1 Title: Effects of long distance translocation on corticosterone and testosterone levels in
2 male rattlesnakes

3
4 Author names and affiliations:

5 Kory H. Heiken^a, George A. Bruschi IV^{a,b}, Sarah Gartland^a, Camilo Escallón^c, Ignacio T.
6 Moore^c, Emily N. Taylor^a

7
8 ^a Department of Biological Sciences, California Polytechnic State University, San Luis
9 Obispo, CA 93401, USA, khheiken@gmail.com

10 ^b Present address: School of Life Sciences, Arizona State University, Tempe, AZ 85287,
11 USA, bruschi@gmail.com

12 ^c Department of Biological Sciences, Virginia Tech, Blacksburg, VA 24061, USA,
13 camiloescallon@gmail.com, itmoore@vt.edu

14 Corresponding author: etaylor@calpoly.edu

15
16 Abstract

17 Translocation is an increasingly common conservation tool used to augment declining
18 populations or to remove nuisance animals from areas of human conflict. Studies show
19 that venomous snakes translocated long distances may wander and experience increased
20 mortality. However, potential sub-lethal physiological effects on translocated snakes
21 remain unknown. We conducted an experimental study on free-ranging rattlesnakes to
22 test the hypothesis that long distance translocation is stressful. The glucocorticoid
23 response to translocation was variable among snakes. There was some evidence that
24 translocation may be stressful, as baseline corticosterone levels in most snakes rose
25 following translocation, whereas levels remained consistent in control snakes.
26 Interestingly, testosterone levels rose dramatically following translocation, possibly
27 reflecting effects of interaction with new environmental cues and/or resident snakes, or
28 effects of navigation in a new environment. Corticosterone and testosterone were
29 positively correlated. Our study shows that long distance translocation can affect steroid
30 hormone concentrations in rattlesnakes, a result that should be taken into consideration
31 when managing nuisance snakes or repatriating animals to the wild.

32
33 Key words: stress, translocation, glucocorticoid, corticosterone, testosterone
34

1. Introduction

Translocation, or the relocation of wild animals within their former or current natural range, has long been a popular tool for the management of a wide variety of wildlife, including snakes (Dodd and Seigel, 1991; Cope and Waller, 1995; Fischer and Lindenmayer, 2000; Lenain, 2001; Germano and Bishop, 2008; Kingsbury and Attum, 2009; Troy, 2013). Translocation is increasingly being used as a management or conservation tool to augment a current population to increase its viability, to create a new population in a part of a species' historical range, or to remove nuisance venomous animals from areas with human activity (Plummer and Mills, 2000; King et al., 2004; Kingsbury and Attum, 2009; Roe et al., 2010). Since translocation will likely remain a popular management tool, especially for venomous snakes, it is crucial that its effect on subject animals be evaluated (Sullivan et al., 2015). Snakes appear to tolerate repeated short distance translocation (e.g., 250m translocations) with few behavioral or physiological effects, including a lack of effect on circulating androgens and glucocorticoids (Holding et al., 2014). However, long distance translocation, in which snakes are moved outside their home ranges and often exhibit increased rates of movement through the environment and mortality (Reinert and Rupert, 1999; Hardy et al., 2001; Nowak et al., 2002; King et al., 2004; Butler et al., 2005; Brown et al., 2008; Sullivan et al., 2015), is more commonly performed. Few studies have examined potential sub-lethal effects of long distance translocation, including possible negative impacts on the physiology of translocated snakes.

Elevated blood plasma concentration of corticosterone (CORT), the primary glucocorticoid hormone of reptiles, has been commonly used as an indicator of physiological stress (Moore and Jessop, 2003; Tokarz and Summers, 2011). In response to an acute stressor, a rapid elevation in circulating concentrations of CORT can benefit the animal by mobilizing energy stores and shifting physiological processes to best cope with the stressor. However, chronically elevated CORT concentrations can become detrimental to the animal (Dickens et al., 2010), as prolonged elevation of CORT can result in immunosuppression (Dhabhar and McEwen, 1997; French et al., 2007; Dickens et al., 2010), decreased cognitive ability (Bodnoff et al., 1995; McEwen and Sapolsky, 1995; de Kloet et al., 1999; Mendl, 1999), and decreased secretion of reproductive hormones, such as testosterone (T) in male reptiles (Moore et al., 1991; Manzo et al., 1994; Moore et al., 2000a; Moore and Jessop, 2003; Jones and Bell, 2004; Tokarz and Summers, 2011). Thus, chronic physiological stress can result in tradeoffs that adversely affect fitness. Although one study on rattlesnakes showed that short distance translocation has no major effects on baseline CORT, stress-induced CORT, or T concentrations (Holding et al., 2014), long distance translocation exposes animals to the potential stressor of introduction to a novel environment in addition to capture, handling, and transportation. Thus, long distance translocation has the potential to generate conditions of chronic stress, in which CORT concentrations remain elevated for days or weeks (Dickens et al., 2010).

Our main goal in this study was to test the hypothesis that long distance translocation causes chronic physiological stress in western rattlesnakes, *Crotalus*

oreganus. We were specifically interested in the weeks immediately following translocation because habituation could potentially occur if animals survive in the new environment for long periods of time. A ‘Before-After Control-Impact’ experimental design was used, where translocated snakes were compared to a dedicated control group that remained at the control site for the entire study duration, but where the translocated group also served as its own control prior to the act of translocation. We used baseline CORT concentration and the CORT response to an acute confinement stressor as metrics of stress. We predicted that snakes subjected to translocation would exhibit higher baseline CORT and a greater CORT response relative to control snakes. We also predicted that, among the translocated snakes, both baseline CORT and the CORT response would be greater post-translocation than pre-translocation. We also investigated the effects of translocation on T concentrations in males. Elevated T is associated with mating behavior and spermatogenesis in several rattlesnake species and other pit vipers (Schuett, 1997; Schuett et al., 2002; Taylor et al., 2004; Schuett et al., 2005; Graham et al., 2008; Lind et al., 2010). If long distance translocation is a stressor in rattlesnakes, then we predicted that translocation would reduce T concentrations. According to our hypothesis, the increase in CORT and decrease in T should result in a negative relationship between CORT and T following translocation.

