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**Rodent Responses To Drought In The Southwestern
United States: Resource Utilization Strategies
And Effects To Keystone Resources**

by

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B.A., Biology, Grinnell College, 1988
M.S., Wildlife and Fisheries Science, University of Arizona, 1992

DISSERTATION

Submitted in Partial Fulfillment of the
Requirements for the Degree of

**Doctor of Philosophy
Biology**

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RODENT RESPONSES TO DROUGHT IN THE SOUTHWESTERN UNITED STATES: RESOURCE UTILIZATION STRATEGIES AND EFFECTS TO KEYSTONE RESOURCES

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ABSTRACT

The role of abiotic and biotic factors in regulating community and population dynamics is a central question of ecological inquiry. In arid environments, the degree of abiotic versus biotic regulation varies, based on gradients of environmental stressors and associated biological responses. Plant-herbivore interactions help shape these communities through foraging effects and actions of consumers. In this dissertation I investigated consumer-producer interactions during resource scarcity, and their implications for plants and herbivores.

The North American monsoon supplies vital pulses of moisture to the southwestern United States, including Sonoran desert, arid grassland, and montane communities. Gunnison's prairie dog (*Cynomys gunnisoni*) is a primary consumer inhabiting a diversity of the region's grasslands. I evaluated abiotic limitation in arid grassland and montane populations of *C. gunnisoni* during a multi-year drought using

stable isotope analysis to quantify foraging niche widths. Niche widths declined under periods of drought stress. Prairie dogs at the montane site exhibited seasonal shifts in dietary niche width during favorable growth periods for more nutritious plants using the C_3 photosynthetic pathway. Production of offspring was positively correlated with C_3 plant use. Investigation of body condition as a mechanistic link between C_3 forage use and reproductive output revealed no differences in body condition between sites. Body condition improved after emergence from hibernation, except in montane females, who exhibited evidence of early-season reproductive investment. Despite similar body condition and initial population densities, montane *C. gunnisoni* reached densities up to 20x those of the prairie site. The link between plant nutritional quality and demographic parameters suggests bottom-up regulation within this reportedly disease-limited species.

In the Sonoran Desert, white-throated woodrats (*Neotoma albigula*) supplement their herbaceous diets with succulents such as the saguaro cactus (*Carnegiea gigantea*). The massive saguaros store water in their tissues for annual production of flowers, fruits, and stem growth that feeds desert consumers during drought periods. Saguaros with high levels of herbivory (>20% of the surface) produced fewer flowers and fruits than similar plants with no herbivory. These findings suggest that periodic use of saguaros by *N. albigula*, such as during extended droughts, can reduce long-term reproductive capacities of this keystone desert resource.

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INTRODUCTION

Plant-herbivore interactions play vital roles in shaping communities and ecosystems. Herbivory and its associated plant responses influence the composition, diversity, productivity, heterogeneity, competitive advantages, and energy flow within biological communities (Ehrlich and Raven 1964, Brown and Heske 1990, Ritchie et al. 1998, Agrawal 1999, Rausher 2001, Loranger et al. 2012). Mammals make up the largest-bodied and most conspicuous class of herbivores (Huntly 1991), and each of these consumers must obtain energy to meet lifetime metabolic needs from primary productivity within a single home range. Effects of herbivory are further exacerbated in social or herd-forming mammals whose clumped distributions are influenced by conspecifics (Coppock et al. 1983, McNaughton 1984, Fryxell 1991, Steuter et al. 1995).

Mammalian herbivores influence biological communities by constructing or modifying physical components of their environments (Jones et al. 1994). Mammals may function as keystone species with disproportionately large effects on their communities through actions as ecosystem engineers, prey for higher-level consumers, or apex predators (Mills et al. 1993, Power et al. 1996, Sinclair 2003, Soule et al. 2005). Prairie dogs (*Cynomys spp*.) and their expansive burrow systems are prime examples of ecosystem engineers, and have been documented to support life history needs of 117 vertebrate species (Kotliar et al. 1999). Through engineering or foraging, mammalian herbivores influence biological communities by direct and cascading effects that transform ecosystem structure, composition, and function (Gutierrez et al. 1997, Olff and Ritchie 1998, Davidson et al. 2012).

In addition to top-down effects on the ecosystem, mammalian herbivore communities are influenced by bottom-up forces (Meserve et al. 2001). Temperature shifts and other abiotic perturbations alter community composition and population dynamics of temperate mammalian consumers (Humphries et al. 2004). Arid environments experience temporal heterogeneity of abiotic inputs that stimulate primary productivity and generate temporary surpluses of energy driving producer and consumer dynamics (Noy-Meir 1973). How consumers acquire, store, and allocate energy to survive periods of variable or unpredictable resource availability is a central question of ecological inquiry (Noy-Meir 1974, Hirshfield and Tinkle 1975, Wiens 1977, Lindström 1999, Liow et al. 2009, Bergeron et al. 2011).

The implications of drought periods on the foraging ecology a primary consumer, Gunnison's prairie dog (*C. gunnisoni*), are investigated in Chapter 1. *Cynomys gunnisoni* is an herbivore that feeds on leaves of grasses and forbs throughout its active season (Rayor 1985). Prairie dogs obtain the energy necessary to complete their life cycle by consuming plants within a shared territorial radius surrounding the underground burrow system that serves as the social, reproductive, and protective hub for each family group or coterie (Hoogland 1995). Without a specialized diet of foods such as seeds or fruits, *C. gunnisoni* must meet its metabolic needs by assimilating nutrients from quantities of relatively low-energy foods that are limited by *C. gunnisoni* gut capacity and body size (Demment and Van Soest 1985). Maintaining a threshold of consumed forage quality is therefore necessary to sustain these small generalist herbivores (Sinclair et al. 1982). The temperate grasslands inhabited by *C. gunnisoni* are dominated by herbaceous vegetation comprised of plants using both the C_3 and C_4

photosynthetic pathways. The two plant functional types contain structural differences that render the C_3 plants as having more nitrogen, less fiber, and being more digestible, leading to the hypothesis that C_3 plants are preferred energy resources for primary consumers based on these nutritional advantages (Caswell et al. 1973). Multiple studies have evaluated the C_3 hypothesis within laboratory environments and short-lived consumers (e.g., Heidorn and Joern 1984, Barbehenn et al. 2004). However, empirical determination of fitness benefits to longer-lived consumers from selective foraging on C_3 plants is more difficult to demonstrate. I evaluated evidence of selective herbivory and the C_3 hypothesis in two *C. gunnisoni* populations that occurred along a gradient of environmental conditions and C_3/C_4 plant productivity. Stable carbon and nitrogen isotopes ratios from *C. gunnisoni* body tissues represented dietary niches that were tracked over time at both an arid and more mesic study site. As drought conditions that inhibited growth of the more water-limited C_3 plants progressed over multiple years, grassland composition changed. I monitored trends in energy assimilation from C_3 and C_4 plants in *C. gunnisoni* tissues to determine consumer responses to changes in available forage quality, and quantified reproductive output as an index of fitness associated with different levels of C_3/C_4 plant utilization.

In Chapter 2, I explore mechanistic links that translate improved forage resource quality into greater reproductive success and higher population densities. Reproductive output is associated with parental body condition across a broad spectrum of consumers (Bonnet et al. 1998, Fokidis et al. 2007, Risch et al. 2007), and I predicted that groups of *C. gunnisoni* with access to higher-quality energy resources would exhibit improved body condition and greater abundances of juvenile prairie dogs. Under the stress of drought

conditions, adult *C. gunnisoni* must effectively allocate limited energy resources between reproductive costs and maintaining somatic body condition for survival and future reproduction (Williams 1966, Reznick 1985). A positive relationship between body condition and reproductive success indicates benefits to adult *C. gunnisoni* from energy reserves to support reproductive processes including ovulation, spermatogenesis, breeding, parturition, lactation, and other forms of parental investment. Conversely, reproduction may occur as a trade-off to the condition of parents. This strategy is adaptive when lifetime fitness is increased through energy allocation toward short-term reproductive success. However, perceived resource stress that projects risks to future survival and recruitment may dictate a more conservative energy allocation strategy of foregoing reproductive investment (Stephens and Krebs 1986). These resource allocation decisions thereby confer important implications not only for *C. gunnisoni* population dynamics, but for the full species assemblages inhabiting the prairie dog ecosystem.

Chapter 3 addresses an additional aspect of the interaction between mammalian herbivores and the plants that supply them with vital resources. In the Sonoran desert of North America, energy sources available during the hot and dry periods between pulses of monsoon-driven primary productivity are highly valuable to consumers. One such resource is the saguaro cactus (*Carnegiea gigantea*), which uses reduced evaporative leaf area, stem photosynthesis, and water storage within its massive tissues to support survival and annual reproductive cycles under extreme desert conditions (Steenbergh and Lowe 1977). Consequently, saguaro flowers, fruits, and tissues provide energy and resources for desert herbivores at times when few other energy sources may be available (Fleming and Valiente-Banuet 2002). When herbivores such as the white-throated woodrat

(*Neotoma albigula*) penetrate the outer stem tissue during foraging, the photosynthetic capacity of the columnar *C. gigantea* is permanently reduced through an epidermal wound response the plant generates to prevent further injury or infection (Steelink et al. 1967). We quantified herbivory on individual *C. gigantea* plants, and evaluated its effects on saguaro reproductive output. Because *C. gigantea* is a keystone resource for desert consumers and lives for hundreds of years, impacts from desert foragers may persist for decades following herbivory. This system demonstrates how changes in precipitation, climate, or other abiotic conditions alter relationships among primary consumers and their foraging resources, and create long-lasting impacts that permeate throughout biological communities.

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CHAPTER 1

Abiotic limitation and the C$_3$ hypothesis: isotopic evidence from Gunnison's prairie dog during persistent drought

Abstract

Gunnison's prairie dog (*Cynomys gunnisoni*) is a herbivore that ranges from desert grasslands to high-montane meadows, and is limited by disease across much of its range. The importance of abiotic drivers to the population dynamics of the species is poorly known. We employed stable isotope analysis to investigate energy assimilation patterns as indicators of abiotic limitation in arid grassland and montane populations of *C. gunnisoni* during a multi-year drought. Standard ellipse areas of plasma and red blood cell carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) isotope values, representing population-level dietary niche widths, declined during years and seasons of drought stress at both study sites. Prairie dogs at the montane site, but not the desert grassland site, maintained seasonal shifts in dietary niche width corresponding to periods of favorable growth conditions for the more nutritious C$_3$ plants. Production of offspring was strongly and positively correlated with C$_3$ resource use as indicated by $\delta^{13}C$ values in metabolically active tissues (plasma and red blood cells), but not with $\delta^{13}C$ values in adipose tissues used for long-term energy storage, or with foraging niche widths. These findings demonstrate that assimilation of energy from C$_3$ plants is associated with increased

reproductive output, and that drought conditions importantly constrain the resource base available to *C. gunnisoni*. The link between plant nutritional quality and demographic parameters highlights the role of abiotic regulation within this reportedly disease-limited species.

Key Words

abiotic regulation; drought; Gunnison's prairie dog; niche width; stable isotope analysis

Introduction

The roles of abiotic versus biotic factors in regulating community and population dynamics represents a line of inquiry that has led to an understanding of some of the most fundamental principles and processes in ecology (Grinnell 1917, Whittaker et al. 1973, Tilman et al. 1981, Dunson and Travis 1991, Jones et al. 1994, Sexton et al. 2009). The relative importance of bottom-up regulation represents one aspect of this query that has been debated for over a century (Forbes 1887, Hairston et al. 1960, Carpenter et al. 1985, Hunter and Price 1992, Power 1992, Bunnell et al. 2013). Within New World deserts and arid grasslands, the degree of bottom-up regulation of biological communities changes over time based on gradients of abiotically-driven environmental stressors and cascading relationships among dynamic consumer populations (Brown and Ernest 2002, Meserve et al. 2003). This phenomenon is illustrated in populations of Gunnison's prairie dog (*Cynomys gunnisoni*), a ground-dwelling herbivore of the family *Sciuridae* that lives in colonies of related individuals within grasslands ranging from arid prairies and high desert regions (Travis et al. 1995, Davidson et al. 2014) to mesic montane grasslands (Fitzgerald and Lechleitner 1974). Montane *C. gunnisoni* experienced catastrophic population declines following the introduction and spread of sylvatic plague (*Yersinia*

pestis) in North America (Lechleitner et al. 1968, Rayor 1985a, Cully et al. 1997).

Abiotic regulation of *C. gunnisoni* is less well documented but suggested for the more

arid grassland habitats of this species, where drier soils and lower primary productivity

have been associated with reduced numbers of sylvatic plague vectors, hosts, and disease

outbreaks (Parmenter et al. 1999).

A rangewide conservation assessment of *C. gunnisoni* concluded that only

montane populations of the species warranted additional management protections to

preclude disease-related extirpation (USFWS 2008). Prairie populations of *C. gunnisoni*

experience abiotically-driven declines during drought (Davidson et al. 2014) that provide

evidence for bottom-up regulation outside of the montane range. High growing-season

temperatures and reduced water availability during drought limit the growth of plants

utilizing the C_3 photosynthetic pathway (Ehleringer and Björkman 1977, Pearcy et al

1981), which are hypothesized to be preferred forage for primary consumers over less

nutritious C_4 plants (Caswell et al. 1973). The mechanistic basis for this C_3 hypothesis

arises from structural differences between leaf tissues from C_3 and C_4 plants, with C_4

plants having lower nitrogen content, more fiber and silica, lower digestibility, and

reduced macronutrient availability for herbivores compared to C_3 plants (Caswell et al.

1973, Landa and Rabinowitz 1983, Wilson et al. 1983, Scheirs et al. 2001). These

anatomical differences between C_3 and C_4 plant tissues have been investigated for

relationships to consumer selection and nutrient utilization in grasshopper diets (Heidorn

and Joern 1984, Barbehenn et al. 2004a); and to abundance, growth, and intake rates for

insects consuming C_3 and C_4 grasses (Boutton et al. 1978, Barbehenn and Bernays 1992,

Barbehenn et al. 2004c). Implications to body condition and population dynamics from

isotopically-observed diet shifts between C_3 and C_4 plants are inferred within larger and longer-lived consumers (Bearhop et al. 2004, Codron et al. 2006, Warne et al. 2010, Hahn et al. 2013, Seamster et al. 2014), but have rarely been evaluated through measurement of demographic parameters. In prairie dogs, heavier adult breeding-season body masses are associated with individuals exhibiting increased reproductive success (Hoogland 2001). Reduced availability of high-quality (e.g., C_3) forage within arid grassland habitats could thereby serve as a mechanism for explaining the consistently low recruitment of *C. gunnisoni* documented by Davidson et al. (2014) during periods of resource stress.

The maturing field of isotopic ecology has contributed novel approaches to understanding the role of biotic and abiotic factors in population regulation (LaPointe 1997, Marra et al. 1998). Resource use can be tracked using distinct carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) isotope ratios among food sources, and these isotopic ratios are incorporated into a consumer's tissues through its diet (DeNiro and Epstein 1978, DeNiro and Epstein 1981). Isotopic indicators of abiotically-limited systems include behavioral shifts in foraging toward intermittently-available resources, changes in dietary niche of consumers following pulses of abiotic inputs (Darimont and Reimchen 2002, Orr et al. 2015), and reduced dietary specialization of individual consumers in environments that lack consistent availability of preferred food sources (Darimont et al. 2009, Murray and Wolf 2013).

In this study, we investigate desert grassland and montane-dwelling populations of *C. gunnisoni* for evidence of abiotic versus biotic regulation by comparing isotopically-based indicators of resource assimilation and diet quality to observed

abundances of newly emerged offspring. We hypothesized that *C. gunnisoni* inhabiting a lower-elevation arid grassland (prairie) environment would exhibit resource utilization patterns indicative of abiotic limitation when compared to a population from the more mesic, disease-limited montane portion of the species' range. Specifically, we predicted that prairie populations of *C. gunnisoni* would show greater seasonal similarity in dietary niche width (i.e., range of tissue stable isotope values) than montane *C. gunnisoni*, which are able to shift to preferred food sources during more dependable annual pulses in primary productivity. We also predicted that the limited availability of distinct forage resources in prairie populations would result in a narrower dietary niche breadth than in populations of montane *C. gunnisoni*. Periods of drought and abiotic resource stress should similarly reduce availability of preferred resources and constrain population-level dietary niche breadth. Finally, we evaluated whether changes in dietary niche width and composition of assimilated energetic resources within body tissues were associated with offspring abundance in prairie and montane *C. gunnisoni*.

Methods

Study sites

We conducted research on *C. gunnisoni* colonies at Sevilleta National Wildlife Refuge, located 85 km south of Albuquerque, New Mexico, USA, and at Vermejo Park Ranch, situated ~300 km to the northeast between the towns of Raton and Cimarron, New Mexico. Sevilleta colonies were located at 1650 m elevation and considered to be prairie populations of *C. gunnisoni*. Prairie dogs at Sevilleta inhabited Chihuahuan Desert grasslands dominated by blue grama (*Bouteloua gracilis*) and other warm-season (C_4) grasses. Sevilleta receives an average of 25 cm of precipitation annually, with the

majority falling during the late summer monsoon period (Muldavin et al. 2008). Vermejo

prairie dogs were located at 2220 m, within the montane portion of the *C. gunnisoni*

range. Vermejo habitats consisted of long (30–40 km), canyon-bounded grasslands that

transitioned from short-grass prairie to montane meadows. A mixture of primarily C_3

forbs and other grasses complemented abundant *B. gracilis*, which was the most common

plant species present within the Vermejo grasslands. Annual precipitation at the Vermejo

colonies averages ~50 cm, and includes summer monsoonal thunderstorms that

contribute an estimated 29–42% of the total annual precipitation (Legler 2010). The same

summer storm systems associated with the North American monsoon therefore impact

both study sties, but monsoonal activity becomes less intense as it moves northward to

Vermejo from its sources of moisture in the Gulf of California and eastern Pacific Ocean

(Adams and Comrie 1997). Based on relationships between precipitation and net primary

productivity (NPP) developed by Sala et al. (1988), Vermejo experiences a projected 2.2x

increase in NPP compared to Sevilleta during an average year.

Weather measurements

We obtained precipitation data for the Sevilleta colonies from the Sevilleta Long-

Term Ecological Research (LTER) project's Deep Well weather station

(http://sev.lternet.edu, dataset SEV001), and from the National Oceanic and Atmospheric

Administration station Cimarron 4 SW, located 30 km southwest of the Vermejo site.

Precipitation seasons were summer (June-August) and non-monsoonal months

(September–May). We compared seasonal precipitation totals to means at each site from

the 20-year (1990–2009) period prior to study initiation, which represented the time

frame when detailed meteorological data were available from the Deep Well station.

