

Negative impacts of invasive plants on conservation of sensitive desert wildlife

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Abstract. Habitat disturbance from development, resource extraction, off-road vehicle use, and energy development ranks highly among threats to desert systems worldwide. In the Mojave Desert, United States, these disturbances have promoted the establishment of nonnative plants, so that native grasses and forbs are now intermixed with, or have been replaced by invasive, nonnative Mediterranean grasses. This shift in plant composition has altered food availability for Mojave Desert tortoises (*Gopherus agassizii*), a federally listed species. We hypothesized that this change in forage would negatively influence the physiological ecology, immune competence, and health of neonatal and yearling tortoises. To test this, we monitored the effects of diet on growth, body condition, immunological responses (measured by gene transcription), and survival for 100 captive Mojave tortoises. Tortoises were assigned to one of five diets: native forbs, native grass, invasive grass, and native forbs combined with either the native or invasive grass. Tortoises eating native forbs had better body condition and immune functions, grew more, and had higher survival rates (>95%) than tortoises consuming any other diet. At the end of the experiment, 32% of individuals fed only native grass and 37% fed only invasive grass were found dead or removed from the experiment due to poor body conditions. In contrast, all tortoises fed either the native forb or combined native forb and native grass diets survived and were in good condition. Health and body condition quickly declined for tortoises fed only the native grass (*Festuca octoflora*) or invasive grass (*Bromus rubens*) with notable loss of fat and muscle mass and increased muscular atrophy. *Bromus rubens* seeds were found embedded in the oral mucosa and tongue in most individuals eating that diet, which led to mucosal inflammation. Genes indicative of physiological, immune, and metabolic functions were transcribed at lower levels for individuals fed *B. rubens*, indicating potential greater susceptibility to disease or other health-related problems. This study highlights the negative indirect effects of invasive grasses, such as red brome, in desert ecosystems, and provides definitive evidence of a larger negative consequence to health, survival, and ultimately population recruitment for Mojave Desert tortoises than previously understood.

Key words: annual plants; *Bromus rubens*; diet; gene transcription; *Gopherus agassizii*; habitat disturbance; immune function; invasive; Mojave Desert; Mojave Desert tortoise; nutrition.

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INTRODUCTION

Habitat disturbance and human land use have degraded desert habitats worldwide. Disturbances from military activities, off-road vehicle use, energy and urban development, and increased wildfire frequency and intensity have directly impacted desert habitats, changing the composition of plants in desert ecosystems around the globe (Brooks et al. 2004, Rahlao et al. 2009, Gioria and Pyšek 2016, Thomas et al. 2016). Nonnative grasses are now common invaders that cause irreversible alterations to ecosystem structure and function and fire regimes in many arid regions (D'Antonio and Vitousek 1992). In the Mojave Desert, United States, invasive Mediterranean grasses such as *Bromus* spp. have dramatically increased (Brooks 1999, DeFalco et al. 2003, Salo 2005, Drake et al. 2015), altering wildfire regimes (Brooks et al. 2004, Brooks and Matchett 2006), changing community dynamics (Billings 1990, D'Antonio and Vitousek 1992), impeding perennial shrub establishment (Rinella et al. 2015), and reducing the structural stability of soils (Soulard et al. 2013). *Bromus* grasses in the southwestern United States have been linked to declines in species richness and abundance in small mammals (i.e., cheatgrass, Litt and Pearson 2013), birds (Earnst and Holmes 2012), and lizards (Steidl et al. 2013), causing physical injury to livestock and other wildlife foraging on them (Crampton 1974, McCray and Bloom 1984, Currie et al. 1987, Bossard et al. 2000, Medica and Eckert 2007). Even with this information, few studies have quantified the effects of *Bromus* spp. and other introduced species on the nutrition or health of wildlife (Batzli 1986, Nagy et al. 1998, Hazard et al. 2009, 2010, Gray and Steidl 2015).

Changes in plant communities can have substantial impacts on herbivore populations, as available forage and nutrition are major drivers of animal health and survival. Nutrient-rich food bolsters healthy immune systems and resources needed for growth, resistance to disease, and other key functions, whereas malnutrition often limits growth (Huitu et al. 2003), reduces reproductive output (Henen 1997, 2002, Cook et al. 2004), causes immune deficiencies, and increases susceptibility to infection and other health-related effects (Gregor and Hotamisligil 2011, Schlaudecker et al. 2011, MacIver et al. 2013, Saucillo et al. 2014).

Acquiring adequate nutrition is particularly challenging for herbivores in desert environments where low primary plant productivity is often compounded by limited water availability and by high concentrations of electrolytes during the dry season (Minnich and Shoemaker 1970, Nagy and Medica 1986, Peterson 1996a, b).

The Mojave Desert tortoise (*Gopherus agassizii*) is a medium-sized herbivorous reptile that occurs throughout the Mojave Desert in habitats invaded by nonnative plants. It is currently listed as a threatened species under the US Endangered Species Act and suffers from continued habitat degradation and loss and other human-related impacts that can cause direct tortoise mortality and can negatively affect desert plant communities (USFWS 1994, 2011, Tracy et al. 2005). Tortoises acquire sufficient energy, protein and amino acids, minerals, vitamins, water, and other components from selected food plants and plant parts when available, with the majority of energy coming from carbohydrates and through fermentation of fiber in the plants they eat (Bjorndal 1987). Tortoises modify their activity and feeding behavior relative to periods of plant growth, climatic and environmental conditions, and their own physiology. They select food species based on individual preference (Esque 1994), requirements for nutritive quality (Ofstedal 2002, but see Tracy et al. 2006), mineral content (Nagy and Medica 1986, McArthur et al. 1994, DeFalco 1995), and the phenological condition of the plants (Esque et al. 2014, Jennings and Berry 2015).

The general forage preferences and nutritional ecology of tortoises and other North American tortoise species have been well studied (reviewed in Esque et al. 2014, Jennings and Berry 2015). In undisturbed systems, the majority of their diet consists of a mix of native annual forbs and grasses, and herbaceous perennial shrubs (Esque 1994, DeFalco 1995, Nagy et al. 1998, Jennings 2002, Van Devender et al. 2002, Tracy et al. 2006, Esque et al. 2014, Jennings and Berry 2015). Of these, spring annual native forbs and grasses are believed to be the most important food sources in most years (Jennings 2002, Esque et al. 2014, Jennings and Berry 2015), although the role of summer annual plants in Mojave Desert tortoises has not been substantiated (Esque et al. 2014).

Because of the prevalence of surface disturbances and increased wildfire frequency in the

Mojave Desert, tortoise habitat is often dominated by invasive *Bromus* grasses, with *B. rubens* widespread throughout the eastern Mojave Desert (Brooks 1999). Despite the wealth of knowledge on tortoise diet, limited information is available on how invader-induced shifts in vegetation composition influence the physiology, immune function, and health of tortoises and other herbivorous reptiles (Boarman 2002). To quantify these shifts in plant-based diets on the overall health of Mojave Desert tortoises, we studied captive neonate and yearling tortoises that were fed one of five dietary treatments (an invasive grass, a native grass, a mixture of four native forbs, and mixtures of the grasses with native forb diets) at a captive research facility in Clark County, Nevada, United States.

