

# Physiological and Behavioral Effects of Repeated Handling and Short-Distance Translocations on Free-Ranging Northern Pacific Rattlesnakes (*Crotalus oreganus oreganus*)

MATTHEW L. HOLDING,<sup>1,4</sup> JULIUS A. FRAZIER,<sup>1</sup> SCOTT W. DORR,<sup>1</sup> SLOANE N. HENNINGSEN,<sup>2</sup> IGNACIO T. MOORE,<sup>3</sup>  
AND EMILY N. TAYLOR<sup>1</sup>

<sup>1</sup>Department of Biological Sciences, California Polytechnic State University, San Luis Obispo, California, USA  
<sup>2</sup>398 Carilla Ln., Columbus, Ohio, USA

<sup>3</sup>Department of Biological Sciences, Virginia Tech, Blacksburg, Virginia, USA

**ABSTRACT.**—Translocation, a management and conservation strategy used commonly in which animals are moved from their sites of origin to other localities, has proven controversial. We examined the physiological and behavioral impacts of repeated handling and short-distance translocation on rattlesnakes, which are often translocated from areas of human use because of a perceived threat to people. Northern Pacific Rattlesnakes (*Crotalus oreganus oreganus*) were radiotracked for 2 months, during which time one of three treatments was imposed weekly: translocation, walk and release at that day's capture site (handling control), and undisturbed control. At both the beginning (spring) and the end (summer) of the study, blood samples were obtained before and after an acute handling stressor, and plasma concentrations of corticosterone (CORT) and testosterone (T) were determined. All rattlesnakes showed a CORT stress response, but baseline and stressed concentrations of neither hormone were affected by either translocation or handling. However, the response of both hormones to stress differed between spring and summer, with a greater increase in CORT and a detectable decrease in T occurring in summer. Activity range size was affected by translocation, whereas no effects on snake behaviors recorded during observer approach were detected. Rattlesnakes appear quite resilient to the potential impacts of frequent handling or short-distance translocation.

Resolving conflicts between humans and animals through animal translocation reduces the chances of immediate animal mortality, but can cause lasting impacts in the form of translocation-induced stress (Dickens et al., 2010). The stress associated with capture and handling is often studied through assay of the circulating concentrations of "stress" hormones, most often glucocorticoids (Dunlap and Wingfield, 1995; Moore et al., 2000; Dickens et al., 2010). Marked stress reactivity, or a rise in corticosterone (CORT) concentrations in response to acute stress, has been documented in reptiles and quantifying levels of this hormone can provide insight regarding the sensitivity of a species or population to both acute and chronic stressors (Moore et al., 1991; Dunlap and Wingfield, 1995; Tokarz and Summers, 2011). Temporary elevations of CORT can be adaptive, aiding an organism in mitigating the impacts of an acute stressor (Dunlap and Wingfield, 1995). However, chronic elevations of CORT can result in loss of energy reserves, diminished body condition, altered reproductive output, a suppressed immune system, a reduced ability to escape from predators or defend territories, as well as a diminished ability to mount an adequate adrenocortical response to acute stressors (Moore et al., 1991; Guillette et al., 1995; Moynihan, 2003; French et al., 2008). Additionally, high CORT concentrations may lower testosterone (T), thus negatively affecting male reproductive activities (reviewed in Tokarz and Summers, 2011).

Translocation is popular as a conservation strategy for repatriation efforts or removal of reptiles, especially venomous species, from areas of conflict with humans (Dodd and Seigel, 1991; Reinert, 1991; Nowak et al., 2002; Butler et al., 2005). Numerous field experiments have been conducted to assess how translocation affects the movements and survival of rattlesnakes (Reinert and Rupert, 1999; Plummer and Mills, 2000; Nowak et al., 2002; Butler et al., 2005; Brown et al., 2008, 2009). In general, short-distance translocation, or translocation