Vegetation sampling

We monitored available vegetation 2x/year at three 1-ha plots per study area during pre- and post-monsoon periods. Sevilleta LTER monitoring provided vegetation data (http://sev.lternet.edu, dataset SEV129) from plots established at Sevilleta prairie dog colonies, and we established equivalent randomly-located plots at Vermejo colonies. At each plot, we determined total vegetative cover by species (Daubenmire 1959) and by functional group (C_3 versus C_4/CAM photosynthetic pathways) for all plants present within 0.25 m^2 vegetation sampling frames. We measured total standing biomass by clipping vegetation samples from a 24% subsection of the sampling frame at 6 of the 12 cover measurement points. Biomass sampling locations were directly adjacent to the vegetative cover plots, and rotated directionally to prevent clipping on any given quadrat during consecutive sampling seasons. We placed clipped vegetation samples in paper bags and weighed them on site using a portable electronic balance with a precision (readability) of 0.1 g SD (Scout Pro, Ohaus Corporation, Parsippany, New Jersey, USA).

To quantify the range in stable carbon and nitrogen isotope values within plants, we collected common plants from each study site over a range of moisture conditions. Collections began during the pre-monsoon period of 2011, and extended through the 2013 post-monsoon period to capture variation among years of poor and strong monsoon influences on moisture availability. All plants collected were identified to species and photosynthetic pathway (C_3 or C_4) to assess isotopic variation within and between plant functional groups.

Prairie dog sampling

We captured prairie dogs from 2010–2012 at four trapping plots (~1 ha each) within a complex of adjacent colonies that overlapped vegetation plots in each study area (Davidson et al. 2014). Capture and sampling methods followed all applicable institutional and national guidelines for care and use of animals, including protocol 10-100465-MCC approved by the University of New Mexico's Institutional Animal Care and Use Committee. High trap success rates dictated use of a reduced numbers of traps (30/plot) at Vermejo to prevent excessive above-ground exposure of captured prairie dogs. Capture periods occurred during the spring (post-emergence and pregnancy; April), early summer (pre-monsoon; June), and late summer (following monsoon initiation; August–early September) seasons. For all captured prairie dogs, we recorded age class (juvenile or adult) and weight to ± 0.1 g using a portable electronic balance. We collected blood samples (~50 µl) for isotopic analysis from adult and late-summer juvenile *C. gunnisoni* by clipping the distal end of the toenail on the lateral hind digit (Hoogland 1995), which provided blood flow directly into capillary tubes for 60–90 seconds before coagulation occurred. We sampled adipose tissue non-destructively (Baker et al. 2004) using 16 ga, 6–9 cm standard bevel-tip biopsy needles (Products Group International, Inc., Lyons, Colorado, USA) inserted under the skin and into fat stores deposited on top of the lower dorsal musculature. Prior to release of each *C. gunnisoni*, we uniquely marked captured animals with hair dye for within-season identification of individuals during counts and trapping efforts, and with ear tags and passive integrated transponder (PIT) tags to identify individuals that were recaptured in different seasons (Schooley et al. 1993, Hoogland 1995).

We estimated the number of offspring produced at each study site through counts obtained from direct observations of prairie dogs at trapping plots (Facka et al. 2008) during June, soon after *C. gunnisoni* juveniles first emerge from their burrows and become surface active (Hoogland 1999). We observed *C. gunnisoni* from a portable blind located adjacent to the plot at an elevated vantage point, or from a constructed platform in flat terrain (Facka et al. 2008). Observers arrived at count sites before sunrise and prior to the emergence of *C. gunnisoni* from their burrows, and monitored the colony for ~3 hours. Observation periods included systematic scans of the entire plot conducted every 30 minutes, beginning and ending from marked points at the edge of the plots. We observed each prairie dog plot 1–2 times during June, and completed annual counts of all plots at a study site within a period of ≤5 days. From observations, we determined maximum above-ground counts of adult and juvenile *C. gunnisoni*, and calculated the proportion of juveniles present within each count.

Laboratory procedures

We separated whole blood into plasma and red blood cells following procedures from Warne et al. (2010). Removal of foreign or cloudy materials (e.g., lipids) from blood occurred by sectioning capillary tubes as necessary to remove impurities, or to prevent mixing from any lysis of blood cells, prior to loading samples into tin capsules for isotope processing. We washed adipose tissue samples (0.6–0.8 mg) in distilled water and ethanol, and examined them under a hand lens to ensure that no connective or other tissue was attached to the sample. Plant samples were dried in an oven at 60 C for 24 hours, and ground into 1.0–1.5 mg samples of homogenized tissues from individual plants before loading into tins for isotopic analysis.

We measured carbon and nitrogen isotope ratios through continuous flow isotope ratio mass spectrometry at the University of New Mexico Center for Stable Isotopes (UNM-CSI), using a Costech ECS 4010 elemental analyzer coupled to a Thermo Finnigan Delta Plus mass spectrometer via a ConFlo II interface. Isotope ratios are reported in standard delta (δ) notation in parts per thousand (‰) relative to isotopic standards (Vienna Pee Dee Belemnite [VPDB] for carbon, atmospheric air for nitrogen), as:

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

where R represents the ratio of heavy to light isotopes ($^{13}C/^{12}C$ or $^{15}N/^{14}N$). Average analytical precision based on routine analysis of laboratory standards at UNM-CSI was ≤ 0.1 ‰ SD. Laboratory standards were calibrated against NBS 21, NBS 22 and USGS 24 for $\delta^{13}C$. We considered extreme $\delta^{13}C$ or $\delta^{15}N$ values >3 interquartile ranges from the first or third quartiles to represent outliers (Tukey 1977) that resulted from processing errors, and removed those values from further analysis.

Statistical analyses

We compared the proportion of C_4 plant cover and the mass of clipped vegetation among seasons and years using a mixed effects linear model (Zuur et al. 2007). The mixed effects model tested for differences at each site using plot number as a fixed effect to account for repeated measures of vegetation plots over time. We used the logit transformation (= log [y_{C4} / (1− y_{C4})], where y_{C4} = proportion of C_4 plants) on all vegetative cover proportion data (Warton and Hui 2011), and the square root transformation on all plant biomass data, in order to meet assumptions for linear

modeling. Bonferroni procedures controlled experimentwise error rates for post-hoc multiple comparisons among time periods.

We analyzed and compared plant $\delta^{13}C$ values from arid (pre-monsoon periods and the 2011 monsoon failure year) and more mesic periods to quantify temporal variation in plant $\delta^{13}C$ values. Carbon:nitrogen (C:N) ratios in plant tissues were calculated from quantities of combusted carbon and nitrogen, calibrated to a laboratory standard value. We used *t*-tests to evaluate differences in $\delta^{13}C$ values between dry and wet periods for each photosynthetic pathway, and for differences in C:N ratios between C_3 and C_4 plants as an index of nutritional quality.

To estimate dietary niche breadth, we used the Stable Isotope Analysis in R package (SIAR) to calculate and compare Stable Isotope Bayesian Ellipses in R (SIBER) metrics (Jackson et al. 2011) among seasons and years. Standard ellipse areas (SEAs) from $\delta^{13}C$ and $\delta^{15}N$ bivariate plots represented foraging niche widths based upon the range of C_3 (lower $\delta^{13}C$ values) versus C_4 (higher $\delta^{13}C$ values) plant carbon assimilated, and the variation in $\delta^{15}N$ in plants associated with differences in nitrogen fixation, moisture stress, plant parts, or other plant physiological traits (Dawson et al. 2002). Calculated SEAs for different tissues provided niche width metrics from distinct time frames, based upon projected continuous turnover rates for plasma (half life = 3–4 days) and red blood cells (half life = 28–30 days) (Hobson and Clark 1993, Hilderbrand et al. 1996). We tested for population-level differences in foraging niche width among seasons (spring, pre-monsoon, post-monsoon) and years, and between consecutive seasons, by generating Bayesian estimates of plasma and blood isotope SEAs using 10,000 posterior draws. Two-tailed probabilities of differences in SEAs between groups were calculated as

2x the proportion of Bayesian ellipse sizes for one group (time period) that were smaller than SEAs for its comparison group. We considered differences to be significant at $P <$ 0.05 if SEAs were larger for one group in >97.5% or <2.5% of the draws relative to the comparison group.

To evaluate the assimilation of preferred resources as energetic capital reserves, we analyzed $\delta^{13}C$ ratios in adipose tissue. Prairie dogs do not turn over these fatty tissue deposits at fixed intervals, but may retain lipid stores as long-term energy storage for hibernation or periods of energetic deficit (Thompson et al. 1993, Lehmer and Van Horne 2001). We tested for changes in adipose tissue $\delta^{13}C$ values among seasons and years using Kruskal-Wallis tests to account for heterogeneous variances among $\delta^{13}C$ values from different sampling periods. Where overall differences among time periods existed, we evaluated pairwise comparisons of seasonal and yearly mean $\delta^{13}C$ values using Dunn's test (Dunn 1964). We tested for relationships between early-summer abundance of juvenile *C. gunnisoni* at each study site and dietary niche width from plasma and blood samples, yearly $\delta^{13}C$ values in all tissues, and total standing plant biomass from off-colony vegetation plots, using Pearson product-moment correlation analysis. We eliminated post-monsoon period plasma samples from these yearly $\delta^{13}C$ estimates because plasma has rapid turnover, and during late summer reflects foods assimilated after the June counts of *C. gunnisoni* adults and juveniles.

Results

Precipitation

Annual precipitation was below average at both sites during all three years of the study (Fig. 1). Seasonal precipitation at Sevilleta was below the long-term average for

every season during the study period (Fig. 2), including a near complete failure of summer monsoons during 2011, when precipitation was 80% less than the long-term seasonal mean. Vermejo experienced five consecutive seasons of below-average precipitation beginning post-monsoon 2010. Precipitation during all monsoonal periods at Vermejo was at least 28% less than the long-term average, including two consecutive seasons in 2011 with precipitation inputs that were at least 45% less than the long-term average.

Vegetation

Vegetative cover at Sevilleta was dominated by perennial C_4 grasses, with C_4 plants comprising >80% of total vegetative cover during the entire study period. *Bouteloua gracilis* was the most abundant of these grasses, averaging 55% of the total vegetative cover from plots at Sevilleta. No consistent increase in C_4 plant cover was observed following post-monsoon periods (Fig. 2). There were no significant differences in proportion of C_4 cover by season at Sevilleta ($P = 0.44$), but differences among years were significant ($P < 0.001$), with proportion of C_4 cover increasing in 2011 compared to 2010. Total standing biomass of vegetation from clipped samples showed similar patterns, with no significant seasonal patterns ($P = 0.41$), but a significant year effect ($P < 0.001$). Standing biomass was greater in 2010 than in 2011 and 2012.

Bouteloua gracilis was also the most abundant plant species at Vermejo, averaging 59% of vegetative cover for the duration of the study period. However, C_4 vegetation at Vermejo initially comprised <50% of the total cover when the study began in 2010. By the 2011 pre-monsoon season, C_4 plants at Vermejo represented >80% of total vegetative cover, and remained at or near that level for the duration of the study.

Vermejo experienced significant seasonal ($P = 0.001$) and year ($P < 0.001$) effects on the proportion of C_4 vegetation. Proportional C_4 plant cover increased during the post-monsoon season, and was greater in 2011 and 2012 than 2010. Standing biomass of sampled vegetation also showed significant differences both by season ($P = 0.018$) and year ($P < 0.001$). Standing biomass was greater post-monsoon than pre-monsoon, and exhibited an annual pattern of 2010 > 2012 > 2011.

Plants with C_3 carbon fixation had $\delta^{13}C$ values (mean ± SD) of -26.7 ± 1.5‰, and $\delta^{13}C$ for C_4 plants was -13.8 ± 1.0‰ (Appendix S1: Table S1). Plant $\delta^{13}C$ values differed significantly between dry and wet periods for C_3 plants ($t = 2.33$, $P = 0.03$), but not for C_4 plants ($t = -1.36$, $P = 0.19$). Tissues from C_3 plants had significantly different C:N ratios from C_4 plants ($t = -2.52$, $P = 0.02$).

Prairie dog sampling and tissue stable isotopes

We collected tissue samples from 319 captures of *C. gunnisoni* at Sevilleta (n = 77) and Vermejo (n = 242), representing 214 distinct individuals and 105 between-season recaptures. Standard ellipse areas of $\delta^{13}C$ and $\delta^{15}N$ in plasma (Fig. 3) and red blood cells (Fig. 4) from Sevilleta *C. gunnisoni* showed no significant differences in comparisons between seasons. At Vermejo, seasonal SEAs of plasma isotopes were smallest during the pre-monsoon season and significantly different from both spring ($P = 0.008$) and after monsoon initiation ($P = 0.01$). Standard ellipse areas from red blood cell isotopes at Vermejo showed a pattern that was shifted one season later from plasma isotopes. Seasonal SEAs were largest pre-monsoon and smallest during the post-monsoon season, although no pairwise differences between individual seasons were significant. Yearly SEAs of plasma isotopes at Sevilleta peaked during 2010, and were significantly different

from both 2011 ($P = 0.006$) and 2012 ($P = 0.012$). Plasma isotope SEAs at Vermejo were also highest in 2010, and differed significantly between 2011 ($P = 0.024$) and 2012 ($P = 0.002$). Red blood cell isotope SEAs showed no significant differences between years at Sevilleta, but at Vermejo were greatest in 2010 and significantly different from 2011 ($P < 0.001$) and 2012 ($P = 0.018$). Plasma isotope SEAs were larger at Vermejo than Sevilleta in all seasons and years, and red blood cell isotope SEAs were larger at Vermejo in all years but failed to show the same pattern among seasons.

Both plasma and blood isotope SEAs exhibited no significant differences in pairwise comparisons between consecutive seasons at Sevilleta (Table 1). However, plasma SEAs at Vermejo differed between pre-monsoon and post-monsoon seasons in 2010 ($P = 0.049$), monsoon season 2010 and post-emergence (spring) 2011 ($P = 0.003$), post-monsoon season 2011 and spring 2012 ($P < 0.001$), and pre-monsoon and post-monsoon seasons in 2012 ($P = 0.040$). Blood isotope SEAs from Vermejo showed less variation between consecutive seasons, with the only significant differences between the 2011 post-monsoon and 2012 spring emergence periods ($P = 0.011$).

Samples of adipose tissue had significantly different $\delta^{13}C$ values at both sites by year ($P < 0.001$ Sevilleta, $P < 0.001$ Vermejo), and by season ($P = 0.03$) at Sevilleta (Table 2). Seasonal $\delta^{13}C$ values from Sevilleta adipose tissue were greater (more similar to C_4 plant tissues) during the post-monsoon than the pre-monsoon season. Adipose tissue $\delta^{13}C$ values were greater in 2012 compared to 2010 and 2011 at Sevilleta, and followed the chronological sequence of $2012 > 2011 > 2010$ at Vermejo.

Production of offspring

Juvenile *C. gunnisoni* comprised 8–25% of the total number of individuals observed at Sevilleta during June 2010–2012, and 55–68% of animals counted at Vermejo. The proportion of juveniles in the population was more strongly correlated with yearly $\delta^{13}C$ values than with SEAs from both plasma ($r_{\delta13C} = -0.744$, $r_{SEA} = 0.037$) and red blood cells ($r_{\delta13C} = -0.890$, $r_{SEA} = 0.397$) (Fig. 5). Correlation between the proportion of juveniles and yearly $\delta^{13}C$ values in adipose tissue ($r_{\delta13C} = 0.392$) was weaker than for the two metabolically active tissues with fixed turnover intervals. The lowest proportion of juveniles observed occurred during 2011 at both sites, when $\delta^{13}C$ values in *C. gunnisoni* plasma and red blood cells peaked at both Sevilleta and Vermejo. Correlation between proportion of juveniles observed and yearly mean standing vegetation biomass ($r_{mass} = 0.187$) was also weak.

Discussion

The extended drought conditions present during this study provided a unique opportunity to observe responses of consumers to periods of persistent resource stress. Over the course of our three-year study, only a single season of precipitation (pre-monsoon 2010) could have been classified as average (Fig. 1). On both study sites, all periods following the first summer exhibited a decline in available standing biomass as drought conditions persisted. Reduced moisture availability precluded any apparent growth pulses of the more nutritious C_3 photosynthetic group of forage plants (Fig. 2). Sevilleta vegetation showed little seasonal or annual variation in cover by plant photosynthetic group, with C_4 perennial grasses consistently comprising the majority of vegetative cover during all seasons and years. Vegetation at Vermejo shifted from an

initial majority of C_3 plant cover to a C_4-dominated composition in response to extended

drought. Unlike Sevilleta, Vermejo experienced a detectable seasonal increase in C_4

vegetation during the summer post-monsoon period, but still showed a pattern of

decreasing C_3 plant cover over the three years of the study. We hypothesized that periods

of reduced overall primary productivity and a decrease in more palatable, cool-season C_3

vegetation would create evidence of abiotic limitation within colonies of the strict

herbivore *C. gunnisoni*. We also predicted that these constraints on the availability of

forage resources would in turn result in narrower foraging niches, and shifts in energy

assimilation away from the more nutritious C_3 food sources. In the following discussion

we interpret *C. gunnisoni* foraging niche width data and composition of energy stores to

assess whether drought-related resource stress was associated with population-level

indicators of abiotic regulation, including differences in reproductive output.

Temporal changes in foraging niche

At Sevilleta, both the proportion of C_4 vegetative cover and *C. gunnisoni* foraging

niche width (as indicated by SEAs of tissue $\delta^{13}C$ and $\delta^{15}N$ values) failed to show

significant seasonal variation in any of the tissue types analyzed. Seasonal and yearly

foraging niche widths were smaller at the consistently C_4-dominated Sevilleta site than at

Vermejo (Fig. 3, 4), where more pronounced seasonal influences on vegetation

composition provided *C. gunnisoni* with increased opportunities for dietary shifts.

Differences in foraging niche widths between study sites were particularly pronounced in

plasma, which experiences rapid carbon turnover and confines plasma SEAs to the short-

term variation in δ-space of carbon and nitrogen from available plant tissues. Plasma

SEAs at Sevilleta were on average 33% smaller by season and 45% smaller by year than for corresponding time periods at Vermejo.