2. Methods

2.1 Study area

Two sites on Vandenberg Air Force Base (VAFB), in California, U.S.A., were chosen based on the criteria of maximizing distance and habitat similarity between them. Both sites have similar vegetation, lack water sources like lakes or ponds, and are adjacent to the coast and therefore have very similar climates. Site one (N 34.60887°, W -120.52702°) is located on the southern part of the base, at the southern end of Arguello Road, Santa Barbara County, California. Site two (N 34.87266°, W -120.60091°) is located on the northern part of the base, approximately 1,500 meters heading 19° northeast from the intersection of Cuatro Road and Point Sal Road. Sites one and two served respectively as control and translocation sites and were separated by a straight-line distance of 30 kilometers.

2.2 Study snakes

Nineteen adult male *C. oreganus* (range: 77-98 cm snout to vent length, SVL, mean = 84.8 cm, SD = 5.3 cm) were captured during visual searches at the control site between 19 April 2012 and 9 July 2012. Upon capture, each snake was transported to the California Polytechnic State University, San Luis Obispo campus. Snakes were anesthetized by way of isoflurane (Halocarbon Production Corp., U.S.A.) inhalation and intracoelomically implanted with 11 or 13.5g radio-transmitters (model SI-2, Holohil Systems Ltd., Carp, Ontario, Canada). While snakes were under anesthesia, SVL ($\pm$ 0.5cm) and body mass ($\pm$ 1g) were recorded. Following surgery, snakes were given at least one day to recover, and were then released at their original capture locations. Each snake was randomly assigned to a translocated or control treatment group (see below).

Once returned to the field, each snake was tracked via radio telemetry. Prior to translocation, all snakes were tracked every other day, while after translocation, all snakes were tracked every day. Due to one field mortality, one unexplained disappearance, and three snakes that remained irretrievable underground, the sample size was reduced to N = 14 individuals (n = 7 control snakes and n = 7 translocated snakes). All snake losses occurred prior to translocation. Collection and experimental use of the snakes were authorized by the California Department of Fish and Game (# SC-8159), California Polytechnic State University (IACUC protocol # 1203), and Vandenberg Air Force Base.

2.3 Translocation

Translocation from the control site (site one) to the translocation site (site two) was considered a long distance translocation procedure because the 30 km distance between the two sites far exceeds the average total distance moved by all of our study snakes at the control site during the period prior to translocation, as well as the average total distance moved ($2470.3 \text{ m} \pm 283.4$) by *C. oregonus* from a nearby population during the spring mating season when they actively search for mates (Putman *et al.*, 2013).

Translocations began on 25 August 2012, 45 days after the last study snake was released in the field at the control site, and ended on 29 August 2012. Translocated snakes were transported inside cloth bags in buckets and released at the translocation site into suitable covered habitat. To decrease the likelihood of social interactions among study snakes, release locations were separated by distances of 24-75 m.

2.4 Hormone sampling

Just prior to translocation and again at the conclusion of the study (ca. two weeks after translocation), blood was collected in the field from all 14 snakes for the purpose of quantifying plasma concentrations of T and CORT. Blood was collected from the caudal vein with heparinized syringes. During each of the two sampling periods (before translocation: 25-28 August 2012; after translocation: 7-8 Sept 2012), two samples were collected, the first to obtain baseline hormone concentrations and the second after the application of an acute confinement stressor to measure hormonal stress reactivity. We collected baseline blood samples as quickly as possible (mean = 3.8 min, range 1-12 min). Sampling time (time of day) and time-to-bleed (# min from capture until blood draw) were recorded. Following collection of the baseline blood sample, snakes were subjected to an acute stressor in the form of one hour of restraint in a cloth bag inside an opaque 9.5 liter plastic bucket (Holding et al., 2014). Blood samples were stored in an opaque bag, out of sunlight, while in the field. Taylor and Schuett (2004) showed that such storage does not impact steroid hormone concentrations. Samples were stored at 4°C and centrifuged within 24 hours of collection to separate the plasma, which was stored at -20°C until radioimmunoassay.

2.5 Radioimmunoassay

Plasma CORT and T concentrations were quantified using a standard radioimmunoassay procedure, following extraction and chromatographic separation (see

Lind *et al.*, 2010). A volume of 10 µl of plasma was used in all instances. For individual extraction efficiency determination, we equilibrated each sample overnight with 2,000 counts per min (CPM) of tritiated steroid. Each sample was extracted with 5 ml of distilled dichloromethane with the dichloromethane phase removed and dried in a warm water bath, under a stream of nitrogen gas, and re-suspended in 10% ethyl acetate in isooctane. For the removal of neutral lipids and isolation of individual steroids, all samples were transferred to diatomaceous earth (Celite, Sigma) columns for chromatographic separation. Neutral lipids and other steroids were eluted with 2 ml of isooctane and discarded. Testosterone was eluted with 2 ml of 20% ethyl acetate in isooctane, while CORT was subsequently eluted with 2.5 ml of 50% ethyl acetate in isooctane. Samples were then dried in a 40° C water bath under nitrogen gas, re-suspended in 600 µl phosphate buffered saline, and maintained overnight at 4°C.

Individual extraction efficiencies for each steroid were determined from 100 µl of the sample while 200 µl of the sample was allocated to each of two duplicates for the assay. Mean recoveries were 60.36% for T and 58.12% for CORT. Serial dilutions for the standard curves were performed in triplicate (T curve range = 500-1 pg; CORT curve range = 2000-4 pg). All samples were incubated overnight with 100 µl of antiserum (T: T-3003, Wien Laboratories, Succasunna, NJ; CORT: Esoterix Endocrinology, Calabasas Hills, CA) and 100 µl of tritiated (³H) steroid. Unbound steroid was separated using dextran-coated charcoal and the bound steroid decanted into scintillation vials. Samples were counted on a liquid scintillation counter, and final concentrations corrected for

individual extraction efficiency. Intra-assay coefficients of variation (CV) were 3.04% for T and 11.33% for CORT.

