

Does translocation influence physiological stress in the desert tortoise?

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Keywords

desert tortoise; *Gopherus agassizii*; translocation; stress; Mojave Desert; corticosterone.

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Editor: Karina Acevedo-Whitehouse
Associate Editor: Torsten Nygaard Kristensen

Received 5 October 2011; accepted 22 March 2012

doi:10.1111/j.1469-1795.2012.00549.x

Abstract

Wildlife translocation is increasingly used to mitigate disturbances to animals or habitat due to human activities, yet little is known about the extent to which translocating animals causes stress. To understand the relationship between physiological stress and translocation, we conducted a multiyear study (2007–2009) using a population of desert tortoises (*Gopherus agassizii*) near Fort Irwin, California. Blood samples were collected from adult tortoises in three treatment groups (resident, translocated and control) for 1 year prior to and 2 years after translocation. Samples were analyzed by radioimmunoassay for plasma total corticosterone (CORT), a glucocorticoid hormone commonly associated with stress responses in reptiles. CORT values were analyzed in relation to potential covariates (animal sex, date, behavior, treatment, handling time, air temperature, home-range size, precipitation and annual plant production) among seasons and years. CORT values in males were higher than in females, and values for both varied monthly throughout the activity season and among years. Year and sex were strong predictors of CORT, and translocation explained little in terms of CORT. Based on these results, we conclude that translocation does not elicit a physiological stress response in desert tortoises.

Introduction

Urbanization and increased renewable energy development in the Mojave Desert are rapidly consuming and altering desert landscapes inhabited by the desert tortoise. The Mojave population of the desert tortoise (*Gopherus agassizii*) north and west of the Colorado River is listed as a threatened species under the federal Endangered Species Act due to a number of threats largely associated with human impacts (USFWS, 1990; 1994; 2011). Currently, there are more than 50 proposed projects to establish sustainable power installations, requiring translocation of desert tortoises from over a million acres of federal lands (BLM/DOE, 2011). Experimental translocations have been conducted to inform resource managers about the costs and benefits of these activities (Field *et al.*, 2007; Nussear *et al.*, 2012). Translocated tortoises have been shown to have reproductive effort and survivorship equivalent to those in resident tortoises (Field *et al.*, 2007; Esque *et al.*, 2010; Nussear *et al.*, 2012). However, the physiological stress associated with translocation has not been quantified in desert tortoises.

While the physiological stress response is complex and involves a variety of endogenous mediators, the glucocorticoid hormones [corticosterone (CORT) or cortisol] generated via the hypothalamic-pituitary-adrenal (HPA) axis are most often associated with the stress response. Glucocorticoids are a class of steroid hormones released from the adrenal glands during a wide variety of stress stimuli and conditions, including harsh weather, animal manipulation (Axelrod & Reisine, 1984; Romero & Wikelski, 2001), and disturbances in habitat (Wingfield *et al.*, 1998; Moore & Jessop, 2003). The release of these hormones is under the control of adrenocorticotrophic hormone (ACTH) released from the anterior pituitary, which is in turn under the control of other hypothalamic hormones (Dallman & Bhatnagar, 2004).

Capture, handling, transport, restraint and release of wild animals into novel environments (activities associated with translocation) are known to cause acute and sometimes-chronic stress in vertebrate species (Romero & Reed, 2005; Delehanty & Boonstra, 2009; Adams *et al.*, 2010). In particular, CORT has been correlated with both acute and chronic stress making CORT a 'biomarker' of an altered

physiological state in some species (Gregory *et al.*, 1996; Jessop *et al.*, 2004). Changes in CORT may have cascading impacts on immune function or other physiological factors (Tracy *et al.*, 2006); however, there are no studies on changes in CORT relative to translocation in desert tortoise populations. Translocation may cause acute stress in desert tortoises; however, it is unknown whether acute stress is important to the immune competence or if translocation causes chronic stress to tortoises as they adjust to new environments. Population supplementation resulting from translocation may also have unknown physiological consequences on resident tortoise populations.

The time associated with the cascade of events within the HPA axis from the time the animal detects a stressful stimulus to measurable alterations of glucocorticoids in the blood varies by species. Previous work on wild red-eared slider turtles (*Trachemys scripta elegans*) noted that there was no change in glucocorticoids in the first 10 minutes of capture, but levels significantly increased within 30 minutes (Cash, Holberton & Knight, 1997). This lag in glucocorticoid response is consistent with research on other reptiles (Gist & Kaplan, 1976; Dunlap & Wingfield, 1995), including free-living loggerhead turtles (*Caretta caretta*, Gregory *et al.*, 1996), captive American alligator (*Alligator mississippiensis*; Lance & Lauren, 1984; Gregory *et al.*, 1996) and wild gopher tortoises (*Gopherus polyphemus*; Ott *et al.*, 2000; Kahn, Guyer & Mendonca, 2007).

Because handling animals to collect blood can induce stress that could influence our ability to detect a response to translocation, we conducted a laboratory experiment to quantify the time required for captive desert tortoises to elicit elevated glucocorticoid concentrations (total plasma CORT) after a single dose injection of ACTH. Such 'challenge' studies use ACTH to stimulate the adrenal glands to release CORT, bypassing other steps of the HPA axis that would require exogenous stressors for activation (e.g. handling stress, poor environmental conditions or translocation stress). Results from this experiment guided our blood collection and animal handling protocols in the field.