We hypothesized that a reduction in available energy and nutrients and increased fiber typically found in grass-dominated diets (McArthur et al. 1994) combined with physical mucosal damage due to *Bromus* impactions (Medica and Eckert 2007) would impede growth and immune competency as measured by gene transcription in juvenile tortoises. Specifically, genes and immune pathways that regulate growth and protein synthesis (Cathepsin L [CL]; Zhou et al. 2008), calcium metabolism (Calmodulin [CaM]; Li et al. 2004), and overall nutritional and neuroendocrine functions (Leptin; Otero et al. 2005; Appendix S1: Table S1) would likely be lower for individuals foraging solely on grass diets with limited nutrients available (Kilberg et al. 2005, Bowen et al. 2014, 2015a, b). Here, we provide the first comprehensive evidence of the impacts of nonnative grasses on desert tortoises using an integrated assessment method that includes direct measures of forage nutrition content, growth, clinical condition, immune function, and survival. Our integrated assessment method improves health monitoring for this species of concern by combining physical and molecular health metrics (Acevedo-Whitehouse and Duffus 2009, Bowen et al. 2015a, b).

METHODS

Diet treatments

We created five different diets that were fed to 100 captive tortoises. Tortoises were assigned to one of five diets: invasive grass, native grass, native forbs, and native forbs combined with either the

invasive or native grass. We used the invasive non-native annual Mediterranean grass *Bromus rubens* (red brome), a native annual grass *Festuca octoflora* (six-week fescue), and a mixture of four native annual forbs *Camissonia claviformis* (brown-eyed evening primrose), *Eschscholzia californica* (California poppy), *Malacothrix californica* (desert dandelion), and *Plantago ovata* (indian wheat); treatments hereafter will be referred to as *Bromus*, *Festuca*, forbs, *Bromus* and forbs, and *Festuca* and forbs, respectively. Diets were grown in a glasshouse using grass seed collected from natural areas in Clark County, Nevada, United States, and forb seed obtained commercially from Comstock Seed (Gardnerville, Nevada, USA). The plant seeds for each diet were mixed and grown in 19-L pots filled with a 3:1 mix of coarse sand to mulch and maintained in a glasshouse near ambient conditions.

Study animals and experimental design

Neonate (approximately 0.5 yr) and yearling (approximately 1.5 yr) Mojave desert tortoises, collectively referred to as juveniles, were chosen as the focus of this study because they grow rapidly and were more likely than adults to respond to differences in diet over the span of the research project (Morafka et al. 2000). Dietary influences may be more severe for juveniles than adults, thus reducing survival rates for this threatened species. For this study, we used both neonate ($n = 66$) and yearling ($n = 34$) tortoises to increase the sample size, statistical power, and detection of potential dietary effects. We acquired tortoises by incubating eggs collected from captive gravid adult females in Clark County, Nevada, United States, in 2011 and 2012. Immediately after hatching, tortoises were housed in 15 outdoor pens (2.23 m²) within a predator-proof enclosure. Each pen included an artificial burrow, a native shrub, and a shallow water dish that was filled three times per week (Fig. 1). Tortoise cohorts within pens were randomly assigned to one of the five diet treatments, providing three replications of each diet. Six to seven tortoises were assigned to each pen using a stratified random design to minimize cooccurrence of related individuals and even distributions of neonate and yearling age classes within each pen.

Prior to the experiment, all tortoises were fed a commercial diet (Natural Grassland Tortoise Food; ZooMed, San Luis Obispo, California,



Fig. 1. (Left) Photograph of a typical 2.23-m² (4' by 6') enclosure housing six to seven neonate and yearling Mojave Desert tortoises (*Gopherus agassizii*). Enclosures include a nonforage shade bush, burrow, water basin, and two forage pots that were replaced weekly. (Right) Photograph of juvenile tortoises foraging on the native grass (*Festuca octoflora*).

USA) specifically designed for tortoises twice per week during months of activity. Starting 1 April 2013, forage was supplied ad libitum to tortoises by placing two pots of the selected diet into recessed holes within each pen such that the plants were flushed with the ground (Fig. 1). Pots were replaced weekly with stock from the glasshouse through late July 2013. We conducted all handling and experiments using animals according to Institutional Animal Care and Use Committee guidelines (U.S. Geological Survey WERC 2012-03 and University of California-Davis WERC-2007-02) and under the appropriate state (Nevada Division of Wildlife Permit #S33762) and federal (U.S. Fish and Wildlife Service TE-030659) permits.

Plant nutrients

Representative plant samples ($n=6$) of *Bromus*, *Festuca*, and forbs were clipped at ground level

three times during the study: early, when all plants were green (April 19); midway through the experiment and after grasses were dry, but forbs remained green (June 13); and late, when all plants were dry and senesced (July 12). Forage samples were analyzed by Chandler Analytical Laboratories (Chandler, Arizona, USA) for moisture content, fat, crude protein (CP), nonstructural carbohydrates (NSC), neutral detergent fiber (NDF), acid detergent fiber (ADF), total digestible nutrients (TDN), total mineral content (Ash), calcium (Ca), chlorine (Cl), potassium (K), magnesium (Mg), sodium (Na), phosphorus (P), sulfur (S), copper (Cu), iron (Fe), manganese (Mn), molybdenum (Mo), and zinc (Zn). The mean and standard error of each analyte were calculated for each sampling period (Table 1). Data were collected on a dry weight basis, except for moisture content. Statistical analyses, including analysis

Table 1. Plant analyses for nutrient and fiber contents for the invasive nonnative grass (*Bromus rubens* or *Bromus*), a native grass (*Festuca octoflora* or *Festuca*), and a mixture of four native annual forbs (*Cammissonia claviformis*, *Eschscholzia californica*, *Malacothrix californica*, and *Plantago ovate* or forbs).

