_{tissue\text{-}diet\ (Mazuri)}$ (uptake diet)	$\Delta^{13}C_{tissue\text{-}diet\ (Zoomed\ diet)}$* (elimination diet)
Mazuri diet	-21.9 ± 0.0	--	--
Zoomed diet	-25.0 ± 0.1	--	--
Plasma	-20.9 ± 0.2	1.0 ± 0.2	1.6 ± 0.4
RBC	-21.7 ± 0.3	0.2 ± 0.3	0.8 ± 0.2
Scute keratin	-21.1 ± 0.1	0.8 ± 0.1	0.6 ± 0.3

* The listed $\Delta^{13}C_{tissue\text{-}diet}$ values for tortoises eating the pre-switch diet (Zoomed diet) are based on the mean tissue values after 10 months of feeding on this diet.

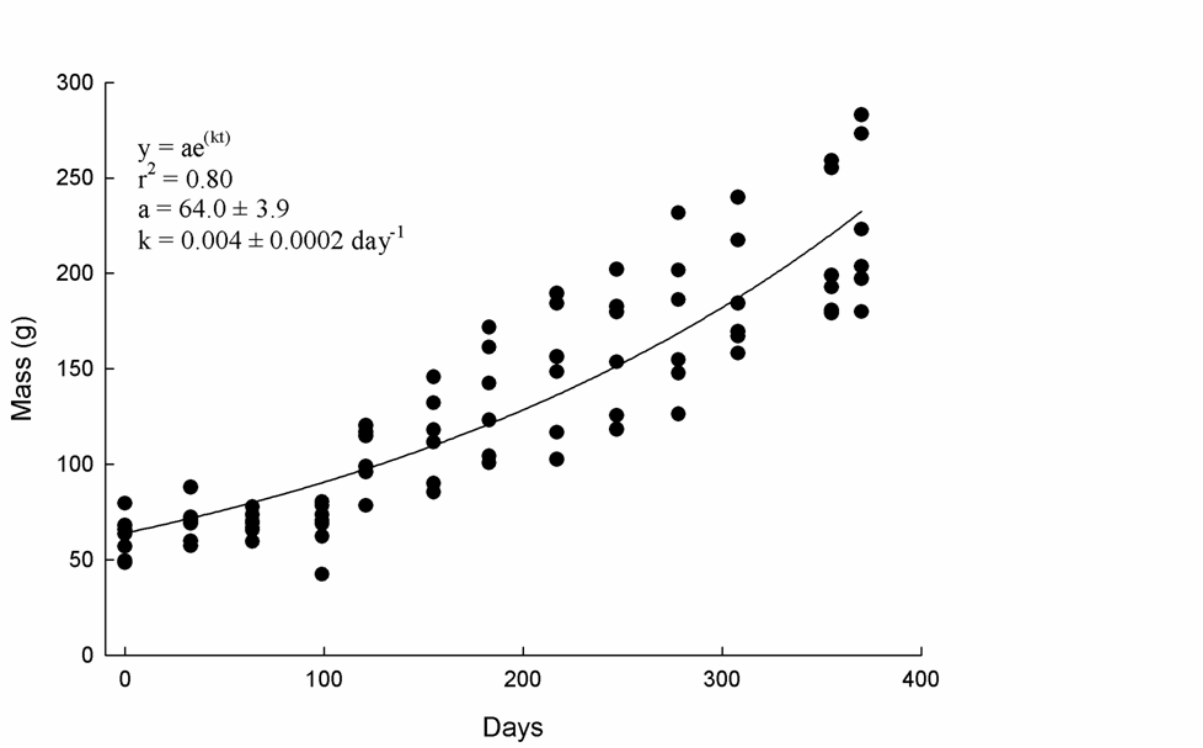

Figure 2.1. Growth in juvenile desert tortoises *Gopherus agassizii*, after the diet switch over a 371 day experiment. Each point represents the mass of an individual tortoise (N = 7 per date for first 100 days, thereafter N = 6). Juvenile tortoise growth is well characterized by an exponential function ($y = ae^{(kt)}$; $r^2 = 0.80$; $k = 0.004$ g*day^{-1}).

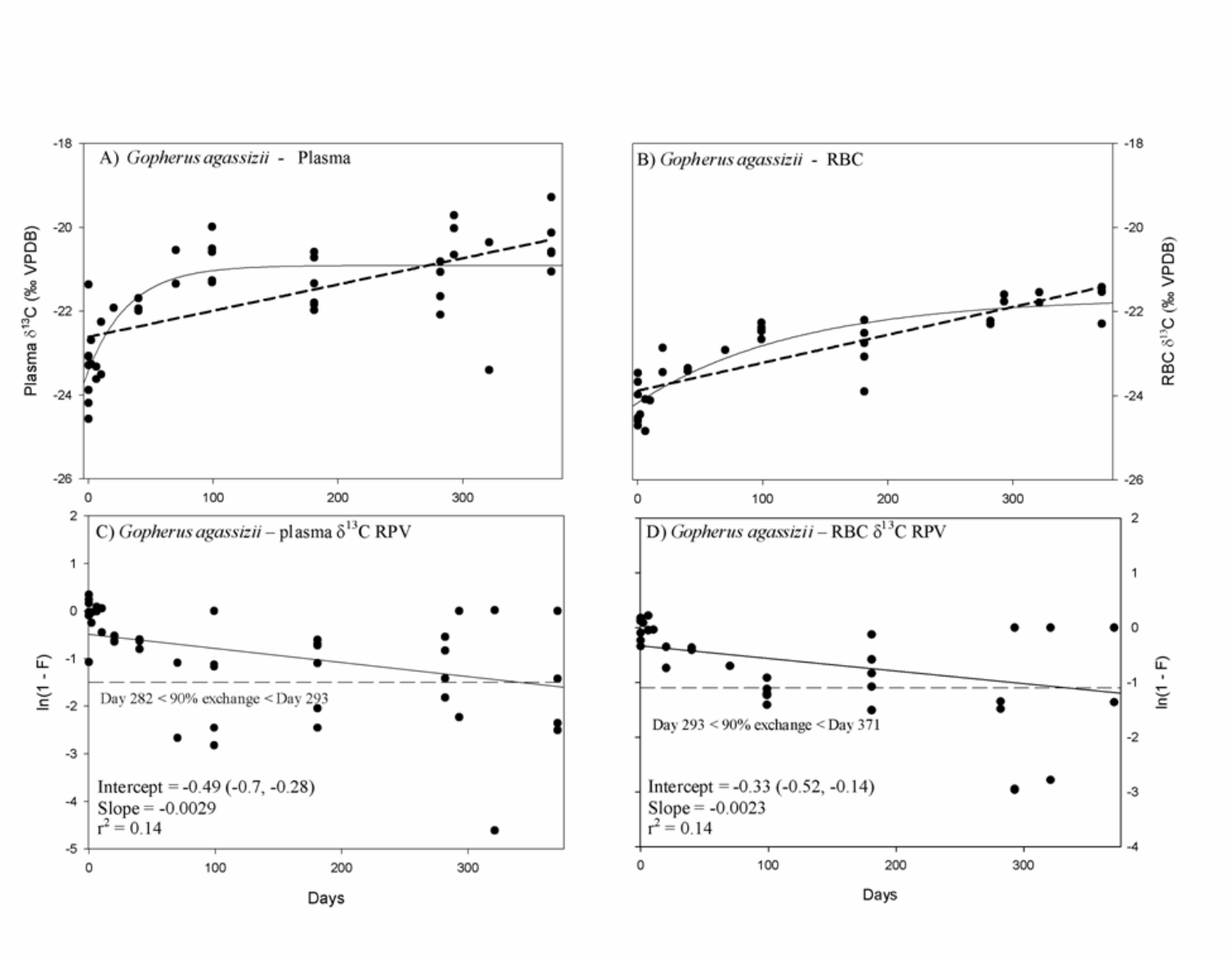
A) Gopherus agassizii - Plasma
Plasma δ13C (‰ VPDB)
B) Gopherus agassizii - RBC
RBC δ13C (‰ VPDB)
C) Gopherus agassizii – plasma δ13C RPV
ln(1 - F)
Day 282 < 90% exchange < Day 293
Intercept = -0.49 (-0.7, -0.28)
Slope = -0.0029
r2 = 0.14
Days
D) Gopherus agassizii – RBC δ13C RPV
Day 293 < 90% exchange < Day 371
Intercept = -0.33 (-0.52, -0.14)
Slope = -0.0023
r2 = 0.14
Days

Figure 2.2. Changes in the $\delta^{13}C$ values of *Gopherus agassizii* blood plasma (A) and RBC (B) during a 371 day diet switch experiment. Data best suits a one-compartment model (solid curve). Dashed lines (A, B) represent the fit if growth is the sole determinant of carbon incorporation rates ($k = 0.004\ g*day^{-1}$). The negative intercepts on the RPV plots (C, D) support the use of two-compartment models, but AICc comparisons support the use of one-compartment models for plasma and RBC.

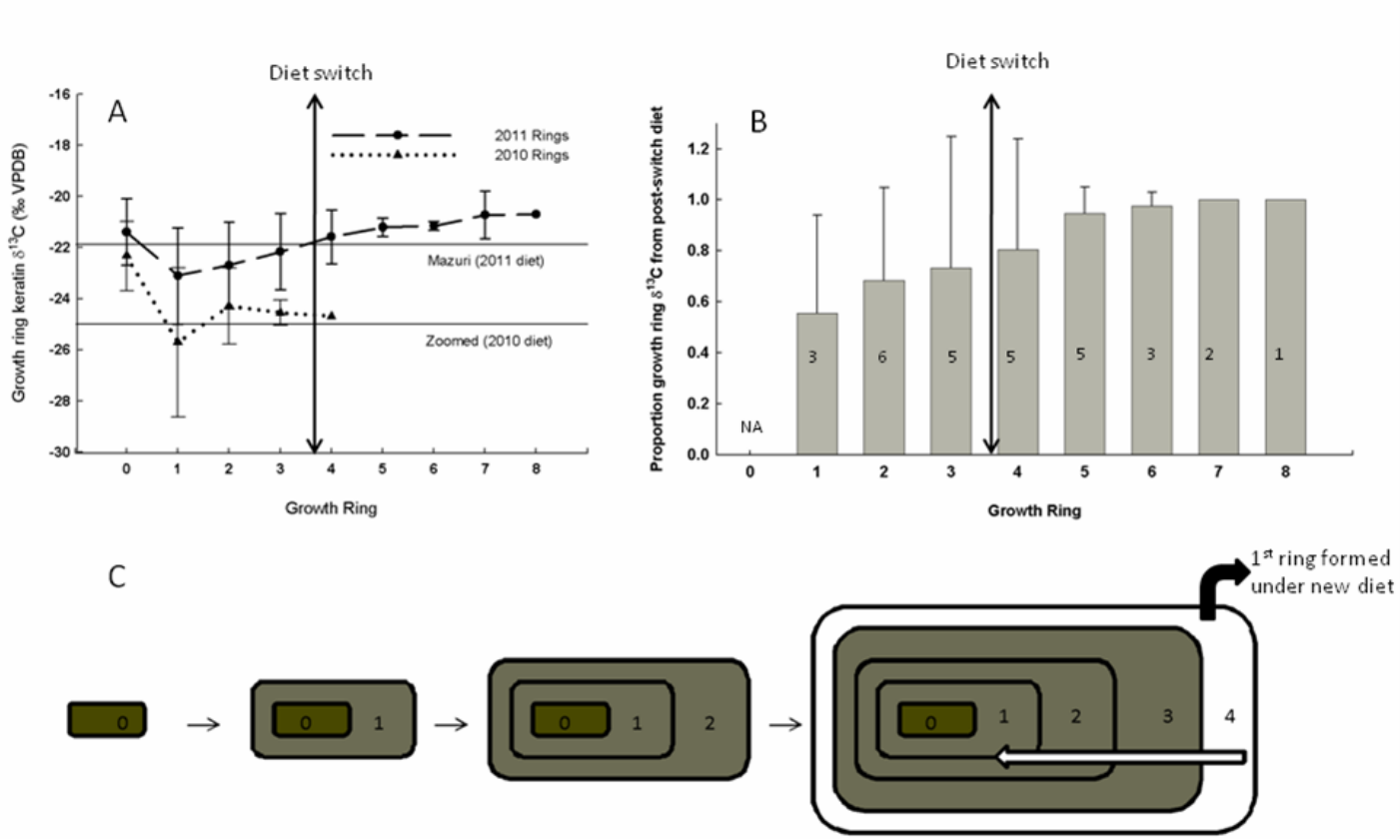
A
Diet switch
2011 Rings
2010 Rings
Mazuri (2011 diet)
Zoomed (2010 diet)
Growth ring keratin δ13C (‰ VPDB)
Growth Ring
B
Diet switch
Proportion growth ring δ13C from post-switch diet
NA
3
6
5
5
5
3
2
1
Growth Ring
C
1st ring formed under new diet
0
1
2
3
4