To quantify the effects of translocation on stress physiology in wild desert tortoises, we studied translocated, resident and control tortoises at the US Army National Training Center (NTC) at Fort Irwin, California and surrounding public lands. In particular, we studied levels of plasma total CORT each month throughout the period of animal activity (April–October) for 1 year prior and 2 years subsequent to translocation. We hypothesized that translocation of wild tortoises may cause a chronic physiological stress response in translocated animals, and to a lesser extent, resident animals.

Methods

Laboratory ACTH experiment

To quantify the rate at which desert tortoises could elicit an increased CORT response to handling, we conducted an ACTH laboratory stimulation test on captive adult desert

tortoises using a modified protocol from Lance & Rostal (2002) with increased sampling frequency. We used 11 captive adult tortoises [5 males (M) and 6 females (F)] housed at the University of Nevada-Reno. We injected half of the tortoises (3M : 3F) with 1 µg Cortrosyn® (synthetic ACTH, Henry Schein, Reno, NV, USA) in 200 µL of physiological saline per kg of body mass via the jugular vein (Jacobson, Schumacher & Green, 1992). Our control group of tortoises (2M:3F) was injected with equal volumes of saline via the jugular vein. Blood samples (100 µL) were collected via subcarapacial venipuncture (Hernandez-Divers, Hernandez-Divers & Wyneken, 2002) prior to treatment injections (0 minutes) and repeated at 5, 10, 15, 20, 30 and 60 minutes postinjection.

Field research

Study area

The study area was in the north-central Mojave Desert, near Barstow, San Bernardino County, California. Wild desert tortoises were translocated from a ~94 km² area of designated critical habitat for the desert tortoise (USFWS, 2011) in the southern portion of the NTC. Tortoises were moved to a 1000 km² translocation area also in critical habitat on public lands located in Superior-Cronese Desert Wildlife Management Area (USFWS, 1994) administered by the US Department of the Interior – Bureau of Land Management (Fig. 1). The translocation area was bounded on the south by Interstate 15 and on the north by the southernmost boundary of the NTC. Within the translocation area, we established release plots ($n = 4$) measuring 1.6 km² as suitable habitat for release for translocated tortoises (Heaton *et al.*, 2008). The study area was typified by Mojave Desert scrub (Turner, 1982). Vegetation ranged from sparse mixed-shrub communities in the rockier areas, to a dominant assemblage of creosote bush (*Larrea tridentata*)/white bursage (*Ambrosia dumosa*) at middle elevations, and halophytic *Atriplex* spp. assemblages on the margins of Coyote Dry Lake at the lowest elevation in the study area.

Field study

We monitored 45 (27M : 18F) tortoises at the NTC and 179 (107M : 72F) tortoises in the translocation area at least monthly using radio telemetry (Holohil Systems Ltd, Carp, Ontario, Canada). During each animal encounter, data were collected to record the sex, date and time of capture, geographic location, animal handling time (minutes required to collect a blood sample), air temperature, microhabitat location (burrow, open scrub, vegetation), and behavior (basking, eating, resting, walking, inactive; Ruby & Niblick, 1994).

Translocation

We translocated adult tortoises on March 27, 2008 from the southern NTC to four release areas within the translocation