Plant analyte	Abbr.	April			June			July		
		<i>Bromus</i>	<i>Festuca</i>	Forbs	<i>Bromus</i>	<i>Festuca</i>	Forbs	<i>Bromus</i>	<i>Festuca</i>	Forbs
Wet mass	Wet (g)	87.75 (13.24)	63.83 (5.91) ^b	159.30 (8.88) ^c	35.66 (3.35)	47.90 (9.06) ^b	322.16 (50.09) ^c	0.00 (NA)	0.00 (NA)	0.00 (NA)
Dry mass	Dry (g)	24.79 (3.52)	13.03 (2.63) ^b	27.76 (2.61)	33.11 (3.16)	28.87 (2.92) ^b	60.98 (4.69) ^c	24.85 (2.23) ^a	16.37 (0.63) ^b	30.18 (2.15)
Moisture	Moist (%)	71.51 (0.99) ^a	80.30 (2.22)	82.66 (1.17) ^c	10.53 (0.92) ^a	36.45 (5.14) ^b	79.89 (2.98) ^c	7.38 (0.12)	7.59 (0.14) ^b	36.07 (10.45) ^c
Ash	Ash (%)	11.41 (0.18) ^a	13.50 (0.61) ^b	19.70 (0.73) ^c	13.38 (0.32) ^a	9.81 (0.64) ^b	16.40 (0.70) ^c	16.35 (0.28) ^a	12.63 (0.49) ^b	15.19 (0.60)
Neutral detergent fiber	NDF (%)	53.55 (0.68) ^a	48.39 (1.52) ^b	29.76 (1.60) ^c	58.89 (0.52)	57.91 (0.64) ^b	29.85 (1.47) ^c	55.82 (0.60)	54.57 (1.32) ^b	41.08 (1.20) ^c
Acid detergent fiber	ADF (%)	27.60 (0.79)	25.78 (1.02) ^b	21.56 (0.68) ^c	30.32 (0.66)	31.92 (0.49) ^b	24.26 (1.19) ^c	29.80 (0.60)	28.45 (1.22)	30.97 (1.05)
Crude protein	CP (%)	18.78 (0.51) ^a	28.81 (2.28) ^b	22.83 (1.00) ^c	15.59 (0.38)	13.89 (0.71) ^b	21.09 (1.49) ^c	15.80 (0.72)	15.50 (0.88) ^b	18.30 (0.63) ^c
Nitrogen	N (%)	3.01 (0.08) ^a	4.61 (0.36) ^b	3.65 (0.16) ^c	2.49 (0.06)	2.22 (0.11) ^b	3.37 (0.24) ^c	2.53 (0.12)	2.48 (0.14) ^b	2.93 (0.10) ^c
Fat	Fat (%)	2.80 (0.10)	2.81 (0.06)	2.81 (0.09)	1.30 (0.06) ^a	2.25 (0.12) ^b	3.55 (0.21) ^c	1.66 (0.09) ^a	2.26 (0.10) ^b	2.72 (0.11) ^c
Calcium	Ca (%)	1.24 (0.10)	1.24 (0.05) ^b	2.34 (0.13) ^c	1.61 (0.05) ^a	1.13 (0.10)	1.46 (0.22)	2.01 (0.09) ^a	1.63 (0.05)	1.95 (0.13)
Phosphorus	P (%)	0.30 (0.02) ^a	0.43 (0.03) ^b	0.31 (0.02)	0.23 (0.01) ^a	0.21 (0.01) ^b	0.34 (0.03) ^c	0.24 (0.01) ^a	0.19 (0.01) ^b	0.24 (0.02)
Calcium to phosphorus ratio	CA:P	4.34 (0.59)	2.95 (0.12) ^b	7.84 (0.85)	7.05 (0.48) ^a	5.41 (0.49)	4.34 (0.75) ^c	8.38 (0.41)	8.62 (0.43)	8.20 (0.47)
Magnesium	Mg (%)	0.34 (0.02)	0.35 (0.01) ^b	0.52 (0.03) ^c	0.45 (0.02)	0.36 (0.08)	0.48 (0.03)	0.55 (0.02) ^a	0.40 (0.01) ^b	0.60 (0.05)
Sulfur	S (%)	0.28 (0.01)	0.37 (0.01) ^b	0.61 (0.02) ^c	0.67 (0.04) ^a	0.39 (0.02)	0.41 (0.05) ^c	0.70 (0.04) ^a	0.50 (0.03)	0.48 (0.05) ^c
Potassium	K (%)	2.90 (0.24)	3.70 (0.10)	4.20 (0.21) ^c	2.05 (0.10) ^a	1.67 (0.32) ^b	4.36 (0.37) ^c	2.27 (0.10) ^a	1.81 (0.06) ^b	3.52 (0.19) ^c
Sodium	Na (%)	0.18 (0.02) ^a	0.12 (0.02) ^b	0.69 (0.04) ^c	0.85 (0.06) ^a	0.47 (0.05)	0.37 (0.10) ^c	0.98 (0.06)	0.89 (0.09) ^b	0.37 (0.05) ^c
Chloride	Cl (%)	2.32 (0.24)	2.03 (0.06) ^b	2.50 (0.06)	2.92 (0.13) ^a	2.02 (0.07)	1.62 (0.19) ^c	3.29 (0.11) ^a	2.28 (0.28) ^b	1.25 (0.10) ^c
Iron	Fe (ppm)	90.83 (15.25) ^a	288.68 (39.0)	256.6 (18.85) ^c	62.83 (3.47) ^a	148.00 (25.37)	153.40 (19.58) ^c	70.27 (3.90)	90.24 (11.68)	112.62 (9.56) ^c
Manganese	Mn (ppm)	26.14 (1.23) ^a	37.27 (1.94) ^b	49.42 (4.45) ^c	18.57 (0.68) ^a	15.07 (0.92) ^b	52.24 (7.79) ^c	19.76 (1.15)	20.10 (1.45) ^b	38.74 (1.44) ^c
Copper	Cu (ppm)	8.35 (0.40) ^a	15.16 (0.98) ^b	8.61 (0.39)	6.51 (0.20) ^a	7.53 (0.30)	8.64 (0.46) ^c	6.16 (0.23)	6.26 (10.28) ^b	5.02 (0.24) ^c
Zinc	Zn (ppm)	54.84 (2.09) ^a	93.05 (2.44)	120.07 (11.01)	56.67 (1.26)	61.60 (3.54) ^b	186.38 (29.40) ^c	48.33 (1.84)	44.50 (2.64) ^b	133.68 (15.07) ^c
Molybdenum	Mo (ppm)	4.80 (0.23)	4.76 (0.42) ^b	2.36 (0.10) ^c	4.81 (0.37) ^a	3.66 (0.29)	2.95 (0.41) ^c	5.29 (0.25)	5.59 (0.44) ^b	2.19 (0.19) ^c
Total digestible nutrient	TDN	69.48 (0.45) ^a	72.92 (1.01) ^b	85.35 (1.07) ^c	65.92 (0.35)	66.57 (0.42) ^b	85.29 (0.98) ^c	67.83 (0.30)	69.29 (1.03)	81.05 (NA)
Nonstructural carbohydrates	NSC	13.46 (0.54) ^a	6.49 (1.54) ^b	24.89 (1.16) ^c	10.84 (0.27) ^a	16.14 (0.79) ^b	29.11 (1.14) ^c	10.38 (0.27) ^a	15.05 (0.63) ^b	22.71 (0.34) ^c

Notes: Six plant samples were collected from each group on 19 April, 13 June, and 12 July 2013. Values represent the mean and standard error for each plant analyte. Within each sampling period, differences ($P \leq 0.05$) between groups are noted with superscripted letters (*Bromus* and *Festuca*^a, *Festuca* and forbs^b, *Bromus* and forbs^c). NA, not applicable.

of variance, Tukey means comparison tests, and *t* tests, were performed using the R statistical software (v3.2.1, R Development Core Team 2015).

Animal measurements

Tortoises were assessed monthly (April–July) to document growth, overall health, and clinical abnormalities or injuries. Growth was quantified by measuring the shell plastron length (PL) between the inner notches of the gular and anal scutes and recorded to the nearest 0.01 mm using digital calipers. Growth was analyzed with a series of linear mixed-effects models (R Package lme4 ver 1.1-10) with tortoise individual and clutch as random effects to account for repeated measures. Candidate models were compared using Akaike's information criterion corrected for small sample sizes (AICc; Burnham and Anderson 2002) using the package MuMIn (v1.15.1) in R (R Development Core Team 2015).

We assessed tortoises for general characterizations of health and body condition. Assessments included the animal's general posture, respiration, face (with specific attention to the eyes, periocular tissue, nares, mouth, tongue, and oral mucosa), skin, and shell for any clinical signs of disease, abnormalities, damage, or discoloration (USFWS 2015). We looked for discharge from the cloaca, eyes, external nares, and mouth and examined the skin for evidence of ulceration, erythema, swelling, or discharge. Throughout the experiment, all plant debris (specifically, *B. rubens* grass seeds including palea, lemma, and awn) found to be impacting the mucosal lining of the eye, tongue, choana, or oral cavity was noted and removed when possible with tweezers during each observation.

The animal's body condition score (BCS) was used to assess overall muscle condition and fat stores with respect to skeletal features of the head and limbs (Bowen et al. 2015b, USFWS 2015; N. Lamberski, *personal communication*). The BCS was classified as either under condition (atrophied forelimbs and a palpable or visible sagittal crest), adequate condition (palpable muscle mass on the forelimbs and head with no visible sagittal crest), or over condition (prominent musculature on the forelimbs and head). Once classified, a numeric score was assigned to provide a more precise assessment (under: 1–3, adequate: 4–6, over: 7–9).