Variation in C_3/C_4 plant cover, total standing biomass, and *C. gunnisoni* foraging niche width at Sevilleta followed yearly patterns that appeared to reflect annual precipitation totals and the cumulative increase in severity of drought conditions. In contrast, Vermejo continued to show seasonal shifts in plant photosynthetic group cover, total biomass, and *C. gunnisoni* seasonal foraging niche width against a backdrop of decreasing C_3 plant cover that persisted over multiple years of drought. Vermejo *C. gunnisoni* translated this available seasonal variation in plant growth to expanded foraging niches during spring and post-monsoon periods (Table 2), which are typical times for growth pulses in C_3 and C_4 plants, respectively. These results support our hypotheses that prolonged droughts at arid locations (e.g., Sevilleta) limit the opportunity to consume nutritious C_3 resources, and thereby constrict the dietary niche widths of *C. gunnisoni* under environmentally stressful conditions.

We also considered whether the effects of water availability on plant water use efficiency and carbon discrimination in C_3 and C_4 plants could explain the observed trends in $\delta^{13}C$ of consumer tissues and their relationships to *C. gunnisoni* population dynamics (Fig. 5). Based on the $\delta^{13}C$ values for C_3 and C_4 plants in our study areas, the annual variation in rapid-turnover tissues (range = 3.5‰ plasma $\delta^{13}C$, Fig. 5) corresponded to a 27% shift in C_3/C_4 plant assimilation in *C. gunnisoni* tissues. While arid conditions may influence carbon isotope discrimination in C_3 plant tissues through demands for increased water use efficiency (Tieszen 1991, Ehleringer et al. 1992), discrimination factors in desert plant species are highly constrained within water-limited

systems, and δ^{13}C values exhibit a reduced range of variability (Wolf and Martínez del Río 2000, Codron et al. 2005, Symes et al. 2011, Orr et al. 2015). In contrast to C$_3$ plants, carbon isotope discrimination in C$_4$ plants may decrease under more mesic conditions, although the magnitude of such shifts is smaller (Bowman et al. 1989, Madhavan et al. 1991, Peisker and Henderson 1992). Because δ^{13}C values shift in opposite directions in C$_3$ and C$_4$ plants with increasing water stress, dietary mixing of C$_3$ and C$_4$ plants dampens effects from moisture-related variation in carbon isotope discrimination to observed δ^{13}C values in consumer tissues. In our study, the difference in δ^{13}C values between dry and moist conditions was −1.5‰ in C$_3$ plants, and +0.5‰ in C$_4$ plants (Appendix S1: Table S1), corresponding to an apparent diet shift in C$_3$/C$_4$ plant carbon assimilation of 4–12%. Yearly *C. gunnisoni* tissue δ^{13}C values shifted in a direction consistent with changes in C$_3$ plant δ^{13}C values under moisture limited conditions, with isotopic foraging niches extending toward the more positive δ^{13}C values during the driest year (2011) at both study sites (Fig. 3). Temporal changes in carbon isotope discrimination in plants could therefore contribute to observed shifts in δ^{13}C among years, and overestimate apparent diet shifts. However, seasonal foraging niches showed an opposite pattern, and exhibited a shift toward more positive δ^{13}C values under the site-by-season combination with the most mesic conditions (post-monsoon seasons at Vermejo) (Fig. 3, 4). Moisture-related variation in carbon isotope discrimination would thus serve to reduce the observed magnitude of shifts in consumer tissue δ^{13}C values, and underestimate seasonal diet changes. Our observed shifts in consumer isotopic foraging niches can therefore not be explained simply by temporal variation in plant δ^{13}C values, but indicate selective

foraging among plant groups (i.e., C_3 and C_4 photosynthetic pathways) with distinct $\delta^{13}C$ values when preferred forage resources are available.

Under food-limited conditions, individual foragers may be forced to utilize available resources, whether or not they represent preferred sources of energy assimilation (Araújo et al. 2008). Shifts in resource availability can lead to niche partitioning among specialized individuals adapted to different portions of the total population niche (Van Valen 1965), or to more generalized individual foraging that creates greater intrapopulation niche overlap when the total available niche width decreases (Roughgarden 1972, Murray and Wolf 2013). Our study involved population-level sampling and was not designed to assess changes in individual dietary specialization over time, but persistent drought produced decreases in population-level foraging niche widths that strongly indicate generalist foraging strategies with opportunistic utilization of moisture-limited forage resources.

Changes in both consumer resource use (selection) and resource availability have been identified as mechanisms associated with foraging niche width expansion. Niche width increases when optimal foragers selectively add intermittently-available preferred food sources to their diets, which can result in demographic benefits to consumer populations (Ostfeld and Keesing 2000, Yang et al. 2008). Alternatively, expanded foraging niches may reflect increased diversity of available food sources (Darimont et al. 2009, Jaeger et al. 2010), even in generalist foragers consuming food resources in proportion to availability. In our study, expansion of consumer foraging niches occurred when conditions favored increases in C_3 plant growth (Fig. 3, 4). The observed timing of niche width expansion signals an adaptive resource utilization strategy by *C. gunnisoni*

and supports the C_3 hypothesis, with primary consumers capitalizing on the nutritional advantages of C_3 over C_4 plants (Barbehenn et al. 2004a).

Foraging resources and reproductive output

As an obligate hibernator inhabiting montane grasslands, *C. gunnisoni* experiences pronounced temporal variability in energetic demands for meeting its life history requirements. The high metabolic costs associated with breeding, pregnancy, and lactation pose particular energetic challenges for *C. gunnisoni* during spring (Hoogland 2003). Increased adult body masses and condition prior to parturition are associated with larger litter sizes in prairie dogs and other sciurids (Hoogland 2001, Fokidis et al. 2007, Risch et al. 2007), and availability of food resources can thereby affect production of prairie dog offspring (Rayor 1985b). We found a strong positive correlation between the number of juveniles present and the quantity of C_3 carbon assimilated by adult *C. gunnisoni* to support our hypothesis relating C_3 diet quality to juvenile recruitment (Fig. 5). While other tests of the C_3 hypothesis have focused largely upon abundances or growth rates of insects on C_3 and C_4 grasses (Boutton et al. 1978, Barbehenn and Bernays 1992, Barbehenn et al. 2004b), our results provide strong evidence of how differences in relative nutritional quality of C_3/C_4 grasses and forbs may affect the demography of longer-lived consumers.

Although juvenile *C. gunnisoni* abundance was associated with $\delta^{13}C$ values in tissues with regular turnover, we found no evidence to support predicted relationships between numbers of juvenile prairie dogs to total foraging niche width, or to adipose tissue $\delta^{13}C$ values (Table 2). Variation in the $\delta^{15}N$ component of observed foraging niche widths may reflect changes in plant tissue $\delta^{15}N$ during periods of high soil nitrogen

turnover (Kielland et al. 1998) or other factors unrelated to consumer metabolic demands. Similarly, $\delta^{13}C$ values in stored lipids reflect composition of food sources assimilated during periods when capital energy stores are accumulated, and may not replicate the isotopic composition of vegetation during times of peak energetic requirements for reproductive output. While consumers inhabiting environments characterized by periods of low or intermittent resource availability are predicted to fuel reproduction using capital energy stores (Bonnet et al. 1998), seasonal limitations in energetic resources may require delaying accumulation of capital energy reserves and somatic growth to post-reproductive periods (Jonsson 1997). The association between increased abundance of juveniles and assimilation of carbon from C_3 plants, which exhibit peak growth that coincides with the *C. gunnisoni* reproductive period, highlights the importance of seasonally-available C_3 food sources as income energy sources to support reproduction. In contrast, the isotopic composition of fat stores appears largely unrelated to reproductive success, and may reflect accumulation of available resources during periods of surplus energetic resources (e.g., late summer and early fall).

Implications for population persistence

Understanding how resource dynamics fuel reproduction and other demographic processes is vital to our understanding of the role of abiotic regulation in the persistence of *C. gunnisoni* populations. Climate models for the southwestern United States project increases in mean annual temperatures of $>3^{\circ}C$ by the end of the century, along with decreases in annual precipitation (Seager et al. 2007, Gutzler 2013). Projections for this region include disproportionate seasonal decreases in precipitation and moisture conditions during winter (Christensen et al. 2004). Because early season C_3 plant growth

is linked to winter precipitation, future conditions of decreased precipitation and soil moisture would further reduce C_3 plant productivity in these arid grasslands (Muldavin et al. 2008). Sylvatic plague is also promoted by wet winters and springs (Gage and Kosoy 2005), and under future climate scenarios may play a reduced role in the limitation of *C. gunnisoni* populations. Climate-driven changes to future habitat conditions project an increased role of bottom-up regulation in *C. gunnisoni* populations, by dampening seasonal availability of the more nutritious C_3 plants. Our research identifies demographic consequences associated with shifts in the assimilation of carbon from C_3 vegetation, and demonstrates important conservation concerns for the persistence of *C. gunnisoni* populations within moisture-limited grassland environments.

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Tables

Table 1. Bayesian estimates of standard ellipse areas (SEA) for consecutive season $\delta^{13}C$ and $\delta^{15}N$ values in *Cynomys gunnisoni* plasma and red blood cells at Sevilleta National Wildlife Refuge and Vermejo Park Ranch

Season†	Plasma SEA (‰^{2})‡		Red blood cell SEA (‰^{2}) ‡	
	Sevilleta ($n = 64$)	Vermejo ($n = 222$)	Sevilleta ($n = 65$)	Vermejo ($n = 181$)
2010 Pre-monsoon	4.48	2.39	4.56	4.99
2010 Post-monsoon	3.40	5.98*	2.68	4.67
2011 Spring	2.55	1.90**	1.28	2.87
2011 Pre-monsoon	1.70	2.25	3.92	1.84
2011 Post-monsoon	1.63	1.57	1.53	1.77
2012 Spring	1.89	4.52***	4.04	4.94*
2012 Pre-monsoon	1.33	4.27	1.86	5.52
2012 Post-monsoon	1.65	2.52*	3.61	3.60

†*Cynomys gunnisoni* sampling seasons were April (spring), June (pre-monsoon), and August–September (post-monsoon)

‡Significant differences between consecutive (current versus previous) season SEAs are indicated as: *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$

Table 2. Seasonal and yearly δ^{13}C values (mean ± SD‰, VPDB) in adipose tissue samples from *Cynomys gunnisoni* at the Sevilleta and Vermejo study sites

Time period	Sevilleta		Vermejo	
	δ^{13}C	*n*	δ^{13}C	*n*
Season†				
Spring	—	0	−21.0 ± 1.5[c]	20
Pre-monsoon	−22.4 ± 3.1[a]	33	−19.4 ± 3.5[c]	54
Post-monsoon	−20.6 ± 2.9[b]	22	−19.9 ± 3.4[c]	62
Year				
2010	−23.0 ± 2.4[d]	21	−23.1 ± 3.0[f]	29
2011	−22.4 ± 3.2[d]	19	−20.0 ± 2.1[g]	56
2012	−18.9 ± 2.1[e]	15	−17.9 ± 2.9[h]	51

†*Cynomys gunnisoni* sampling seasons were April (spring), June (pre-monsoon), and August–September (post-monsoon)

[a-h]Shared letters within study areas indicate no significant differences in adipose tissue δ^{13}C values among time periods (Kruskal-Wallis test of rank values with Dunn's test of pairwise comparisons)