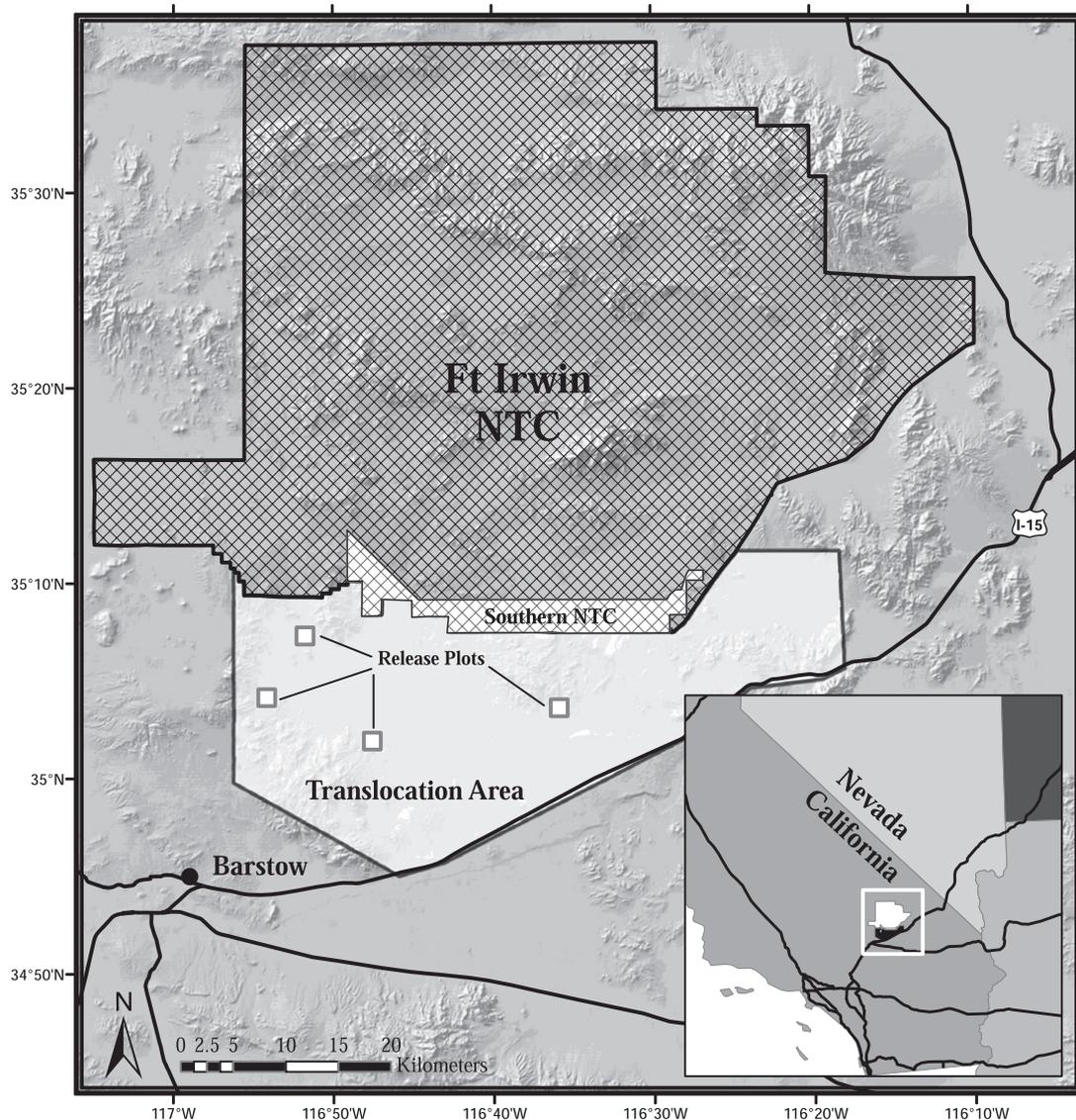


Figure 1 Desert tortoise study area at Ft. Irwin National Training Center (NTC) (US Department of Defense Lands) and adjacent Translocation Area (Bureau of Land Management Lands). The NTC is represented by hashed lines and includes a highlighted Southern NTC where tortoises were removed from in 2008. The Translocation Area is represented by a light gray polygon and includes four release plots designated as translocation release areas on 27 March 2008.

area (Fig. 1). Each tortoise was transported to the release area by a vehicle. Release sites ranged in distance from 9 to 30 km from the point of capture on the NTC. Tortoises were taken to a predetermined point, hydrated for 30 minutes, and released in the shade of a nearby shrub or in a natural burrow. Approximately equal numbers of female and male tortoises were placed within each release area and were spaced minimally 400 m away from tortoises of the same sex.

Experimental design

We had three experimental treatments in the study: 'translocated', 'resident' and 'control' tortoises. Tortoises that

originated in the NTC and were moved to new locations were 'translocated' tortoises, and retained that designation throughout this study, including prior to translocation, in order to provide before and after comparisons on the same animals. Prior to 27 March 2008, tortoises in the translocation area were considered 'residents' if they resided within a 200-m buffer of the four release areas (Fig. 1). All tortoises that were located outside the buffer of the designated release plots in the translocation area were considered 'controls'. Because translocated animals were released in unfenced areas, the 'control' population was potentially influenced as they expanded from their release sites. When translocated tortoises were estimated to have overlapped with controls, the status of the control animal was changed to 'resident'

because they were no longer spatially separated from the translocated tortoises. We created a monthly estimate of the distribution of translocated animals by calculating a kernel use distribution for all translocated animals using R statistical software (v 12.1.2, R Development Core Team, 2010) and the package *adehabitat* (v 1.8.2, Calenge, 2006). We used the ad hoc method to calculate a smoothing parameter (h) and a 95% confidence level.

Animal home-range

To explore the potential influence of animal home-range on CORT, we used telemetry locations to calculate a yearly home-range for each tortoise using the package *adehabitat*. We calculated an individual-based smoothing parameter (h) using the least square cross-validation method and home range area based on a 95% confidence level.

Annual plant production and precipitation

Spring annual plants are the most important food source for desert tortoises in most years across the Mojave Desert (Jennings, 2002). We used randomly selected transects to quantify spring annual vegetation production. We estimated above-ground biomass within 20 quadrats (1 m²) along each 200-m transect (Andariese & Covington, 1986; Tausch, 1989) from 18 June to 21 June 2007 ($n = 30$ transects), 17 March to 9 April 2008 ($n = 99$ transects) and 23 March to 9 April 2009 ($n = 110$ transects). At each quadrat, annual vegetation was subjectively ranked on a scale of 0 to 10 by visual estimation, where 0 = absolute absence of any annual vegetation and 10 = complete cover within each quadrat. Above-ground annual biomass was clipped and sorted by phenological condition (i.e. current years growth and previous years growth) from two representative quadrats (10%) along each transect. Production samples were dried in a convection oven at 50°C for 48 hours to a constant mass and weighed. Calibration curves were constructed separately for each phenological condition and for each observer. The mass of each clipped quadrat was regressed against the above estimates to generate an equation expressing rank as a function of measured plant biomass (Singh, Madan & Vasudevan, 1990). We used the calibration curve to estimate biomass for each quadrat, and then we calculated the average production for each transect.