Figure 2.3. Growth ring keratin $\delta^{13}C$ values for growth rings added while being fed two isotopically distinct diets. A) Tortoises accrued two to three growth rings (one animal had four) after 10 months on the 2010 diet. $\delta^{13}C$ values for these rings, and the additional rings grown after 371 days on the 2011 diet were are plotted for comparison. The distal most ring present at day 0, before the diet switch (ring 3; only one animal had 4 rings) is significantly enriched (paired t-test, $t = -3.7$, $P < 0.01$) after 371 days on the new diet, while the neonatal scute (ring 0), ring 1, and ring 2 show non-significant trends towards carbon enrichment. B) Two source mixing model solving for the relative proportion of the 2011 diet in growth ring keratin of old rings sampled after 371 days on the new diet. Significant carbon dilution is noted in rings one through three that were formed under the 2010 diet (mean 73%, 68%, and 55% for rings 3, 2, and 1, respectively). The neonatal scute (ring 0) is not included because its initial $\delta^{13}C$ value reflects unknown maternal inputs. (Mixing model; $\delta^{13}C_{\textbf{(keratin)}} = p(\delta^{13}C_{\textbf{(2011 diet)}}) + (1\text{-}p)(\delta^{13}C_{\textbf{(2010 diet)}}) + \Delta$; Δ = apparent keratin discrimination factor (*G. agassizii* = 0.8‰)). Sample sizes are listed within the bars, and all error bars represent 95% confidence intervals. C) Hypothetical schematic illustrating in gray rings 1 – 3 grown under the 2010 diet, and the first ring (ring 4) in white, formed after the diet switch. A significant proportion of carbon from the new diet is incorporated into previously laid down growth rings, i.e. there is 'carbon creep.'

Chapter 3: Desert tortoise (*Gopherus agassizii*) dietary niche breadth across a precipitation gradient

(Co-author B.O. Wolf)

Abstract

Current climate change models project a general warming and drying trend for much of the southwestern U.S. Consequently, it remains critical to understand the range of responses species may have to this change. Environmental variation is one factor known to influence consumer resource use, making it an important variable to consider when seeking understanding of how a species responds to climatic variation. The tortoise (*Gopherus agassizii*) presents a useful opportunity to measure differences in resource use between and within populations because it occurs in a variety of desert habitats under a varying precipitation regime. In particular, we were interested in how fluctuations in precipitation might influence the patterns of plant resource use. To address this, we used stable isotope analyses from series of tortoise growth ring sequences from individuals across a precipitation gradient to characterize dietary breadth and the use of C_4/CAM *vs*. C_3 plant resources. C_3 plants are generally more nutritionally profitable compared to C_4 plants, which leads to important growth and fitness consequences for the consumer using these resources. We found that dietary specialization decreases with successively drier and less vegetated sites, and that juvenile tortoises have a narrower dietary breadth relative to adult tortoises. Additionally, male tortoises have less specialized diets compared to females. Use of C_4/CAM plant resources was variable, but in the more mesic sites with the most dependable summer rainfall desert tortoises increasingly

foraged on C_4/CAM resources as they grew larger. Use of C_4/CAM plant resources was erratic through growth for animals in drier sites with less summer precipitation. Our results highlight how individual consumer plant resource use is bounded under a varying regime of precipitation and plant productivity, and informs some predicted effects under a changing climate.

Introduction

As Earth's climate warms, it becomes increasingly important to understand how species may respond. The accurate and predictive modeling of species distributions depends on an understanding of the full breadth of species responses to the biotic, and abiotic or physical environment. Species distributions occur within a bounded set of physical and biological parameters, a relationship termed the niche. The exact boundaries delineating the niche may vary between individuals and among populations for many reasons, including environmental variation. Understanding how the physical environment impacts the relationship that organisms have with their surroundings, at the scale of the individual or population lends important insights into the factors affecting species' distributions and the ability of species to persist and adapt to a landscape dominated by humans.

The desert tortoise (*Gopherus agassizii*) is a long-lived herbivorous reptile that occurs over a wide range of arid habitats in the American southwest. Consequently, this species presents an ideal opportunity to examine how the niche (here we examine resource use as a niche proxy) changes across a diversity of habitats receiving variable precipitation. In the Sonoran Desert of Arizona, it is typically restricted to isolated, xeric mountain ranges with infrequent occurrence in the inter-mountain plains and valleys (Van Devender 2002; Averill-Murray and Averill-Murray 2005; Fig. 1). This hot, water-limited environment produces significant physiological challenges for animals inhabiting this region. Sparse and unpredictable rainfall results in limited and highly variable plant productivity. How then does this species take full advantage of such a highly variable resource environment to maximize survival and growth? Foraging observations and scat

analyses of desert tortoises have shown that they feed on a wide variety of grasses, forbs, and shrubs (Woodbury and Hardy 1948; Hansen et al. 1976; Oftedal 2002; Tracy et al. 2006). Individuals within a population, however, frequently specialize on a small subset of the available plant resources (Hansen et al. 1976; Tracy et al. 2006). Although these studies provide important insight into desert tortoise feeding patterns over short periods of time, they still provide only a limited view of the diet of an animal that can live in excess of sixty years (Germano 1992, 1994; Curtain et al. 2008).

Here, we examine the lifetime dietary history of tortoise populations by taking advantage of the information embedded in the keratinized scute rings on the tortoise's shell. Because tortoises grow via the sequential addition of distinctly marked growth rings in a fashion similar to tree rings, a single cross-section of an individual growth ring sequence contains dietary and growth information integrated over an individual's lifespan (Fig. 2). We use analyses of carbon and nitrogen stable isotopes in scute keratin to characterize the isotopic niche of individuals and populations for up to the last four decades across a precipitation gradient.

We use stable isotope analyses to describe dietary breadth of plant resource use. Significantly, plants vary in their photosynthetic pathways (C_3, C_4, CAM), which leads to differences in tissue carbon isotope ratios. C_3 and C_4/CAM plant resources have non-overlapping carbon isotope ratios, and because consumers are isotopically coupled to their diet, consumer use of these discrete ecosystem compartments can be tracked in systems with both plant photosynthetic groups. The bimodal annual precipitation characteristic of the Sonoran Desert results in seasonally distinct pulses of vegetation. In

winter, this plant biomass is largely characterized by C_3 plant production. In contrast, summer plant growth is generally through the C_4/CAM pathway.

A herbivore's decision to graze on C_3 or C_4/CAM plants has important physiological and fitness consequences. Tortoises in the Sonoran Desert forage among an annually variable selection of C_3 forbs, C_3 shrubs, C_4 grasses, and succulent CAM plants such as cacti. For tortoises in this environment, C_3 grasses, C_4 forbs, and cacti are relatively insignificant dietary components on most sites studied (Van Devender et al. 2002). The majority of a Sonoran Desert tortoise's diet is made up of C_4 grasses, C_3 mallows, and C_3 desert vine (Van Devender et al. 2002). Due to differences in structural anatomy, C_3 plant tissues are generally more digestible and yield more energy than C_4 plant tissues (Barbehenn et al. 2004*a*,*b*). Additionally, C_3 forbs have superior nitrogen and water yields relative to grasses (Meienberger et al. 1993; Nagy et al. 1998; Hazard et al. 2009). Desert tortoises face considerable challenges balancing their nitrogen, water, and energy budgets in an often harsh and unpredictable environment, which means the choice to forage on C_3 *vs.* C_4/CAM plants may have important repercussions for growth and reproduction.

The isotopically distinct plant resource groups present in the Sonoran Desert allow the tracking of consumer nutrient use and dietary niche breadth across variable environments. Because individuals vary in their degree of dietary specialization, a single population can contain both specialists and generalists (Van Valen 1965). Specialists use a narrow spectrum of resources, while generalists utilize a wider variety of resources (Roughgarden 1972; Kassen 2002). Sometimes this inter-individual variability may be due to the unique requirements or phenotypes of different age classes or sexes (Polis

1984; Slatkin 1984; Schoener 1986). However, even individuals of the same sex and similar age can display distinct levels of resource specialization (Bolnick et al. 2002; Bolnick et al. 2003). Stable isotope analyses can help characterize the degree of individual dietary specialization within the dietary niche. By exploring the variability in plant resource use in tortoise populations currently occurring over a range of habitats with different climatic conditions, we can better understand how these populations may respond to the climate changes projected for the future.

We characterize the dietary niche and plant use by tortoises across a precipitation gradient in the Sonoran Desert of Arizona, USA. We follow the conceptual model outlined in Bolnick et al. (2002) and Newsome et al. (2009) where the total niche width of a population is determined by the isotopic 'space' occupied by individuals. Thus, total niche width (TNW), or total isotopic variance, is the sum of the isotopic variation within an individual (within individual component; WIC) and the variation among individuals (between individual component; BIC). Conceptually, TNW accommodates the breadth of resources used by all individuals within a population, BIC describes the variability of resource use among individuals, and WIC estimates the average individual dietary niche width (Bolnick et al. 2003). Thus, the ratio WIC/TNW describes the level of specialization within a population. A value of 1 indicates all individuals use all available resources (i.e., generalist), and a value of 0 describes a population of complete specialists, each using a single resource type (Roughgarden 1972; Bolnick et al. 2002).

In this case, a higher index of dietary specialization suggests that tortoises graze on a variety of plant resources occupying a more extensive portion of the available spectra of carbon isotope ratios. It follows that members of a generalist population can

be thought of as using the full extent of available resources (i.e., generalists within a generalist population; high BIC, high WIC), or as using a subset of the available resources (i.e., specialists within a generalist population; high BIC, low WIC) (Newsome et al. 2009; Vander Zanden et al. 2010).

In this work, we address several questions that are particularly relevant given the projected shifts towards a warmer and drier climate that desert organisms like tortoises will experience. Specifically, how does tortoise dietary specialization change across a gradient of increasing aridity and temperature? We assume that as rainfall decreases and plant resource availability declines, tortoises will adopt a more generalized feeding strategy. Second, does the degree of tortoise dietary specialization (WIC/TNW) vary with age class or sex? We suspect that spatial and seasonal differences in activity and ecology between juveniles and adults may increase juvenile tortoise dietary specialization. Furthermore, seasonal differences in activity between male and female tortoises may lead to significant differences in the dietary niche.

Methods

Study Area

We chose eight locations in the Sonoran Desert of Arizona that represent a gradient of increasing aridity and temperature (Fig. 1). These were all part of an Arizona Game and Fish Department long-term tortoise monitoring project used to study tortoise ecology and track tortoise abundance. Each was approximately 2.6 km^2. Precipitation varied by more than two fold across the gradient (Table 1). Two sites situated within 5 km shared similar vegetation communities but differed in their fire history. The burned site experienced a fire in 1994, whereas the unburned site has never been burned. This comparison

presented the opportunity to gain potential insights into how fire affects the plant resource use of desert tortoises.

Tortoises and tortoise tissue collection

This work was part of a larger study of the stable isotope ecology and growth of desert tortoises across multiple long-term tortoise monitoring plots within Arizona. In this region, tortoises are most common on boulder-strewn slopes with desert vegetation. Additionally, these animals are cryptic and remain in rock shelters for long periods of the year. Tortoise study sites were visited during seasons known to be optimal for tortoise activity. Habitats likely to harbor tortoises, such as rocky ridges and desert washes, were thoroughly searched for these animals as part of a larger effort to monitor populations of desert tortoises within Arizona. Indeed, recent work suggests that Sonoran Desert populations of the desert tortoise are distinct enough to warrant species status (*Gopherus morafkai*), but here we include all desert tortoises as *Gopherus agassizii* (Murphy et al. 2011).

Tortoise shells grow by the peripheral addition of keratinized growth rings, which remain distinct for many years after deposition (Cagle 1946; Carr 1952; Fig. 2). Serially produced keratin in tortoise growth rings is relatively inert, and records the stable isotope signature of the plants eaten during the time of development. Thus we can examine plant resource use over growth within and among tortoise populations (See Murray and Wolf 2012 for discussion of the non-stasis of proximate growth rings.). The nitrogen stable isotope ratios ($\delta^{15}N$) recorded within tortoise annuli probably indicate regional plant nitrogen isotope ratios that are explained by site-specific precipitation patterns and local soil properties (e.g., Handley et al. 1999; Hartman 2011; Ugan and Coltrain 2011) in

these almost exclusively herbivorous reptiles, but carbon stable isotope ratios ($\delta^{13}C$) in the keratin of these tortoise shells are reflects a foraging strategy incorporating variable amounts of plant resources belonging to the C_3 and C_4/CAM photosynthetic pathways (Fig. 3).