Tortoises that received a $BCS \leq 2$ were removed from the study and returned to husbandry care. For the purposes of calculating survivorship, tortoises removed from the experiment were considered as a fatality. Clinical abnormalities (i.e., impacted *Bromus* seeds, pale coloration in the tongue and oral mucosa) and changes in body condition were calculated each month and analyzed with a series of linear mixed-effects models with tortoise individual and clutch as random effects to account for repeated measures and compared using AICc.

Animal blood collection and gene transcription

We measured an individual tortoise's change in physiological status using gene transcription by quantifying the amount of messenger RNA (mRNA) that is transcribed for targeted genes in response to intrinsic and extrinsic stressors. Blood was extracted from each tortoise via subcarapacial venipuncture (Hernandez-Divers et al. 2002) using a 0.96-cm, 26-gauge needle and 1-mL syringe immediately before (March) and after (July) the experiment. A 0.1-mL sample of whole blood was placed immediately into an RNeasy Animal Protect collection tube (Qiagen, Valencia, California, USA) and frozen at -80°C . In the laboratory, RNA extractions and cDNA synthesis were performed as described in Bowen et al. (2015b) on 25 randomly selected yearling tortoises (five from each diet) both before and after the experiment.

PCR primers developed for Mojave Desert tortoises were used to amplify 11 genes of interest and one ribosomal housekeeping gene within each sample (see Bowen et al. 2015b). Gene transcription cycle threshold values (C_T) were measured for the housekeeping gene (18S) and the genes of interest: CaM; aryl hydrocarbon receptor (AHR); Mx1; heat-shock protein 70 (HSP70); serum amyloid A; myeloid differentiation factor 88 (MyD88); CD9; superoxide dismutase (SOD); ATF; CL; and leptin (Lep; Appendix S1: Table S1) from each sample in duplicate using real-time PCR. Amplifications were conducted on a StepOnePlus Real-Time PCR System (Thermo Fisher Scientific, Hanover Park, Illinois, USA). Gene transcription measures were normalized by subtracting the average 18S housekeeping ribosomal gene C_T crossing value from the gene of interest crossing value for each tortoise.

We analyzed the qPCR gene transcript data using normalized C_T values. These values are inversely proportional to the amount of subject mRNA in the sample such that the lower the normalized value, the more the transcripts present. A change in normalized value of 2 is approximately equivalent to a fourfold change in the amount of the transcript. The total RNA concentration present within each blood sample was calculated using a NanoDrop Absorbance Spectrophotometer (Thermo Scientific, Wilmington, Delaware, USA) and used as a covariate in the analyses. We evaluated normalized gene transcript profiles for each gene of interest (Appendix S1: Table S1) and RNA concentration to examine potential differences in immune function between dietary treatments. We calculated the change (ΔC_T) in transcript values for each gene throughout the experiment by subtracting the normalized C_T value preexperiment (March) from the normalized C_T value postexperiment (July) for each tortoise.

The geometric means and 95% confidence intervals were calculated for each gene in each diet group. We used conventional mean responses per forage type (*Bromus*, *Festuca*, forbs, *Bromus* and forbs, and *Festuca* and forbs) with data assessed for statistical significance between classification ranks using Kruskal–Wallis with Dunn’s multiple comparison tests (Hollander and Wolfe 1973, R package stats v3.2.2). Transcription responses between diets were compared using a nonparametric multivariate analysis of variance (permutation MANOVA: Anderson 2001, R package vegan v2.3-1; Oksanen et al. 2011). Principal components analysis (PCA: Chatfield and Collins 1980) was then used on transformed variables, normalized to have a mean of zero and a standard deviation of one, as a data reduction technique to identify orthogonal factors and the variables that loaded highly in each factor. We performed a nonmetric, multivariate, multidimensional scaling (NMDS: Kruskal and Wish 1978, R package vegan v2.3-1; Oksanen et al. 2011) ordination with the Bray–Curtis similarity measure in conjunction with agglomerative hierarchical cluster analysis for statistical and graphical representation of individual tortoises clustered by similarity in transcription and not by predefined groups such as diet. Statistical comparisons of individuals grouped by clusters were made using SIMPROF (similarity profile

analysis), which is a similarity profile permutation test for significance among a priori, unstructured clusters of samples (Clarke et al. 2008; R package clustsig v1.1). Statistical significance was based on P values ≤ 0.05 .

Animal survivorship

Animal survivorship was evaluated each month using a logistic-exposure model (Shaffer 2004, R Package nestsurvival 0.5 by Herzog 2010). A logistic-exposure model is an extension of the generalized linear model that aids in modeling survival in terms of specific explanatory variables (both continuous and categorical) when you have individuals die at various ages or time frames. We evaluated survivorship relative to dietary treatment, month, animal size, cohort, and two-way interactions between treatment and covariates. Candidate models were compared using AICc in R.

RESULTS

Plant nutrients

Plant nutrients varied widely among *Bromus*, *Festuca*, and forb diets (MANOVA $F_{2,53} = 21.36$, $P < 0.01$) and among months (MANOVA $F_{2,53} = 8.68$, $P < 0.01$). The moisture content decreased throughout the experiment for all diet types. In April, the two grasses (*Bromus* and *Festuca*) had considerably less Ca, Mg, S, K, Na, Mn, Mo, Zn, NSC, and TDN than forbs; also higher contents of NDF in *Bromus* and CP, N, Cu in *Festuca* were found (Table 1). As the plant phenology changed and individual plants started to senesce by June and July, more moisture, CP, N, Fat, P, Mg, K, Mn, NSC, Cu, Zn, and TDN were measured in the forbs compared with either grass species (Table 1). Fe contents were found to be drastically lower in *Bromus* than forbs and *Festuca* in April and June.

Tortoise growth

Growth rates for tortoises varied significantly among diet treatments ($F_{4,93} = 7.55$, $P < 0.01$) and among months ($F_{3,275} = 29.28$, $P < 0.01$), but not between neonate and yearling size classes ($F_{1,93} = 1.84$, $P = 0.18$). Tortoises grew on average 0.71 ± 0.04 mm per month (ranging from -1.33 to 3.44 mm). Tortoises foraging on forbs and *Festuca* and forb diets grew more each month than