Winter precipitation is positively correlated with the growth of desert tortoises (Medica *et al.*, in press) as well as annual plant production (Beatley, 1974). To examine the potential influence of these two factors on CORT in tortoises, we modeled these factors to create continuous raster GIS layers for the study area. Winter precipitation (November to April) was estimated over the study area using a 30-m cell size grid. The raster layer was generated from point data using a spatial inverse distance weighted interpolation (Nalder & Wein, 1998) of data from a pool of 928 climate stations from the National Climate Data Center (NCDC) website (<http://www.ncdc.noaa.gov/>).

Plant biomass was modeled using the biomass estimates from the sampling transects (see above), with potential covariates of MODIS (MODerate-Resolution Imaging Spectroradiometer; Oak Ridge National Laboratory Distributed Active Archive Center, 2010) MOD13Q1 Global 250 m 16-day index of the Enhanced Vegetation Index for May and January of each year, and the winter precipitation layer for the relevant year as described previously. We used spatial general linear models (package *geoR* v 1.6–34) to assess the best model fit, and conventional kriging was used to calculate spatial predictions for each year. Akaike information criterion (AIC) was used to identify the best model of plant biomass for each year (Burnham & Anderson, 2002).

The raster GIS layers produced for spring plant biomass and winter precipitation were intersected with individual tortoise home-ranges for each year, and the average of each was calculated for each tortoise home range.

Blood collection

To assess CORT, we collected 1793 blood samples from wild desert tortoises (779 in 2007, 550 in 2008 and 394 in 2009). Blood samples were collected monthly between early April and October of each year from available tortoises using a 3.81 cm, 23- or 25-gauge needle, and 3 cc sterile syringe coated in sodium heparin via subcarapacial venipuncture (Hernandez-Divers *et al.*, 2002). Some animals (~3%) were not sampled each month due to our inability to extract the animal from deep cover sites or successfully collect tissue. Blood samples were transferred to lithium heparin microtainers and stored on ice for no longer than 5 hours. Plasma was separated using centrifugation with a centrifugal force of 1318 $\times g$ and stored in liquid nitrogen until moving to an ultracold freezer (–70°C).

We recorded notes during blood collection describing the sample color, consistency and unusual characteristics. We estimated the percentage of packed red blood cells (hematocrit) and amount of lymphatic fluid present by visual inspection, and assigned each sample to one of four categories: free (0%), low (> 0–15%), medium (> 15–30%) or high (> 30%).

Radioimmunoassay

The concentration of total CORT in plasma was measured using a commercial radioimmunoassay kit (#07–120102, MP Biomedical, Costa Mesa, CA, USA). This assay was highly specific, had low cross-reactivity with other steroids, did not require steroid extraction steps and included a corticosteroid-binding globulin blocking agent. This allows the concentration of CORT to be measured without additional chemical or heating steps to denature binding proteins. Plasma samples were thawed on ice and diluted 1:25 or 1:50 with the kit diluent. CORT titers (in ng per mL) were calculated using the *drc* package in R (Soerensen *et al.*, 2007) from a standard curve generated in each assay. The average inter- and intra-assay coefficients of variation were calculated using data from a pooled sample of 10 individual

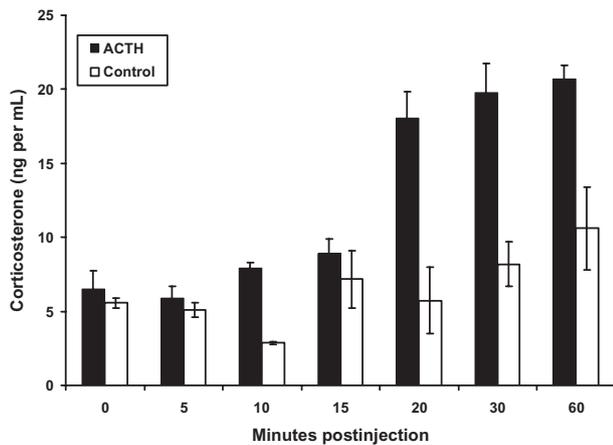


Figure 2 Plasma total corticosterone ng per mL (mean \pm 1 SE) in captive adult desert tortoises (*Gopherus agassizii*) injected with synthetic adrenocorticotrophic hormone (ACTH—solid bars) and saline-control (open bars).

male and female tortoises which were analyzed within and across 20 separate assays. The average inter- and intra-assay coefficients of variation for laboratory analyses were 22.2 and 9.9%, respectively.

CORT analyses

We analyzed CORT (ng per mL) with covariates hypothesized to influence stress levels in desert tortoises using linear mixed-effects models with tortoise ID entered as a random variable to account for repeated measurements of individuals. We modeled CORT relative to experimental treatment, sex, month and year of sample collection, air temperature, number of needle insertions required to collect sample, handling time, tortoise behavior, tortoise location, animal home-range, and the average annual plant production and precipitation within the animal's home range. A factor was also included to account for the relative quantity of lymph present in each sample represented by categories. All samples with a handling time > 15 min were excluded from analyses. Model selection was conducted using AIC with bidirectional stepwise selection to evaluate models including different factors and covariates. We made comparisons among models and estimated the relative importance of different parameters by using normalized Akaike weights (w_i ; Burnham & Anderson, 2002). Models were ranked according to a corrected AIC (AICc).