We sampled growth rings from 53 wild desert tortoises between 2007 and 2010 (See Fig. 1 for a breakdown of sampled tortoises by site.). These data were supplemented with six shells opportunistically salvaged from dead tortoises found on several of the sites. Scute strips were obtained using a razor saw (Revell 88-6964) to cut thin cross-sections ~15 mm wide from the 2nd or 3rd costal scutes of tortoises immediately after encountering them in the field during area-constrained tortoise surveys (Fig. 2). The keratinized strips were carefully situated to bisect all of the growth rings starting at the neonatal scute. Thus, they included all of the keratin extending from the dorsal surface of the shell to the bony carapace. The entire process took several minutes, and tortoises showed minimal discomfort beyond being physically detained. All tortoise handling followed approved protocols mandated by the Arizona Game and Fish Department, and was done under the appropriate state (SP594732), federal (SAGU-2007-SCI-0007), and institutional IACUC (09-100244-MCC) permits and guidelines (Averill-Murray 2000).

Precipitation data

To characterize the precipitation for each site, we used the PRISM climate mapping system (http://www.prism.oregonstate.edu/). The UTM coordinate of the southeast corner of each site was entered and the program provided a site-specific precipitation estimate for a one kilometer grid resolution. Data are presented as mean values using precipitation data from 1950 to 2010. Because peak tortoise activity in the

Sonoran Desert occurs during periods of summer precipitation, we computed metrics of mean annual, winter (November through April), and summer (May through October) precipitation for each of the sites (Averill Murray et al. 2002; Martin and Van Devender 2002).

Characterization of plant resources

Plant tissues were collected in both 2009 and 2010. We collected tissue from 88 plant species from six of the eight sites. Sampling was an arbitrary affair taking advantage of the sporadic appearance of many forbs and grasses, and we did not sample equally between all the sites. In most cases, we sampled multiple stems, leaves, and flowers from several individual plants of each species. We dried all plant tissues in a drying oven at 55°C (VWR #1390FM), and homogenized dried plants with a mortar and pestle to create an amalgamation of the sampled plant parts before analyzing the carbon and nitrogen stable isotope ratios in several aliquots (ca. 1.0 mg) of dried plant homogenate for each plant species. We identified plants to the species level and grouped them according to photosynthetic pathway (i.e., C_3, CAM, or C_4).

We characterized the abundance and composition of vegetation on all of the sites. We gathered data on annual plant coverage and species identity on each of the eight plots during 2009 and 2010. In 2010 we measured the perennial plant cover on all sites. Most of the eight sampling sites have established transects for taking vegetation data. Due to the remote location of the sites and the variable timing of precipitation, we did not standardize the timing of vegetation measurements between the sites, although we always collected plant data on the burned and unburned sites within the same two day period. We estimated site-specific perennial plant cover and species composition by tallying the

species identity and coverage (m) along five 100 m line-intercept transects in tortoise habitat (Averill-Murray 2000). We calculated annual plant cover and diversity via 20 cm x 50 cm Daubenmire plots placed every 10 m along each of the five transects (Averill-Murray 2000).

Stable isotope analyses

Before processing, we scrubbed samples and removed scute keratin surface contaminants with a 2:1 chloroform/methanol wash. Under a dissecting scope (Nikon SMZ800), we separated all of the individual growth rings from the tortoise scute cross-sections with a razor blade, as described in Murray and Wolf (2012). Tortoise growth ring samples and plant tissues were analyzed for carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) using a continuous flow isotope ratio mass spectrometer (Thermo-Finnigan IRMS Delta Plus) connected to a Costech ECS 4010 Elemental Analyzer in the UNM Earth and Planetary Sciences Mass Spectrometry lab. The precision of these measurements was ± 0.1‰ SD based on repeated measurements of internal lab standards. All sample runs included regularly-spaced lab standards (soy $\delta^{13}C$ = -27.2‰ VPDB; $\delta^{15}N$ = 2.8‰ AIR) that were calibrated against international standards. All values are reported using delta notation (δ) in parts per thousand (‰) as $\delta X = (R_{sample}/R_{standard} - 1) * 1000$. The R_{sample} and $R_{standard}$ represent the ratio of heavy to light isotopes (e.g. $^{13}C/^{12}C$ or $^{15}N/^{14}N$) for the sample and standard.

We corrected for the diet-to-tissue discrimination (Δ) that occurs during tissue keratin synthesis in desert tortoises by subtracting the experimentally determined carbon (0.8‰) and nitrogen (2.55‰) discrimination factors from tortoise growth ring $\delta^{13}C$ and $\delta^{15}N$ values before plotting mean population/gender isotope ratios in bivariate plots with

plant tissue isotope ratios (Fig. 3; Murray and Wolf 2012). We estimated the percent dietary use of C_4/CAM vs. C_3 plant resources using a two-end-point mixing model (Martínez del Rio and Wolf 2005):

$$\delta^{13}C_{(keratin)} = p(\delta^{13}C_{(C4/CAM)}) + (1\text{-}p)(\delta^{13}C_{(C3)}) + \Delta;$$

where p is the fraction of C_4/CAM plant resources assimilated in tortoise scute keratin, and Δ is the keratin carbon discrimination factor (*G. agassizii* = 0.8‰).

Estimates of dietary niche metrics

To determine tortoise dietary breadth, we used the program IndSpec1 (Bolnick et al. 2002). This allowed us to partition the total niche width (TNW) into the variation within an individual (WIC) and the variation among individuals (BIC) for tortoise populations using $\delta^{13}C$ ratios. We employed a bootstrapped Monte Carlo analysis (N = 1000) to assess the robustness of our results. This analysis tested the null hypothesis that tortoises at each site were dietary generalists with random growth ring carbon isotope chronologies. This hypothesis was then tested against a hypothetical group of tortoises with the same number of individuals having the same number of growth rings foraging on the population's diet. IndSpec1 calculates the percentage of the hypothetical populations that had a WIC/TNW value lower than that actually observed. We used $\delta^{13}C$ and not $\delta^{15}N$ ratios because we expect that any nitrogen variability is likely to be due to regional differences in background nitrogen ratios rather than a reflection of different trophic levels in these herbivorous tortoises. Additionally, plant nitrogen isotope values are known to be correlated strongly with site specific soil and precipitation characteristics (Handley et al. 1999; Hartman 2011; Ugan and Coltrain 2011), an assumption supported

by variance components analyses that showed most of the total keratin nitrogen isotopic variability to be determined by sampling site.

Results

Sampling Sites

We sampled living desert tortoises from eight populations situated within progressively drier and hotter habitats within Arizona's Sonoran Desert (Table 1; Fig.1). The wettest site (Wickenburg Mts.; 406 mm) had two and a half times the mean annual precipitation relative to the driest site (New Water Mts.; 158 mm). This difference in annual precipitation translated into a three-fold difference in annual net primary productivity (ANPP; calculated via Webb et al. 1978; Fig. 1) available as tortoise forage (WM; 140 $g*m^{-2}$ vs. NW; 46 $g*m^{-2}$). The coefficient of variation for seasonal summer (May – October) precipitation, a measure of unpredictability, significantly increased as mean annual summer rainfall decreased across sites ($y = -7.1x + 431.5$; $df = 7$; $r^2 = 0.79$; $p = 0.002$; Fig. 5A). The coefficient of variation for winter and annual precipitation did not significantly increase with decreasing rainfall when the Wickenburg Mts. site was included. When the Wickenburg Mts. site was removed the coefficient of variation significantly increased as both winter and annual rainfall decreased across sites. Estimates of ANPP sharply decrease with increasing annual rainfall coefficient of variation (including WM; $y = -2.9x + 183.5$; $r^2 = 0.28$; $p = 0.2$; excluding WM; $y = -4.0x + 212.5$; $r^2 = 0.94$; $p = 0.000$; Fig. 5B). The Wickenburg Mts. site is a site where tortoises are at lower densities relative to other monitoring sites (C. Jones, pers. comm.), and air temperatures were cooler here relative to the other eight sites between November 2009 and April 2010 (Tukey's HSD; $p < 0.05$). Indeed, the cold-sensitive saguaro cactus

grows at lower densities here, and only on the warmer south and west-facing slopes (pers. obs.). As might be expected, measures of vegetation cover were also correlated with the quantity of site precipitation. Perennial plant cover increased as annual rainfall increased ($y = 0.4x + 6.8$; $r^2 = 0.5$; df = 7; P = 0.03). During 2010, the number of summer days reaching high temperatures of at least 40°C (tortoises cease voluntary activity at this temperature; Vaughan 1984) was significantly higher as mean annual precipitation declined ($y = -10.6x + 371$; $r^2 = 0.66$; df = 7; P = 0.008).

Plant analyses

We isotopically characterized 88 plant species, and separated these into plant functional groups based upon species growth form and photosynthetic pathway (i.e., C_3 herbaceous annual vs. C_3 shrub: Fig. 4; Appendix 1). Plant functional groups growing in desert tortoise habitat occupied distinct positions in plotted stable isotope space with $\delta^{13}C$ values between -27.6‰ and -12.5‰ VPDB, and $\delta^{15}N$ values ranging from 2.3‰ to 4.0‰ AIR. There is a higher level of isotopic variation among plant functional groups/photosynthetic pathways, compared to within plant functional groups. These data show that desert tortoises select plant resources from among a diverse array of plants with disparate stable isotope values, particularly for carbon. This variability in resource isotope character thus provides an ideal background for investigating tortoise use of two disparate ecosystem compartments of plant resources (C_3 and C_4/CAM) across an environmental gradient.

Desert tortoise niche breadth across an environmental gradient

Our analysis of carbon and nitrogen stable isotopes in 1,378 growth ring sequences from 59 tortoises across 8 sites showed that tortoises fed on a mixed diet of

plants distributed among the available photosynthetic pathways and functional groups. Moreover, we found substantial variation in the use of plant resources across years for individual tortoises (Fig. 6). At one extreme, tortoise populations used 86% C_3 plants (Maricopa Mts. site), compared to 61% C_3 plants at the other extreme (Burn site; Fig. 1). Across all populations, desert tortoise dietary niche breadth tended to be almost equally comprised of isotopic variability within an individual's diet (WIC; 3.6) as well as dietary variation among tortoises (BIC; 4.7; Table 2). Mean tortoise niche width (TNW) was 9.6, and the degree of dietary specialization, estimated by the relationship WIC/TNW was 0.38, meaning that there was dietary specialization occurring, i.e., a reduction in inter-individual overlap in resource use. Differences in niche metrics occurred in tortoise populations inhabiting sites over an east-west gradient of warmer and drier environments. An adult tortoise's diet (but not juvenile diet) became more generalized (WIC/TNW approaches 1.0) as a site's ANPP and precipitation decreased and became more stochastic (i.e., a higher coefficient of variation; Fig. 5C), however the total niche width decreased as environmental unpredictability increased ($y = -1.6x + 76.3$; $r^2 = 0.47$; $df = 7$; $P = 0.04$). Additionally, among individual (BIC; $y = 0.34x - 1.04$; $r^2 = 0.78$; $df = 7$; $P = 0.002$) and within individual (WIC; $y = 0.36x + 3.57$; $r^2 = 0.62$; $P = 0.013$) variability increased as the population TNW increased.

Diet and tortoise life stage and sex

The dietary niche and degree of specialization varied significantly during ontogeny. Additionally, male and female tortoises had different patterns of plant resource use (Table 2). Mean female tortoise carbon isotope values (n = 511; -20.6‰) were significantly enriched over mean male carbon values (n = 546; -21.1‰), which

were themselves enriched over juvenile tortoise carbon values (n = 203; -22.2‰; Tukey's HSD; $P < 0.05$). Despite these differences, male, female, and juvenile tortoises had a similar degree of dietary specialization at 0.56, 0.55, and 0.49, respectively when all of the growth rings in an individual's chronology were included. However, mean desert tortoise growth ring widths increased from the first ring grown after hatching, to the 11^{th} successive ring, at which point there was a sharp decline in ring width throughout the rest of the growth chronology of the individual (Fig. 7). During the growth period encompassing the first 11 rings young female and male tortoises had more specialized diets (males = 0.22; females = 0.22; combined = 0.25) relative to the more generalized niche during the accretion of all rings past the eleventh ring (males = 0.45; females = 0.35). Additionally, the TNW for rings 1 – 11 (males = 5.1; females = 4.9; combined = 5.2) was significantly more restricted than the TNW for either males (10.5) or females (18.5) for growth periods after ring 11. Desert tortoises also had a higher degree of among and within-individual dietary variability for growth and feeding occurring during the addition of rings > 11 compared to growth during the juvenile and sub-adult period, and male tortoises had a narrower dietary breadth metrics for all rings beyond the 11^{th}, relative to females (Table 2).