2.6 Data analysis

For clarity, “treatment” refers to translocated versus control snakes, and “time” refers to before vs. after translocation. We investigated the effects of treatment and time on the following dependent variables: baseline CORT and T, stressed CORT and T (after application of the acute confinement stressor), and CORT and T reactivity (the difference between stressed and baseline CORT or T). The baseline sample from one snake in the translocation treatment group, before translocation, was lost while in the field. However, the other data for that snake were used in all hormone analyses. Unless otherwise noted, all data were analyzed with repeated-measures ANOVA. In all analyses of CORT and T, time-to-bleed and sampling time were investigated as possible covariates, but were not significant in any models (see below).

A mixed effects regression model was used to investigate possible relationships between CORT and T (baseline, stressed, and reactivity or the difference between the two). Individual snake was included as a random effect to account for dependence between repeated measures, and CORT was the explanatory variable and T the response variable. Transformations (natural log, square root, or cube root) of the response variable were carried out as necessary to meet the assumptions of normality (assessed via the Shapiro-Wilk test) and equality of variance (assessed via Bartlett’s and Levene’s tests). All presented data (tables and figures) are untransformed. *Post-hoc* pair-wise

comparisons (Tukey's HSD, $\alpha = 0.05$) were only carried out following a significant F-test result for the treatment group by time interaction, since only two groups were compared in all main effects tests. All analyses were carried out using JMP Pro 10.0.1 (SAS Institute Inc., Cary, North Carolina, U.S.A.).

3. Results

3.1 Corticosterone (CORT)

In the analysis of baseline CORT (prior to application of the acute stressor), the main effect of treatment was significant, with the translocated group being on average 2.3 times higher ($66.70 \text{ ng/ml} \pm 12.98$ vs. $28.98 \text{ ng/ml} \pm 6.56$) than the control group (Table 1, Figure 1a). The treatment by time (before or after translocation) interaction and the main effect of time were not significant, largely due to high variance in the translocated group CORT data. Though the interaction term was not significant, the divergence in CORT between the two groups was greater after translocation occurred, with more snakes in the translocated group increasing baseline CORT (Figure 1a). There was also a significant effect of treatment on stressed CORT concentrations, with levels in the translocated group 2.4 times higher than the control group ($344.68 \text{ ng/ml} \pm 43.99$ vs. $145.29 \text{ ng/ml} \pm 32.86$, Table 1, Figure 1b), but there was no effect of time or its interaction with treatment. Baseline CORT was a significant covariate, as was the baseline CORT by time interaction. Regarding the CORT response (stressed CORT minus baseline CORT), the main effect of treatment group was significant, with the translocated group 2.3 times higher than the control group ($264.58 \text{ ng/ml} \pm 40.45$ vs.

116.31 ng/ml $\pm$ 28.88, Table 1, Figure 1c), while the treatment by time interaction and the main effect of time were not significant. Baseline CORT and its interaction with time were also included as covariates in the analysis of the stress response.

3.2 Testosterone (T)

In the analysis of baseline T (prior to application of the acute stressor), the main effect of treatment and the treatment by time interaction were significant, while the main effect of time (before or after translocation) was not significant (Table 2, Figure 2a). Tukey's HSD *post-hoc* testing found that the two groups were not significantly different before translocation, while the translocated group had 7.3 times higher baseline T post-translocation (48.63 ng/ml $\pm$ 9.35 vs. 6.65 ng/ml $\pm$ 1.78, Figure 2a). Post-translocation, individual variation in baseline T was much greater in the translocated group than the control group. The analysis of stressed T included baseline T as a significant covariate. The main effects of treatment and time and their interaction were not significant (Table 2, Figure 2b). While it can be seen in Figure 2b that there appears to be an interaction, the trends in stressed T were explained by the very similar trends in baseline T. In the analysis of the T response (change from baseline to stressed condition), the main effects of treatment and time and their interaction were not significant (Table 2, Figure 2c). Post-translocation, there was a great deal of individual variation in T response within the translocated group.

3.3 The relationship between CORT and T

A significant but weak positive relationship between baseline CORT and baseline T was observed ($R^2 = 0.19$, $F_{1, 22.93} = 4.48$, $P = 0.045$, Figure 3a), as well as a significant but weak positive relationship between stressed CORT and stressed T ($R^2 = 0.16$, $F_{1, 22.64} = 4.76$, $P = 0.04$, Figure 3b). There was no relationship between the magnitude of the CORT response and the T response ($R^2 = 0.00$, $F_{1, 24.97} = 0.05$, $P = 0.82$, Figure 3c).

4. Discussion

We found mixed support for the hypothesis that long distance translocation is physiologically stressful to rattlesnakes in the CORT results. There was a significant main effect of treatment on CORT levels, apparently driven by increased CORT in a number of snakes following translocation, in contrast to control snakes where CORT levels remained low. The large amount of variation, especially in the control group, rendered the interaction term non-significant, which technically would lead us to conclude that CORT levels in snakes in the translocated and control groups did not change differently over time. It is important to note, however, that the sample size of seven snakes in each group may have been too small to detect an effect of translocation. It appears that individual snakes may have responded differently to translocation; it is common to observe high variability among free-ranging snakes in variables ranging from CORT levels (Lind et al., 2010) to spatial ecology (Putman et al., 2013). Furthermore, it is possible that the snakes had a large initial CORT response to translocation that had dampened by the time we collected blood samples two weeks later. Given the strong main effect of treatment and the observation that mean CORT increased over two-fold in

the translocated group and did not change in the control group, the possibility that translocation is a stressor to the snakes receiving this treatment cannot be excluded.