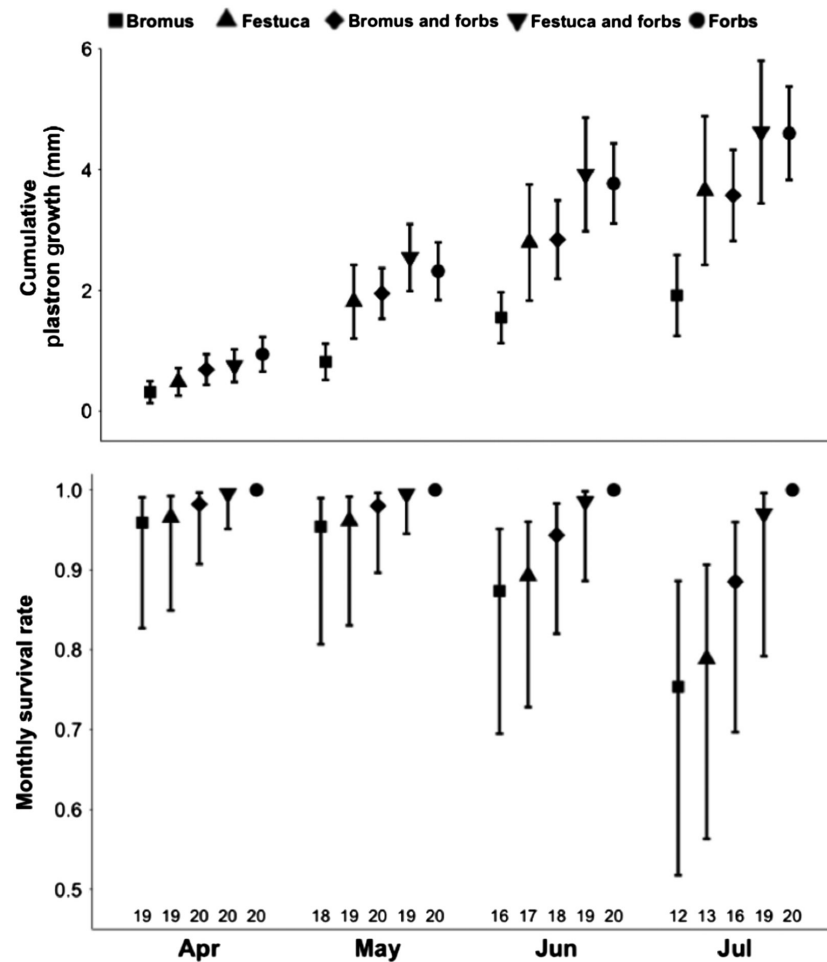


Fig. 2. Mean \pm 95% confidence interval for cumulative growth measurements (top) and monthly survival rates (bottom) for neonate and yearling Mojave Desert tortoises (*Gopherus agassizii*) each month (April–July) foraging on five dietary treatments. At the end of the experiment, tortoises foraging on diets with native forbs grew more than tortoises consuming only the invasive nonnative grass (*Bromus*) or native grass (*Festuca*). The survival rate decreased for tortoises foraging on the nonnative (*Bromus*) and native (*Festuca*) grasses compared with individuals consuming native forbs. The sample size used to calculate growth and survival each month is stated at the bottom of the graph.

individuals consuming either the *Bromus*, *Festuca*, or *Bromus* and forb diet ($F_{4,93} = 7.55$, $P < 0.01$; Fig. 2 top). Growth was significantly lower in individuals consuming only the nonnative *Bromus* grass (Fig. 2 top) throughout the experiment.

Tortoise health

Overall body condition (measured by the change in BCS each month) declined in tortoises consuming only invasive (*Bromus*) or native (*Festuca*) grass diets compared with those eating diets entirely composed of or containing native

forbs ($F_{4,94} = 2.77$, $P = 0.03$); the decline was most apparent in June and July ($F_{3,262} = 5.81$, $P < 0.01$). Tortoises eating only grass lost more muscle mass and fat ($F_{1,262} = 15.31$, $P < 0.01$) and experienced muscular atrophy, a condition that was not seen with individuals foraging on native forbs.

Impacted *Bromus* seeds were found embedded within the oral mucosa, tongue, and choana in individuals consuming only *Bromus* plants starting in May (4% or 21%), June (8% or 47%), and July (13% or 93%) and those foraging on a mixture of *Bromus* plants and forbs in May (3% or

15%), June (1% or 5%), and July (8% or 44%), with subsequent inflammation observed in 28% of those individuals. *Bromus* seeds were also found embedded in the palpebral and periocular membranes on occasion throughout the experiment. During oral examinations each month, the membranes of the oral cavity and tongue appeared pale in coloration for tortoises foraging only on grasses (*Bromus* or *Festuca*) than diets with native forbs ($F_{4,95} = 2.36$, $P = 0.06$), possibly related to an overall state of anemia in these individuals.

Clinical abnormalities around the cloaca were also observed for tortoises foraging on all diets containing grass species (*Bromus*, *Festuca*, *Bromus* and forbs, and *Festuca* and forbs) and included dried fecal matter and staining from frequent diarrhea. The majority of these abnormalities were observed in the *Festuca* diet ($F_{4,95} = 4.85$, $P < 0.01$), increasing each month of the experiment. During the last two months of the study, six

(32%) individuals eating *Festuca* were observed with fecal staining compared with only three (16%) in the *Bromus* and forb diet, two (12%) in the *Bromus* diet, and one (6%) in the *Festuca* and forb diet. Cloacal abnormalities were not observed for individuals foraging on native forbs throughout the experiment.

Gene transcription

Prior to the experiment, normalized gene transcript profiles (C_T values) and total RNA concentration (RNA) indicative of immune and physiological condition were similar for all individuals (MANOVA $F_{4,23} = 0.30$, $P = 0.99$). However, after four months of foraging on experimental diets, the C_T values for gene transcript profiles and RNA concentration in yearling tortoises were notably different among diets (MANOVA $F_{4,22} = 3.09$, $P = 0.01$; Table 2). We observed similar patterns when evaluating the

Table 2. Geometric mean normalized cycle threshold (C_T) transcription values for 11 genes of interest and total RNA concentration in 25 yearling (1.5 yr old) desert tortoises (*Gopherus agassizii*) before (Pre) and after (Post) the experiment.

Gene	Sample	Range	<i>Bromus</i>	<i>Festuca</i>	Forbs	<i>Bromus</i> and forbs	<i>Festuca</i> and forbs
SAA	Pre	13.03–20.76	17.56	17.31	16.93	16.31	17.54
SAA	Post	13.06–20.98	19.41	17.23	17.40	17.53	17.83
HSP70	Pre	10.75–15.72	14.07	13.46	14.18	13.35	13.81
HSP70	Post	9.30–15.72	14.08	12.69	13.06	13.93	13.56
MX1	Pre	17.01–24.27	20.64	20.36	18.91	19.65	19.87
MX1*	Post	17.01–23.23	21.96	19.45	19.46	19.58	20.63
CD9	Pre	10.89–15.91	14.31	13.26	13.37	13.21	14.06
CD9	Post	8.58–18.67	14.98	14.66	12.56	13.36	12.86
SOD	Pre	9.77–15.27	12.44	12.27	11.87	11.42	12.66
SOD**	Post	6.35–14.03	13.43	12.24	10.93	11.43	11.23
AHR	Pre	14.70–23.70	17.64	16.44	16.33	16.37	17.25
AHR	Post	12.38–18.22	17.04	16.13	15.70	16.20	15.91
MDF88	Pre	12.79–18.29	17.41	16.32	15.47	15.46	16.81
MDF88**	Post	13.19–18.29	17.81	16.68	16.78	16.65	16.30
CaM	Pre	9.94–15.49	13.41	12.28	12.61	12.05	12.49
CaM**	Post	6.78–13.92	12.17	11.57	10.02	11.10	10.16
ATF	Pre	10.43–22.40	14.78	15.37	14.00	14.65	14.58
ATF*	Post	6.94–20.97	13.65	8.25	9.86	11.41	11.14
CL	Pre	15.24–22.32	19.14	18.21	19.33	18.81	19.55
CL*	Post	12.08–21.43	18.60	17.94	15.71	17.35	16.30
Lep	Pre	13.86–21.67	16.80	15.51	15.16	16.02	16.34
Lep**	Post	11.78–16.97	15.97	14.97	13.95	15.09	14.72
RNA concentration	Pre	3.20–105.30	21.28	16.34	9.92	14.83	17.96
RNA concentration**	Post	5.20–54.80	12.98	16.82	39.54	34.39	15.51

Notes: Tortoises were fed one of five diets from April to July 2013. Note the smaller the mean value, and the higher level of transcript for 11 genes. Higher RNA concentration implies an overall increase in mRNA gene expression within individuals. ** indicates significant difference ($P \leq 0.05$) and *marginal difference ($P \leq 0.09$) between diets.