Figures
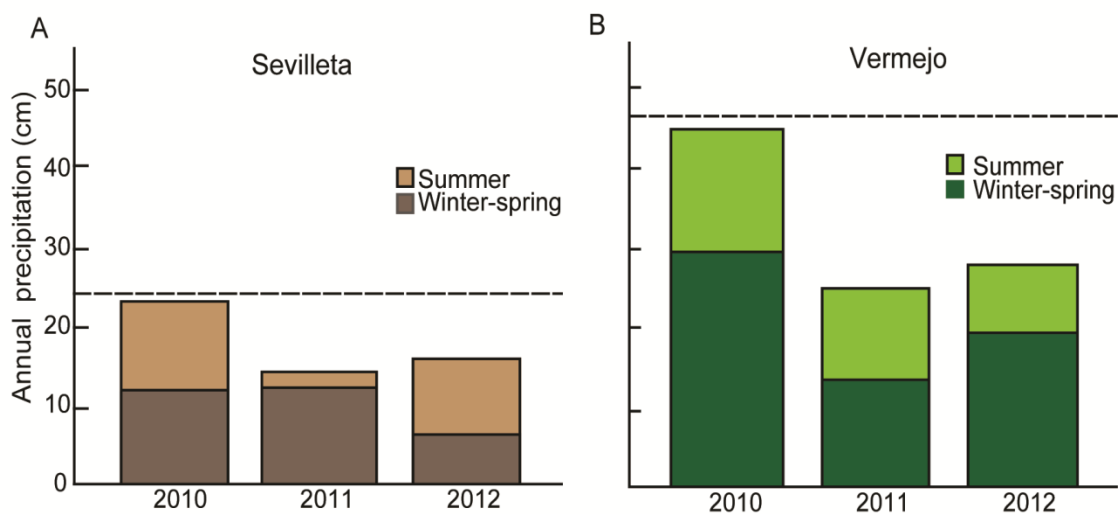
Fig. 1. Annual precipitation at (A) Sevilleta National Wildlife Refuge and (B) Vermejo Park Ranch. Precipitation years were defined as September–August, the timing of precipitation considered most relevant to growth of vegetation available to prairie dogs during sampling periods. Dashed lines represent the long-term (20-year) average annual precipitation at each site
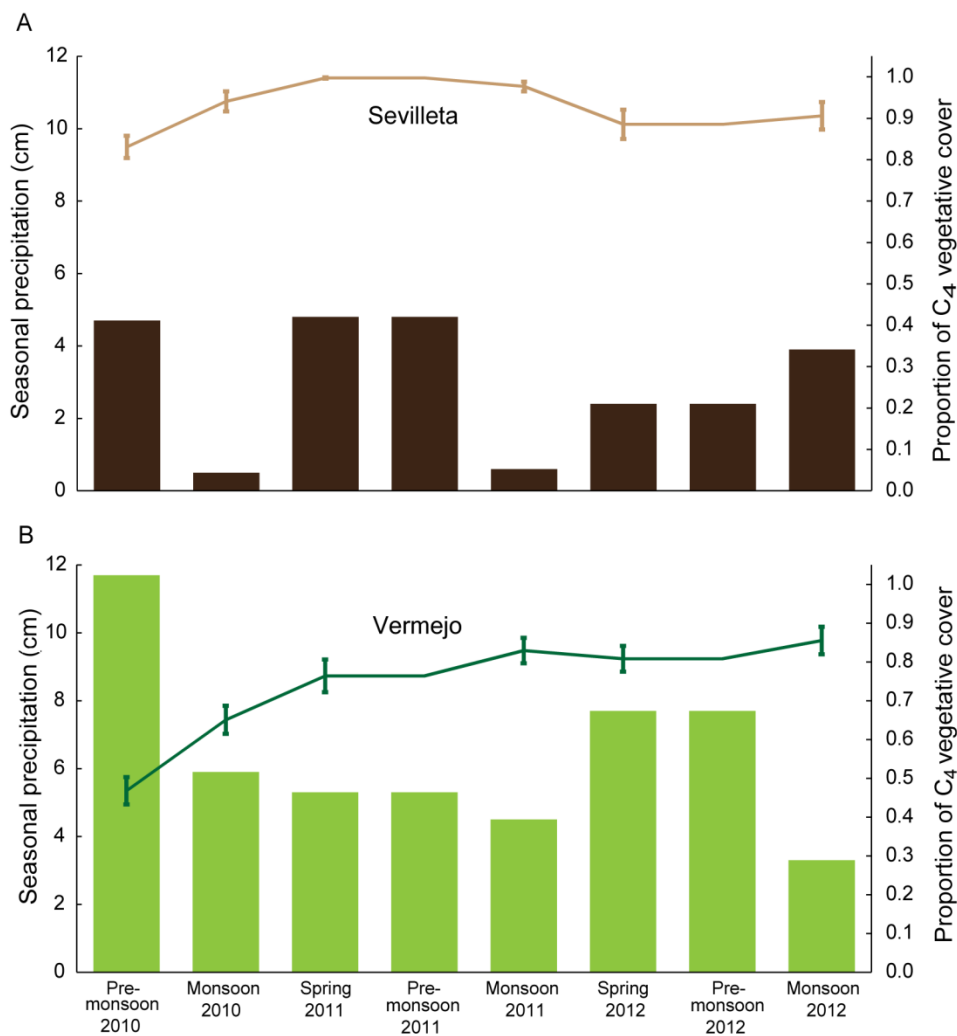
Fig. 2. Seasonal precipitation (bars, left axis) and mean proportion of total vegetative cover from C_4 plant species ($\pm$ 1 SE, line on right axis) at vegetation plots on the (A) Sevilleta and (B) Vermejo *Cynomys gunnisoni* study sites. Vegetation measurements were collected 2x/year, prior to and after the initiation of the summer monsoon growth period
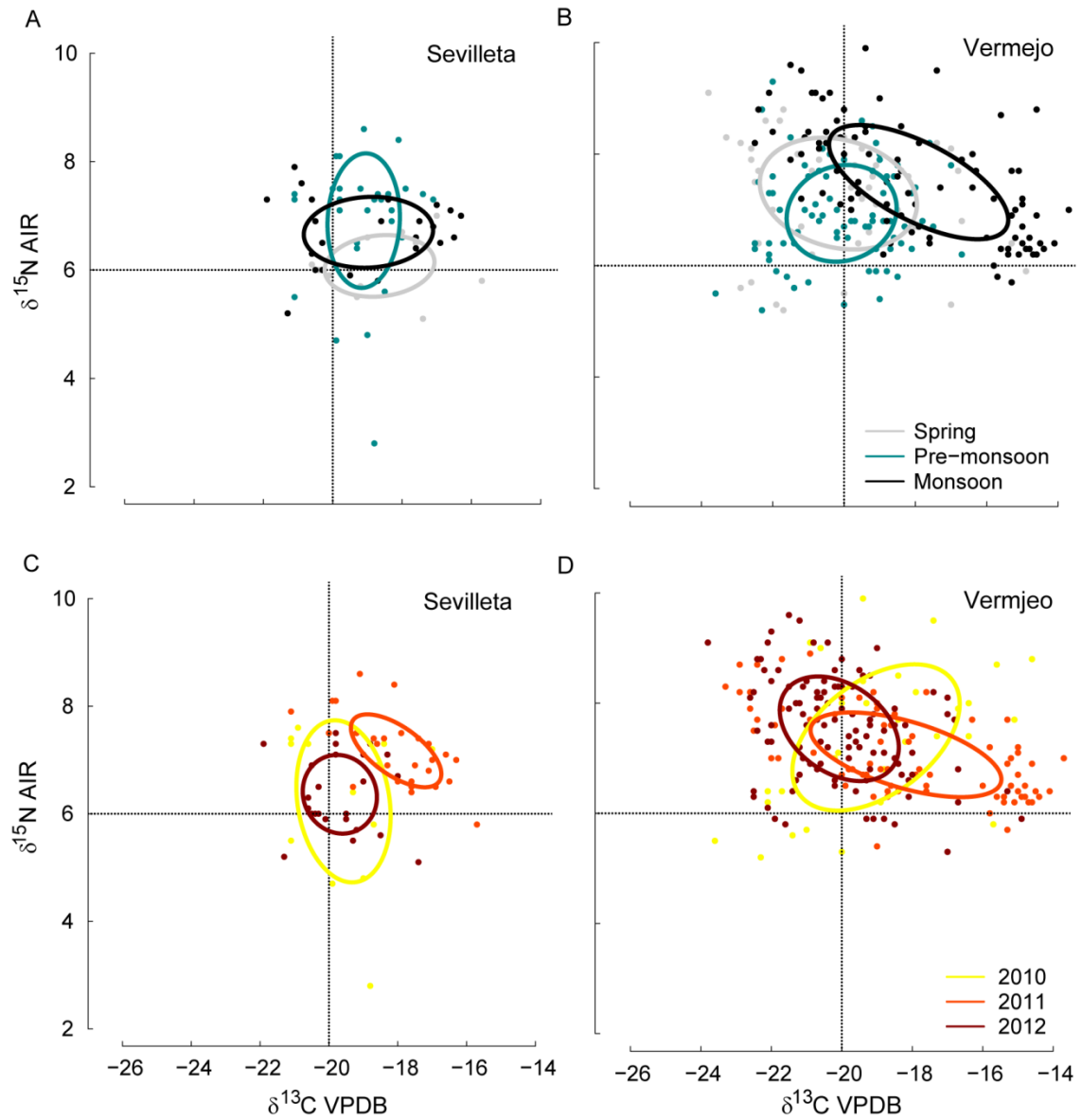
Fig. 3. Standard ellipses of (A–B) seasonal and (C–D) yearly $\delta^{13}C$ and $\delta^{15}N$ values from plasma in *Cynomys gunnisoni* at Sevilleta National Wildlife Refuge and Vermejo Park Ranch
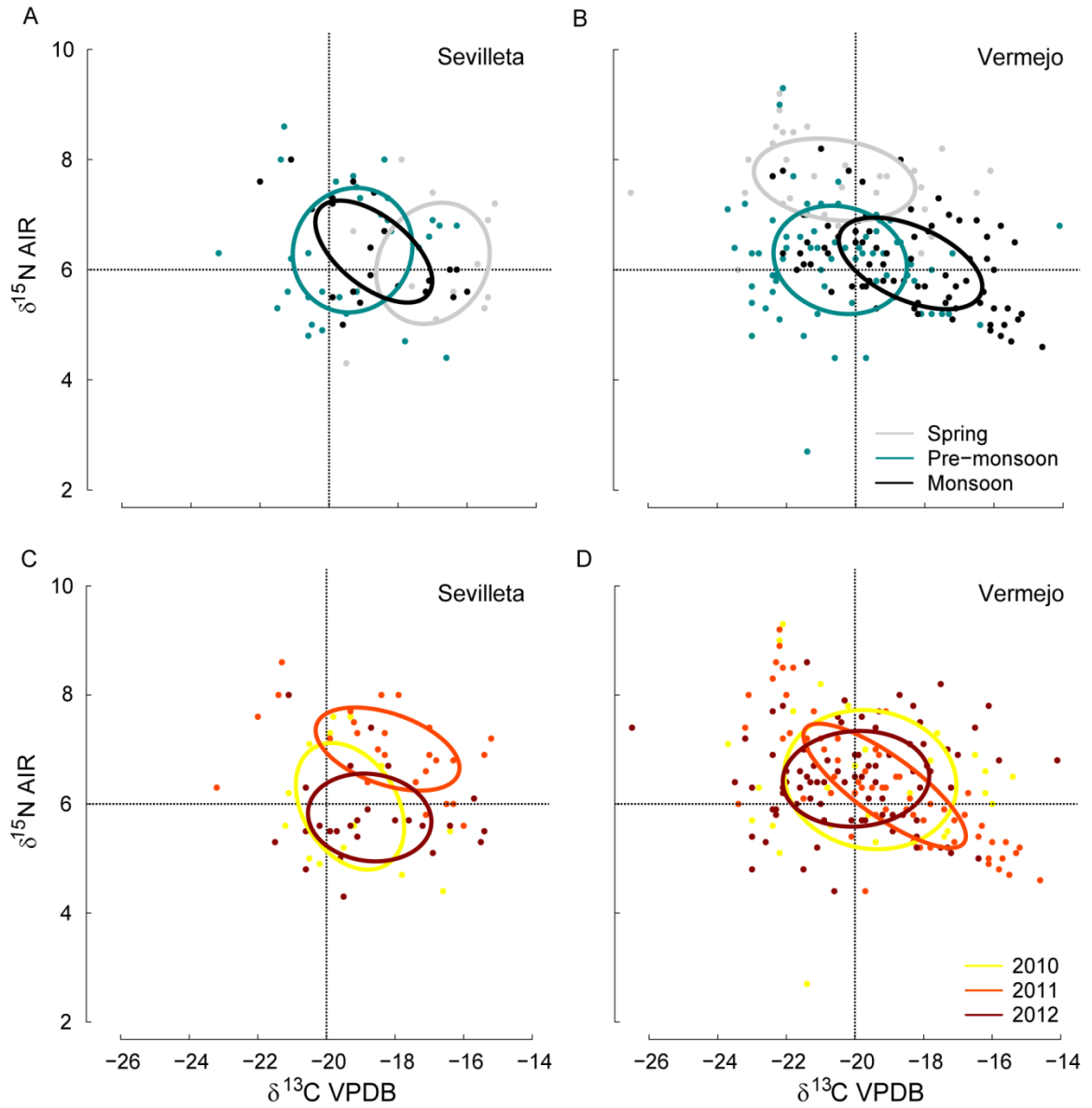
Fig. 4. Standard ellipses of (A–B) seasonal and (C–D) yearly δ^{13}C and δ^{15}N values from red blood cells in *Cynomys gunnisoni* at Sevilleta National Wildlife Refuge and Vermejo Park Ranch
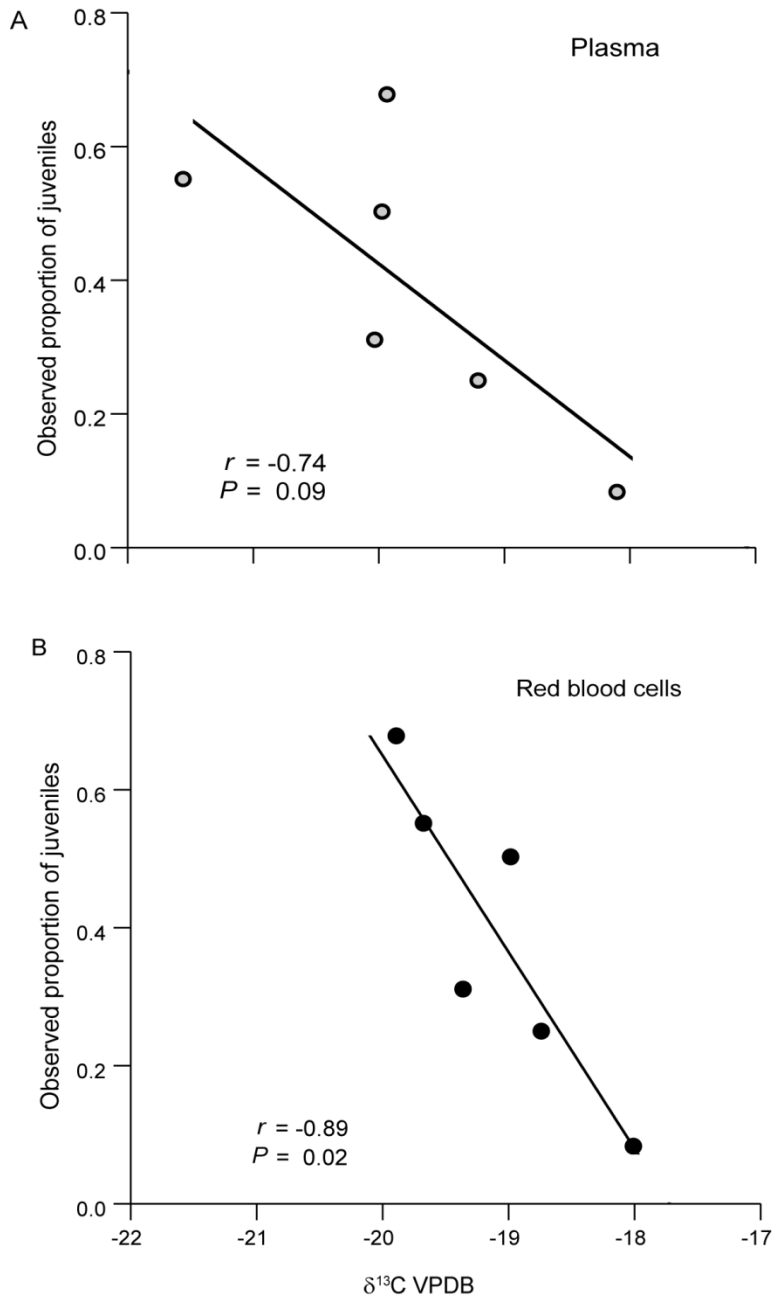
Fig. 5. Observed proportion of juvenile *Cynomys gunnisoni* present during above-ground counts and yearly δ^{13}C values of *C. gunnisoni* (A) plasma and (B) red blood cells

Appendix

Supplemental Appendix S1 (Table S1). C_3 and C_4 plant $\delta^{13}C$ values and carbon:nitrogen ratios (C:N) during dry and mesic periods at Sevilleta National Wildlife Refuge and Vermejo Park Ranch

Plant Species	$\delta^{13}C \pm SD$ (‰ VPDB)	C:N	n	Site†
C_3 plants, dry periods				
Artemesia dracunculus	-26.6	8.8	1	VPR
Chenopodium incanum	-25.5	9.7	1	Sev
Chenopodium leptophyllum	-27.0	13.4	1	VPR
Chenopodium watsonii	-28.2	27.0	1	VPR
Elymus trachycaulus	-27.9	14.3	1	VPR
Grindelia squarrosa	-28.3 ± 0.3	30.1	2	VPR
Gutierrezia sarothrae	-25.4 ± 0.5	21.6	2	Sev
Proboscidea parviflora	-24.0	6.8	1	VPR
Ratibida columnifera	-26.0	16.7	1	VPR
Senna bauhinioides	-25.0 ± 0.5	9.8	2	Sev
Sphaeralcea coccinea	-26.1	13.3	1	VPR
Sphaeralcea hastulata	-25.5 ± 0.8	14.3	2	Sev
All samples (pooled)	-26.2 ± 1.4	16.3	16	

C$_3$ plants, mesic periods

Chenopodium incanum	-24.7	6.4	1	VPR
Pascopyrum smithii	-27.9 ± 0.4	11.9	2	VPR
Solanum elaeagnifolium	-28.9 ± 0.1	14.4	2	Sev
Sphaeralcea coccinea	-27.7 ± 0.1	9.2	2	VPR
All samples (pooled)	-27.7 ± 1.4	11.1	7	

C$_4$ plants, dry periods

Bouteloua eriopoda	-15.6 ± 0.3	40.1	2	Sev
Bouteloua gracilis	-13.3 ± 0.7	35.9	2	VPR
Chamaesyce albomarginata	-14.5	20.6	1	Sev
Chamaesyce serpyllifolia	-14.6 ± 0.1	24.6	2	Sev
Pleuraphis jamesii	-14.3	33.1	1	Sev
Portulaca oleracea	-12.9 ± 0.7	13.2	2	VPR
Salsola collina	-12.8	7.7	1	VPR
Salsola tragus	-15.3	7.8	1	Sev
All samples (pooled)	-14.1 ± 1.1	24.7	12	

C$_4$ plants, mesic periods

Amaranthus biltoides	-12.9	8.4	1	VPR
Bouteloua gracilis	-13.9 ± 1.0	28.6	4	Sev, VPR

Muhlenbergia wrightii	-13.8 ± 0.3	13.2	2	VPR
Pectis angustifolia	-13.6 ± 0.1	23.6	2	Sev
Pleuraphis jamesii	-14.9 ± 0.1	29.5	2	Sev
Portulaca oleracea	-12.4 ± 0.2	22.3	2	Sev
Salsola collina	-13.1 ± 0.6	6.5	2	VPR
All samples (pooled)	-13.6 ± 0.9	20.9	15	

Note: Samples from dry periods were collected prior to the onset of monsoon seasons and in late summer during the 2011 monsoon failure year, and mesic period samples were obtained during post-monsoon seasons

†Sev = Sevilleta National Wildlife Refuge, Socorro County, New Mexico; VPR = Vermejo Park Ranch, Colfax County, New Mexico

CHAPTER 2

Gunnison's prairie dogs (*Cynomys gunnisoni*) maintain body condition, not population size, under extended drought conditions

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Abstract

Gunnison's prairie dogs (*Cynomys gunnisoni*) inhabit temperate grasslands experiencing seasonal and interannual variability in primary productivity, and survive periods of negative energy balance using internal reserves. We investigated relationships of resource use and energy assimilation to population demographics in two populations of *C. gunnisoni*. Both the arid lower-elevation (prairie) and more mesic higher-elevation (montane) populations experienced persistent drought conditions that began following study initiation. We documented no overall differences in body condition between the two *C. gunnisoni* populations, but found seasonal changes in body condition differed between sites. *Cynomys gunnisoni* from both sites improved condition after emergence from hibernation, except for females at the montane site, who maintained a more stable condition and exhibited evidence of early-season investment in reproduction. Despite

similar body condition and initial population densities, montane *C. gunnisoni* populations

reached densities up to 20x those of the prairie site, increasing each year with

recruitment. Our results suggest that consumers may be forced to choose between

reproductive and somatic investment during extended droughts, and that maintenance of

body mass and long-term energy stores may be independent of conditions that support

population recruitment.

Key words

body condition, *Cynomys gunnisoni*, Gunnison's prairie dog, population density, resource

allocation, stable isotope analysis

Introduction

A central question in ecology is how consumers obtain and allocate energy to

meet life-history needs and maximize fitness (Lack 1968; Smith and Fretwell 1974;

Charnov 1976; Drent and Daan 1980; Brown 1988). Long-term survival requires

successful trade-offs in energy allocation among processes that support organismal

maintenance, growth, and reproduction (Gittleman and Thompson 1988; Lindström 1999,

Kooijman 2000; Brown et al. 2004). Consumers must match metabolic demands with the

availability of energy sources (Thomas et al. 1996; Both et al. 2006). In temporally

heterogeneous environments, aligning resource availability with consumer metabolic

needs can lead to modified life history strategies or changes in community structure

(Paine 1966; Weltzin and Tissue 2003; Chesson et al. 2004; Yang et al. 2008). One

strategy for coping with periodic resource scarcity is to decouple periods of maximum

energy availability and peak metabolic demand by accumulating reserves of "capital"

energy sources that can be mobilized when "income" sources are insufficient to meet reproductive or other critical life history needs (Jonsson 1997; Bonnet et al. 1998).

Mammals of the family Sciuridae establish energetic reserves that are available during hibernation or periods of winter resource scarcity, and the presence of endogenous energy stores can play a vital role in gaining reproductive advantages and maximizing fitness (Fokidis et al. 2007; Risch et al. 2007). Cyclical changes in body condition follow seasonal patterns of energy accumulation and subsequent depletion of energy reservoirs (McLean and Towns 1981; Körtner and Heldmaier 1995; Buck and Barnes 1999; Humphries et al. 2003). Gunnison's prairie dog (*Cynomys gunnisoni*) is a herbivorous ground squirrel of temperate North American grasslands that exhibits these patterns (Hoogland 2003). *Cynomys gunnisoni* is an obligate hibernator with iteroparous reproduction and life expectancy in the wild of up to five years (Hoogland 2001). Northern and higher-elevation (montane) *C. gunnisoni* habitats are characterized by extended periods of cold followed by temperature-induced snowmelt and spring green-up that provide abundant forage throughout the short growing season (Longhurst 1944, Fitzgerald and Lechleitner 1974). Mesic soil conditions in montane *C. gunnisoni* habitats help trigger vector-mediated cycles of sylvatic plague (*Yersinia pestis*) (Parmenter et al. 1999; Stapp et al. 2004; Nakazawa et al. 2007), and montane populations *of C. gunnisoni* have experienced catastrophic declines since the introduction and spread of *Y. pestis* in North America (Rayor 1985a; Cully et al. 1997; Friggens et al. 2010). In contrast, prairie *C. gunnisoni* inhabit warmer, arid grasslands where primary productivity tracks seasonal precipitation patterns including dry spring conditions that limit cool-season plant growth, and coincide with suppressed recruitment in *C. gunnisoni* populations (Davidson et al.

2014). Monsoonal precipitation during the summer stimulates growth of the dominant warm-season herbaceous vegetation (Muldavin et al. 2008), but varies in intensity among days, seasons, and multi-year periods (Gutzler 2004; Vera et al. 2006).

The seasonal activity cycle of *Cynomys gunnisoni* begins in early spring, with breeding following soon after emergence for hibernation. Endogenous reserves promote post-emergence reproductive success in mammals by providing energy to support reproductive activities from ovulation through weaning in adult females (Humphries et al. 2003) and restoration of gonadal condition for spermatogenesis in males (Barnes 1996). Within populations of *C. gunnisoni*, heavier adult breeding-season body masses are associated with individuals exhibiting increased reproductive success (Hoogland 2001). Facultative or non-hibernating black-tailed prairie dogs show similar relationships between adult body mass, juvenile recruitment, and population trajectories (Facka et al. 2010). Heavier females are more attractive to males (Hoogland 1998), and incur fitness advantages compared to females with lower body masses. Body condition thereby provides a conceptual mechanism linking energy availability, body condition, reproduction, and population dynamics.

Previous research quantifying reproductive output in prairie and montane populations of *C. gunnisoni* found a positive relationship between juvenile abundance and assimilation of energy from C_3 plants (Hayes et al. 2016). Plants using the C_3 photosynthetic pathway provide more nutritious and digestible tissues than C_4 plants (Caswell et al. 1973; Landa and Rabinowitz 1983; Wilson et al. 1983; Scheirs et al. 2001). In this study, we evaluate the role of *C. gunnisoni* body condition in linking the availability of high quality energy resources to population demographics. We investigated the assimilation of energy from forage resources relative to resource availability, and hypothesized that energy assimilation from higher

quality forage resources would be associated with improved body condition. We also hypothesized that population-level differences in body condition would influence population densities and reproductive success on *C. gunnisoni* observation plots. By evaluating relationships among availability of energy resources, body condition, and population dynamics, we provide an improved understanding of life history strategies associated with the growth and persistence of *C. gunnisoni* populations.