Other studies examining effects of translocation on hormone levels are scarce. Drake et al. (2012) used a model selection approach in a multi-year study to show that long distance translocation treatment was not a good predictor of baseline CORT in Desert Tortoises (*Gopherus agassizii*), suggesting that translocation may not result in changes in CORT in that species. Given that inter-population variation in CORT response to an acute stressor has been documented in Western Fence Lizards (*Sceloporus occidentalis*) and Common Garter Snakes (*Thamnophis sirtalis*) (Dunlap and Wingfield, 1995; Moore et al., 2001; Moore and Jessop, 2003), it may be difficult to make generalizations at any taxonomic level in reptiles. Holding et al. (2014) found that repeated short distance translocation did not affect baseline CORT, stressed CORT, or CORT response in another population of *C. oreganus*. Short distance translocation is generally considered to be a better approach for snakes than long distance translocation, likely because snakes are translocated within their home ranges and recognize their surroundings. Our data showing increased CORT levels in some of the long distance translocated snakes, combined with the results of Holding et al. (2014) showing no effect of short distance translocation on CORT levels, supports the notion that short distance translocation is less stressful to snakes.

Contrary to our prediction, we found a significant increase in baseline T in translocated snakes. While there appeared to be a similar association for stressed T, the variation in stressed T can be explained by baseline T (e.g., those snakes with higher

baseline T also had higher stressed T as the result of a similar T response). There are several possible explanations for the increase in T post-translocation. First, the translocated snakes may have encountered resident male and/or female snakes or their chemical (e.g., pheromone) signatures, and it is possible that such encounters could stimulate increased T secretion. For example, aggressive interactions among males could potentially stimulate elevated T (e.g., Gleason et al., 2009; but see Schuett et al., 1996), and exposure to new females or their pheromones can elevate T in male animals (Macrides et al., 1975; Maruniak and Bronson, 1976; Richardson et al., 2003). Since we did not witness any social interactions between resident snakes and our study snakes, it is difficult to do more than speculate on the social ramifications of any such interactions. Alternatively, the elevated T levels might reflect a positive relationship between T and spatial learning and memory. It has been demonstrated in mice (Pyter et al., 2006), rats (Sandstrom et al., 2006; Leonard and Winsauer, 2011; Spritzer et al., 2011; McConnell et al., 2012; Hawley et al., 2013), and humans (Janowsky et al., 1994; Postma et al., 2000; Silverman et al., 1999; Cherrier et al., 2001; 2007; Burkitt et al., 2007) that baseline T is positively correlated with spatial learning and memory (but see Naghdi et al., 2003; 2005; Khorshidahmad et al., 2012). Because translocated snakes were in a completely novel environment, any movements that they made required them to learn new topography and remember where key beneficial habitat features (e.g., rodent burrows and other suitable cover) were located. It is possible that the act of navigating through a new environment stimulated the release of T in our snakes. Undoubtedly, further study is needed to

elucidate the relationship between baseline T and spatial learning and memory, especially in reptiles.

Overall, we failed to find support for our prediction that there would be a negative relationship between CORT and T. Instead, we found a weak positive relationship between baseline CORT and T and between stressed CORT and T. This positive relationship between baseline CORT and T is incongruent with the findings of other studies on rattlesnakes, in which a negative relationship (Lutterschmidt et. al., 2009) or no relationship (Taylor et al., 2004; Holding et al., 2014) was found. However, a positive association between baseline CORT and T was found in male Red-spotted Garter Snakes (*Thamnophis sirtalis*) (Moore et al., 2000b) as well as in birds that were administered exogenous T (Ketterson et al., 1991). The relationship between CORT and T is context-dependent and variable in reptiles (Moore and Jessop, 2003). While elevated baseline T may be vital for mating and may aid in spatial learning and memory, especially following long distance translocation, an associated elevation of baseline CORT may mobilize energy reserves to fuel exploratory movements or energetically costly reproductive behaviors, such as mate searching (Ashton, 2003; Moore and Jessop, 2003; Jenkins and Peterson, 2005; Putman et al., 2013).

While ours is not the first study of the effects of long distance translocation on stress physiology in reptiles (Drake et al., 2012; Holding et al., 2014), it is the first on snakes and the first to investigate a relationship between long distance translocation and steroid hormones associated with reproduction. Our study on *C. oreganus* helps elucidate how human-wildlife interactions can impact stress physiology and androgen levels, and

provides valuable information for the mitigation of conflict between this widespread venomous species and humans, with which it frequently comes into contact. By studying some of the same spatial and physiological variables, our study facilitates comparison with other short distance translocation (Brown et al., 2008; Brown et al., 2009; Holding et al., 2014) and long distance translocation (Reinert and Rupert, 1999; Nowak et al., 2002; Butler et al., 2005; Brown et al., 2008) studies on snakes, allowing better-informed management decisions. Additionally, though *C. oreganus* is a species of little conservation concern, our results may be tentatively extrapolated to inform management of threatened rattlesnake species, such as Eastern Massasaugas (*Sistrurus c. catenatus*) and Eastern Diamondbacks (*C. adamanteus*). We advocate using short distance translocation whenever feasible when managing rattlesnakes, since negative impacts on the snakes appear to be minimized in this method.

In summary, we found some support for the hypothesis that long distance translocation causes stress in rattlesnakes, as measured by increased circulating CORT levels. We found an unprecedented association between long distance translocation and elevated baseline T, which may be affected by interactions with conspecifics or their chemical signatures, movements, the extent of navigation, or CORT concentrations. We also found evidence for a positive relationship between CORT and T that is incongruous with other studies on rattlesnakes (Taylor et al., 2004; Lutterschmidt et al., 2009), highlighting the fact that the relationship between CORT and T is not iron-clad and can vary by season and taxon (Moore et al., 2000a, 2000b; Moore and Jessop, 2003). Due to such variability, coupled with the fact that the magnitude of the CORT stress response

varies so dramatically in reptiles (Dunlap and Wingfield, 1995; Moore et al., 2001; Moore and Jessop, 2003; Drake et al., 2012; Holding et al., 2014), further study of the effects of translocation on stress and sex steroid hormones in a variety of reptile taxa is warranted. The importance of integrating physiology into conservation biology (Stevenson, 2006; Tracy et al., 2006; Wikelski and Cook, 2006) and the importance of translocation to reptile conservation (Dodd and Seigel, 1991; Germano and Bishop, 2008) are established ideas. Given the paucity of studies conducted to date on how translocation affects animals physiologically, it is crucial that researchers continue to examine the effects of translocation on snakes, especially given that translocation may only increase in prevalence as a management strategy for these and other reptiles.