Results

ACTH laboratory experiment

Plasma CORT did not increase significantly in ACTH-injected animals until 20 minutes postinjection ($F_{1,29} = 0.56$, $P = 0.04$; Fig. 2) and there was no significant increase in CORT levels in saline-injected animals during the experiment. At the end of the experiment (60 minutes postinjec-

tion), ACTH-injected animals had an average increase of 392% ($\pm 129\%$), while control animals averaged only a 45% ($\pm 27\%$) increase. This result suggests that blood collection using subcarapacial venipuncture induces minimal stress on captive tortoises in the first 15 minutes from the onset of the initial handling of the animal.

Annual plant production

Annual plant production (mean g per $m^2 \pm$ SE) was estimated in 2007 ($n = 30$, 1.7 ± 1.8 g m^{-2}), 2008 ($n = 99$, 11.5 ± 5.4 g m^{-2}) and 2009 ($n = 110$, 16.4 ± 5.4 g m^{-2}). There was significantly less annual plant production in 2007 compared with 2008 and 2009 ($F_{2,60} = 47.80$, $P < 0.01$).

CORT concentration among wild desert tortoises

The best model explaining CORT in tortoises included sex and year while accounting for the amount of lymph present in the samples (model 1; Tables 1 and 2). None of the other

Table 1 Models considered and ranked according to AICc and change in AICc (Δ AICc). w_i is Akaike weight. Where models were similarly performing, the model with the fewest factors was preferred. Factor abbreviations are: Lymph, lymph presence in sample (none, low, medium, high); Sex, sex of animal; Month, month in which blood sample was collected; Year, year in which blood sample was collected; Treatment, treatment group spatially assigned to tortoise (control, resident, translocated); NeedleInsert, number of needle insertions required to collect sample; HandleMin, animal handling time (minutes) required to collect sample; Tair, ambient air temperature at time of sample collection; Behavior, animal behavior (resting, eating, etc); and Location, animal location (burrow, open, etc). Includes all data from 2007 to 2009

Model	AICc	(Δ AICc)	w_i
1. {Lymph + Sex + Year}	3455.21	0.00	0.90
2. {Lymph + Year}	3460.84	5.63	0.05
3. {Lymph + Sex + Year + Treatment}	3463.18	7.97	0.02
4. {Lymph + Sex + Year + Month}	3463.92	8.70	0.01
5. {Lymph + Sex \times Year}	3464.09	8.88	0.01
6. {Lymph + Sex + Year \times Treatment}	3464.63	9.42	< 0.01
7. {Lymph + Sex + Year + Tair}	3467.71	12.50	< 0.01
8. {Lymph + Sex + Year + Behavior}	3469.51	14.30	< 0.01
9. {Lymph + Year \times Treatment}	3469.86	14.65	< 0.01
10. {Lymph + Sex \times Year + Treatment}	3472.05	16.84	< 0.01
11. {Lymph + Sex + Year + Location}	3473.67	18.46	< 0.01
12. {Lymph + Sex \times Month + Year}	3481.84	26.63	< 0.01
13. {Lymph + Treatment}	3689.68	234.47	< 0.01
14. {Lymph + Sex}	3689.90	234.69	< 0.01
15. {Lymph + Sex + Month}	3695.71	240.50	< 0.01
16. {Lymph + Location}	3703.33	248.12	< 0.01
17. {Lymph + Month}	3707.12	251.91	< 0.01
18. {Lymph + Behavior}	3708.40	253.19	< 0.01
19. {Lymph + Tair}	3709.26	254.05	< 0.01
20. {Lymph + NeedleInsert}	3709.98	254.77	< 0.01
21. {Lymph + HandleMin}	3710.37	255.16	< 0.01
22. {Lymph + Sex \times Month}	3713.65	258.44	< 0.01
23. {Null}	3717.49	262.28	< 0.01