Methods

Study Sites

We studied *C. gunnisoni* colonies at Sevilleta National Wildlife Refuge, 85 km south of Albuquerque, New Mexico, USA, and Vermejo Park Ranch, located ~300 km to the northeast between the towns of Raton and Cimarron, New Mexico. Colonies occurred at 1650 m elevation on Sevilleta, and represented prairie populations of *C. gunnisoni*. Precipitation at Sevilleta averages 26 cm annually, with 53% falling during the summer monsoonal period and 47% during winter (Muldavin et al. 2008). Mature warm-season (C_4) grasses such as blue grama (*Bouteloua gracilis*) dominated Sevilleta's Chihuahuan Desert vegetation (Collins and Xia 2015). Vermejo colonies fell within the montane portion of the *C. gunnisoni* range, and were located at 2220 m elevation. Annual precipitation at the Vermejo colonies averages ~50 cm, and includes summer monsoonal thunderstorms that account for an estimated 29–42% of the total annual precipitation (Legler 2010). Vermejo habitats consisted of long (30–40 km), canyon-bounded grasslands characterized by a mixture of primarily C_3 forbs and grasses that complemented abundant *B. gracilis*, which was the most common plant species present within the Vermejo grasslands. The same North American monsoon summer storm systems therefore impact both study sties, but monsoonal activity becomes less intense

and contributes a smaller proportion of total annual precipitation as it moves northward

from its sources of moisture in the Gulf of California and eastern Pacific Ocean (Adams

and Comrie 1997).

Animal capture and sampling

We captured prairie dogs from 2010–2012 at 4 trapping plots (~1 ha each) within

a complex of *C. gunnisoni* colonies at each study area (Davidson et al. 2014; Hayes et al.

2016). Capture and sampling methods followed applicable guidance for animal care,

including American Society of Mammalogists guidelines for use of wild mammals in

research, and protocol 10-100465-MCC approved by the University of New Mexico's

Institutional Animal Care and Use Committee. Capture periods occurred during spring

(post-emergence; April), early summer (pre-monsoon; June), and late summer (following

monsoon initiation; August-early September). We recorded weight of each captured *C.*

gunnisoni to ±0.1 g using a portable electronic balance (Scout Pro, Ohaus Corporation,

Parsnippany, New Jersey, USA), and measured hind foot length (Schulte-Hostedde et al.

2005) to the nearest 1 mm. We collected blood samples (~50 µl) for isotopic analysis

from adult and late-summer juvenile *C. gunnisoni* by clipping the distal end of the toenail

on the lateral hind digit (Hoogland 1995), which provided blood flow directly into

capillary tubes for 60–90 seconds before coagulation occurred. We sampled adipose

tissue non-destructively (Baker et al. 2004) using 16 ga, 6–9 cm standard bevel-tip biopsy

needles (Products Group International, Inc., Lyons, Colorado, USA) inserted under the

skin and into fat stores deposited on top of the lower dorsal musculature.

We counted *C. gunnisoni* each season by observing colonies from constructed

platforms and portable blinds in flat terrain (Facka et al. 2008), or from elevated vantage

points located adjacent to the observation plots. Observers arrived at count sites before

sunrise and prior to the emergence of *C. gunnisoni* from their burrows, and monitored the

colony for ~3 hours until activity declined. Observers counted all prairie dogs above

ground at 30 minute intervals by systematically scanning between marked endpoints at

the edge of a plot. We counted prairie dogs at each plot during 1–2 days of observation

periods per season, and completed seasonal counts of all plots at a study site within a

span of $\leq$5 days. For each observation period, we determined the maximum number of

adult *C. gunnisoni* observed above ground on the plot (Severson and Plumb 1998). We

counted the number of *C. gunnisoni* juveniles present on plots during pre-monsoon

observations, when pups had been recently weaned and emerged from underground

burrows. By the post-monsoon period, juveniles and adults could not be reliably

separated when viewing from the visual observation points, and all *C. gunnisoni* observed

were counted as adults.

Vegetation measurements

We monitored available vegetation during pre- and post-monsoon periods at 3 1-

ha grids that overlapped *C. gunnisoni* sampling plots within each study area. At each

plot, we determined total plant cover by species (Daubenmire 1959) and by functional

group (C_3 versus C_4 or crassulacean acid metabolism [CAM] photosynthetic pathways)

using 0.25 m^2 vegetation sampling frames. Sevilleta Long Term Ecological Research

monitoring provided vegetation data (http://sev.lternet.edu, dataset SEV129) from plots at

Sevilleta prairie dog colonies. We monitored plant species composition at equivalent

randomly-located plots on Vermejo colonies, and calculated relative abundance of plant

functional groups (Hayes et al. 2016). Presence of large grazing herbivores at Vermejo

(elk , *Cervus canadensis* and American bison, *Bos bison*) that were absent from Sevilleta dictated the use of relative (versus absolute) functional group abundances to obtain unbiased comparisons of plant growth between sites.

Laboratory procedures

We extracted plasma for isotope analysis from blood samples following procedures from Warne et al. (2010). When foreign or cloudy materials (e.g., lipids) were present, sectioning of capillary tubes allowed for the removal impurities before loading samples into tin capsules for processing. We washed adipose tissue samples (0.6 - 0.8 mg) in distilled water and ethanol, and examined them under a hand lens to ensure that no connective or other tissue was attached to the sample.

We measured carbon and nitrogen isotope ratios through continuous flow isotope ratio mass spectrometry at the University of New Mexico Center for Stable Isotopes (UNM-CSI), using a Costech ECS 4010 elemental analyzer coupled to a Thermo Finnigan Delta Plus mass spectrometer via a ConFlo II interface. Isotope ratios are reported in standard delta (δ) notation in parts per thousand (‰) relative to isotopic standards (Vienna Pee Dee Belemnite for carbon, atmospheric air for nitrogen), as:

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

where *R* represents the ratio of heavy to light isotopes ($^{13}C/^{12}C$ or $^{15}N/^{14}N$). Average analytical precision based on routine analysis of laboratory standards at UNM-CSI was ≤ 0.1 ‰ SD. Laboratory standards were calibrated against NBS 21, NBS 22 and USGS 24 for $\delta^{13}C$.

Analyses

We standardized all maximum above ground counts of *C. gunnisoni* as densities per hectare. Variation in *C. gunnisoni* densities was tested using a mixed effects linear model (Zuur et al. 2007) for repeated measures of plots, with a diagonal covariance matrix including study area and season as factors. We tested for differences between study areas and end of year densities (post-monsoon seasons, when all *C. gunnisoni* present on plots were counted as adults) using *t*-tests, and controlled experimentwise error rates using Bonferroni procedures.

We compared the proportion of C_4 plant cover and the mass of clipped vegetation among seasons and years within each study area using a linear mixed model to account for repeated measures of plant composition and quantity at fixed vegetation plots. We used the logit transformation (= log $[y_{C4} / (1- y_{C4})]$, where y_{C4} = proportion of C_4 plants) on all vegetative cover proportion data (Warton and Hui 2011) in order to meet assumptions for linear modeling. Bonferroni procedures controlled experimentwise error rates for post-hoc multiple comparisons among time periods.

We calculated the proportional contribution of plant carbon to animal tissues from C_3 versus C_4 and CAM photosynthetic pathways using a one-isotope, two-source Bayesian mixing model (Stable Isotope Analysis in R; Parnell et al. 2010). Plant samples collected during the study period supplied $\delta^{13}C$ values for plant tissues that served as endpoints for the mixing model (mean $\pm$ SD = –14.0 $\pm$ 1.0‰ C_4 plants, -26.7 $\pm$ 1.5‰ C_3; Hayes et al. 2016). We obtained diet-to-tissue discrimination factors ($\Delta^{13}C$) from previous studies of carbon isotope assimilation in rodents. These $\Delta^{13}C$ values were 0.6‰ for plasma (Yoneyama et al. 1983), and -2.7‰ for adipose tissue based on results from

Tieszen and Boutton (1989). We tested for seasonal and yearly differences in contributions of C_3 and C_4 plants to *C. gunnisoni* tissue carbon by generating Bayesian estimates of mean proportion of C_3/C_4 carbon in plasma and adipose tissue based on 500,000 model iterations (with a burn-in of 50,000 runs). Seasons and years were considered significantly different from each other when the intervals containing 95% of modeled source proportions did not overlap between time periods.

The mean proportion of C_3/C_4 carbon from *C. gunnisoni* on a sampling plot was compared to the proportion of each plant functional group present on those plots using linear regression. We calculated 95% confidence intervals of regression slopes to test whether use of C_3/C_4 plant resources shifted proportionally to plant availability (slope = 1). To meet linear regression assumptions, we considered extreme $\delta^{13}C$ values >1.5 interquartile ranges from the first or third quartiles to represent outliers (Tukey 1977) that were removed from further analysis.

We estimated body condition by developing a mass-length regression equation for captured *C. gunnisoni*, and considered residuals of individual mass-length ratios as indices of body condition (Le Cren 1951; Reist 1985; Krebs and Singleton 1993; Stevenson and Woods 2006). The condition index compared total body mass to hind foot length, a structural body measurement that asymptotes to its mature size by ~2 months of age in prairie dogs (Pizzimenti and McClenaghan 1974). We performed ordinary least squares regression on mass-length data plotted on a log-log scale to linearize the data, and calculated residuals of individual points from the regression line as metrics of overall body condition (Jakob et al. 1996; Schulte-Hostedde et al. 2005). Inconsistent foot length measurements from recaptured prairie dogs ($\geq$ 2.5 mm difference) were considered to be

process errors and removed from body condition analyses. Relationships between

individual body condition and δ^{13}C and δ^{15}N values from *C. gunnisoni* tissues were

evaluated using stepwise linear regression ($P \leq 0.05$ to enter, $P \geq 0.10$ to remove). We

used analysis of variance to evaluate individual *C. gunnisoni* body condition by season

and study area, using sex as a covariate to account for sexually dimorphic differences in

body size (Pizzimenti and Hoffman 1973; Hoogland 2003). At the plot level, we

compared mean body condition to total plot densities during current seasons and from

subsequent sampling periods using linear regression.

We created an index of proportional population growth on each plot from births

and recruitment (λ_R) by calculating changes in observed plot densities following periods

of annual emergence of *C. gunnisoni* juveniles. Normalized values for λ_R were calculated

as:

$$\lambda_R = (N_{adults} + N_{juv.}) / N_{adults}$$

where $N_{juv.}$ is obtained directly from counts of juveniles during June (pre-monsoon), and

estimated during August-September (post-monsoon) by the increase in adult-sized

animals on a plot since June. Values of λ_R were not calculated from spring counts

because no juvenile (young of year) *C. gunnisoni* were present during those observation

periods. We considered extreme λ_R values associated with low adult densities observed

on plots as outliers resulting from sampling error, and excluded them from further

analysis. We used linear regression to predict λ_R from mean seasonal body condition

indices for adult *C. gunnisoni*, and for changes in body condition from the previous

season. Relationships among body condition and other variables were significant when

regression coefficients were different from zero at the $\alpha = 0.05$ level. To test for

relationships specific to the reproductive segment of *C. gunnisoni* populations, we conducted linear regression of λ_R values and mean body condition indices for adult females on a plot.

Results

We captured 319 *C. gunnisoni* from Sevilleta (n = 77) and Vermejo (n = 242), representing 214 distinct individuals and 105 between-season recaptures. A total of 296 of these captures produced mass and length measurements that generated body condition indices, after outliers and any inconsistent measurements from recaptured animals were removed. Prairie dog masses were predicted by the equation:

$$\log (\text{mass, g}) = 2.48* \log (\text{hind foot length, cm})_+ 1.01$$

($r^2 = 0.26$, $F = 102.3$, $P < 0.001$). Individual condition indices (residuals) for adult prairie dogs ranged from −0.23 to 0.24. Adult body masses ranged from 430.5 to 1301.6 g, and the minimum and maximum condition indices corresponded to a difference between observed and predicted body masses of −304 and 468 g, respectively. Considering sex a covariate ($F = 10.9$, $P = 0.001$), body condition varied by season ($F = 5.8$, $P = 0.004$), with condition indices increasing each season following emergence from hibernation. Differences in condition indices ($\Delta_{resid.}$) during the late summer were significantly different from both spring ($\Delta_{resid.} = 0.043$, $P = 0.007$) and early summer ($\Delta_{resid.} = 0.038$, $P = 0.003$; Fig. 1). Body condition did not vary significantly by study area ($F = 3.6$, $P = 0.06$), but there was a significant season by study area interaction ($F = 4.4$, $P = 0.01$).

Mean seasonal densities of *C. gunnisoni* observed on plots ranged from 2.1–10.3 ($\bar{x} = 4.1$, SD = 3.7, total plot range = 0.0–15.0) adults/ha at Sevilleta, and 11.9–49.6 ($\bar{x} =$

32.8, SD = 15.8, total plot range = 7.9–74.0) adults/ha at Vermejo (Fig. 2). Both the

maximum densities at Sevilleta and minimum densities at Vermejo occurred during the

first season of the study. June plot counts produced mean observed densities of 0.8–4.7

($\bar{x}$ = 1.7, SD = 2.9, total plot range = 0.0–10.0) juveniles/ha at Sevilleta, and 14.8–71.3 ($\bar{x}$

= 42.4, SD = 31.3, total plot range = 11.9–95.2) juveniles/ha at Vermejo. Mean end-of-

year plot densities differed between areas during each year of the study ($t \geq 4.2$, $P < 0.01$

for each year). Study area ($F = 220.4$, $P < 0.001$) and season ($F = 5.2$, $P = 0.01$) both had

significant effects on observed densities of adult *C. gunnisoni*, with greater numbers

observed at Vermejo than Sevilleta and during the late summer compared to spring

sampling periods.

Composition of vegetation on *C. gunnisoni* plots varied over the period of the

study. Changes in proportion of C_3 cover among seasons were not significant at Sevilleta

($F = 0.6$, $P = 0.44$), but Vermejo plots exhibited significant seasonal differences ($F =$

16.8, $P = 0.001$) with proportionally more C_3 vegetation pre-monsoon than post-

monsoon. Proportion of C_3 vegetative cover changed significantly among years at both

Sevilleta ($F = 26.6$, $P < 0.001$) and Vermejo ($F = 50.0$, $P < 0.001$). The proportion of C_3

vegetative cover was highest at both sites in 2010, and then decreased in subsequent

years of the study.

The proportion of tissue carbon assimilation in montane (but not prairie) *C.*

gunnisoni plasma from C_3 plants varied among seasons, and was lower during late

summer than in spring at the Vermejo site (Table 1). Both prairie and montane *C.*

gunnisoni showed yearly variation in assimilated C_3 carbon, with lowest proportion of C_3

carbon during 2011 compared to 2010 or 2012 (Table 1). Isotopic composition of

adipose tissue also changed at both sites, shifting toward increased contributions of C_4 plant carbon during each progressive year of the study (Table 2). Contributions of C_3 plant carbon to adipose tissue declined during later seasons at the prairie site, with the proportion of C_3 carbon dropping from the pre-monsoon to post-monsoon season (Table 2). At the montane site, the proportion of C_3 plant carbon in adipose tissue decreased from spring to early summer, but did not continue to decline into the post-monsoon period (Table 2).

Overall, the proportion of C_4 plant carbon in *C. gunnisoni* plasma increased with C_4 plant availability ($r_{plasma} = 0.34$, $P = 0.03$; Fig. 3). The 95% confidence interval for the regression slope (C.I. $= 0.02$–0.52) was between 0 (no relationship) and 1 (forage use $=$ availability). The proportion of C_4 carbon in adipose tissue showed no significant relationship to the proportion of C_4 vegetation present on a plot ($r_{fat} = 0.19$, $P = 0.28$), and the 95% confidence interval for the regression slope (-0.20–0.65) included zero, indicating no predictive relationship between the variables.

The relationship of body condition indices to carbon and nitrogen isotope values in tissues was significant (negative) only for $\delta^{13}C$ values in adipose tissue ($t = -2.1$, $P = 0.04$), but had low predictive power ($r^2 = 0.04$). Plot-level means of adult *C. gunnisoni* body condition showed no significant relationship to total plot densities during current ($t = -0.56$, $P = 0.58$, $r^2 = 0.006$) or subsequent ($t = -1.3$, $P = 0.20$, $r^2 = 0.04$) seasons of *C. gunnisoni* sampling. However, adult body condition showed a significant inverse relationship to the index of reproductive investment (λ_R), based on seasonal means for each plot ($t = -2.3$, $P = 0.033$, $r^2 = 0.13$). For the reproductive segment of the population (adult females), λ_R had significant inverse relationships to mean body condition ($t = -2.1$,

$P = 0.045$, $r^2 = 0.13$) and to changes in body condition from the previous season ($t = -2.6$,

$P = 0.014$, $r^2 = 0.23$; Fig. 4).

Discussion

Densities, body condition, and preferred resources

We documented dramatic differences in *C. gunnisoni* population densities

between two study sites located at different points along a moisture gradient within the

same region. Although observed densities from the two sites did not differ at the

initiation of the study, population trajectories diverged so that greater abundances of *C.*

gunnisoni were observed at the more mesic site during all subsequent seasons (Fig. 2).

Reduced densities were found at the more xeric site, which experiences periods of

precipitation-limited primary productivity (Muldavin et al. 2008; Thomey et al. 2011)

that force consumers to shift patterns of resource utilization (Warne et al. 2010). Based

on our previous findings linking *C. gunnisoni* reproductive output to carbon assimilation

from more nutritious C_3 forage plants (Hayes et al. 2016), we hypothesized that *C.*

gunnisoni body condition would decline under conditions of resource scarcity, and that

reduced body condition would be observed during population declines. However, we

found no support for the relationship between body condition and study area, even though

plots at the more mesic site (Vermejo) always had greater abundances of juvenile *C.*

gunnisoni than plots at Sevilleta. These differences in recruitment led to mean plot

densities of adult *C. gunnisoni* that grew to over 20x greater at Vermejo than Sevilleta by

the end of the study.