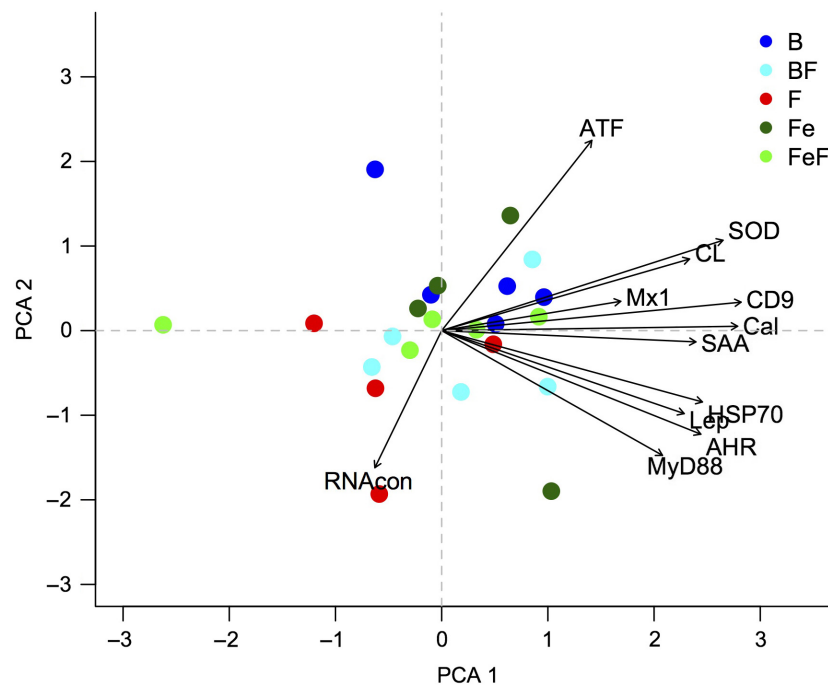


Fig. 3. Principal components analysis (PCA) using the change in normalized transcription values for 11 genes of interest and RNA concentration throughout the experiment (Δ = July C_T or RNA concentration – March C_T or RNA concentration). Yearling Mojave Desert tortoises (*Gopherus agassizii*) foraged on five dietary treatments from April to July 2013. Diets are represented as B = *Bromus*, Fe = *Festuca*, F = forbs, BF = *Bromus* and forbs, and FeF = *Festuca* and forbs.

change in gene transcripts (ΔC_T) and RNA (ΔRNA) throughout the experiment (MANOVA $F_{4,22} = 1.97$, $P = 0.05$).

Transcription levels for all genes and RNA concentration were higher in tortoises eating native species (*Festuca*, forbs, *Festuca* and forbs) than diets with *Bromus* after the experiment (Table 2). Statistical analyses using a priori diet groupings indicated significant differences in four genes (SOD, MDF88, CaM, and Lep) and RNA as well as marginal differences in three genes (Mx1, ATF, and CL; Table 2). Throughout the experiment, the ΔRNA significantly increased for individuals eating native forbs. Genes important for calcium metabolism, shell formation, and growth (i.e., CaM; Li et al. 2004) as well as overall nutritional condition and neuroendocrine and immune functions (i.e., Lep; Otero et al. 2005) were down-regulated in yearling tortoises eating only grass diets (*Bromus* or *Festuca*).

PCA revealed four principal components with eigenvalues greater than unity that together explained 74.8% of the total variance within

our data set. The first principal component was related to the 11 genes of interest (ΔC_T) with roughly equal loadings for each gene (Fig. 3). The second principal component was related to the ΔRNA concentration. NMDS and cluster analysis identified overall groupings of individuals that were mostly represented by their assigned diet (Fig. 4). SIMPROF identified primary significant differences among five distinct clusters, consisting of two to nine individual tortoises in each cluster (Fig. 5). Individuals identified in distinct clusters were generally represented by their assigned diet. For example, cluster two (yellow) consisted mostly of animals foraging on grass diets including four of the five tortoises evaluated in the *Bromus* diet (Fig. 5). Clusters one (red) and five (purple) were mostly comprised of animals that had native forbs in their diet (Fig. 5).

Survivorship

Diet ($Z = 9.86$, $P < 0.01$) and month ($Z = -3.70$, $P < 0.01$) strongly influenced animal survival during this experiment. Survival was lowest in

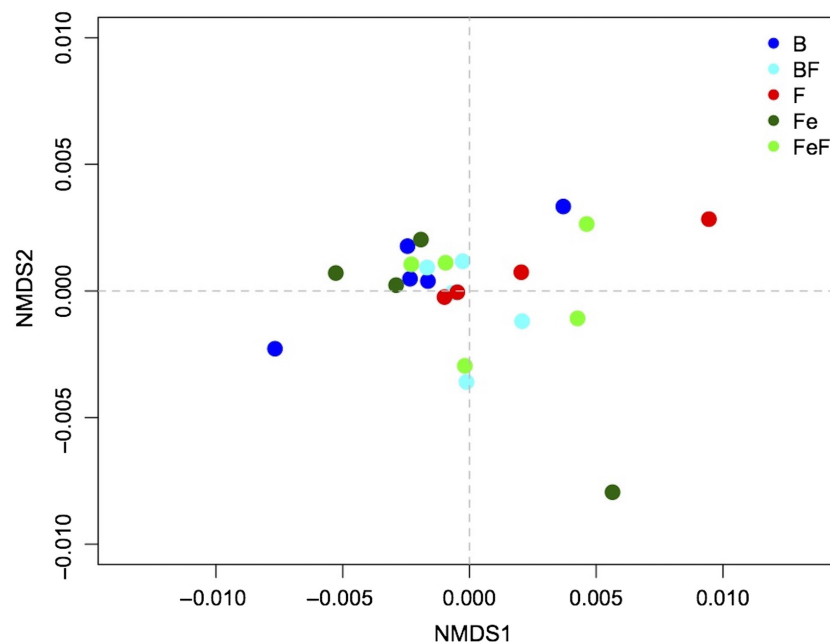


Fig. 4. Multivariate, nonmetric multidimensional scaling (NMDS) two-dimensional plot of the change in normalized transcription cycle threshold values (Δ = July C_T – March C_T) throughout the experiment. Yearling Mojave Desert tortoises (*Gopherus agassizii*; $n = 25$) foraged on five dietary treatments (B = *Bromus*, Fe = *Festuca*, F = forbs, BF = *Bromus* and forbs, and FeF = *Festuca* and forbs) from April to July 2013. NMDS axis 1 differentiated zones with native forb in diets, while grass diets (*Bromus* and *Festuca*) were important in differentiating axis 2.

the grass-only diets (*Bromus* and *Festuca*) and highest in the forbs and *Festuca* and forb diets (Fig. 2 bottom), highlighting the importance of the nutrients and dietary fibers found in native species, particularly native forbs. Survival decreased each month as temperatures increased and plants senesced and dried. Individuals were found dead and/or emaciated and pulled from the experiment within most diets (*Bromus* = 7 [36.8%], *Festuca* = 6 [31.6%], *Bromus* and forbs = 4 [20%], and *Festuca* and forbs = 1 [5%]) except native forbs (forbs = 0 [0%]).