5. Acknowledgements

T. Frazier helped with collection and transport of snakes, and T. Sorrells and Alyssa Semerdjian helped with the daily tracking of snakes. S. Steinmaus, J. Walker, and U. Lund provided expert statistical advice. J. LaBonte and R. Evans helped with logistics, equipment, and navigation on Vandenberg Air Force Base. Members of Cal Poly's Physiological Ecology of Reptiles Laboratory (PERL) and two anonymous reviewers provided valuable comments on earlier drafts of this manuscript. This work was funded by the United States Air Force, the Chicago Herpetological Society, the California Polytechnic State University Department of Biological Sciences, and the U.S. National Science Foundation (NSF grant IOS-1145625 to ITM). The funding agencies had no role

in study design, in the collection, analysis and interpretation of data, in the writing of the report, or in the decision to submit the article for publication.

6. Literature Cited

Ashton, K.G., 2003. Movements and mating behavior of adult male Midget Faded Rattlesnakes, *Crotalus oreganus concolor*, in Wyoming. *Copeia* 2003, 190-194.

Bodnoff, S.R., Humphreys, A.G., Lehman, J.C., Diamond, D.M., Rose, G.M., Meaney, M.J., 1995. Enduring effects of chronic corticosterone treatment on spatial learning, synaptic plasticity, and hippocampal neuropathology in young and mid-aged rats. *J. Neurosci.* 15, 61-69.

Brown, J.R., Bishop, C.A., Brooks, R.J., 2009. Effectiveness of short-distance translocation and its effects on Western Rattlesnakes. *J. Wildl. Manage.* 73, 419-425.

Brown, T.K., Lemm, J.M., Montagne, J., Tracey, J.A., Alberts, A.C., 2008. Spatial ecology, habitat use, and survivorship of resident and translocated red diamond rattlesnakes (*Crotalus ruber*), in: Hayes, W.K., Beaman, K.R., Cardwell, M.D., Bush, S.P. (Eds.), *The Biology of the Rattlesnakes*. Loma Linda University Press, Loma Linda, California, pp. 377-394.

402 Burkitt, J., Widman, D., Saucier, D.M., 2007. Evidence for the influence of testosterone
 403 in the performance of spatial navigation in a virtual water maze in women but not in men.
 404 Horm. Behav. 51, 649-654.

405 Butler, H., Malone, B., Clemann, N., 2005. Activity patterns and habitat preferences of
 406 translocated and resident tiger snakes (*Notechis scutatus*) in a suburban landscape. Wildl.
 407 Res. 32, 157-163.

408 Cherrier, M.M., Asthana, S., Baker, L.D., Plymate, S., Matsumoto, A., Peskind, E.,
 409 Raskind, M.A., Brodtkin, K., Bremner, W., Petrova, A., Latendresse, S., Craft, S., 2001.
 410 Testosterone supplementation improves spatial and verbal memory in healthy older men.
 411 Neurol. 57, 80-88.

412 Cherrier, M.M., Matsumoto, A.M., Amory, J.K., Johnson, M., Craft, S., Peskind, E.R.,
 413 Raskind, M.A., 2007. Characterization of verbal and spatial memory changes from
 414 moderate to supraphysiological increases in serum testosterone in healthy older men.
 415 Psychoneuroendocrinol. 32, 72-79.

416 Cope, W.G., Waller, D.L., 1995. Evaluation of fresh-water mussel relocation as a
 417 conservation and management strategy. Regulated Rivers: Res. Manag. 11, 147-155.

418 de Kloet, E.R., Oitzl, M.S., Joëls, M., 1999. Stress and cognition: are corticosteroids
 419 good or bad guys? Trends Neurosci. 10, 422-426.

420 Dhabhar, F.S., McEwen, B.S., 1997. Acute stress enhances while chronic stress
 421 suppresses cell-mediated immunity in vivo: a potential role for leukocyte trafficking.
 422 Brain Behav. Immunity 11, 286-306.

423 Dickens, M.J., Delehanty, D.J., Romero, L.M., 2010. Stress: an inevitable component of
 424 animal translocation. Biol. Cons. 143, 1329-1341.

425 Dodd, K.C., Seigel, R.A., 1991. Relocation, repatriation, and translocations of
 426 amphibians and reptiles: are they strategies that work? Herpetologica 47, 336-350.

427 Drake, K.K., Nussear, K.E., Esque, T.C., Barber, A.M., Vittum, K.M., Medica, P.A.,
 428 Tracy, C.R., Hunter Jr., K.W., 2012. Does translocation influence physiological stress in
 429 the desert tortoise? Anim. Cons. 15, 560-570.

430 Dunlap, K.D., Wingfield, J.C., 1995. External and internal influences on indices of
 431 physiological stress I. Seasonal and population variation in adrenocortical secretion of
 432 free-living lizards, *Sceloporus occidentalis*. J. Exp. Biol. 271, 36-46.

433 Fischer, J., Lindenmayer, D.B., 2000. An assessment of the published results of animal
 434 relocations. Biol. Cons. 96, 1-11.

435 French, S.S., McLemore, R., Vernon, B., Johnston, G.I.H., Moore, M.C., 2007.
 436 Corticosterone modulation of reproductive and immune systems trade-offs in female tree
 437 lizards: long-term corticosterone manipulations via injectable gelling material. J. Exp.
 438 Biol. 210, 2859-2865.

439 Germano, J.M., Bishop, P.J., 2008. Suitability of amphibians and reptiles for
 440 translocation. *Cons. Biol.* 23, 7-15.