The apparent disassociation of *C. gunnisoni* abundance and body condition in our

study leaves the observed positive relationship between reproductive output and use of

high-quality forage resources in search of a mechanistic explanation. In both populations, relative abundance of C_3 plants declined over the years of the study, as extended drought conditions persisted. Assimilation of carbon from C_3 plants in *C. gunnisoni* adipose tissue, which does not turn over on a fixed interval but serves as a reservoir for long-term energy storage, also declined over years at both study sites (Table 2). Relative abundance of C_3 and C_4 plants continued to show expected seasonal shifts at Vermejo, but exhibited no detectable pulse of productivity from perennial warm-season grasses following the monsoonal period at Sevilleta. Carbon isotope composition of *C. gunnisoni* plasma, a rapid-turnover tissue, reflected these shorter-term trends in available vegetation. Plasma from Vermejo *C. gunnisoni* tracked the site's post-monsoon spike in availability of C_4 plant carbon, while plasma from individuals at Sevilleta displayed a less variable carbon isotope signature (Table 1). The proportion of C_4 plant carbon in plasma increased when C_4 plants had greater relative abundance, but at a reduced rate than would be expected if energy assimilation from C_3 and C_4 forage plants occurred strictly as a function of plant availability (Fig. 3). These observations portray *C. gunnisoni* foraging as responsive to plant availability, with some active selection that slows the transition toward a lower quality (C_4-dominated) diet during periods of scarcity for higher-quality energy sources.

Resource allocation and energetic trade-offs

We hypothesized that prairie dogs consuming preferred diets (e.g., selective folivory with a diversity of grasses and forbs; Rayor 1985b; Shalaway and Slobodchikoff 1988; Hoogland 1995) that include C_3 plants should achieve an improved body condition with increased energetic reserves, which support vital life history functions. However,

we found little to no relationship between *C. gunnisoni* tissue δ^{13}C and δ^{15}N ratios and body condition. As an alternative life history strategy, high-quality energy resources may be translated to income energy sources. Observed low levels of recruitment and marked population declines in desert grassland populations of *C. gunnisoni* during years of recurring spring droughts (Davidson et al. 2014) suggest an important role of income energy sources in reproductive success. Growth pulses of the more digestible C_3 plants occur earlier in the growing season and under cooler and more mesic conditions than for C_4 plants, at similar times to energetically-demanding reproductive activities such as parturition and lactation. The coinciding periods of high-quality forage plant growth and increased reproductive investment (in fetuses or offspring) offers further evidence of *C. gunnisoni* as income breeders.

Resource scarcity stimulates adoption of a risk-averse reproductive strategy, where parents adjust their reproductive effort based upon perceived risk of surviving future periods of environmental stress (Stephens and Krebs 1986). This strategy is predicted when short-term prospects for marginal increases in reproductive investment cannot be anticipated without parental costs that result in future decreases in fitness (Williams 1966; Reznick 1985). Risk-sensitive reproduction leads to diminished reproductive investment under poor environmental conditions, but subsequent improvements in resource conditions and energy availability are not immediately allocated to maternal reproductive condition (Bårdsen et al. 2008).

Following the risk sensitive reproductive allocation hypothesis, iteroparous consumers forego reproduction when drought conditions create resource scarcity and risk to adult survival. Risk-sensitive reproduction is adaptive for consumers in temporally

heterogeneous environments who experience repeated periods of energetic resource stress within their reproductive lifetimes (Boyce 1980). Patterns of body condition and reproductive output are predicted by the "selfish mother" hypothesis, where parents reduce care and investment in offspring during resource scarcity in favor of their own survival and subsequent reproductive opportunities (Festa-Bianchet and Jorgenson 1998). These resource allocation systems are most frequently observed in populations of large-bodied, long-lived mammalian herbivores inhabiting environments characterized by variable ecological conditions over seasonal or interannual periods (Gaillard et al. 2000). Gunnison's prairie dogs survive periods of potential energetic stress through obligate winter hibernation, when they rely upon endogenous energy stores and experience cyclical depletion of body mass until spring emergence. Overall, *C. gunnisoni* in our study improved their body condition throughout the active season to reach peak condition in the fall (Fig. 1), presumably to accumulate capital energy stores for winter hibernation. The significant interaction in body condition between sex and study area highlights differences in this pattern for female *C. gunnisoni* at the montane site, who achieved highest body condition following spring emergence and displayed no evidence of body condition increases later in the active season (Figure 1). Because our condition index was based upon masses of *C. gunnisoni* relative to structural body size, increases in calculated body condition could reflect internal growth of fetuses during pregnancy, which coincided with our spring sampling period. Increases in the presence and number of fetuses would generate upwardly-biased estimates of female somatic condition, but also demonstrate differential reproductive allocation early in the active season. The montane population exhibited higher early-season body condition and greater densities of

juvenile *C. gunnisoni* observed during post-parturition periods, resulting in greater total population densities during each year of the study (Fig. 2). These findings provide strong evidence of differential reproductive allocation between populations, despite the lack of any observed effect of study area on body condition.

Evidence of *C. gunnisoni* reproductive output that follows a risk-sensitive reproductive allocation must also be reconciled with the body of literature indicating increased reproductive success with improved parental body condition. These relationships are documented across a wide range of mammal species (Millar 1977; Clutton-Brock 1991), and within multiple species of prairie dogs (Hoogland 2001; Facka et al. 2010). We found an inverse relationship between adult body condition and reproductive output, where populations with greater reproductive allocation experienced reductions in body condition (Fig. 4). When looking strictly at maternal effects, adult female condition and changes in body condition were inversely related to quantity of reproductive output. Investment in reproduction thereby generates an apparent cost to body condition. Increased reproductive investment in the form of larger litter sizes and increased lactation results in body mass losses and condition reductions in female sciurids that may be evident through the duration of the active season (Millesi et al. 1999). Although we were unable to document the fitness consequences of condition reductions at the individual level, previous research has shown that *C. gunnisoni* females suffer reduced overwinter survival after rearing young when compared to non-reproducing females (Rayor 1985b).

Reproductive investment decisions made under a risk-sensitive allocation paradigm are condition-dependent, relative to a threshold for future risk of starvation or

other factors that reduce lifetime fitness (Lindström 1999). Exceeding the condition threshold does not result in an immediate allocation of reproductive resources in a risk-averse strategy, even though seasonal thresholds of endogenous reserves may be safely crossed during other times of the year (Monteith et al. 2013). Because we did not continuously record body condition, our observed differences in reproductive output could reflect condition-dependent responses during a critical time period when we did not sample. Site-level differences in female condition cycles began early in the active season (Fig. 1), suggesting that condition-dependent reproductive allocation was determined prior to our spring sampling period. Reproductive allocation decisions made at spring emergence must consider body condition relative to some threshold for surviving the post-reproductive period, and can only project resource conditions later in the active season based on cues available upon emergence. The limited reproductive output of the Sevilleta *C. gunnisoni* population suggests that it existed at or near some resource or environmental condition threshold which precluded successful reproductive investment. We found no evidence that achieving somatic body condition comparable to a more productive population was able to mitigate early-season reproductive investment decisions made by the Sevilleta *C. gunnisoni* population.

Our study evaluated population-level effects of life history strategies regarding assimilation of foraging resources, and did not address specific mechanisms used in reproductive resource allocation decisions. While most energetic trade-offs among physiological processes are hormonally-mediated (Stearns 1989), the proximate cues utilized by individual *C. gunnisoni* to distribute energy among somatic maintenance and reproductive activities are less clear. However, the role of high-quality forage

availability should not be dismissed. Energy assimilation in *C. gunnisoni* at the more

productive montane population tracked seasonal pulses in vegetation growth and

composition, while no seasonal patterns were evident at the prairie site. As a result, the

montane *C. gunnisoni* experienced a relative increase in C_3 plant assimilation during the

earlier portions of the active seasons. The reproductive segment of this montane

population (adult females) was the only demographic group that did not reach minimum

condition levels during spring, consistent with early-season reproductive investment.

Conversely, spring droughts at the arid grassland site were associated with low

recruitment and population declines in *C. gunnisoni* (Davidson et al. 2014). Although

body condition was unrelated to the isotopic composition of *C. gunnisoni* tissue, evidence

remains for a direct relationship between C_3 forage availability and population growth

through recruitment. Manipulative experiments addressing the relative availability of C_3

and C_4 forage plants, and the role of these income energy sources in reproductive output

of *C. gunnisoni*, provide fertile ground for future research.

Conservation implications

Reproductive output of *C. gunnisoni* is associated with winter precipitation and

spikes in C_3 plant productivity (Hayes et al. 2016). Future climate projections for the

southwestern United States include decreases in winter precipitation and moisture

(Christensen et al. 2004), creating conditions less favorable to growth of more nutritious

C_3 plants. These projected changes in climate thereby create greater threats to *C.*

gunnisoni reproductive output than to adult survival. Declines in prairie dog populations

associated with changes in climate and primary productivity may not be as readily

detected as catastrophic population losses that have been attributed to disease events

(Lechleitner et al. 1968; Cully and Williams 2001). This is especially true if observations are made outside of seasons when abundance of juveniles can be readily determined.

Despite the drought conditions experienced during our study, montane populations of *C. gunnisoni* appeared to maintain population recruitment, unlike the more southerly prairie populations. These montane populations may be more resistant to climate shifts that serve to reduce primary productivity at the desert grassland site. Pulses of C_3 plant growth in montane habitats follow more predictable periods of spring warming and release of moisture from snowpack than in the monsoon-dominated environments, where consumer populations fluctuate with changes in precipitation and moisture availability associated with variation in El Nino Southern Oscillation cycles (Brown and Ernest 2002; Meserve et al. 2003). Although montane habitats of *C. gunnisoni* support more mesic soils that increase numbers of sylvatic plague vectors, host, and outbreaks, prairie *C. gunnisoni* may experience more pervasive threats to their persistence. Growth pulses of the more palatable C_3 plants were undetectable during drought periods at the arid grassland site. Still, Sevilleta *C. gunnisoni* maintained somatic condition under precipitation and moisture regimes experienced during the study, but experienced depressed reproductive output during resource-limited periods. Vulnerability of prairie dog colonies to drought has been previously documented within southern New Mexico (Facka et al. 2010; Davidson et al. 2014), and is further evidenced through our results. Risk-sensitive reproductive resource allocation and limitations in forage conditions that lead to foregoing or deferring of reproduction (Rayor 1985b) may further exacerbate population vulnerability. Risk-sensitive resource allocation buffers large-bodied, long-lived herbivores against climate impacts by precluding investment in

reproductive efforts that trade future reproductive condition for limited short-term increases in fecundity (Bårdsen et al. 2008). However, this life history strategy is successful only when cycles of resource heterogeneity occur at sufficiently short intervals for resource conditions to change between breeding periods. Multi-year drought periods reduce opportunities for shorter-lived animals to maximize their lifetime reproductive success. Changing climates with reduced winter precipitation and more arid conditions suppress cool-season C_3 plant growth that provides an income energy source during post-hibernation breeding seasons, and may serve as a greater threat to population persistence than the disease events that were previously postulated as limiting factors for *C. gunnisoni*.

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Tables

Table 1. Seasonal† and yearly proportions of C_3 plant carbon in plasma sampled from *Cynomys gunnisoni* at Sevilleta National Wildlife Refuge and Vermejo Park Ranch, New Mexico.

	Sevilleta		Vermejo	
	Proportion C_3 carbon	95% credible interval	Proportion C_3 carbon	95% credible interval
Season				
Spring	0.41[a]	0.37-0.46	0.55[b]	0.52-0.57
Pre-monsoon	0.48[a]	0.45-0.51	0.53[b]	0.51-0.54
Post-monsoon	0.47[a]	0.44-0.51	0.36[c]	0.34-0.38
Year				
2010	0.52[d]	0.48-0.56	0.49[f]	0.45-0.53
2011	0.38[e]	0.35-0.41	0.40[g]	0.39-0.42
2012	0.52[d]	0.49-0.56	0.47[f]	0.46-0.48

†*Cynomys gunnisoni* sampling seasons were April (spring), June (pre-monsoon), and August–September (post-monsoon)

[a-g]Shared letters within study areas indicate no significant differences in carbon isotope composition of *C. gunnisoni* plasma among time periods (overlapping 95% credible intervals for Bayesian estimates of proportion of C_3 plant carbon in animal tissues)

Table 2. Seasonal† and yearly proportions of C_3 plant carbon in adipose tissue sampled from *Cynomys gunnisoni* at Sevilleta National Wildlife Refuge and Vermejo Park Ranch, New Mexico.

	Sevilleta		Vermejo	
	Proportion C_3 carbon	95% credible interval	Proportion C_3 carbon	95% credible interval
Season				
Spring	--	--	0.33[c]	0.30-0.36
Pre-monsoon	0.52[a]	0.49-0.55	0.17[d]	0.14-0.19
Post-monsoon	0.33[b]	0.30-0.37	0.17[d]	0.19-0.21
Year				
2010	0.55[e]	0.51-0.59	0.54[g]	0.51-0.57
2011	0.53[e]	0.48-0.58	0.25[h]	0.14-0.19
2012	0.16[f]	0.12-0.20	0.04[h]	0.02-0.06

†*Cynomys gunnisoni* sampling seasons were April (spring), June (pre-monsoon), and August–September (post-monsoon)

[a-h]Shared letters within study areas indicate no significant differences in carbon isotope composition of *C. gunnisoni* plasma among time periods (overlapping 95% credible intervals for Bayesian estimates of proportion of C_3 plant carbon in animal tissues)
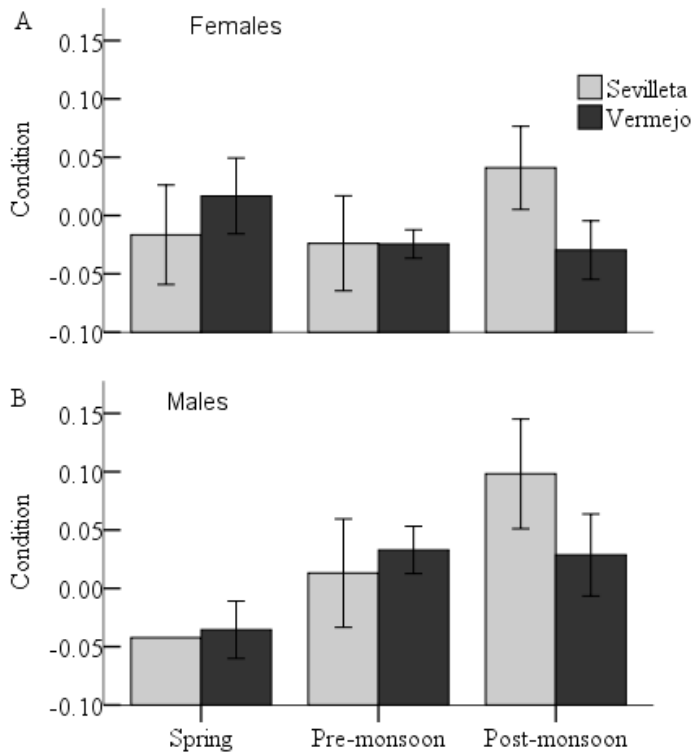
Figures

Figure 1. Mean body condition by season and study area for 298 female (A) and male

(B) *Cynomys gunnisoni* at Sevilleta National Wildlife Refuge and Vermejo Park Ranch.

Body condition indices were calculated as residuals from a mass-length regression

equation derived from measurements of captured *C. gunnisoni*. Error bars represent 95%

confidence intervals for mean condition indices by sex, site, and season
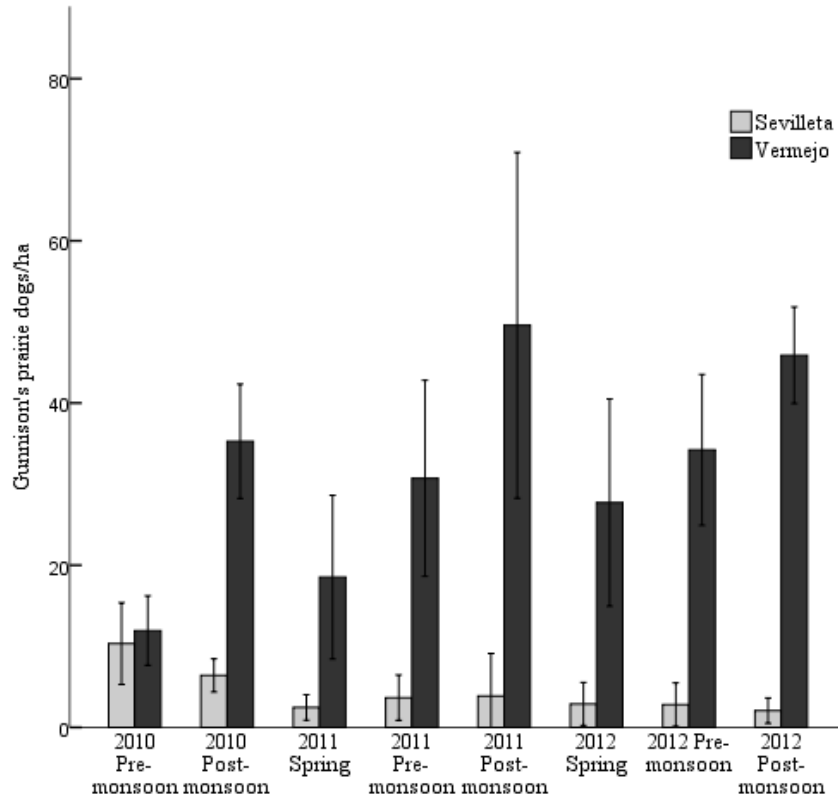
Figure 2. Mean densities of *Cynomys gunnisoni* observed on sampling plots at Sevilleta