DISCUSSION

While considerable attention has been given to the impacts of direct threats to desert tortoises such as off-road vehicle use and solar and urban development (Doak et al. 1994, Tracy et al. 2006, USFWS 2011, Darst et al. 2013), relatively little is known about the indirect effects these and other disturbances have on Mojave Desert tortoises such as the spread of invasive plant species. Our study finds that juvenile Mojave Desert tortoises

thrived on a diet of native plants (forbs or combined forbs and grass) by gaining mass, maintaining positive clinical condition, appropriately functioning molecular immune responses, and achieving high survival rates. In contrast, adding an invasive annual grass such as red brome *Bromus rubens* to diets resulted in dramatic negative consequences on the growth, health, immune function, and survival. Tortoises foraging only on the native annual grass *Festuca octoflora* also displayed poor general condition and health, although this species does not generally occur as a monoculture in the Mojave Desert and tortoises naturally forage on combinations of native forbs and grasses when available (Jennings 2002, Esque et al. 2014, Jennings and Berry 2015).

What accounts for the superiority of the forb-based diets for desert tortoises? Results from the plant nutrient analyses reveal that native annual forbs are essential to supply the nutritional and energetic requirements for younger tortoises. Our results are similar to previous research documenting declines in growth for both juvenile and adult tortoises foraging on grass diets

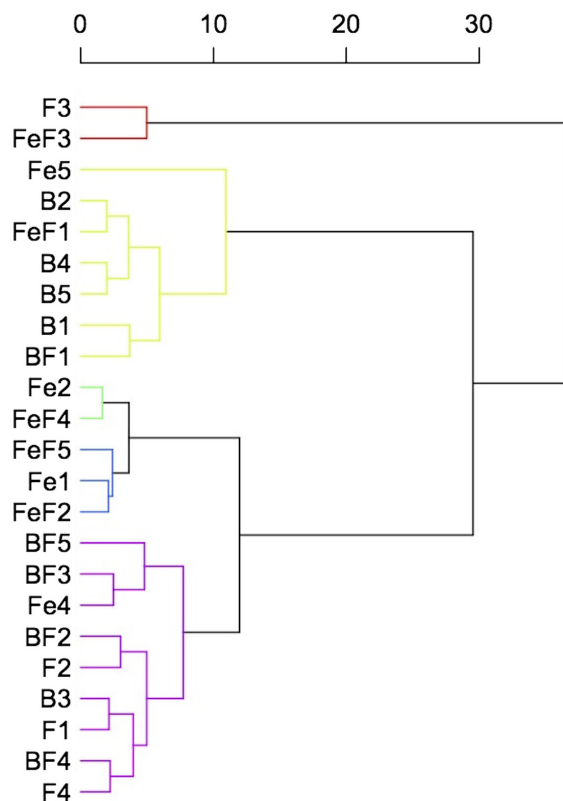


Fig. 5. Similarity profile analysis (SIMPROF) with Euclidean distance and ward linkage showing five significant clusters using normalized transcript values for 11 genes of interest for yearling Mojave Desert tortoises (*Gopherus agassizii*). The numbers along the left correspond to diet (B = *Bromus*, Fe = *Festuca*, F = forbs, BF = *Bromus* and forbs, and FeF = *Festuca* and forbs) and unique tortoise number (1–5) within each diet. Number of tortoises in each cluster = 1 (2), 2 (7), 3 (2), 4 (3), 5 (9) = 23 total tortoises.

(Ofstedal and Allen 1996, Nagy et al. 1997, 1998, Hazard et al. 2009, 2010). In our study, growth and body condition for tortoises were positively correlated with higher moisture and mineral contents, NSC, protein, and TDN, all characteristics that were found in the diets of the group eating native annual forbs. Generally, plant species such as native forbs that are high in protein and low in fiber are also high in energy content, calcium, magnesium, phosphorus, and potassium (Tracy et al. 2006). McArthur et al. (1994) found that plants with higher water content were generally correlated with better nutritive and mineral content, and high protein was correlated with high

fat content; protein was negatively correlated with ADF (primarily compounds for structural materials) and NDF (hemicellulose, indigestible cellulose, and lignin representing the fibrous bulk). Plant nutrient analyses from our study support this finding; native annual forb diets had higher moisture content, fat, protein, and overall nutrients such as nitrogen, phosphorus, potassium, magnesium, copper, zinc, manganese with reductions in NDF and ADF than either invasive or native grass (*B. rubens* or *F. octoflora*) diet.

Invasive Mediterranean annual grasses are fully integrated into western North American landscapes, and in the Mojave and Sonoran deserts have drastically modified the vegetation composition (Beatley 1966, Brooks 1999). Although annual invasive grasses can have nutritional values similar to native grasses (Hazard et al. 2009, 2010), they can dominate ecosystems (Brown and Minnich 1986, Brooks 1999, Brooks and Esque 2002, Esque et al. 2010), reducing plant diversity and ultimately lowering overall nutritional availability (Ofstedal 2002, Ofstedal et al. 2002, Tracy et al. 2006). By most accounts, native perennial and annual grasses are important components in tortoise diets, and all North American tortoises eat substantial amounts of them (Mushinsky et al. 2003, Esque et al. 2014). In addition, many African tortoises consume both perennial and annual grasses as a large portion of their diets (Kabigumila 2001). Grasses may be consumed in some locations because of their high relative abundance at times when more nutritionally beneficial species are not available: they are less important when forbs are readily available (Garner and Landers 1981, MacDonald and Mushinsky 1988, Esque 1994, Scalise 2011). However, for desert tortoises that live in environments with limited food and scarcity of water, the low nutritional value and highly indigestible fiber content of grasses suggest that even a native grass diet alone may not provide sufficient energy and nutrients needed for key life periods such as growth or immune functions (McArthur et al. 1994, Basiotis 2007).

Effects of nutrition on physiological and immune functions

The immunosuppressive effects of nutritional deficiencies in most vertebrates are well documented (Weston and Memon 2009, Saucillo et al.

2014, Iyer et al. 2015), and can have a lasting effect on the overall health in most organisms. Malnutrition produces morphological and functional changes in virtually all organs (Hulsewe et al. 1999). The severity of nutrition-related dysfunction depends in part on the rate of cellular proliferation and renewal, and on protein synthesis in the tissue, making the lymphoid and gastrointestinal systems most vulnerable. Thus, deficiencies in energy, protein, iron, folic acid, vitamin A, vitamin D, pyridoxine, and zinc can have profound adverse effects on the mucosal and systemic immunity with implications for infection and disease (Berdanier 1996, Fraker and Telford 1996, White et al. 1996, Ferguson et al. 2015). In our study, we demonstrated that the gene transcription levels (molecular immune functions) of young tortoises eating diets low in Zn, Fe, S, and K (grass-only diets) were reduced relative to other diet groups, potentially limiting important metabolic and immune functions needed during critical life stages.

Many clinical and experimental studies of human and laboratory animals have shown that immune-deficient or immune-suppressed organisms tend to have defective wound healing (Uba et al. 2004), higher mortality rates, shorter lifespans and develop more severe infectious diseases and cancer (Brandau and Gilbert 2007, Norlin et al. 2008, Acevedo-Whitehouse and Duffus 2009). During our study, tortoises with decreased immune function (lower gene transcription levels) exhibited limited growth and increased mortality. Results from previous gene transcription research (Bowen et al. 2015b) suggest that the immune and physiological functions of our study subjects were compromised across all individuals eating the invasive grass (*B. rubens*). Conceivably, young animals with developing and stressed immune function would not be able to repair or survive the physical injuries inflicted by ingesting *B. rubens*. Further study of the effects of invasive food plants such as red brome on adults would clarify effects of this invasive grass on immune and physiological function in desert tortoises.