Desert tortoises showed significant increases in their use of C_4/CAM plants as they grew larger on those sites that experienced reliable annual monsoons, which were also the most mesic sites (Fig. 8). Juvenile tortoises almost tripled their incorporation of C_4/CAM resources through their growth trajectories on the WM, WSB, and the R-B sites, while almost doubling their use of this resource on the R-U site. Tortoises occurring in

the ET, NW, MM, and ST sites that experienced weaker and less predictable monsoons, showed little significant, systematic change in C_4/CAM plant resource use as they grew.

Fire and desert tortoise dietary niche

Desert tortoises on the burned site used vegetation differently than those on unburned sites. Mean growth ring $\delta^{13}C$ values were enriched by over 1‰ in tortoises occupying the burned site relative to the unburned site (-19.5‰ *vs*. -20.7‰ VPDB; two sample t-test, t = -3.7, P = 0.000). This is indicative of an almost 10% increase in dietary integration of C_4/CAM plant resources for tortoises on the burned site. Scute ring $\delta^{15}N$ values were also depleted for tortoises on the burned plot compared to animals living on the unburned site (6.5‰ *vs*. 7.4‰ AIR; two-sample t-test, t = -5.5, P = 0.000). A sharp separation in foraging strategies was apparent for tortoises living in burned vs. unburned habitats. Desert tortoises on the unburned plot can be classified as relative generalists (WIC/TNW = 0.57) within a more restricted population niche (TNW = 18.7; BIC = 5.5). In contrast, those animals on the previously burned land were more specialized (WIC/TNW = 0.36) within a generalist population (TNW = 38.4; BIC = 15.2). Precipitation inputs and temperature regimes do not significantly differ between the proximate burned and unburned plot, but some estimates of vegetative cover differ. Perennial plant cover was higher (158 m) on the unburned site relative to the unburned site (114 m), but observed annual forb diversity was similar on the two sites (15 and 13 species for the burned and unburned sites). The observed diversity and coverage (cm^2) of grasses was higher on the burned site, however. We documented seven species of grass for the burned site and four species for the unburned site. In one year of measurement (2009), the quantity of grass cover on the two sites was similar, but in 2010, there was

five times more grass cover on the burned site (4,860 cm^2) compared to the 880 cm^2 growing on the unburned site.

Desert tortoises living on the neighboring burned and unburned sites in the Rincon Mountains used similar quantities of C_4/CAM plant resources during the first few years of juvenile growth, but during development of successive growth rings, animals on the burned site showed increasingly higher utilization of C_4/CAM plant resources (burn plot slope = 2.0 ± 0.2; unburn plot slope = 0.9 ± 0.1) ultimately incorporating ~60% of their growth ring carbon from these plant sources (Fig. 8). Desert tortoises on the unburned plot also progressively incorporated more of these plant resources as they accrued rings, but this was at a lower rate than that of burn-dwelling tortoises (~40%).

Discussion

Overall, desert tortoises are highly individualistic specialized herbivores (Oftedal 2002; Oftedal et al. 2002; Tracy et al. 2006). The observed specialization index of 0.38 found here is significantly lower than virtually all vertebrates and invertebrates in a recent review by Araújo et al. (2011) who found a mean individual specialization of 0.66 ± 0.21 across taxa. Indeed, we find a greater degree of dietary variability between individuals than within an individual's ontogeny (Table 2).

Moreover, we find significant differences in resource use between populations of desert tortoise related to differences in habitat aridity and productivity. Desert tortoise dietary specialization significantly increases as habitats become wetter, cooler, and more vegetated. Additionally, we find important differences in resource utilization between tortoise size classes (juvenile *vs*. adult) and between males and females. As desert tortoises grow, the dietary niche expands some 2-4 times, dietary breadth increases, and

the variability between individuals and within individuals expands. In general, male desert tortoises had narrower dietary niches and a lower level of dietary specialization relative to females. Juvenile tortoises of both sexes had equally reduced dietary breadths and high dietary specialization relative to adults. This disparity may be due to the less intense nutritional demands and more restricted activity season for male tortoises, and the constraints imposed by juvenile tortoise physiology.

The desert mountain ranges inhabited by the desert tortoise in Arizona become successively drier as one moves from the southeastern to the southwestern part of the state. This gradient is largely due to the less reliable and weaker summer rainfall experienced as the distance from the Mexican core of the North American Monsoon System increases in the more southwesterly portions of the Sonoran Desert (Crimmins 2006; Table 1). Further, the stochastic nature of moisture inputs increases as one moves further west, accompanied by a concomitant increase in temperature and a decrease in perennial plant cover.

This strong environmental gradient is reflected in the dietary choices made by tortoises; at the eastern and relatively productive and wet end of the range, tortoises are highly specialized (index of specialization = 0.36), while those living in the hottest and driest habitats to the west are much more generalized (index of specialization = 0.74). Desert tortoises in the Sonoran Desert are most active during the period of summer rains, with an additional smaller peak in activity during wet springs (Averill- Murray et al. 2002; Martin and Van Devender 2002). Tortoise activity may also be sensitive to high temperatures, and one studied population was not noted to be active at air temperatures above 40°C (Vaughan 1984). These observations suggest that tortoises have less time for

foraging and accumulating nutrients in severe environments. Tortoises likely have fewer opportunities to be active and forage in the more arid portion of their Sonoran Desert distribution due to constraining climatic conditions, and when they are active they must feed in a patchier, less vegetated landscape. For example, the ANPP of the driest site is 46 $g*m^{-2}$ *vs*. 140 $g*m^{-2}$ at the most mesic site. One of the basic tenets of optimal foraging theory is that animals should minimize costs, and maximize intake while foraging. It follows then that in environments where food plant encounter rates are lower, such as in some of the more arid habitats occupied by tortoises, individuals might not be as selective in their foraging choices. Conversely, desert tortoises in habitats with a higher diversity and quantity of plant resources can afford to be more selective in their feeding choices and choose the most physiologically advantageous resources, as desert tortoises are known to do when patchily distributed, but nutritionally superior plant species sprout (Oftedal 2002). In short, optimal foraging theory states that when tortoises encounter profitable plant resources at a high rate, then the degree of diet specialization should be higher than when these same resources are encountered at lower rates (Pyke et al. 1977).

We observed that as site-specific tortoise niche width increased across sites, individual tortoises were more specialized in their feeding habits, and their intra- and inter-individual dietary variation increased at similar rates. For example, the two driest sites with the least perennial vegetation (ET and NW), and probably with the lowest plant resource encounter rates, hosted tortoises with the narrowest potential dietary niches, but within these bounds they showed very high dietary generalization (i.e., tortoises here cannot afford to be as selective in their foraging criteria; Table 2). The increase in BIC

that accompanies increased niche width is notable because it supports the previously rejected hypothesis by Van Valen (1965) that larger niche widths would be accommodated by a high degree of between individual variation, a hypothesis that received some substantiation in other studies (Bolnick et al. 2003; Bolnick et al. 2007; Araujo et al. 2009).

Juvenile desert tortoises have a narrower dietary breadth, a high degree of specialization, and less variability within and between individuals relative to adult tortoises (Table 2). Tortoise growth is normally more rapid through the first ten years (Germano et al. 1994; Germano 1994; Averill-Murray et al. 2002), and slows as the animal nears reproductive maturity, continuing at a slow rate towards asymptotic size (Ernst et al. 1994; Germano 1994; Averill-Murray et al. 2002). By measuring the width of annuli in desert tortoises over the course of their growth histories, we were able to find a point of inflection between rings 11 and 12 that represents the change from rapid growth during a tortoise's early years of life (between rings 1 to 11) to the steadily decreasing growth rates experienced later in the tortoise's developmental trajectory (rings 12 and beyond; Figure 7). Consequently, we partitioned the tortoise ring sequences to represent these different phases and uncovered important differences. During the early rapid period of growth, juvenile male and female tortoises have equally narrow dietary breadth metrics (index of dietary specialization = 0.22; Table 2) compared to the expanded adult diet breadth. These disparate patterns in plant resource use likely arise from the ecological and physiological differences separating juvenile and adult tortoises.

Juvenile desert tortoises show seasonally different patterns of activity and foraging compared to adult tortoises. For example, juvenile tortoises in the Mojave

Desert are more active during winter and emerge from hibernation earlier relative to larger tortoises (Rautenstrauch et al. 1998; Wilson et al. 1999). Accordingly, we assume that small juvenile Sonoran Desert tortoises are also able to take advantage of cooler conditions in late winter/early spring, when their preferred forbs have just emerged and are accessible to small tortoises.

The physiology of small immature tortoises also constrains the plant resources that they can eat, relative to those available to larger animals (Oftedal et al. 2002; Van Devender et al. 2002). The greater thermal inertia and mobility of large tortoises allow them to access a larger variety of patchily distributed plant species for a greater period of time. The reduced gut capacity and retention times, as well as the smaller and weaker mandibles in immature tortoises limits their foraging to relatively low fiber, leafy forbs whose availability may be temporally and spatially restricted, and which tend to be C_3 plant resources (Meienberger et al. 1993; Tracy et al. 2006). C_4 grasses are a large component of the plant biomass promoted by summer rainfall, but are relatively inaccessible to very small tortoises due to physiological and physical limits. However, as desert tortoises increase in size, C_4 grasses become an important part of their diet. Although C_3 grasses generally are thought to have higher levels of nitrogen relative C_4 grasses, nutritional analyses of C_3 and C_4 grasses from southern Nevada show that C_4 grasses in the growing stage are significantly higher in nitrogen than C_3 grasses in the same area (Oftedal 2002).

Important differences in plant resource are also evident between male and female desert tortoises. Male tortoises have more generalized diets, but more restricted total niche widths and reduced measures of variability among and within individuals relative

to females (Table 2). Patterns of activity are known to be different in male and female tortoises, which probably effects seasonal patterns of foraging and plant use (Bailey 1992; Bulova 1994; Martin 1995; Averill-Murray et al. 2002). For example, within the Sonoran Desert females are more likely to be active and feed in the spring as they yolk up eggs, while both females and males exhibit high levels of activity during the summer rains (Vaughn 1984; Wirt 1988; Averill-Murray et al. 2002). Male tortoises are known to be surface active for a smaller percentage of the year, consequently restricting their access to vegetation available only during those time periods. Additionally, tortoises may subscribe to the nutritional wisdom hypothesis (Westoby 1974; Tracy et al. 2006) whereby individuals make foraging choices in terms of specific nutrients. Adult male tortoises may not have the same nutrient/energetic demands as rapidly growing juveniles and reproductive females (Nagy and Medica 1986; Peterson 1993; Henen 1994, 1997; Nagy et al. 1997).

The dietary integration of C_4/CAM plant resources increases over tortoise growth on those sites experiencing the most precipitation. The relatively mesic sites with reliable summer monsoonal rainfall (R-U, R-B, WSB, and WM; Table 1) all hosted tortoise populations that incorporated successively higher proportions of C_4/CAM plant resources as they grew larger, while at the same time maintaining an important reliance on C_3 plant resources (Figs. 7 and 8). The more xeric, westerly sites had weaker, more unreliable summer periods of rainfall, and a lower diversity of C_4 annual plant species available as forage (Shreve 1964). Consequently, tortoises in these regions (ST, ET, NW) may opportunistically use available C_4/CAM plant resources when they are available during wet summers, but they did not show a regular pattern of increasing reliance on these

forage species as they grew. An alternative pattern of plant use was observed where tortoises integrated C_3 plant resources almost to the exclusion of other plant photosynthetic pathways (MM; Fig. 8).