National Wildlife Refuge (gray shading) and Vermejo Park Ranch (black shading). Error

bars represent 95% confidence intervals for seasonal density estimates
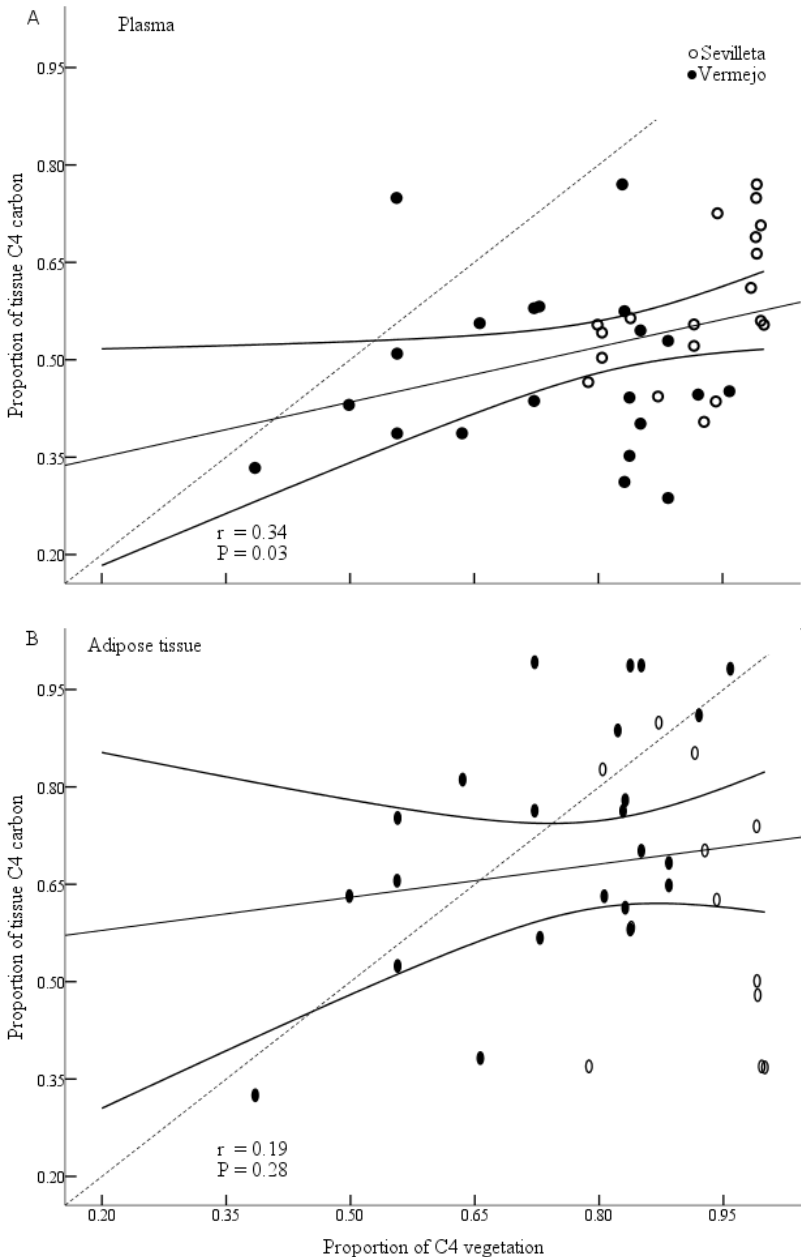
Figure 3. Proportional vegetative cover from C_4 plants on *Cynomys gunnisoni* plots versus the proportion of C_4 plant carbon present in (A) plasma and (B) adipose tissue at Sevilleta National Wildlife Refuge (open symbols) and Vermejo Park Ranch (closed symbols). Dashed lines represent 95% confidence intervals for the best fit regression line. Dotted lines indicate the identity function (slope = 1) where C_4 carbon assimilation in *C. gunnisoni* tissue is directly proportional to availability of C_4 vegetation

Figure 4. Relationship between λ_R (relative change in population size from reproduction) to mean body condition of adult female *Cynomys gunnisoni* (A) and change in adult female body condition from the previous season (B) on sampling plots at Sevilleta National Wildlife Refuge (open symbols) and Vermejo Park Ranch (closed symbols). Dotted lines represent the 95% confidence interval for the best fit regression line
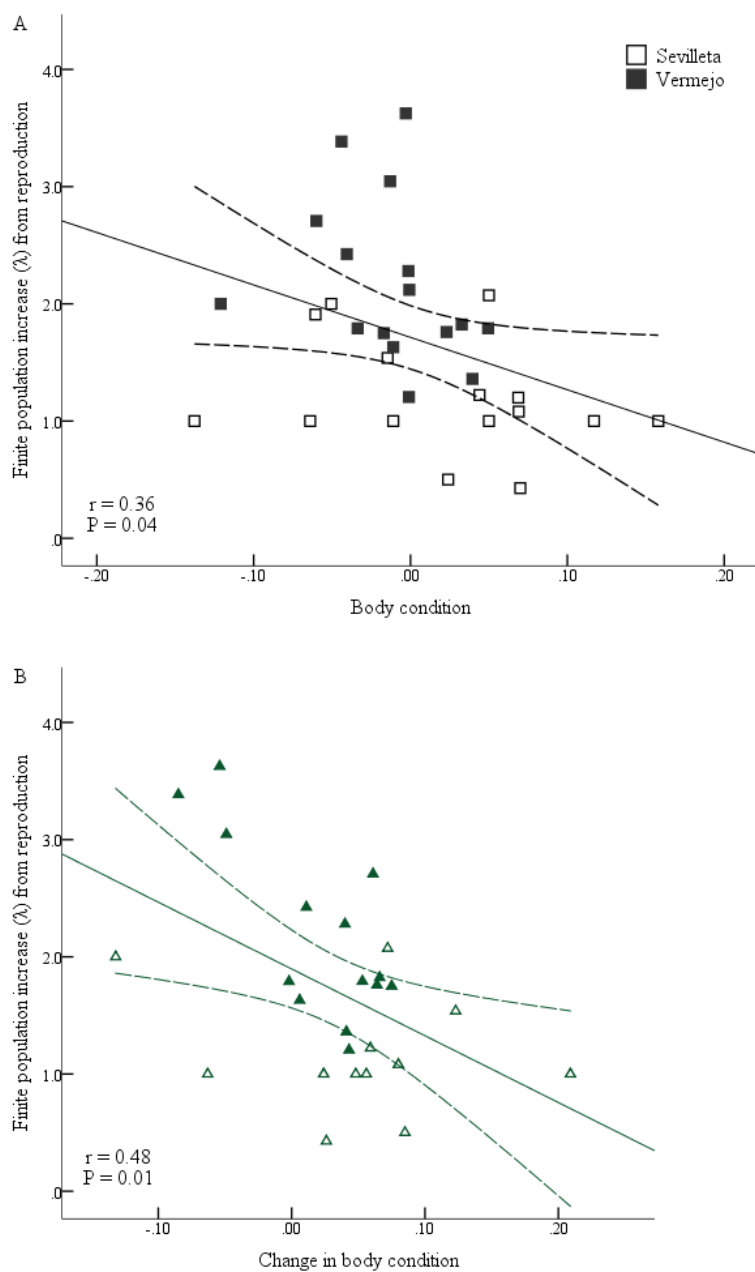
CHAPTER 3

Woodrat herbivory influences saguaro (*Carnegiea gigantea*) reproductive output

Abstract

The saguaro (*Carnegiea gigantea*) is a keystone resource for Sonoran desert consumers of nectar, pollen, fruit, and cactus tissues. Saguaro tissue contains oxalic acid and is unavailable to most consumers. The white-throated woodrat (*Neotoma albigula*) is, however, able to consume foods with high oxalate content, and is strongly associated with desert succulents, primarily cacti of the genus *Opuntia*. *Neotoma albigula* forages secondarily on saguaro tissues, reducing photosynthetic surface area and eliciting an energetically-demanding wound response that reduces energy stores available to fuel reproduction. We observed and quantified *Neotoma* herbivory on saguaros in a low desert environment. Evidence of *Neotoma* grazing was found on 44% of all saguaros surveyed, and 13% of all saguaros had >20% of their surface area affected by *Neotoma* grazing. *Neotoma* herbivory on saguaros was predicted by the number of nearby succulents, presence of *Neotoma* middens, and saguaro age. When comparing similarly sized plants, saguaros with high levels (>20% of surface) of herbivory produced fewer flowers and fruits than plants with no *Neotoma* herbivory. These findings suggest that periodic use of saguaros by *N. albigula*, such as during extended droughts with

conditions unfavorable for *Opuntia* growth and establishment, may reduce long-term reproductive capacities in low-density saguaro populations.

Keywords

Carnegiea gigantea; flower production; fruit production; *Neotoma albigula*; Sonoran desert

Introduction

The saguaro (*Carnegiea gigantea*) is a massive, long-lived columnar cactus that serves as a keystone resource providing breeding substrate, thermal shelter, and nutrient-rich flowers and fruits to Sonoran desert fauna including volant and non-volant mammals, birds*,* and insect consumers of pollen, nectar, and stem tissues (Fleming and Valiente-Banuet, 2002). Reproductive output in saguaro represents a substantial investment of plant energy and resources. Individual saguaros may produce hundreds of fruits, each with ~2250 seeds (Steenbergh and Lowe, 1977), a mass of >25 g, and water content of ~80% (Wolf et al., 2002). Fully hydrated cactus stem tissue may have a water content of $\geq$90%, which in the absence of defenses against herbivory would become highly attractive and vulnerable to desert herbivores (Gibson and Nobel, 1986).

Saguaro populations are limited by recruitment (establishment and regeneration) within portions of the species range (Drezner, 2006). Latitudinal and elevational limits of saguaro distribution are largely determined by catastrophic freezes and subsequent necrosis of stem tissues, particularly near the northern extent of its range (Steenbergh and Lowe, 1976). In contrast to mortality-limited populations, saguaro abundance in the highly-arid northwestern Sonoran desert is related to the frequency of moist, warm-season conditions that promote germination (Turner, 1990). Once saguaros reach a

reproductive maturity threshold (equivalent to 2.2 m in height), establishment of new seedlings is dependent upon the presence of monsoonal moisture conditions for germination (Steenbergh and Lowe, 1977), shading by nurse plants to prevent desiccation of seedlings (Turner et al., 1966), and survival from consumption and physical damage to seeds and seedlings (Niering et al., 1963).

Saguaro establishment occurs episodically at multi-decadal scales (Parker, 1993), with some cohorts being separated by 60 years or more (Drezner and Balling, 2002). Episodes of increased saguaro establishment often coincide with wetter periods, although this relationship becomes less consistent within the more freeze-limited (and less xeric) portions of the species' range (Pierson and Turner, 1998). Factors affecting saguaro recruitment therefore have a disproportionate effect on populations within the germination-limited portions of the species range, such as western Arizona (Drezner, 2008).

Although juvenile saguaros experience substantial mortality from rodent herbivory (Niering et al., 1963), adult cactus tissues are protected against herbivores by the plant's epidermis, rows of spines, and high oxalic content of tissues. The white-throated woodrat (*Neotoma albigula*) overcomes these obstacles to herbivory, and survives without free-standing water through the intake of preformed water from succulent food sources (Schmidt-Nielsen, 1964). *N. albigula* is able to degrade oxalates through the action of its intestinal microbes, and can thereby tolerate secondary plant compounds that may be toxic to other herbivores (Shirley and Schmidt-Nielsen, 1967). Desert-dwelling *Neotoma* shows strong associations with *Opuntia* cacti, and may exhibit numerical responses to changes in *Opuntia* availability (Brown et al., 1972). *Neotoma*

herbivory on saguaros represents use of a secondary food source when primary sources such as *Opuntia* are not readily available (Steenbergh and Lowe, 1977).

While conducting research near the northwestern extent of the Sonoran desert, we observed saguaros with extensive *Neotoma* herbivory. Where herbivory on saguaros occurs, a multi-layered, lignin-based callus tissue seals the wound and protects the plant's internal tissues against evaporative water loss, insect attacks, and necrosis form bacterial infection (Steelink et al., 1967). The wounding response reduces the surface area of photosynthetic tissues and total interception of photosynthetically-active radiation (PAR), which limits CO_2 uptake in cylindrical-stemmed cacti during much of the year (Nobel, 1977). Reduction in photosynthetically-active surface area from *Neotoma* herbivory thereby influences both short-term and long-term availability of energy resources for saguaro growth, maintenance, and reproductive output. Stem tissue damage by non-native herbivores has been documented in association with a >15% reduction in number of flowers and 8% fewer fruits in columnar cacti (Peco et al., 2011). In this study we evaluate the relationship between *Neotoma* herbivory and saguaro reproductive output, by: 1) quantifying the extent of herbivory on saguaros by *Neotoma*, and 2) testing whether herbivory on individual saguaros is associated with reduced flower and fruit production.

Materials and Methods

Site description

We established monitoring points for saguaro reproductive output at ≥50 saguaros at each of 3 transects in southwestern Arizona. Transect locations were at the edge of La Posa Plain, La Paz County, beginning at 425 m elevation; near Kofa Mountain foothills,

La Paz County, 475 m elevation; and near Table Top Mountain foothills, Pinal County, 500 m elevation. All transects occurred near the edge of the Lower Colorado River Valley subdivision of the Sonoran Desert, characterized by hot and arid conditions with low-density vegetation (Shreve, 1951) and saguaro populations with low demographic rates for both "births" (germination under moist and shaded conditions below nurse plants) and deaths (freeze-related mortality) (Drezner, 2006). Characteristic vegetation was low-density shrubs and trees including *Larrea tridentata*, *Ambrosia dumosa*, *Encelia farinosa*, and *Parkinsonia microphylla*, and cacti including *C. gigantea*, *Opuntia bigelovii*, and *Opuntia acanthocarpa*.

Saguaro survey protocols

We established transects 100 m in width, with length determined by quota sampling. Observers walked transects in a randomly-selected direction until 50 saguaros had been identified, and then walked slowly in the opposite direction while thoroughly searching for any additional saguaros that were not located during the first pass along the transect. At each saguaro we recorded plant height (using a graduated telescoping pole), plant diameter at a height of 1 m, number of arms ($\geq$30 cm or with reproductive structures), number of reproductive stems (arms plus the main stem if at a reproductively mature height), and the number of other cacti and *Neotoma* middens present within 10 m, a distance equal to 17–87% of the reported movement radius of *N. albigula* (Brown and Zeng, 1989; Chew and Chew, 1970). We calculated saguaro ages from site specific age–height and growth–precipitation regression equations developed by Drezner (2003).

We assessed *N. albigula* herbivory through a visual classification system to quantify the percentage of damaged plant surface tissues (Turner and Funicelli, 2000),

and a training set of 108 saguaros near the study area that were photographed from multiple directions. *Neotoma* herbivory exhibited characteristic grazing patterns, including circular staircases chewed into the outer stem tissue, long troughs or tunnels of surface tissue excavation, and/or presence of *Neotoma* feces within these excavated pathways (Figure 1). In contrast, tissue damage from lagomorph herbivory was confined to the basal portion of the stem, generally affected a greater width of stem tissue, and in some cases was accompanied by lagomorph feces near the base of the stem. We categorized surface impacts of herbivory (including contiguous areas of tissue necrosis or epidermal collapse) for each saguaro as no *Neotoma* herbivory, $\leq$20% of the surface affected, or >20% of the surface damaged by herbivory.

We conducted visual counts of reproductive structures (flowers and fruits, in any stage of development) present on transect saguaros during peak flowering and fruiting periods (mid-May and mid- to late-June, respectively) in 2008. Two observers using 10x binoculars counted flowers and fruits by standing on opposite sides approximately 10m away from the plant. Observers counted reproductive structures on each side of the stem, and communicated with each other to ensure against skipped or duplicate counts of reproductive structures. In 2009, we counted flower and fruit production on paired, mature saguaros (empirically determined to be $\geq$2.5 m tall) with no *Neotoma* herbivory and with >20% herbivory. Saguaros pairings matched plants on the same transect that had distinct (0 vs. >20%) levels of *Neotoma* herbivory, and were similar in height, age, and number of arms. When mature saguaros with 0 or >20% herbivory could not be paired with other saguaros on the transect, we extended transect surveys to complete

saguaro pairings and recorded information on new saguaros until ~15 ($\pm$3) pairs were
surveyed on each transect.

Statistical analyses

We constructed classification and regression trees (Breiman et al., 1984; Salford
Systems, 2006) to analyze relationships between the presence/absence of *Neotoma*
herbivory and environmental variables (middens and succulents present, saguaro age,
transect), and for analysis of 2008 saguaro reproductive output and plant attributes
(height, diameter, arms, surface herbivory, and transect). We determined variable splits
with the Gini impurity criterion for classification trees and sum of squares for regression
trees, and identified optimal trees from repeated cross-validations to find the smallest
trees whose model errors fell within 1 SE of the minimum error (De'ath and Fabricus,
2000). Variable importance scores reflected changes in misclassification associated with
each variable, expressed on a scale of 0–100 (Breiman et al., 1984). We calculated the
year of establishment by subtracting saguaro age from the year of observation, and
evaluated temporal uniformity of saguaro establishment using Kolmogorov–Smirnov
tests. We used Wilcoxon paired tests to determine whether 2009 flower and fruit
production in saguaros with no *Neotoma* herbivory was significantly increased (α =0.05)
relative to saguaros with >20% surface herbivory, and to test for differences between
reproductive output of individual plants between 2008 and 2009. Paired t-tests (two-
tailed, α =0.05) evaluated differences in all other attributes of paired saguaros (height;
number of reproductive stems, $\sqrt{x + 0.5}$ transformation; diameter; age) (R Development
Core Team, 2011).

Results

Saguaro attributes

We surveyed 158 saguaros on 3 transects in 2008. Saguaro densities on transects ranged from 3.0 to 8.3 plants/ha. Observed saguaros varied in height from 0.3 to 10.0 m. The shortest saguaros observed with flowers and fruits were 3.3 and 2.5 m tall, respectively, which corresponded to a minimum reproductive age of 83 years. The distribution of saguaro establishment was not uniform across years ($D = 3.2$, $P < 0.001$).

Herbivory occurred on 57% of all saguaros observed, and evidence of *Neotoma* herbivory was present on 44% of all saguaros. *Neotoma* herbivory affected 0 to >70% of surface area of individual plants. Thirteen percent of observed saguaros exhibited the highest category of surface herbivory impacts (>20%). The presence or absence of *Neotoma* herbivory on a saguaro was successfully predicted in 65% of all cases, using the number of other cacti present, saguaro age, and the number of *Neotoma* middens present as predictive variables (Figure 2). The predictive model had high specificity (81% of saguaros without herbivory correctly assigned) but relatively low sensitivity (45% correct assignment for plants with *Neotoma* herbivory). No saguaros containing >3 cacti present within 10 m exhibited any evidence of herbivory.

Reproductive output and herbivory

Mean counts of mature saguaro reproductive outputs were 33.1 flowers (range 0–165) and 23.2 fruits (range 0–111) per plant in 2008. Mature saguaros failed to produce flowers and fruits in 27.1% and 24.1%, respectively, of observed cases. Each classification and regression tree for presence/absence or total output of flower and fruit production identified plant height and the number of reproductive stems present as the

variables with the highest importance scores (Figure 3). Importance values for the *Neotoma* herbivory variable were relatively low, but non-zero (5.8–10.4), in the optimal trees for classifying or quantifying reproductive output. None of the optimal regression trees for quantifying or classifying presence of saguaro reproductive output included transect as a predictive variable.

We identified 46 pairs of structurally similar saguaros with distinct categories of herbivory (no *Neotoma* herbivory vs. >20% of surface affected) in 2009. Numbers of flowers and fruits on mature saguaros with no observed *Neotoma* herbivory increased significantly compared to paired saguaros with >20% herbivory (Table 1). No comparisons of other paired saguaro variables approached significance ($0.38 \leq P \leq 0.91$). For individual plants surveyed in both 2008 and 2009, no significant differences in flower ($W = 178$, $P = 0.424$) or fruit ($W = 182$, $P = 0.078$) production occurred between years.