Experimental findings

Our study provides the first comprehensive evaluation of the effects of invasive annual grasses on the overall health and immunity in

Mojave Desert tortoises. We noted declines in growth, overall body condition, gene transcription indicative of immune functions, and survival for individuals foraging solely on grass diets relative to individual tortoises eating native forbs (Kilberg et al. 2005, Bowen et al. 2014, 2015b). Juvenile tortoises foraging on a mixture of native forbs with higher nutritional content (e.g., nitrogen, water, phosphorus) continued to grow, gain muscle and fat mass, and increase their immune function throughout the spring season. Prior to this experiment, we hypothesized that transcription profiles for genes indicative of nutritional (Leptin; Otero et al. 2005) and cellular (SOD, CL; Zhou et al. 2008, Walsh et al. 2010) stress would increase, and those related to calcium regulation and growth (CaM; Li et al. 2004, Chen et al. 2012) would decrease in individuals consuming only grass diets. However, to our surprise, individuals solely eating grass diets experienced a decrease in the amount of transcription for most genes evaluated, likely resulting from the limited nutritional resources available for these physiological processes and possibly from the inability to mount an immune response to injuries acquired when feeding on the invasive red brome.

We noted several clinical conditions in tortoises likely linked to nutrient deficiencies or mechanical injury from their diet. We observed signs of overall dehydration and emaciation (recessed eyes, low body mass, and lethargic behavior), anemia (pale coloration in the tongue and oral mucosa), and thinning of the shell for juvenile tortoises consuming *B. rubens*, and less so in *F. octoflora*. Overall anemia seen in individuals may be linked to low amounts of iron (Fe) and other nutrients found in *B. rubens* throughout the experiment. We also found evidence of diarrhea around the cloaca in tortoises foraging on grass diets. However, this condition was more prominent in juveniles eating the native than invasive grass. Previous studies examining trace mineral deficiencies such as zinc reported subsequent diarrhea (Hambridge 2000). Zinc is ubiquitous in subcellular metabolism, differentiation, and repair, as it is an essential component of the catalytic site or sites of at least one enzyme in every enzyme classification (Fierke 2000). Even a partial understanding of the fundamental importance of zinc in cellular growth and differentiation alerts

us to the special vulnerability to an inadequate supply of zinc of the rapidly growing individual or patient mounting an immune response or requiring tissue repair (Castillo-Duran et al. 1987, Fraker and Telford 1996, Hambridge 2000).

Irritations and inflammation related to impacted *Bromus* seeds in the mucosal lining of the oral cavity, tongue, choana, and palpebral were observed for tortoises consuming the *Bromus* diet. *Bromus* seeds have large splayed spikelet awns that are very rough due to the barb-like hairs that face backward, allowing the seed to catch and lodge like a fish hook. Irritations and impactions from these seeds inside the mouth and eye are commonly reported in livestock and wildlife (Crampton 1974, McCray and Bloom 1984, Currie et al. 1987, Bossard et al. 2000, Medica and Eckert 2007; K. Nussear, K. Drake, and T. Esque, *unpublished data*), likely hindering the foraging behavior and sight for these species. A closely related *Bromus* species (ripgut brome or *B. diandrus*) was found to impact the mucosal linings of the eye in red-shouldered hawks (*Buteo lineatus elegans*) causing them to die as an indirect result of grass seed irritation (McCrary and Bloom 1984). *Bromus* seeds had migrated from the anterior corner of the eye to the nasal area, resulting in both optic and nasal inflammation and fluid drainage.

We recognize that the findings of our study may be narrowed by the limited number of food plants used in this experiment. As a group, Mojave tortoises typically forage on dozens of plant species in given year based on availability (Jennings 2002, Esque et al. 2014, Jennings and Berry 2015), and the potential effects associated with limited diet are unknown. Tortoises have remarkable adaptive capabilities and unique physiological mechanisms for the conservation of water and metabolic energy regulation (Nagy and Medica 1986, Peterson 1996a, b), yet we know little in terms of how they partition resources to self-maintenance activities, including growth, and immune and other physiological functions when they are malnourished or otherwise stressed (Derickson 1976, Zimmerman et al. 2010). Interestingly, juveniles consuming only native grass (*Festuca*) were able to allocate resources toward continued growth albeit less than animals eating native forbs, yet they experienced more than 30% mortality or emaciation

by the end of the experiment. These individuals lost mass, potentially lacking the reserves to maintain basic physiological function (Hazard et al. 2010). Adult turtles and tortoises have been documented to maintain normal activity and body conditions even when they have severe anemia and hypoproteinemia (Christopher 1999, Norton 2005, Tavares-Dias et al. 2009). However, the behavior and physiology of hatchling and juvenile tortoises may differ from adults because of their small body size, increased mass specific metabolic rates, and high demands for protein and macronutrient-rich forage for growth (Naegle and Bradley 1974, MacDonald and Mushinsky 1988, Wilson et al. 1999).

We questioned whether this increase in mortality for individuals eating only *F. octoflora* was associated with diet or a predisposed failure to thrive in this early life stage, as previous studies reported higher mortality ranging from 6% to 40% during in the first year compared with juveniles 1–4 yr (Germano 1994, Nagy et al. 1998, Epperson and Heise 2003). We found similar survivorship between neonate and yearling tortoises within each diet; however, each diet was represented by twice as many neonate (13–14) as yearling (6–7) tortoises and the potential effects of a small sample size may be relevant to our findings. Reference literature and standard reference ranges on most health metrics including clinical conditions, molecular responses (gene transcript patterns), immune functions, growth patterns, and survival rates in relation to diet availability for wild juvenile *Gopherus* tortoises are not available.

Conservation implications

This study provides evidence that invasive nonnative plants such as *Bromus rubens* have a detrimental effect on fitness and survival of desert tortoises. Furthermore, the strong impact of *Bromus rubens* is likely to reduce survival and recruitment rates for sensitive populations throughout the Mojave Desert. Land-use practices and climate change have shifted plant composition and available forage species, changed temperature ranges and rainfall indices, and altered the abundance and distribution of predatory and prey species, as well as pathogens and hosts (Brooks and Berry 2006, MacLeod et al. 2007, Tibbetts 2007, Patz et al. 2008,

Acevedo-Whitehouse and Duffus 2009, Smith et al. 2014). Invasive grasses that displace and dominate native species due to landscape-altering fire or other human disturbances will have adverse effects on the diet, nutrition, and subsequent health of tortoises in the future.

Predicted climatic change will likely exacerbate the negative effects of the spread of invasive nonnative plants on Mojave Desert tortoises. Long periods of drought or above-average winter precipitation associated with climate change or “warm” Pacific Decadal Oscillation regimes are expected to become more frequent, particularly in arid and semiarid ecosystems in the southwestern United States and Mexico (Easterling et al. 2000, Salo 2005, Tagestad et al. 2016). These climatic variations will perpetuate invasions by nonnative Mediterranean grasses, increasing overall plant biomass and susceptibility to wildfires in these regions. Threatened or vulnerable wildlife populations that inhabit invaded habitats are likely to be severely affected by these climatic conditions. For instance, susceptibility to infectious diseases can increase when seasonal food shortages or malnutrition associated with altered diets overlap with periods of high nutritional demands (Jolly and Fernandes 2000, Buehler et al. 2010, Treanor et al. 2015). During extreme climatic events, poor nutrition (i.e., sub-optimal levels of protein, vitamins, and other essential nutrients) and dehydration will lead to depletion of fat reserves, poor body condition (Beldomenico et al. 2008), and decrease innate and acquired immune responses. This decline will reduce resistance to infection, which in turn can impair nutrient absorption owing to altered gut permeability and inflammation and eventual aggravation of the nutritional status (Katona and Katona-Apte 2008). For wildlife, such events will have disastrous consequences for already depleted populations. Further research is required to assess the full impact of invasive plants on native communities and ecosystems.

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