Fire has the potential to affect desert tortoise fitness through direct mortality, as well as through altering the distribution and availability of plant resources (Esque et al. 2003). These negative impacts are intensified by the fact that desert tortoises are likely to remain in their usual home ranges after fire, and they do not tend to leave for nearby unburned habitat (Lovich et al. 2011). Additionally, in burned Sonoran Desert habitats, overall plant cover only reaches pre-fire levels within four decades (roughly two tortoise generations), but species composition remains significantly altered compared to the unburned state (Abella 2009). Sonoran Desert plant species are not considered fire adapted, but the rampant spread of nonnative grasses has resulted in an increase in fire frequency in Sonoran Desert habitats (Brown and Minnich 1986; Schmid and Rogers 1988). These nonnative grasses and the fires they facilitate significantly alter the density and reproductive success of native forbs and grasses, as well as reduce the persistence of native succulents and trees, all of which have the potential to impact desert tortoise plant resource use.

We find significant differences in the species composition and cover of plants between the burned and unburned sites. The burned plot had less perennial plant coverage, more grass cover (significantly more in 2010), less *Opuntia* cacti, and more *Encelia farinosa* and *Calliandra eriophyllum* than the unburned plot. Cacti, such as the measured *Opuntia*, experience heavy mortality rates during and after fires, and shrubby

plants such as *E. farinosa and C. eriophyllum* often emerge as the dominant plants after fires in the Sonoran Desert (Reynolds and Bohning 1956).

Our isotopic analysis suggested that fire did significantly impact the dietary niche of tortoises (Table 2). Desert tortoises on a burned plot incorporated significantly higher levels of C_4/CAM plant resources relative to tortoises on an unburned plot (Table 2). While this may have resulted from site-specific habitat differences, the close proximity, similar elevation and vegetation structure of the sites would seem to make that unlikely. We believe that the observed differences were probably the results of the fire-mediated environmental shifts. We acknowledge, however, that further study is required to validate these results.

Additionally, tortoises in the burned habitat increased their dietary integration of C_4/CAM plants at twice the rate over ontogeny compared to individuals in the unburned habitat (Fig. 7). Previous studies show that CAM resources generally comprise a minor part of tortoise diet in Sonoran Desert tortoise habitat, and because the majority of grass species at this site have C_4 photosynthesis, we interpret the enriched $\delta^{13}C$ ratios in burn-dwelling tortoises as being indicative of higher levels of C_4 grass use. This conclusion is further supported by the prevalence of grasses on the burned site, and the relative rarity of *Opuntia* cacti (the unburned site had three times more coverage of *Opuntia*) on the burned habitat. As a tortoise dietary component, grasses, particularly when dry, yield less energy and nitrogen compared to forbs, so a proliferation of grasses at the expense of forbs may have detrimental long-term fitness effects, especially during physiologically trying periods such as drought (Meienberger et al. 1993; Nagy et al. 1998; Hazard et al. 2009).

There were important differences in population and individual dietary breadths for tortoises living in burned and unburned habitat. As a population, burn-dwelling tortoises had wider dietary niches (38.4) compared to tortoises foraging in nearby unburned upland Sonoran Desert (18.7). However, within the expanded population niche, individuals on the burned-plot tended to be more specialized (WIC/TNW; 0.36 *vs.* 0.57 for burn and unburned plot tortoises), with a concomitant increase in dietary variation among tortoises on the burn site (15.2) compared to animals in an unburned site (5.5). This suggests that individuals on the burned plot are dietary specialists within a generalist population, while tortoises on the unburned site are dietary generalists using a more specialized suite of plant resources. Consequently, on the burned plot inter-individual dietary variation is the principal driver of niche expansion, further supporting the niche variation hypothesis by Van Valen (1965), and more recently documented in diverse taxa from wolves to frogs to gastropods (Bolnick et al. 2007; Araújo et al. 2009; Darimont et al. 2009).

A seemingly expanded population feeding niche on the burned plot was primarily driven by a marked increase in dietary variability among animals. This putative reduction in diet generality may be a result of fire affecting the availability and/or distribution of favored plant resources, effectively limiting a foraging tortoise's feeding choices. Though generally more specialized, the combined inter-individual variability in dietary niche summed to a higher population niche for tortoises living on previously burned Sonoran Desert. This trend may reflect the reduction in plant resources and the altered plant species composition in a landscape not yet recovered from a fire 16 years previously.

Fire may result in important long-term impacts on desert tortoise fitness and growth. Tracy et al. (2006) showed that out of a large number of potentially available forage species, individual desert tortoises tend to specialize on ~ 5 plant species in part due to the physiological costs of re-equilibrating their microbial gut symbionts after a diet change and the concomitant reduction in digestive efficiency after a diet switch. Tortoises on the burned plot that are surrounded by expanses of native and nonnative grass species may face the dilemma of maintaining a stable association of cellulose-fermenting microbes and feeding on the readily available grasses most of the time, or risk sustaining periods of sub-optimal digestive efficiency after changing foraging tactics between grasses and the more nutritionally profitable forbs.

Examining how species respond to a variable environment is crucial as we attempt to predict how organisms will respond to the currently changing global climate. Here, we have demonstrated how individual consumers use resources across climatically variable habitats. We document important differences in plant resource use that are related to both tortoise sex and life stage. In this system, a shift to a warmer and drier climate may lead to a greater reliance on heat adapted and water efficient C_4/CAM plant resources, which will negatively impact the growth and fitness of desert tortoises, particularly C_3 plant-dependent juveniles.

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Table 3.1. Precipitation and vegetative cover by tortoise population.

Site	Annual rainfall (mm)	Summer rainfall (mm)	Winter rainfall (mm)	CV annual rainfall	CV summer rainfall	CV winter rainfall	Perennial plant cover (m)
Rin-U	327	189	137	27.1	35.1	47.9	158
Rin-B	326	189	137	26.9	35.1	47.9	114
West Silverbells	252	135	119	29.7	37.0	49.0	103
San Tan	234	101	132	34.8	47.1	52.2	76
Maricopa	199	90	110	38.3	49.1	56.6	115
Eagletail	176	77	100	41.0	47.6	63.7	90
New Water	158	71	87	41.1	50.3	64.9	21
Wickenburg	406	176	230	38.2	41.7	61.7	139

Table 3.2. Calculated niche metrics 1)WIC – within individual component of isotopic dietary variation 2)BIC – between individual component of dietary variation 3)TNW – total isotopic niche width 4)WIC/TNW – measure of dietary specialization; 1.0 = complete generalist, 0.0 = exclusive specialist. NW - New Water Mts., ET - Eagletail Mts., R-B - Saguaro National Park Burn site, R-U - Saguaro National Park un-burned, WSB - West Silverbell Mts., WM - Wickenburg Mts., MM - Maricopa Mts., and ST - San Tan Mts.

Sex/Site	WIC	BIC	TNW	WIC/TNW
Juvenile	6.3	3.3	12.9	0.49
Male	4.4	3.0	7.8	0.56
Rings 1-11	1.1	4.0	5.1	0.22
Rings > 11	4.8	4.9	10.5	0.45
Female	6.5	3.9	11.8	0.55
Rings 1-11	1.1	3.9	4.9	0.22
Rings > 11	6.5	7.5	18.5	0.35
Pooled	3.6	4.7	9.6	0.38
Rin-U	10.8	5.5	18.7	0.57
Rin-B	13.7	15.2	38.4	0.36
WSB	13.1	11.2	35.2	0.37
ST	20.6	7.6	34.0	0.61
MM	13.6	3.1	18.9	0.72
ET	3.7	3.2	7.0	0.53
NW	3.7	1.2	5.1	0.74
WM	13.3	4.2	19.9	0.67

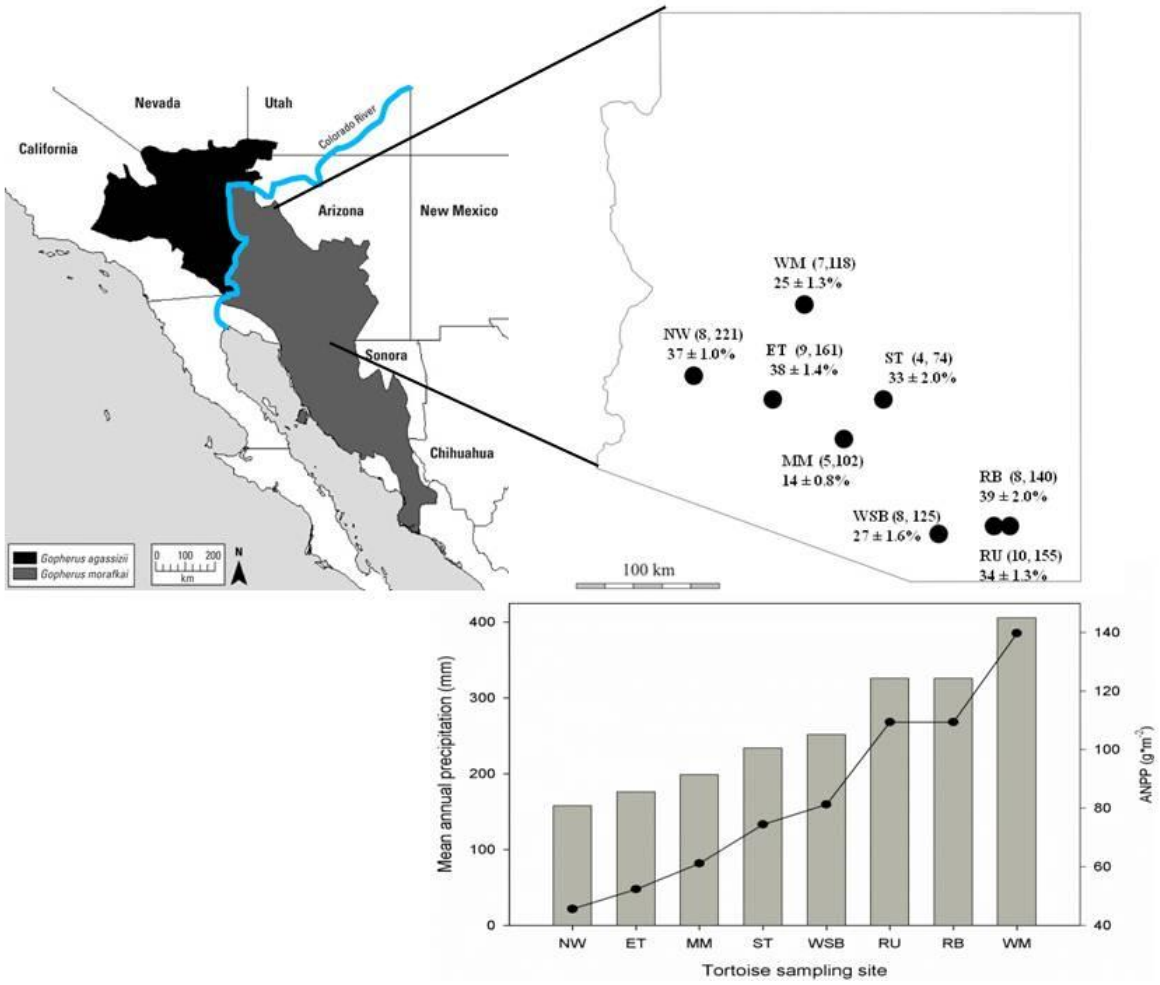

Figure 3.1. Distribution of the desert tortoise followed by the number of tortoises sampled from each sampling site, and the total number of scute growth rings sampled (n,n) for stable isotope analyses for each site. Mean population (± SE) utilization of C_4/CAM plant resources is presented on the map. Estimated annual net primary productivity (ANPP) is calculated using Webb et al. 1978 for each tortoise sampling site. (WM = Wickenburg Mts.; NW = New Water Mts.; ET = Eagletail Mts.; ST = San Tan Mts.; MM = Maricopa Mts.; WSB = West Silverbell Mts.; RB = Rincon Mts. – burned; RU = Rincon Mts. – unburned. Map of tortoise distribution from Murphy et al. 2011