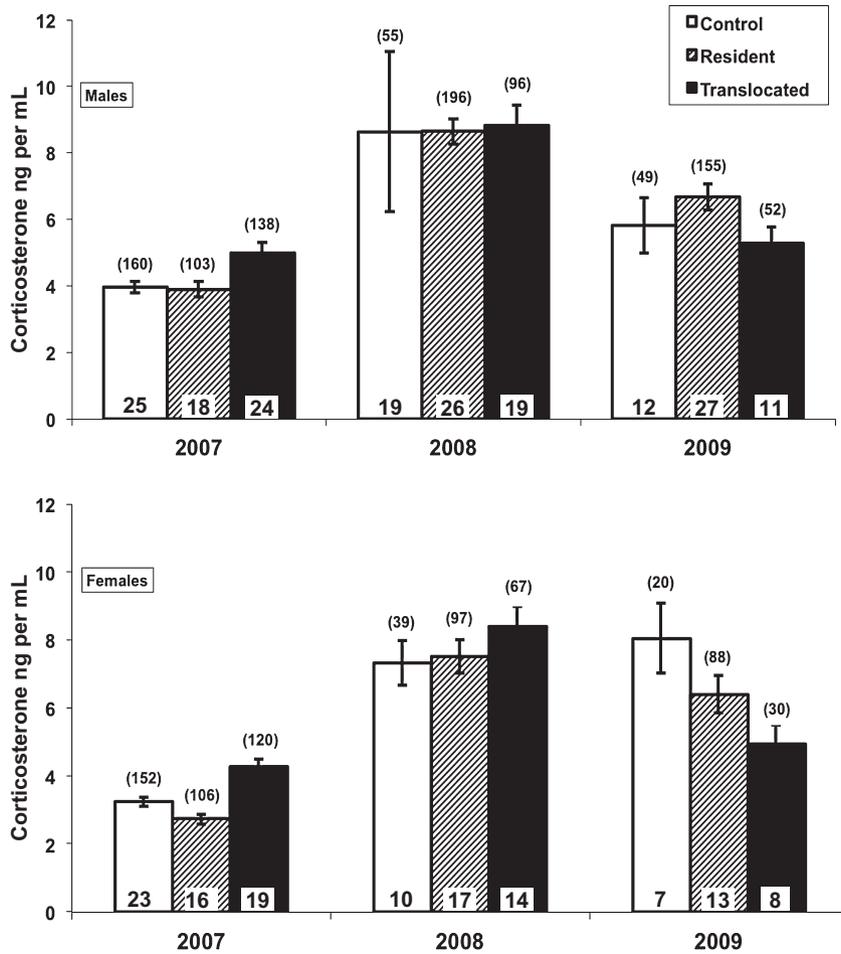


Figure 3 Total plasma corticosterone ng per mL levels (mean ± 1 SE) for male (top) and female (bottom) desert tortoises in 2007–2009 for control, resident and translocated experimental treatment groups. The number of samples collected for each group is stated above the bars, and number of study animals in each group is given within the bar just above the axis. There were no statistical differences among the treatment groups in 2008 and 2009.

covariates or factors of interest (and notably the translocation treatment – see Fig. 3) contributed significantly toward explaining CORT (Table 2). Model weights decreased by more than 0.80 with inclusion of any of the following factors: the experimental treatments, month in which the sample was collected, air temperature; number of needle insertions required to collect sample; tortoise handling time; tortoise behavior; and tortoise location (Table 2).

CORT in male tortoises was generally higher than female tortoises throughout the activity season ($F_{1,174} = 24.95$, $P < 0.01$; Table 3, Fig. 4). CORT concentrations in males did not appear to follow a detectable seasonal trend among years. Female CORT levels increased in late spring (May;

Fig. 4). We were unable to model annual biomass and precipitation across animal home ranges in 2007 due to low production and number of samples taken. However, we analyzed CORT, relative to annual plant production, and precipitation across the animal’s home-range in 2008 and 2009. Neither annual plant production nor precipitation provided a significant contribution to the model (e.g. there was a decrease in model weight over the best model by 0.86 on inclusion), suggesting that other environmental factors (not measured) may play an important role in influencing CORT.

Discussion

We found that translocation of desert tortoises did not result in elevated stress levels (using CORT as a surrogate marker for stress) relative to either resident or control animals. Rather, variations in CORT were best explained by sex and year, while accounting for lymph presence in the sample. Our results are similar to those reported for other species of tortoises (Kahn *et al.*, 2007), and may reflect a general trend in these organisms. Alternatively, the lack of a stress response may suggest that another measure of stress is more important, or that chronic stress associated with

Table 2 ANOVA table showing model coefficients and significance tests for model best describing plasma total corticosterone levels in desert tortoises from 2007 to 2009

Coefficients	numDF	denDF	F value	P value
Intercept	1	1544	3077.3	< 0.01
Lymph	3	1544	460.3	< 0.01
Sex	1	174	16.5	< 0.01
Year	2	1544	111.3	< 0.01

Table 3 Calculated total plasma corticosterone levels (mean \pm 1 SE) for each experimental treatment for female and male desert tortoises for 1 year prior (2007) and 2 years posttranslocation (2008–2009) at Fort Irwin, California