Discussion

Deserts are water-limited systems that produce significant challenges for the plants and animals inhabiting those environments (Noy-Meir, 1973). Extended drought conditions in the Sonoran desert lead to the loss of plants that normally comprise the primary food sources for *N. albigula*, including *Opuntia* spp. and other small cacti (McAuliffe and Hamerlynck, 2010). Shifting of *N. albigula* grazing pressure to saguaro stems results in short-term consequences that are compounded through the life of the saguaro, as reduced surface for PAR interception limits the plant's ability to generate long-term energy stores. We found that *Neotoma* herbivory had significant effects on flower and fruit production of saguaros; counts of flowers and fruits were 31% and 42% lower on heavily-grazed versus ungrazed plants, respectively. Saguaro seeds germinate

only under environmental conditions that occur intermittently in space and in time: under nurse trees or shrubs, and often at multi-decadal scales (Parker, 1993; Pierson and Turner, 1998; Shreve, 1910). Saguaro establishment requires a sufficient seed supply to reach suitable or "safe" sites, and the presence of favorable ambient conditions for seed survival and germination (Andersen, 1989). Because saguaro seeds and seedlings are both subject to high mortality rates from herbivores and abiotic factors (Niering et al., 1963; Steenbergh and Lowe, 1977), decreases in seed output may translate to reduced magnitude of episodic recruitment pulses that are vital to the growth and maintenance of cactus populations. In the following discussion we consider implications to regional saguaro population demographics from the effects of *Neotoma* grazing on reproductive output, and project how increasing regional air temperatures and reductions in seasonal precipitation will affect the persistence of saguaros in the Sonoran desert.

Saguaro densities on our transects ranged from 13 to 36% of mean regional densities documented across the drier, northwestern portion of saguaro range, and were only 2–5% of mean densities where saguaros grow in conditions near their environmental optimum (Drezner, 2006). Saguaro densities were comparable to those reported by Brum (1973) at a low-density site where reproductive output was considered insufficient to prevent population declines. Low saguaro densities and delayed onset of reproduction are consistent with populations where infrequent monsoonal rains limit saguaro recruitment (Brum, 1973; Drezner, 2008). The observed non-uniformity of saguaro ages at our study sites further suggests that recruitment is linked to intermittent periods when conditions are favorable for saguaro establishment.

Assessing the impacts of *N. albigula* herbivory or other factors on reproductive output of saguaros must be viewed in the context of the established relationship between plant size and reproductive output (Parker, 1989; Peco et al., 2011; Schmidt and Buchmann, 1986). Increased plant height is indicative of saguaro age exceeding the maturity threshold, a developmental stage were branching may occur to provide additional reproductive surfaces, increased photosynthetic surface for PAR interception, and increased energy stores. Once saguaros reach a height indicating maturity, they are generally successful in translating energy stores to reproductive output, even during adverse environmental conditions such as drought (Steenbergh and Lowe, 1977; Thackery and Leding, 1929). Our results similarly identified plant height as the strongest predictor of reproductive output among all saguaros surveyed, while comparisons of paired, morphologically-similar plants allowed us to isolate the negative effects to saguaro reproductive output associated with the permanent loss of photosynthetic surface area from *Neotoma* herbivory.

Observed saguaro reproductive output was substantially lower than previously reported (Niering et al., 1963, Schmidt and Buchmann, 1986; Steenbergh and Lowe, 1977; Thackery and Leding, 1929). These differences may be largely methodological, as we did not obtain complete counts of reproductive output throughout the season, but instead quantified reproductive structures present at single visits during peak flowering and fruiting periods. Although direct comparisons of reproductive output among studies are precluded, our study sties exhibited a frequency of mature-sized plants lacking reproductive structures and a delayed timing to maturity that was characteristic of saguaros in marginal habitats undergoing an apparent population decline (Brum, 1973).

The observed reduction in reproductive output from saguaros with high (>20%) levels of

herbivory translates to an estimated loss of 0.48 kg of fruit (containing 0.36 kg of water)

and 41,625 seeds produced per plant annually.

Neotoma herbivory on saguaros was observed regularly at our study sites, and

was associated with presence of nearby *Neotoma* middens (active or currently

unoccupied), lower numbers of other succulents that may serve as primary food and

water sources for N. *albigula*, and saguaro age. *Neotoma* is precluded from subsisting

entirely on non-succulent desert vegetation due to species' water requirements and the

need to avoid potential toxic effects from ingested plant compounds (Karasov, 1989).

Loss of *Opuntia* from the home range of a *Neotoma* requires the animal to abandon its

home range, or find more drought-resistant sources of succulent food to replace *Opuntia*

in its diet. The massive saguaro is one of the few plant species that can survive and

provide a moisture-rich food source under conditions where *Opuntia* may not be able to

persist. Saguaros may experience establishment peaks and/or population increases over

extended periods (>100 years) when other desert plant species exhibit climate-related

population declines (Turner, 1990). Saguaros are the plants most likely to be affected by

diet switching and *N. albigula* herbivory during periodic extended droughts, and may

experience reversed or disrupted mutualistic interactions with grazers that normally serve

as seed dispersers (McCluney et al., 2012). The magnitude of these cascading drought-

related impacts from native herbivore diet-switching exceeds observed depressions in

reproductive output associated with columnar cacti stem tissue damage from multiple

introduced herbivores (Peco et al., 2011).

Climate modeling for the southwestern United States projects increases in mean annual temperatures of $\geq 3^{\circ}C$ by the end of the century, along with decreases in mean annual precipitation (IPCC, 2007). Climate projections for the southwestern United States include disproportionate seasonal decreases in precipitation and moisture conditions during winter (Christensen et al. 2004). Extended drought periods, particularly during winter, hinder the establishment of new *Opuntia* plants (Bowers 2005). The most severe impacts of climate change on Sonoran desert *Opuntia* are expected where *Opuntia* and other cacti currently exist at low densities or under sub-optimal conditions. If future climate conditions are insufficient for *Opuntia* establishment rates to replace mortality of adult plants, our results predict increased frequency of *Neotoma* grazing on saguaro tissues, which is associated with substantial reductions in reproductive output. Diminished saguaro reproductive output reduces the availability of energy and moisture from flowers and fruits that are important resources for Sonoran desert fauna, and has potential demographic implications for saguaro populations.

Acknowledgments

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Tables

Table 1. Characteristics of paired saguaros with no woodrat herbivory and with high (>20%) levels of surface tissue damage.

Saguaro attributes	Mean plant values			Signif.[a]
	No *Neotoma* Herbivory (h_0)	>20% surface herbivory ($h_{>20}$)	Difference ($\bar{x}_{h0} - \bar{x}_{h>20}$)	
N	46	46		
Flowers (n observed)	38.1	26.3	11.7	0.026
Fruits (n observed)	43.7	25.2	18.5	0.008
Height (m)	5.4	5.4	0.02	0.91
Diameter (cm)	39.0	38.9	0.1	0.88
Reproductive Stems[b] (n)	2.0	2.2	-0.2	0.38
Age (years)	129.1	129.3	-0.2	0.91

[a] *P*-values for tests comparing variables between paired plants: $P(h_0 \leq h_{>20})$, Wilcoxon pairs test, for counts of flowers and fruits; $P(\bar{x}_{h0} = \bar{x}_{h>20})$, paired *t*-test, for height, diameter, arms, and age of paired saguaros.

[b] Untransformed values. Transformed values ($\sqrt{x + 0.5}$) were used to meet assumptions of the paired *t*-tests for differences in attributes between saguaro pairs.

Figures

a)

b)

Figure 1. (a) Extensive damage to saguaro from *Neotoma* grazing on succulent stem

tissues. *Neotoma albigula* herbivory reduces the photosynthetic surface area of the plant

and generates a wound response that is necessary to protect grazed saguaro tissues from

desiccation, freezing, and infection of exposed tissue. (b) Damage from *N. albigula*

herbivory is characterized by staircase-like patterns around the stem where feces from the
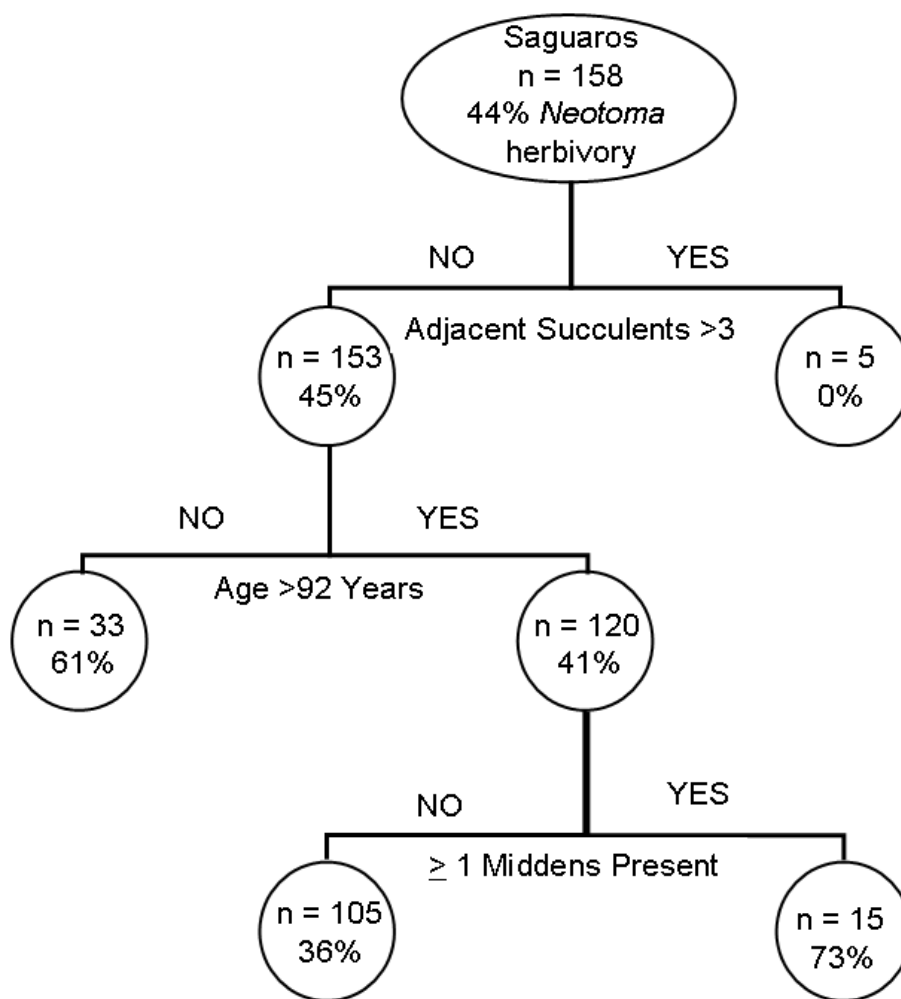
grazing rodents may be found.

Figure 2. Classification tree showing predictive factors for the presence/absence of observed *Neotoma* grazing on saguaros. The branch-defining condition is labeled at each split in the tree, and the number of plants and percentage with *Neotoma* herbivory are given at each node.
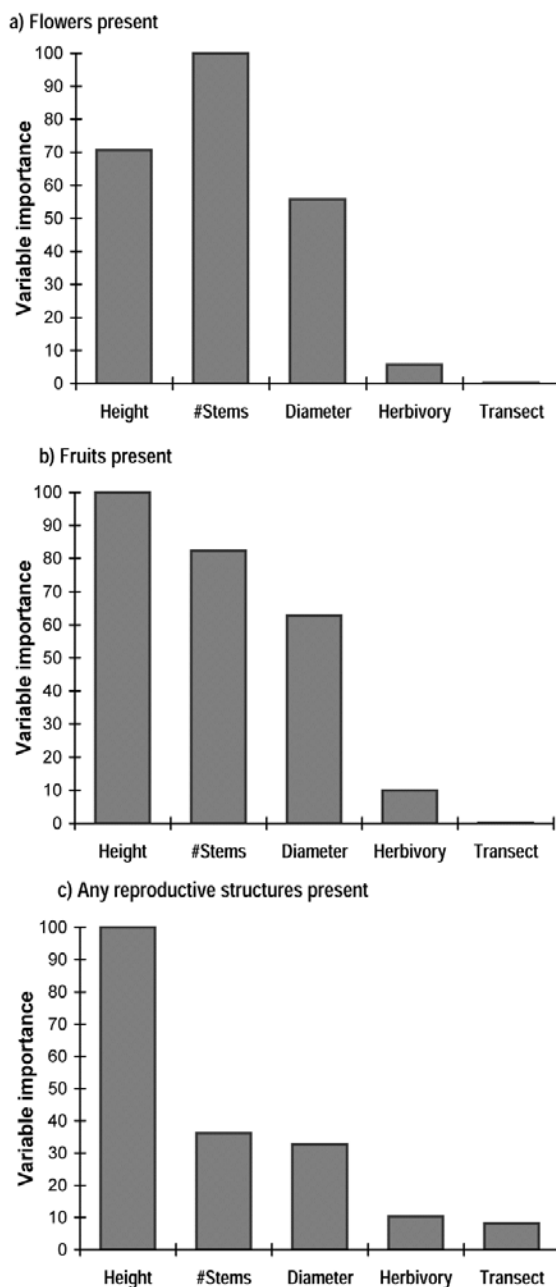
Figure 3. Relative importance values for variables used to predict presence/absence of saguaro (a) flowers, (b) fruits, and (c) any reproductive structures (flowers or fruits). Variables included: total height of the plant (*Height*), plant diameter at 1 m above ground (*Diameter*), the number of arms plus stems above the maturity threshold (*#Stems*), the extent of surface impacts of herbivory, by category (*Herbivory*), and the transect number for each plant (*Transect*).

CONCLUSION

Arid environments challenge producers and consumers through resource scarcity, extreme conditions, and inconsistent or unpredictable pulses of resource availability. Biotic interactions of consumer populations further influence the composition and dynamics of ecological communities within these arid ecosystems. In this dissertation I used emerging stable isotope techniques to track energy flow from producers to primary consumers, and monitored individual and population-level parameters associated with consumer energy assimilation patterns. Research on Gunnison's prairie dog (*Cynomys gunnisoni*) elucidated effects of persistent drought on resource utilization by this grassland herbivore, which subsists on relatively low-energy diets characterized by leafy herbaceous material. Isotopically-indexed dietary niche widths decreased during drought conditions that suppress productivity of cool-season vegetation, including forbs and grasses utilizing the C_3 photosynthetic pathway. These findings portray *C. gunnisoni* as a generalist forager that opportunistically expands its dietary niche to include moisture-limited vegetation, such as C_3 plants, when they become available. Plants with C_3 photosynthesis are hypothesized to be preferred food sources for primary consumers based on increased digestibility and nitrogen content relative to C_4 plants, and have been shown to increase abundance and growth rates of grasshoppers and other insect consumers. This dissertation documents changes in herbivore dietary niche widths during multi-year periods of variable C_3 plant productivity, and links energy assimilation from C_3 plants to increased abundance of *C gunnisoni* juveniles. By combining data regarding uptake of C_3 plant material and changes in demographic parameters of

vertebrate consumers, this research provides some of the strongest empirical support to date for the C_3 hypothesis.

The relationships among C_3 plant productivity, dietary niche widths, and *C. gunnisoni* reproductive output beg the question of mechanistic links between forage quality and reproductive success. Individual reproductive success correlates with parental body condition in a diversity of mammals, including multiple species of prairie dogs. I used isotopic signatures in tissues to test whether *C. gunnisoni* would utilize increased C_3 plant resources when available, and whether populations assimilating increased quantifies of preferred energy resources would achieve improved body condition and greater reproductive output. Although *C. gunnisoni* appeared the select C_3 plants, proportions of C_3/C_4 plant contributions to isotopic tissue composition did not explain *C. gunnisoni* body condition, and increases in mean body condition were not associated with higher-density populations. Instead, *C. gunnisoni* plots with greater reproductive output exhibited reduced body condition, suggesting a trade-off between maintenance of parental condition and energy investment in reproduction. Use of internal energy stores to fuel reproduction (capital breeding) does not appear to be a successful population-level strategy for *C. gunnisoni*. Although reserves of adipose tissue are necessary for survival of *C. gunnisoni* during winter hibernation, reproductive success correlates with assimilation of high-quality forage resources stimulated by growing conditions during periods of *C. gunnisoni* fetal and juvenile growth, and suggests an income energy breeding strategy.

To evaluate implications of plant-herbivore interactions resulting from actions of arid-land consumers, I quantified impacts of herbivory to the Sonoran Desert's iconic

saguaro cactus (*Carnegiea gigantea*). Consumption of saguaro tissue by white-throated woodrats (*Neotoma albigula*) leaves permanent scars on saguaro tissue, thereby reducing photosynthetic surface area that is already limited by the plant's structural adaptations for reduced water loss. Saguaros with high levels of scarring from *N. albigula* herbivory produced fewer flowers and fruits, which provide vital resources for biological consumers during hot and dry periods in the Sonoran Desert. Prevalence of *N. albigula* herbivory on saguaros was inversely related to the presence of other succulents, primarily cholla cacti of the genus *Cylindropuntia*. Desert populations of *N. albigula* show strong associations with *Cylindropuntia*, but may shift foraging to longer-lived saguaros when extended droughts inhibit establishment and reduce densities of the smaller cholla species. Resulting impacts to saguaros and their consumers thereby persist long after drought conditions affecting dynamics of *Cylindropuntia* and woodrats have subsided.

Findings of this dissertation express a series of consistent themes that are relevant to research and management of ecological systems. First, plant-herbivore interactions are complex, and have biological consequences extending beyond a single species feeding upon another. Abiotic inputs and biotic interactions influence keystone resources, which can generate cascading effects to ecological communities. Second, responses to resource scarcity in arid environments may be evidenced well after periods of stress-inducing conditions have ended. Effects to consumer populations can be amplified or extended through energy allocation decisions based on stimuli that project risk to future survival and recruitment, or through physical modifications to long-lived primary producers. Finally, complexities and legacy effects from producer-consumer interactions warrant caution in the ability to replicate or restore perturbed ecological systems and functions.

Changes to producer and consumer populations from biotic or abiotic drivers that include anthropogenic development and climate change cannot be completely foreseen in future no-analog communities, nor can they be immediately reversed following removal of perturbations. Despite our improved understanding of biological processes gained through ecological research conducted over recent decades, guidance from Aldo Leopold's essays on conservation from the 1930s remain prescient today: "If the biota, in the course of aeons, has built something we like but do not understand, then who but a fool would discard seemingly useless parts? To keep every cog and wheel is the first precaution of intelligent tinkering."