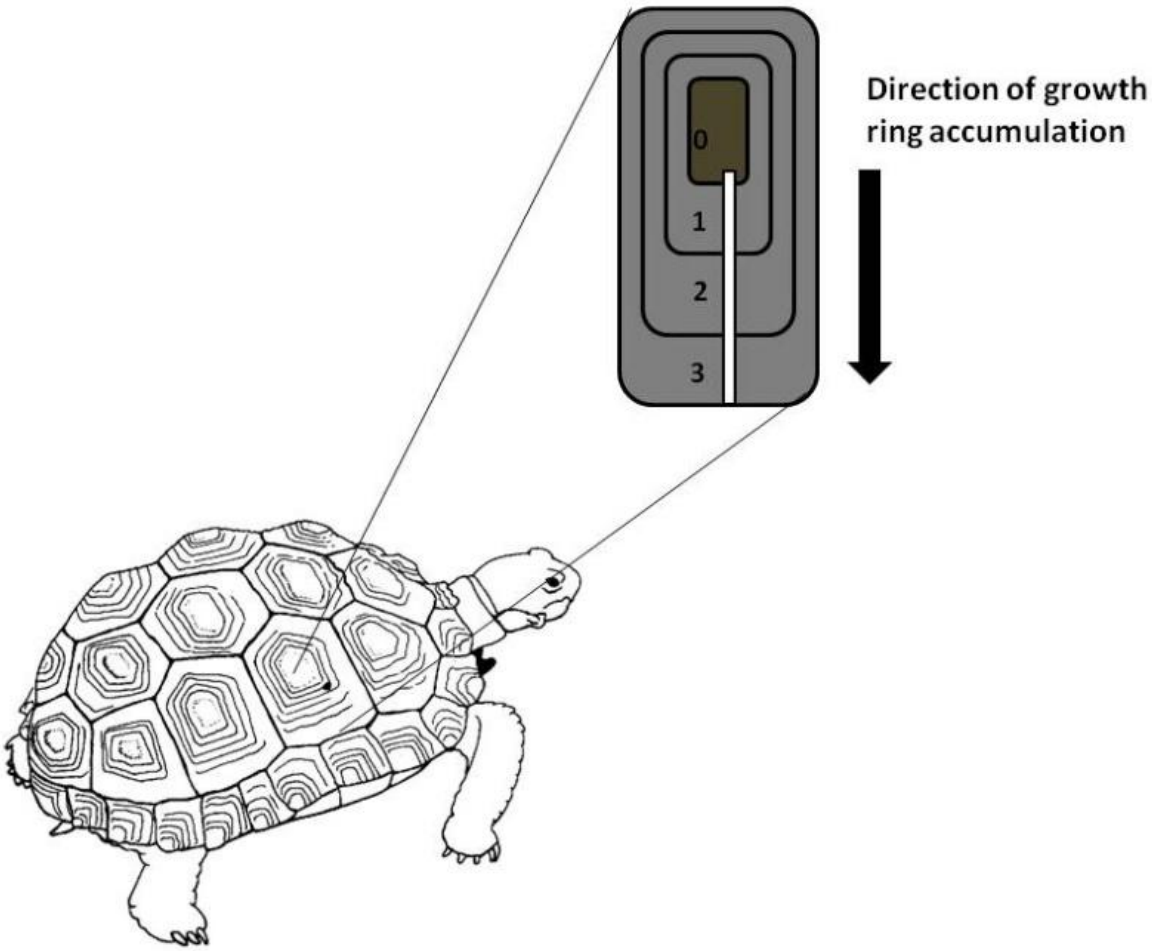

Figure 3.2. A schematic of tortoise growth ring sampling. A thin cross-section of keratinized scute is cut, bisecting all of the growth rings on the 2nd costal scute, and extending from the dorsal surface of the scute to the underlying bony carapace. Tortoises grow via the concentric addition of rings, such that the distal-most ring is the most recently added ring. Here the neonatal scute present at hatching is 0, followed by the sequential addition of rings 1, 2, 3, etc., 3 being the most recently grown ring. (Tortoise diagram used with permission from www.arthursclipart.org

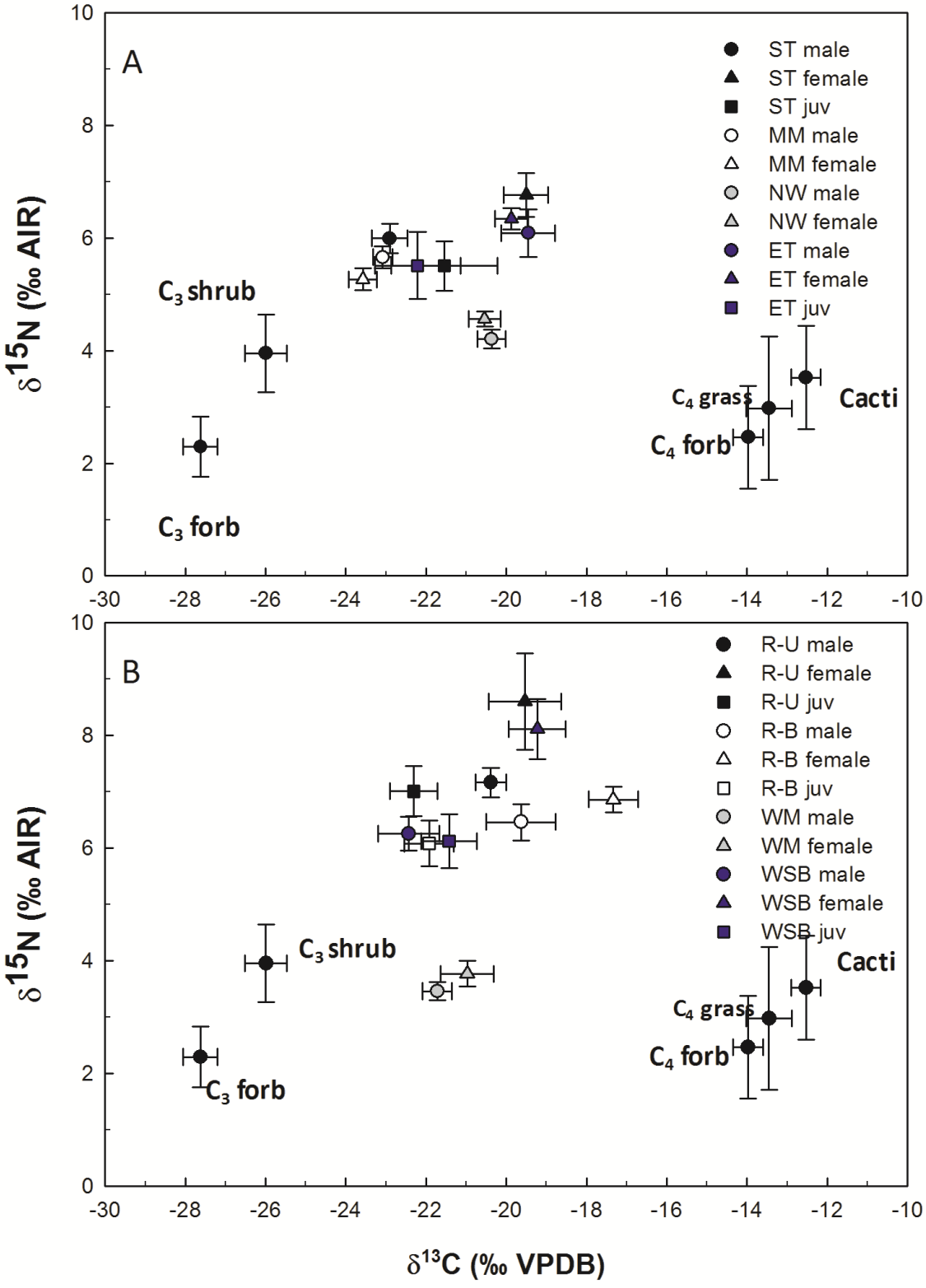
A
B
ST male
ST female
ST juv
MM male
MM female
NW male
NW female
ET male
ET female
ET juv
R-U male
R-U female
R-U juv
R-B male
R-B female
R-B juv
WM male
WM female
WSB male
WSB female
WSB juv
C3 shrub
C3 forb
C4 grass
C4 forb
Cacti
δ15N (‰ AIR)
δ13C (‰ VPDB)

Figure 3.3. Mean desert tortoise growth ring keratin $\delta^{13}C$ and $\delta^{15}N$ values for male, female, and juvenile tortoises captured in 8 populations across Arizona. Scute growth ring carbon and nitrogen values are plotted relative to mean values (± SE) for plant functional groups (88 species; C_3 forbs, C_3 shrubs, C_4 grasses, C_4 forbs, CAM cacti) available as plant resources for grazing desert tortoises. Tortoise scute ring $\delta^{13}C$ (0.8‰) and $\delta^{15}N$ (2.55‰) values have been adjusted by subtracting the appropriate keratin diet-tissue-discrimination factors in tortoises. A) Four xeric sites with less robust summer rainfall. B) Four mesic sites with significant and reliable summer rainfall.

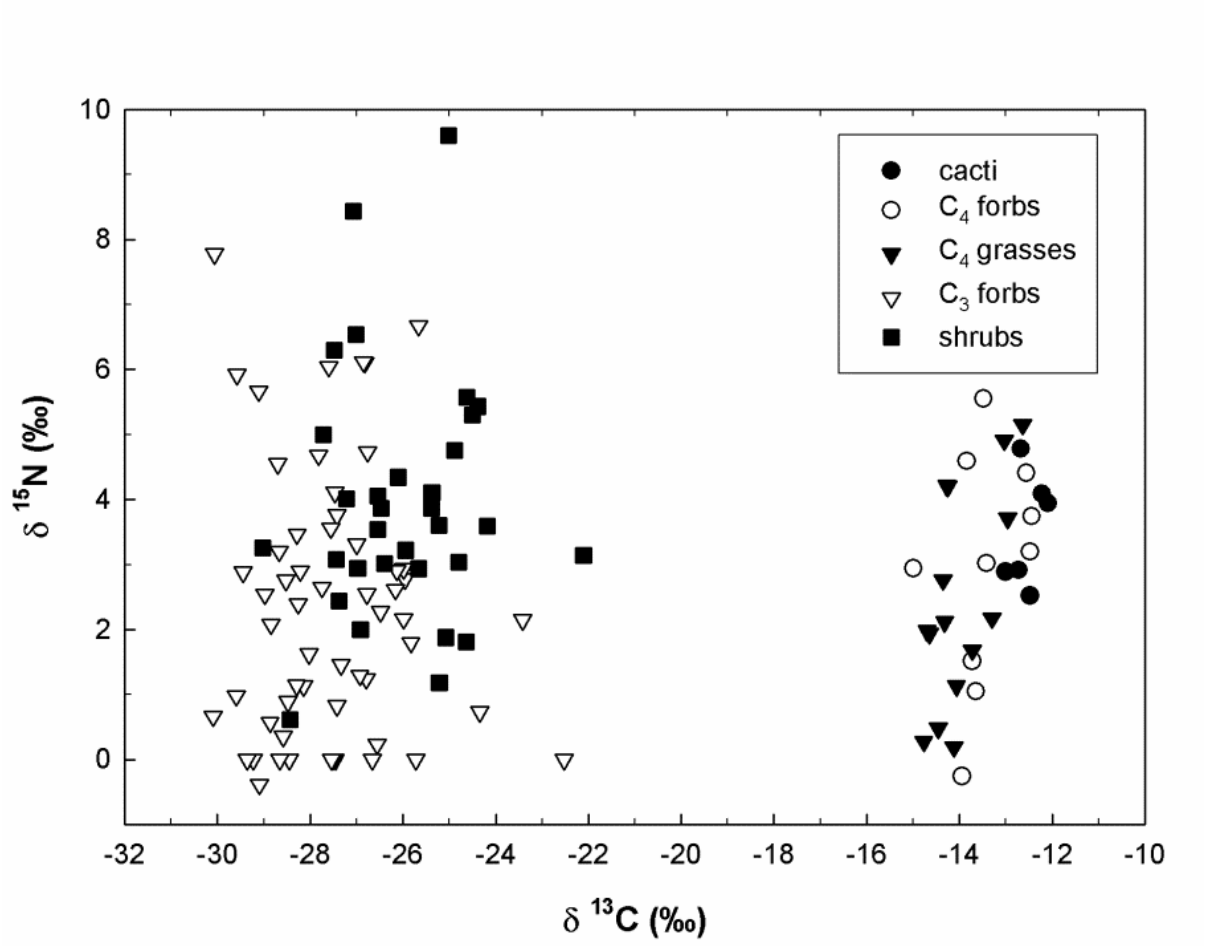

Figure 3.4. Plant $\delta^{15}N$ and $\delta^{13}C$ stable isotope ratios of potential tortoise forage species collected from tortoise habitat in the Sonoran desert of Arizona. See Appendix 1 for a complete listing of plant species.