Year	Experimental treatment	Sex	Mean monthly corticosterone ng per mL \pm SE						
			April	May	June	July	August	September	October
2007	Resident	F	2.45 \pm 0.23	2.53 \pm 0.42	2.56 \pm 0.43	3.30 \pm 0.38	2.94 \pm 0.29	3.11 \pm 0.44	2.16 \pm 0.43
		M	4.09 \pm 0.63	4.19 \pm 0.65	3.11 \pm 0.55	4.03 \pm 0.46	3.79 \pm 0.59	4.36 \pm 0.75	3.61 \pm 0.64
	Control	F	3.36 \pm 0.32	3.03 \pm 0.37	2.73 \pm 0.37	3.46 \pm 0.41	3.00 \pm 0.32	4.02 \pm 0.45	3.17 \pm 0.33
		M	4.11 \pm 0.48	4.76 \pm 0.64	2.84 \pm 0.28	4.36 \pm 0.48	4.01 \pm 0.38	4.21 \pm 0.44	3.41 \pm 0.38
	Translocated	F	3.07 \pm 0.30	6.64 \pm 1.12	4.52 \pm 0.50	4.09 \pm 0.43	3.93 \pm 0.51	4.08 \pm 0.42	3.19 \pm 0.32
		M	5.39 \pm 0.45	7.77 \pm 1.69	4.89 \pm 0.56	3.97 \pm 0.56	5.14 \pm 0.55	4.11 \pm 0.42	3.50 \pm 0.42
2008	Resident	F	7.64 \pm 1.01	9.78 \pm 1.74	8.43 \pm 0.94	9.73 \pm 1.57	7.41 \pm 1.45	4.84 \pm 1.19	5.25 \pm 0.82
		M	11.58 \pm 1.75	9.10 \pm 1.27	9.28 \pm 0.63	8.48 \pm 0.83	9.22 \pm 0.98	5.79 \pm 0.75	8.06 \pm 0.84
	Control	F	7.88 \pm 1.20	11.13 \pm 1.58	6.33 \pm 1.38	6.79 \pm 1.74	6.58 \pm 1.72	3.12 \pm 1.02	7.39 \pm 4.19
		M	10.43 \pm 1.83	9.51 \pm 3.60	7.18 \pm 1.69	4.57 \pm 1.58	7.32 \pm 1.71	5.27 \pm 1.68	8.81 \pm 2.05
	Translocated	F	7.88 \pm 1.00	12.12 \pm 1.38	7.43 \pm 1.14	7.55 \pm 1.55	9.44 \pm 3.47	6.49 \pm 1.07	6.91 \pm 1.42
		M	8.81 \pm 1.18	7.65 \pm 0.92	9.73 \pm 0.88	8.50 \pm 1.46	12.15 \pm 2.96	6.88 \pm 1.20	8.66 \pm 1.94
2009	Resident	F	7.53 \pm 1.45	9.26 \pm 2.61	7.21 \pm 1.22	4.08 \pm 0.89	4.19 \pm 1.27	5.47 \pm 0.86	6.56 \pm 1.25
		M	8.18 \pm 0.92	7.69 \pm 1.46	6.23 \pm 0.93	5.42 \pm 1.02	4.59 \pm 0.87	6.71 \pm 0.82	6.37 \pm 1.02
	Control	F	6.35 \pm 2.10	10.30 \pm 0.64	13.10 \pm 1.11	9.83 \pm 3.92	4.04 \pm 2.18	11.21 \pm 0.64	4.83 \pm 2.23
		M	6.16 \pm 2.35	9.12 \pm 1.38	3.37 \pm 0.85	3.39 \pm 1.00	4.12 \pm 0.79	8.18 \pm 3.79	4.81 \pm 1.56
	Translocated	F	5.55 \pm 0.87	6.33 \pm 1.99	3.03 \pm 0.45	4.07 \pm 1.61	5.89 \pm 2.38	4.18 \pm 1.39	3.75 \pm 0.24
		M	5.82 \pm 1.87	5.95 \pm 1.00	3.93 \pm 0.81	4.49 \pm 1.05	4.61 \pm 1.10	5.72 \pm 1.03	5.96 \pm 1.50

translocation may alter the animal's ability to produce the appropriate glucocorticoid responses (Dickens, Delehanty & Romero, 2010).

Environmental influences such as annual forage and precipitation are thought to influence activity, behavior and metabolism in desert tortoises (Henen *et al.*, 1998). We suspect this dynamic interaction of environmental influences with tortoise physiology and behavior may dictate how they respond to harsh conditions or disturbances. In our experiment, we broadly measured lower CORT in years of low annual forage and precipitation (2007) and higher concentrations in years (2008–2009) with more abundant forage and precipitation (Fig. 3). However, neither habitat variables (i.e. estimated annual biomass production and precipitation), nor behavior and home-range size, significantly explained CORT levels. It seems other environmental or physiological factors outside the scope of this experiment may have influenced CORT. For example, in some reptiles, altered CORT is thought to be associated with changes in metabolism and activity or exposure to external noxious stimuli (Schramm, Casares & Lance, 1999; Kohel *et al.*, 2001) that can stem from a variety of sources.

Male tortoises in our experiment had higher CORT than did females during most months; which is consistent with previous research (Lance, Grumbles & Rostal, 2001). Each year, we observed increases in female CORT in late spring (May) coinciding with periods typically associated with ovulation, egg production, nesting (Rostal *et al.*, 1994; Lance *et al.*, 2001), and increased activity and movement (Inman, Nussear & Tracy, 2009). In contrast to results from Lance *et al.* 2001, we did not find a repeatable seasonal pattern in CORT for male desert tortoises. This may be because our experiment examined a much larger study area, and CORT was potentially influenced by a variety of environ-

mental influences and a greater number of wild tortoises potentially increasing the sampled variation in hormone concentrations.

Measuring stress levels under most field conditions is difficult, and simply capturing the animals to take a blood sample potentially can be stressful to some species (Sapolsky, Romero & Munck, 2000; Romero & Reed, 2005). To minimize problems associated with capture-induced stress, researchers attempt to ensure that blood samples are collected within a few minutes of animal capture (Wingfield *et al.*, 1997; Wingfield & Romero, 2001). Specific animal behaviors such as sequestering themselves in hard-to-reach cover sites, or their ability to physically resist extraction, may increase the time required for capture and blood collection while simultaneously increasing acute stress. Thus, it is important not only to understand the specific physiology of each species when measuring changes in stress relative to animal environment, but also to be cognizant of the context in which the samples were collected. Our ACTH test with captive desert tortoises revealed that ACTH-injected animals did not demonstrate an increase in CORT until approximately 20 minutes postinjection and there was no increase in CORT levels in saline-injected animals within 60 minutes. This comparison indicates that samples collected within 15 minutes of handling and manipulation represent unstressed baseline CORT and that subcarapacial venipuncture induces minimal, if any, measurable stress associated with blood collection within the timeframe that samples were collected in our experiment. However, the time associated with an acute stress response may also be associated with tortoise age, ambient temperature, and animal health or physiological status, and although important, these factors were not addressed in this experiment.