441 Gleason, E.D., Fuxjager, M.J., Oyegbile, T.O., Marler, C.A., 2009. Testosterone release
 442 and social context: When it occurs and why. *Front. Neuroendocrinol.* 30, 460-469.

443 Graham, S.P., Earley, R.L., Hoss, S.K., Schuett, G.W., Grober, M.S., 2008. The
 444 reproductive biology of male Cottonmouths (*Agkistrodon piscivorus*): do plasma steroid
 445 hormones predict the mating season? *Gen. Comp. Endocrinol.* 159, 226-235.

446 Hardy, D.L., Greene, H.W., Tomberlin, B., Webster, M., 2001. Relocation of nuisance
 447 rattlesnakes: problems using short-distance translocation in a small rural community.
 448 *Sonoran Herpetol.* 14, 1-3.

449 Hawley, W.R., Grissom, E.M., Martin, R.C., Halmos, M.B., Bart, C.L.S., Dohanich,
 450 G.P., 2013. Testosterone modulates spatial recognition memory in male rats. *Horm.*
 451 *Behav.* 63, 559-565.

452 Holding, M.L., Frazier, J.A., Dorr, S., Henningsen, S.N., Moore, I.T., Taylor, E.N., 2014.
 453 The hormonal and behavioral effects of repeated handling and short-distance
 454 translocation on free-ranging northern Pacific rattlesnakes (*Crotalus o. oreganus*). *J.*
 455 *Herpetol.* 48, 233-239.

456 Janowsky, J.S., Oviatt, S.K., Orwoll, E.S., 1994. Testosterone influences spatial
 457 cognition in older men. *Behav. Neurosci.* 108, 325-332.

458 Jenkins, C.L., Peterson, C.R., 2005. Linking landscape disturbance to the population
 459 ecology of Great Basin Rattlesnakes (*Crotalus oreganus lutosus*) in the Upper Snake
 460 River Plain. Idaho BLM Technical Bulletin 2005-2007. Idaho State Office, U.S.
 461 Department of the Interior, Bureau of Land Management.

462 Jones, S.M., Bell, K., 2004. Plasma corticosterone concentrations in males of the skink
 463 *Egernia whitii* during acute and chronic confinement and over a diel period. Comp.
 464 Biochem. Physiol. 137, 105-113.

465 Ketterson, E.D., Nolan Jr., V., Wolf, L., Ziegenfus, C., Duffy Jr., A.M., Ball, G.F.,
 466 Johnsen, T., 1991. Testosterone and avian life histories: the effect of experimentally
 467 elevated testosterone on corticosterone and body mass in dark-eyed juncos. Horm. Behav.
 468 25, 489-503.

469 Khorshidahmad, T., Tabrizian, K., Vakilzadeh, G., Nikbin, P., Moradi, S., Hosseini-
 470 Sharifabad, A., Roghani, A., Naghdi, N., Sharifzadeh, M., 2012. Interactive effects of a
 471 protein kinase AII inhibitor and testosterone on spatial learning in the Morris water maze.
 472 Behav. Brain Res. 228, 432-439.

473 King, R., Berg, C., Hay, B., 2004. A repatriation study of the Eastern Massasauga
 474 (*Sistrurus catenatus catenatus*) in Wisconsin. Herpetologica 60, 429-437.

475 Kingsbury, B.A., Attum, O., 2009. Conservation strategies captive rearing, translocation,
 476 and repatriation, in: Mullin S.J., Seigel, R.A. (Eds.), Snakes: Ecology and Conservation.
 477 Cornell University Press, Ithaca, New York, pp. 201-220.

478 Lenain, D., 2001. Is translocation an effective tool to remove predatory foxes from a
 479 desert protected area? J. Arid Env. 48, 205-209.

480 Leonard, S.T., Winsauer, P.J., 2011. The effects of gonadal hormones on learning and
 481 memory in male mammals: a review. Curr. Zool. 57, 543-558.

482 Lind, C.M., Husak, J.F., Eikenaar, C., Moore, I.T., Taylor, E.N., 2010. The relationship
 483 between plasma steroid hormone concentrations and the reproductive cycle in the
 484 Northern Pacific Rattlesnake, *Crotalus oreganus*. Gen. Comp. Endocrinol. 166, 590-599.

485 Lutterschmidt, W.I., Lutterschmidt, D.I., Mason, R.T., Reinert, H.K., 2009. Seasonal
 486 variation in hormonal responses of Timber Rattlesnakes (*Crotalus horridus*) to
 487 reproductive and environmental stressors. J. Comp. Physiol. B 179, 747-757.

488 Macrides, F., Bartke, A., Dalterio, S., 1975. Strange females increase plasma testosterone
 489 levels in male mice. Science 189, 1104-1106.

490 Manzo, C., Zerani, M., Gobbetti, A., Di Fiore, M.M., Angelini, F., 1994. Is corticosterone
 491 involved in the reproductive processes of the male lizard, *Podarcis sicula sicula*? Horm.
 492 Behav. 28, 117-129.

493 Maruniak, J.A., Bronson, F.H., 1976. Gonadotropic responses of male mice to female
 494 urine. Endocrinol. 99, 963-969.

495 McConnell, S.E.A, Alla, J., Wheat, E., Romeo, R.D., McEwen, B., Thornton, J.E., 2012.
 496 The role of testicular hormones and luteinizing hormone in spatial memory in adult male
 497 rats. Horm. Behav. 61, 479-486.

498 McEwen, B.S., Sapolsky, R.M., 1995. Stress and cognitive function. *Cur. Op. Neurobiol.*
 499 5, 205-216.

500 Mendl, M., 1999. Performing under pressure: stress and cognitive function. *Applied*
 501 *Anim. Behav. Sci.* 65, 221-244.

502 Moore, M.C., Thompson, C.W., Marler, C.A., 1991. Reciprocal changes in corticosterone
 503 and testosterone levels following acute and chronic handling stress in the tree lizard,
 504 *Urosaurus ornatus*. *Gen. Comp. Endocrinol.* 81, 217-226.