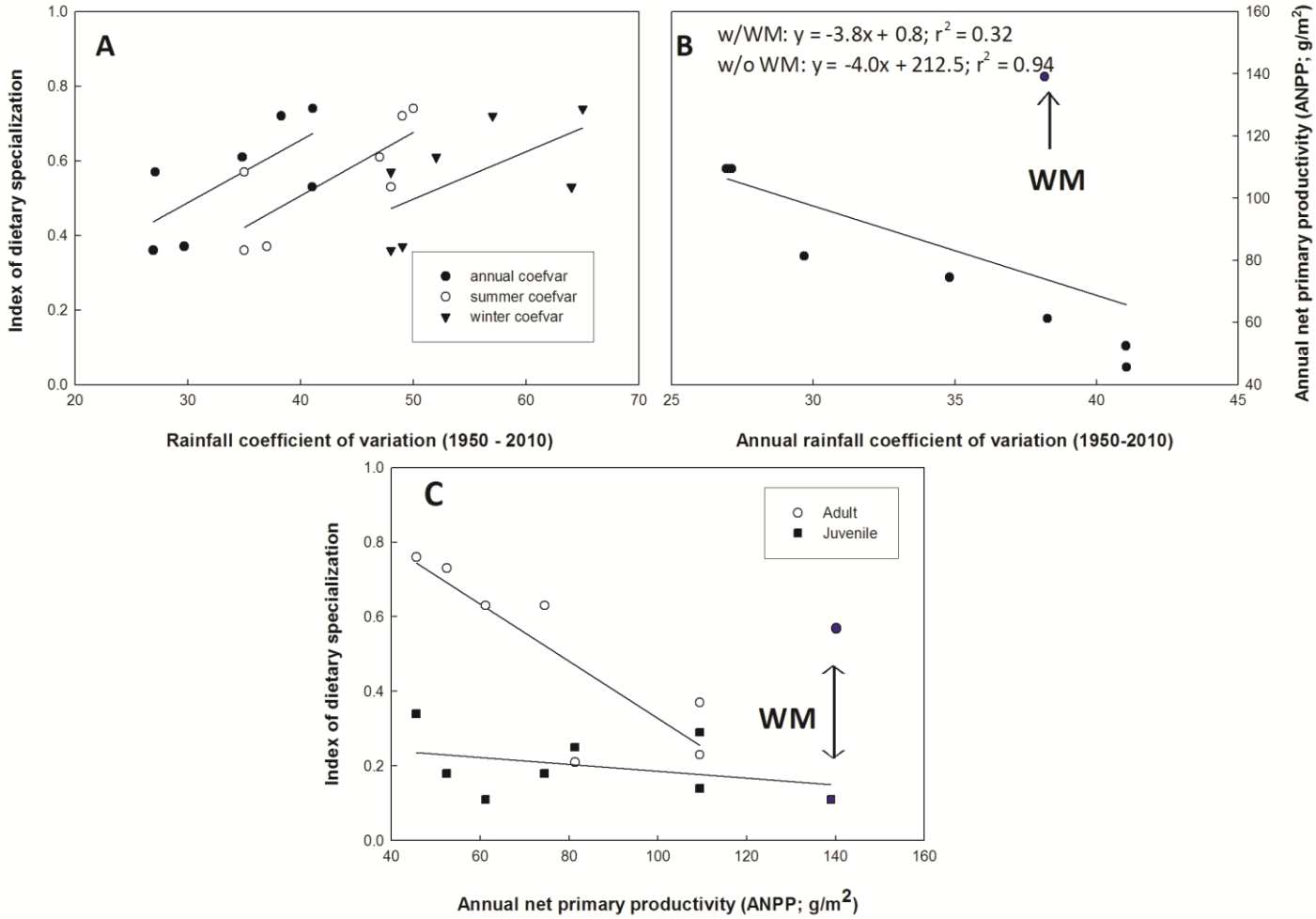
A
Index of dietary specialization
Rainfall coefficient of variation (1950 - 2010)
annual coefvar
summer coefvar
winter coefvar
B
w/WM: y = -3.8x + 0.8; r2 = 0.32
w/o WM: y = -4.0x + 212.5; r2 = 0.94
WM
Annual rainfall coefficient of variation (1950-2010)
Annual net primary productivity (ANPP; g/m2)
C
Adult
Juvenile
WM
Index of dietary specialization
Annual net primary productivity (ANPP; g/m2)

Figure 3.5. A) Desert tortoise dietary specialization (WIC/TNW) versus the unpredictability of site-specific annual, summer (May – October), and winter (November – April) precipitation. Rainfall data interpolated from the PRISM climate mapping system (http://www.prism.oregonstate.edu/) using the UTM coordinates for each tortoise sampling site. B) Site specific annual net primary productivity (ANPP) decreases with increasing stochasticity (coefficient of variation) of annual rainfall C) Desert tortoise dietary specialization (WIC/TNW) for adult (>11 growth rings; w/WM: $y = -3.8x + 0.8$; $r^2 = 0.32$; w/o WM: $y = -7.7x + 1.1$; $r^2 = 0.71$) and juvenile (<=11 growth rings; N.S.) across a gradient of ANPP. The Wickenburg Mts. (WM) site is an outlier due in part to the significantly colder temperatures there.

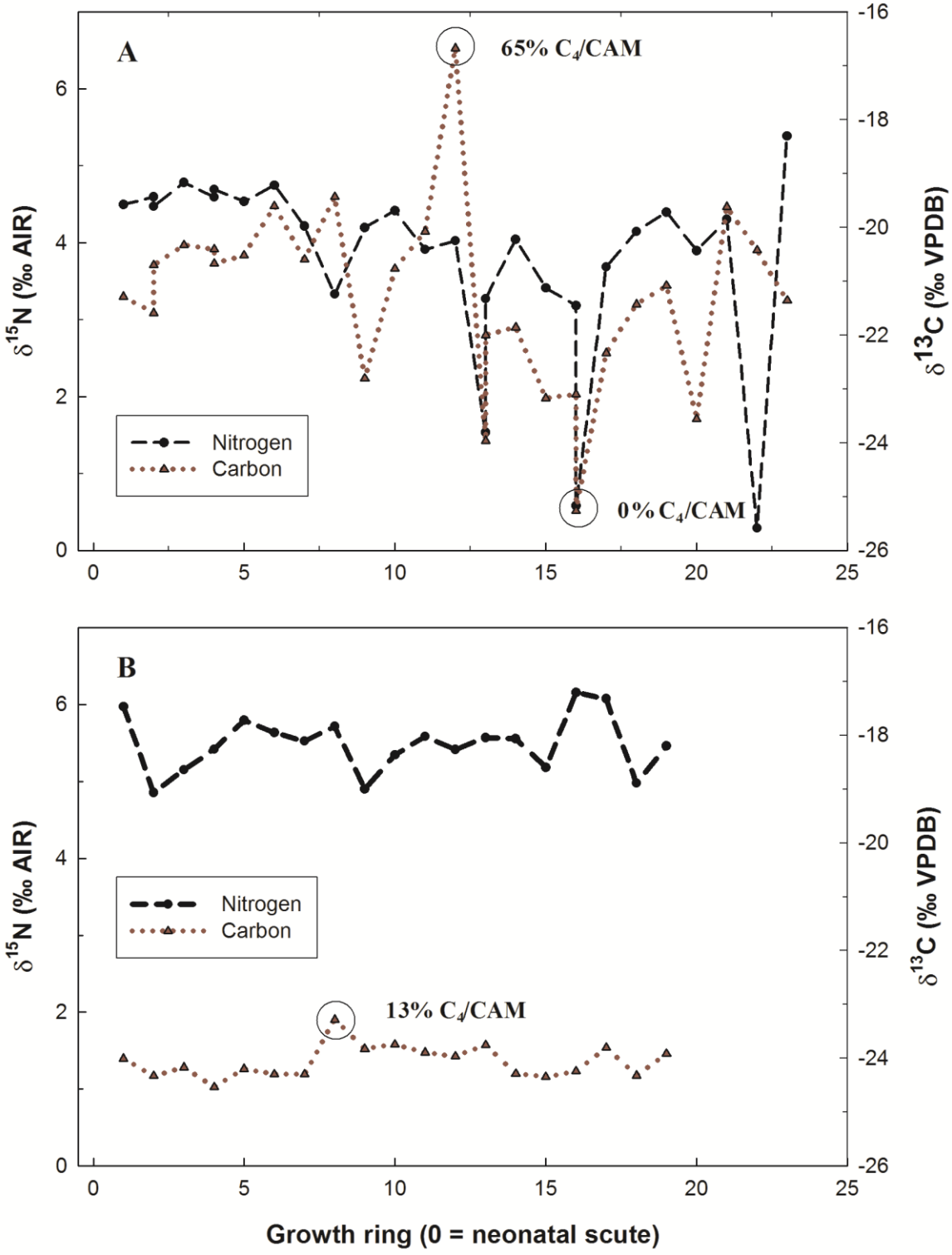
A
65% C4/CAM
0% C4/CAM
Nitrogen
Carbon
δ15N (‰ AIR)
δ13C (‰ VPDB)
B
13% C4/CAM
Nitrogen
Carbon
δ15N (‰ AIR)
δ13C (‰ VPDB)
Growth ring (0 = neonatal scute)

Figure 3.6. The $\delta^{13}C$ and $\delta^{15}N$ ratios from a complete sequence of growth rings from an individual tortoise with large, episodic shifts between incorporating C_3 and C_4/CAM plant resources (A), and an individual tortoise showing a constant and high reliance on C_3 plants across ontogeny (B). Tortoises grow by the successive additions of concentric rings, thus ring 1 would be the ring grown post-hatching, and rings added later in life are numbered consecutively.

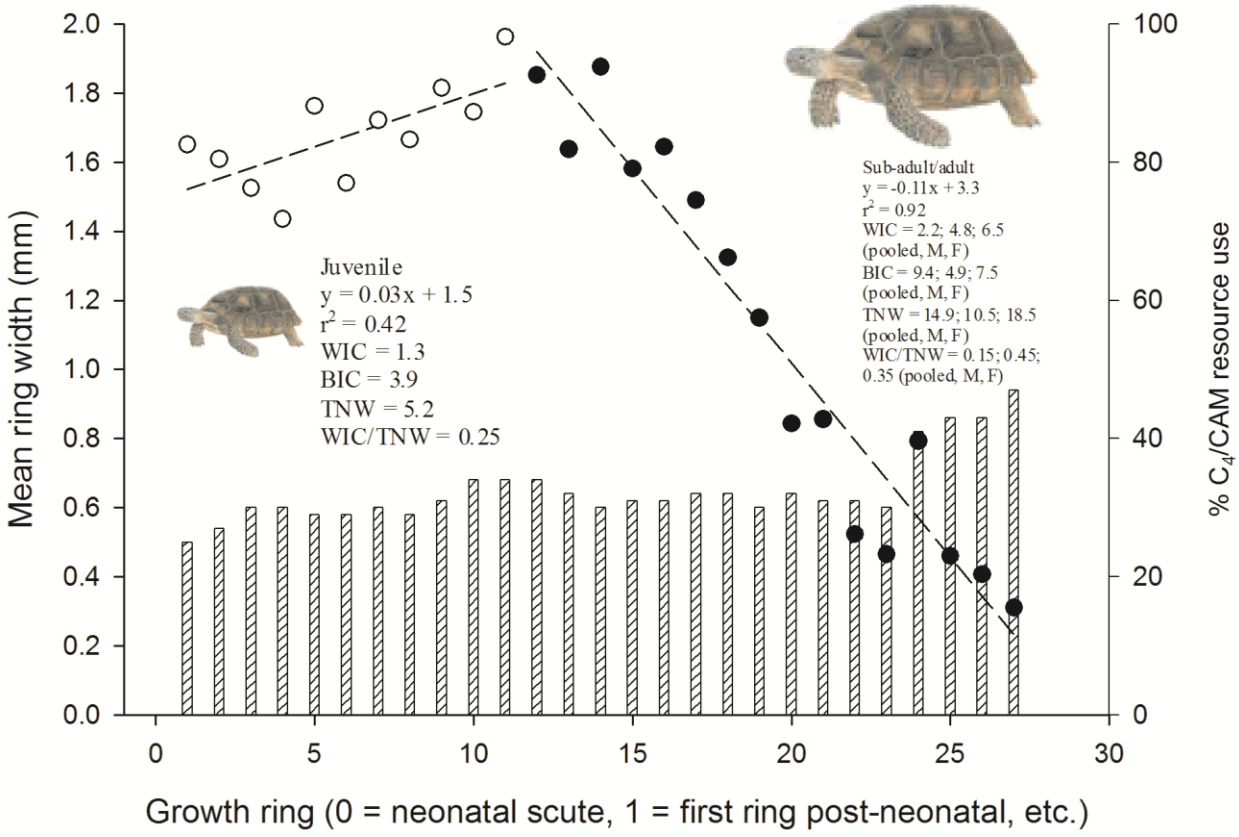

Figure 3.7. Pooled desert tortoise growth ring widths comparing dietary niche metrics on either side of a natural inflection point in the growth patterns between juvenile and subadult/adult animals. Tortoise niche metrics reported for rings 1 – 11, and rings 12 + on all animals, as well as the mean overall use of C_4/CAM plant resources by ring across all populations. Dietary breadth metrics were similar for rings 1 – 11 for both males and females, so the pooled value is reported. Dietary breadth metrics were different for males and females post ring 11, so all values are reported for the sexes combined, males, and then females (i.e., pooled, M, F).