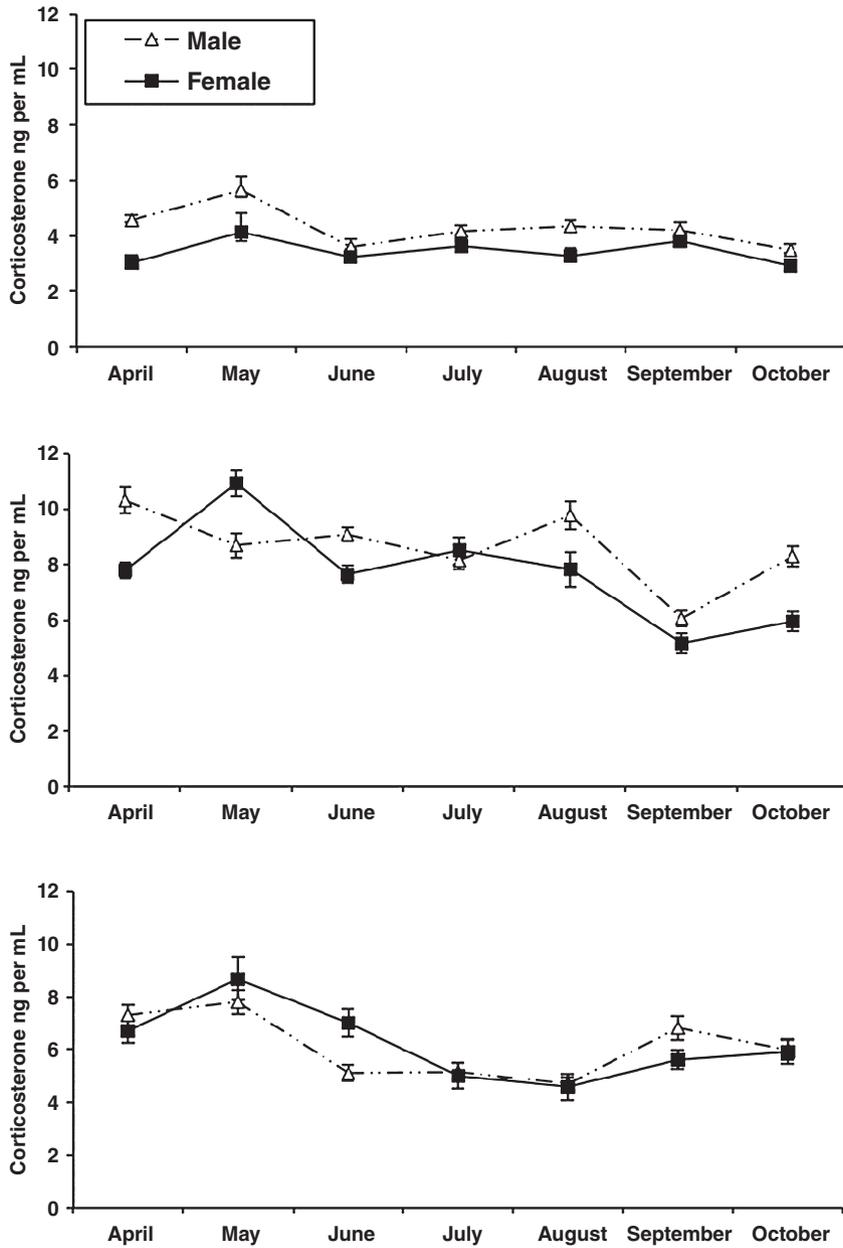


Figure 4 Total plasma corticosterone (mean \pm 1 SE) values each month during the animal activity period for female and male desert tortoises sampled in 2007 (top), 2008 (middle) and 2009 (bottom).

Urbanization, military activities and renewable energy have greatly expanded the human footprint across the Mojave Desert (Leu, Hanser & Knick, 2008) and such activities may exert influence on desert systems in unexpected ways. Because translocation is increasingly considered as a mitigation and minimization tool for the desert tortoise, it is important to understand the effects of these activities on wildlife and their habitats. Many studies have documented physiological changes associated with stress as a result of wildlife translocation (Cabezas *et al.*, 2007; Franceschini *et al.*, 2008; Dickens, Delehanty & Romero, 2009; Linklater *et al.*, 2010). Stress is generally assumed to be an inevitable outcome of translocation because it requires the handling and movement of wild animals. However, we

did not observe a measurable physiological stress response (as measured by CORT) within the first two years after translocation. In fact, the comparison of treatments (resident, control and translocated tortoises) showed no significant differences in stress levels as measured by CORT.

Acknowledgments

We thank B. Jacobs, C. Phillips, M. Walden, F. Chen, R. Inman, M. Kang, D. Essary, A. Walde, P. Woodman, K. Berry, W. Boarman, M. Quillman, C. Everly, R. Jones, R. Averill-Murray, J. Yee, K. Phillips, and numerous others in assisting with data and logistics. We thank L. Bowen, S. Jones, and anonymous reviewers for helpful comments to

improve the quality of this paper. This project was conducted under permits TE-102235 and SCP-011076 and supported by the US Department of Defense, Ft. Irwin, California. Any use of trade, product or firm names in this publication is for descriptive purposes only and does not imply endorsement by the US government.

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