505 Moore, I.T., Jessop, T.S., 2003. Stress, reproduction, and adrenocortical modulation in
 506 amphibians and reptiles. *Horm. Behav.* 43, 39-47.

507 Moore, I.T., Lemaster, M.P., Mason, R.T., 2000a. Behavioural and hormonal responses
 508 to capture stress in the male red-sided garter snakes (*Thamnophis sirtalis parietalis*).
 509 *Anim. Behav.* 59, 529-534.

510 Moore, I.T., Lerner, J.P., Lerner, D.T., Mason, R.T., 2000b. Relationships between
 511 annual cycles of testosterone, corticosterone, and body condition in male Red-spotted
 512 Garter Snakes, *Thamnophis sirtalis concinnus*. *Physiol. Biochem. Zool.* 73, 307-312.

513 Moore, I.T., Greene, M.J., Mason, R.T., 2001. Environmental and seasonal adaptations of
 514 the adrenocortical and gonadal responses to stress in two populations of the male garter
 515 snake, *Thamnophis sirtalis*. *J. Exp. Zool.* 289, 90-108.

516 Naghdi, N., Shahrbanoo, O., Etemadi, R., 2003. The study of spatial memory in adult
517 male rats with injection of testosterone enanthate and flutamide into the basolateral
518 nucleus of the amygdala in Morris water maze. *Brain Res.* 972, 1-8.

519 Naghdi, N., Majlessi, N., Bozorgmehr, T., 2005. The effect of intrahippocampal injection
520 of testosterone enanthate (an androgen receptor agonist) and anisomycin (protein
521 synthesis inhibitor) on spatial learning and memory in adult, male rats. *Behav. Brain Res.*
522 156, 263-268.

523 Nowak, E.M., Hare, T., McNally, J., 2002. Management of “nuisance” vipers: effects of
524 translocation on western diamond-backed rattlesnakes (*Crotalus atrox*), in: Schuett,
525 G.W., Hoggren, M., Douglas, M.E., Greene, H.W. (Eds.), *Biology of the Vipers*. Eagle
526 Mountain Publishing, Eagle Mountain, Utah, pp. 533-560.

527 Plummer, M.V., Mills, N.E., 2000. Spatial ecology and survivorship of resident and
528 translocated hognose snakes (*Heterodon platirhinos*). *J. Herpetol.* 34, 565-575.

529 Postma, A., Meyer, G., Tuiten, A., von Honk, J., Kessels, R.P.C., Thijssen, K., 2000.
530 Effects of testosterone administration on selective aspects of object-location memory in
531 healthy young women. *Psychoneuroendocrinol.* 25, 563-575.

532 Putman, B.J., Lind, C.M., Taylor, E.N., 2013. Does size matter? Testing the effects of
533 body size and sex on spatial ecology parameters of Northern Pacific Rattlesnakes
534 (*Crotalus oreganus oreganus*) in Central California. *Copeia* 2013, 485-492.

535 Pyter, L.M., Trainor, B.C., Nelson, R.J., 2006. Testosterone and photoperiod interact to
 536 affect spatial learning and memory in adult male white-footed mice (*Peromyscus*
 537 *leucopus*). European J. Neurosci. 23, 3056-3062.

538 Reinert, H.K., Rupert Jr., R.R., 1999. Impacts of translocation on behavior and survival
 539 of timber rattlesnakes, *Crotalus horridus*. J. Herpetol. 33, 45-61.

540 Richardson, H.N., Nelson, A.L., Ahmed, E.I., Parfitt, D.B., Romeo, E.D., Sisk, C.L.,
 541 2004. Female pheromones stimulate release of luteinizing hormone and testosterone
 542 without altering GnRH mRNA in adult male Syrian hamsters (*Mesocricetus auratus*).
 543 Gen. Comp. Endocrinol. 138, 211-217.

544 Roe, J.H., Frank, M.R., Gibson, S.E., Attum, O., Kingsbury, B.A., 2010. No place like
 545 home: an experimental comparison of reintroduction strategies using snakes. J. Appl.
 546 Ecol. 47, 1253-1261.

547 Sandstrom, N.J., Kim, J.H., Wasserman, M.A., 2006. Testosterone modulates
 548 performance on a spatial working memory task in male rats. Horm. Behav. 50, 18-26.

549 Schuett, G.W., 1997. Annual cycles of testosterone in male Copperheads, *Agkistrodon*
 550 *contortrix* (Serpentes: Viperidae): relationship to timing of spermatogenesis. Mating and
 551 agonistic behavior. Gen. Comp. Endocrinol. 105, 417-424.

552 Schuett, G.W., Harlow, H.J., Rose, J.D., Van Kirk, E.A., Murdoch, W.J., 1996. Levels of
 553 plasma corticosterone and testosterone in male Copperheads (*Agkistrodon contortrix*)
 554 following staged fights. Horm. Behav. 30, 60-68.

555 Schuett, G.W., Carlisle, S.L., Holycross, A.T., O’Leile, J.K., Hardy Sr., D.L., Van Kirk,
 556 E.A., Murdoch, W.J., 2002. Mating system of male Mojave Rattlesnakes (*Crotalus*
 557 *scutulatus*): seasonal timing of mating, agonistic behavior, spermatogenesis, sexual
 558 segment of the kidney, and plasma sex steroids, in: Schuett, G.W., Hoggren, M.,
 559 Douglas, M.E., Greene, H.W. (Eds.), *Biology of the Vipers*. Eagle Mountain Publishing,
 560 Eagle Mountain, Utah, pp. 515-532.

561 Schuett, G.W., Hardy Sr., D.L., Greene, H.W., Earley, R.L., Grober, M.S., Van Kirk,
 562 E.A., Murdoch, W.J., 2005. Sympatric rattlesnakes with contrasting mating systems show
 563 differences in seasonal patterns of plasma sex steroids. *Anim. Behav.* 70, 257-266.

564 Silverman, I., Kastuk, D., Choi, J., Phillips, K., 1999. Testosterone levels and spatial
 565 ability in men. *Psychoneuroendocrinol.* 24: 813-822.