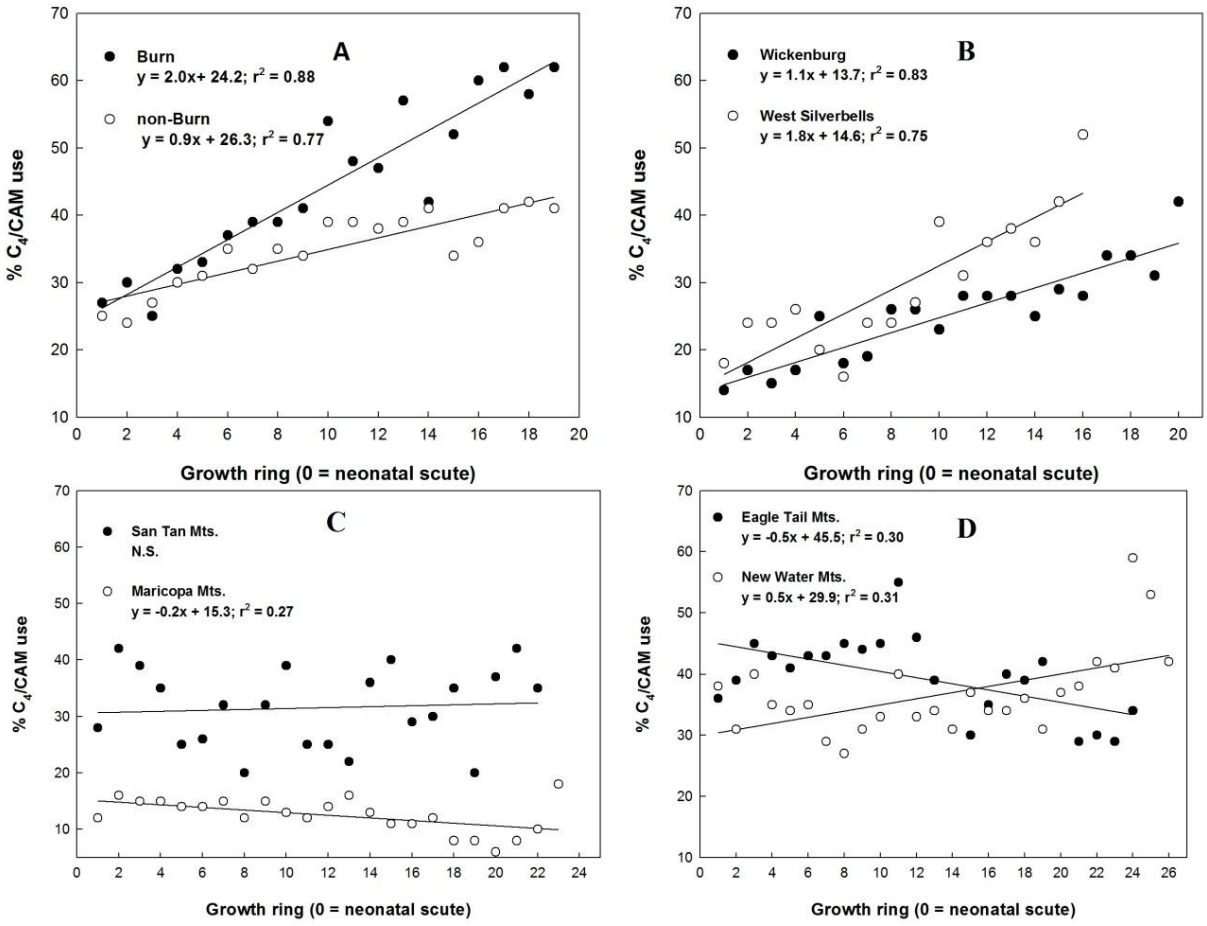

Figure 3.8. The mean utilization of C_4/CAM plant resources across desert tortoise ontogeny (as tortoises grow successive growth rings) for four mesic (A,B) and four xeric (C,D) Sonoran desert sites.

Appendix 1

Sonoran desert plant tissue carbon and nitrogen stable isotope ratios

	^{15}N	^{13}C
Cacti/CAM		
Opuntia englemanni-pads	3.9	-12.1
Cylindropuntia fulgida-flowers	4.1	-12.2
Ferocactus wislizenii-flowers	2.5	-12.5
Cylindropuntia fulgida-joints	4.8	-12.7
Opuntia englemanni-flowers	2.9	-12.7
Opuntia englemanni-fruits	2.9	-13.0
C_4 forbs		
Portulaca oleracea-plant	3.7	-12.4
Chamaesyce hyssopifolia-plant	3.2	-12.5
Portulaca suffrutescens-plant	4.4	-12.6
Chamaesyce albomarginata-plant	5.6	-13.5
Tidestromia lanuginosa-plant	3.0	-13.4
Pectis papposa-flowers	1.0	-13.6
Chamaesyce florida-plant	1.5	-13.7
Allionia incarnata -leaves	4.6	-13.8
Boerhavia coulteri-plant	0.0	-13.9
Pectis papposa-leaves	2.9	-15.0
C_4 grasses (leaf blades)		
Panicum hirticaule	5.2	-12.6
Digitaria californica	3.7	-13.0
Setaria macrostachnya	4.9	-13.0
Eragrostis lehmanniana	2.2	-13.3
Aristida adscensionis	1.7	-13.7
Aristida ternipes	1.1	-14.1
Aristida purpurea	0.2	-14.1
Bouteloua radicosa	4.2	-14.3
Bouteluoa aristoides	4.2	-14.3
Bouteluoa rothrockii	2.1	-14.3
Muhlenbergia porteri	2.8	-14.3
Dasyochloa pulchella	0.5	-14.5
Bouteluoa barbata	1.9	-14.7
Pleuraphis rigida	2.0	-14.7
Tridens mutica	0.3	-14.8
C_3 forbs		
Chorizanthe brevicornu-plant	0.0	-22.5
Camissonia californicus-plant	2.1	-23.4

Calycoseris wrightii-flowers	0.7	-24.3
Lesquerella gordonii-flowers	6.7	-25.7
Lupinus arizonicus-flowers	0.0	-25.7
Sonchus oleraceus-leaves	1.8	-25.8
Eucrypta micrantha-plant	2.8	-26.0
Thymophylla pentachaeta-flowers	2.9	-26.0
Bahia absinthifolia-flowers	2.2	-26.0
Erodium cicutarium-flowers	2.9	-26.1
Acourtia nana-flowers	2.6	-26.2
Phacelia distans-flowers	2.3	-26.5
Calycoseris wrightii-plant	0.2	-26.6
Daucus pusillus-flower/seed	0.0	-26.7
Lesquerella gordonii-plant	4.7	-26.8
Plantago ovatum-flowers	2.5	-26.8
Machaeranthera tagetina-flowers	1.2	-26.8
Lyrocarpa coulteri-flowers	6.1	-26.8
Thysanocarpus curvipes-flower/pod	6.1	-26.9
Astragalus nuttallianus- plant	1.3	-26.9
Pectocarya recurvata- plant	3.3	-27.0
Rafinesquia neomexicana-flowers	1.5	-27.3
Cirsium sp.-leaves	0.8	-27.4
Bahia absinthifolia-leaves	3.8	-27.4
Lotus strigosus-plant	0.0	-27.5
Eriogonum deflexum-plant	4.1	-27.5
Daucus pusillus-plant	0.0	-27.5
Cheilanthes parryi-plant	0.0	-27.5
Siphonoglossa longiflora- plant	3.6	-27.6
Eschscholzia californica-flowers	6.0	-27.6
Zinnia acerosa-flowers	2.6	-27.7
Cryptantha angustifolia-flowers	4.7	-27.8
Machaeranthera tagetina-leaves	1.6	-28.0
Castilleja exserta-flowers	1.1	-28.1
Chaenactis fremontii-flowers	2.9	-28.2
Phacelia distans-plant	2.4	-28.3
Plantago ovatum-plant	3.5	-28.3
Acourtia nana-leaves	1.1	-28.3
Gilia stellata-flowers	0.0	-28.5
Rafinesquia neomexicana-plant	0.9	-28.5
Zinnia acerosa-leaves	2.8	-28.5
Eriophyllum lanosum-flowers	0.4	-28.6
Lupinus arizonicus-leaves	0.0	-28.7
Thymophylla pentachaeta-leaves	3.2	-28.7
Cryptantha angustifolia-plant	4.5	-28.7
Lyrocarpa coulteri-leaves	2.1	-28.8
Castilleja exserta-plant	0.6	-28.9
Chaenactis fremontii-plant	2.5	-29.0

Eriophyllum lanosum-plant	0.0	-29.1
Thysanocarpus curvipes-leaves	5.7	-29.1
Lupinus sparsiflorus-plant	0.0	-29.2
Lupinus sparsiflorus-flowers	0.0	-29.4
Erodium cicutarium-plant	2.9	-29.4
Eschscholzia californica-leaves	5.9	-29.6
Gilia scopulorum-plant/flowers	1.0	-29.6
Mollugo verticillata- plant	7.8	-30.1
Gilia stellata-leaves	0.7	-30.1
Bowlesia incana- plant	0.1	-32.3
C_3 shrubby plants		
Fouqueria splendens-plant	3.1	-22.1
Encelia farinosa-flowers	3.6	-24.2
Ditaxis neomexicana-flowers	5.4	-24.4
Cercidium microphyllum-plant	5.3	-24.5
Prosopsis velutina- plant	1.8	-24.6
Olneya tesota-plant	5.6	-24.6
Sida abutifolia-flowers	3.0	-24.8
Ambrosia dumosa-plant	4.8	-24.9
Celtis pallida-plant	9.6	-25.0
Janusia gracilis-flowers	1.9	-25.1
Encelia farinosa-leaves	1.2	-25.2
Larrea tridentata-leaves	3.6	-25.2
Eriogonum fasciculatum-flowers	3.9	-25.4
Jatropha cardiophylla-plant	4.1	-25.4
Krameria grayi-plant	2.9	-25.7
Eriogonum fasciculatum-leaves	3.2	-25.9
Simmondsia chinensis-plant	4.3	-26.1
Ditaxis neomexicana-leaves	3.0	-26.4
Aloysia wrightii-leaves	3.9	-26.5
Sphaeralcea ambigua-flowers	4.1	-26.5
Aloysia wrightii-flowers	3.5	-26.5
Calliandra eriophylla-leaves	2.0	-26.9
Acacia greggii-plant	6.5	-27.0
Sida abutifolia-leaves	2.9	-27.0
Commicarpus scandens- plant/flower	8.4	-27.1
Hibiscus coulteri-plant	4.0	-27.2
Janusia gracilis-leaves	2.4	-27.4
Sphaeralcea ambigua-leaves	3.1	-27.4
Ambrosia deltoidea-flowers	6.3	-27.5
Ambrosia deltoidea-leaves	5.0	-27.7
Lotus rigidus-leaves	0.6	-28.4
Abutilon incanum-leaves	3.3	-29.0

Summary

In this work I have detailed how the daily activity and nutritional ecology of two species of animal changes across gradients of temperature and precipitation. Animal ecology is not a static affair, but a plastic process shaped by a changing environment. I documented how desert woodrats respond to warmer temperatures by limiting their time spent outside of their dens, and that male woodrats are significantly more active relative to females. Additionally, large male woodrats are less active than small male woodrats (Chapter 1). By using the carbon isotope ratios preserved in growth ring series on the carapace, I estimated the individual dietary specialization in desert tortoises living along a precipitation gradient in the Sonoran Desert of Arizona. Desert tortoises living in areas with the least and most unpredictable rainfall, and consequently the lowest estimates of annual net primary productivity (ANPP), have the most generalized feeding strategies relative to tortoises in moister regions with higher vegetative productivity. This relationship is only seen in larger, sub-adult to adult tortoises. During the juvenile life stage desert tortoises living across this same gradient show equally specialized feeding strategies (Chapter 3). Laboratory validation studies play a critical role in establishing the framework for interpreting consumer diet using tissue stable isotope ratios. In this work I documented the carbon retention times in several tissues and the associated diet-to-tissue discrimination in desert tortoises in the laboratory undergoing a diet switch experiment (Chapter 2). Here, I have explored just some of the ways that animals are able to adjust and respond to a variable environment.