566 Spritzer, M.D., Daviau, E.D., Coneeny, M.K., Engelman, S.M., Prine, W.T., Rodriguez-
 567 Wisdom, K.N., 2011. Effects of testosterone on spatial learning and memory in adult
 568 male rats. *Horm. Behav.* 59: 484-496.

569 Stevenson, R.D., 2006. Ecophysiology and conservation: the contribution of energetics –
 570 introduction to the symposium. *Integr. Comp. Biol.* 46, 1088–1092.

571 Sullivan, B.K., Nowak, E.M., Kwiatkowski, M.A., 2015. Problems with mitigation
 572 translocation of herpetofauna. *Cons. Biol.* 29, 12-18.

573 Taylor, E.N., DeNardo, D.F., Jennings, D.H., 2004. Seasonal steroid hormone levels and
 574 their relation to reproduction in the Western Diamond-backed Rattlesnake, *Crotalus*
 575 *atrox* (Serpentes: Viperidae). Gen. Comp. Endocrinol. 136, 328-337.

576 Taylor, E.N., Schuett, G.W., 2004. Effect of temperature and storage duration on the
 577 stability of steroid hormones in blood samples from western diamond-backed rattlesnakes
 578 (*Crotalus oreganus*). Herpetol. Rev. 35, 14-17.

579 Tokarz, R.R., Summers, C.H., 2011. Stress and reproduction in reptiles, in: Norris, D.O.,
 580 Lopez, K.H. (Eds.), Hormones and Reproduction in Vertebrates, Vol. 3, Reptiles,
 581 Academic Press, pp. 169-213.

582 Tracy, C.R., Nussear, K.E., Esque, T.C., Dean-Bradley, K., Tracy, C.R., DeFalco, L.A.,
 583 Castle, K.T., Zimmerman, L.C., Espinoza, R.E., Barber, A.M., 2006. The importance of
 584 physiological ecology in conservation biology. Integr. Comp. Biol. 46, 1191-1205.

585 Troy, R., 2013. Survival of mountain quail translocated from two distinct source
 586 populations. J. Wild. Manag. 77, 1031-1037.

587 Wikelski, M., Cooke, S.J., 2006. Conservation physiology. Trends Ecol. Evol. 21, 38-45.

7. Tables

Table 1: Repeated measures analyses of baseline and stressed (after the application of an acute stressor) corticosterone (CORT) and the CORT response (stressed minus baseline) in *Crotalus oreganus*. Two treatment groups (translocated and control) were evaluated at each of two time periods (before and after translocation). Significant covariates are included where appropriate.

Response variable	Model term	Test statistic and P-value
Baseline CORT	Treatment	$F_{1,11.77} = 8.59, P = 0.01$
	Time	$F_{1,11.68} = 0.22, P = 0.65$
	Treatment*Time	$F_{1,11.68} = 0.26, P = 0.62$
Stressed CORT	Treatment	$F_{1,11.24} = 10.50, P = 0.008$
	Time	$F_{1,11.57} = 2.17, P = 0.17$
	Treatment*Time	$F_{1,13.27} = 0.95, P = 0.35$
	Baseline CORT	$F_{1,13.12} = 23.58, P = 0.0003$
	Baseline CORT*Time	$F_{1,16.75} = 6.57, P = 0.02$
CORT Response (Stressed minus Baseline)	Treatment	$F_{1,11.41} = 10.45, P = 0.008$
	Time	$F_{1,11.80} = 2.38, P = 0.15$
	Treatment*Time	$F_{1,13.46} = 1.78, P = 0.20$
	Baseline CORT	$F_{1,13.17} = 13.58, P = 0.003$
	Baseline CORT*Time	$F_{1,16.67} = 7.77, P = 0.01$

Table 2: Repeated measures analyses of baseline and stressed (following the application of an acute stressor) testosterone (T) and the T response (stressed minus baseline) in *Crotalus oreganus*. Two treatment groups (translocated and control) were evaluated at each of two time periods (before and after translocation). Significant covariates are included where appropriate.

Response variable	Model term	Test statistic and P-value
Baseline T	Treatment	$F_{1,12.29} = 11.50, P = 0.005$
	Time	$F_{1,12.01} = 0.94, P = 0.35$
	Treatment*Time	$F_{1,12.01} = 8.51, P = 0.01$
Stressed T	Treatment	$F_{1,14.59} = 0.02, P = 0.90$
	Time	$F_{1,12} = 3.81, P = 0.07$
	Treatment*time	$F_{1,14.69} = 1.64, P = 0.22$
	Baseline T	$F_{1,21.96} = 95.50, P < 0.0001$
T Response (Stressed minus Baseline)	Treatment	$F_{1,11.42} = 0.00, P = 0.95$
	Time	$F_{1,11.27} = 1.07, P = 0.32$
	Treatment*Time	$F_{1,11.27} = 0.39, P = 0.55$

8. Figure Legends

Figure 1: Corticosterone (CORT) levels of *Crotalus oreganus* before and after translocation, showing (A) baseline CORT, (B) CORT after an acute stressor, and (C) CORT response (stressed – baseline CORT). The black symbols indicate the translocated group, while the white symbols indicate the control group. The figure shows untransformed data, but data were transformed for analysis to meet assumptions for parametric tests. Error bars are one SEM.

Figure 2: Testosterone (T) levels of *Crotalus oreganus* over time (before and after translocation), showing (A) baseline T, (B) T after an acute stressor, and (C) T response (stressed – baseline T). The black symbols indicate the translocated group, while the white symbols indicate the control group. The figure shows untransformed data, but data were transformed for analysis to meet assumptions for parametric tests. Error bars are one SEM.

Figure 3: Relationship between (A) baseline CORT and baseline T levels, (B) stressed CORT and stressed T levels, and (C) CORT response and T response to an acute stressor in *Crotalus oreganus*. Individual snake was included as a random effect in the regression model to account for repeated measures (before and after translocation). The figure shows untransformed data, but data were transformed for analysis to meet assumptions for parametric tests.

Figures 3