at distances near, or within, an animal's home range (Hardy et al., 2001), imparts fewer negative impacts on behavior and survival (Brown et al., 2008, 2009) than does long-distance translocation, or translocation far beyond the boundaries of the animal's home range (Reinert and Rupert, 1999; Nowak et al., 2002). However, animals translocated only short distances often return quickly to the original capture site and, as a result, can endure multiple translocations (Brown et al., 2008, 2009). Despite the focus on rattlesnakes in studies of translocation, the effects of translocation on their plasma CORT and T concentrations have not been studied. If repeated capture, handling, and movement by humans are stressful events, it is possible that such translocation regimes could result in chronic elevation of CORT, which can have serious negative effects on immune function and reproduction (Guillette et al., 1995; Moynihan, 2003; Dickens et al., 2010), both directly and via the negative effect of increased CORT on plasma T concentrations (e.g., Moore et al., 1991). Translocation could represent both an acute stressor in the form of the capture and translocation event, and a chronic stressor in the form of repeated translocations and the lasting effects of navigating through a potentially unfamiliar area. In addition, the physiological impacts of repeated handling alone merit careful consideration. Investigators often interact closely with wildlife, particularly when characterizing the demography, behavior, and physiology of free-ranging animals, and the necessary handling stress could affect the results of the study (Langkilde and Shine, 2006). Gaining an understanding of the physiological consequences of handling and translocation would be useful for both wildlife managers and herpetologists.

We hypothesized that repeated translocation and handling represent chronic stressors to rattlesnakes. We subjected free-ranging male Northern Pacific Rattlesnakes (*Crotalus oreganus oreganus*) to repeated short-distance translocation and measured its impacts on their baseline plasma CORT concentrations, CORT stress response after 1 h of confinement, and plasma testosterone (T) concentrations. Because translocation and chronic stress can increase energy expenditure, we also measured the body condition of the snakes at the end of the

<sup>4</sup>Corresponding Author. Present address: Department of Evolution, Ecology, and Organismal Biology, The Ohio State University, Columbus, Ohio, USA; E-mail: matthewholding28@gmail.com

experiment. Rattlesnakes use crypsis as an antipredator mechanism, and others have assessed the frequency of risk-averse behaviors in studies of rattlesnake–human interactions (Parent and Weatherhead, 2000; Brown et al., 2009). Here, we also quantified whether repeated short-distance translocation altered the frequency of such risk-averse behaviors by the snakes, including activity and exposure above ground and tendency to rattle when approached. If translocation and handling represent chronic stressors to rattlesnakes, then we predicted that repeatedly translocated and handled animals would have higher baseline plasma CORT, lower CORT stress response, lower plasma T, lower body condition, and a higher frequency of risk-averse behaviors than control snakes.

#### MATERIALS AND METHODS

*Study Design.*—Twenty-two adult male *C. o. oregonus* greater than 80 cm snout–vent length (SVL) were captured on the Chimineas Ranch unit of the Carrizo Plain Ecological Reserve in San Luis Obispo County, California during visual searches between 23 March 2010 and 13 April 2010. Upon capture, we obtained blood samples and then transported the snakes to our laboratory at California Polytechnic State University. We anesthetized snakes with isoflurane, using a variation of the open chamber method of Reinert and Cundall (1982) where snakes were left in plastic restraint tubes. During anesthesia, we recorded mass and SVL and implanted 11-g or 13.5-g SI-2 radiotransmitters (Holohil Systems Ltd., Carp, Ontario, Canada) in the coelom of the individual. The snakes were released at their original capture sites after 1–3 days of postsurgical recovery in the laboratory.

We assigned snakes randomly to one of three experimental groups: translocated (Tr,  $n = 8$ ), handled but not translocated (Hd,  $n = 8$ ), or undisturbed control (Cn,  $n = 6$ ). The Tr snakes received weekly (every 7–8 days) 225-m translocations in the form of a randomly selected, straight-line displacement while being carried in an opaque, white plastic bucket. We chose the 225-m distance as a short-distance translocation because snakes were placed at or near the boundaries of their current home ranges (on the basis of data on home-range size of snakes at the same study site; B. Putman, unpubl. data). The Hd snakes were captured weekly, transported in a white plastic bucket over a straight-line distance of 112.5 m, and then transported back to the site of capture and released. The Hd snakes were therefore captured as often, and transported as far, as the Tr snakes, but were never actually translocated. The Cn snakes were radio-tracked only; thus we only handled these animals at the beginning and end of the study period. We chose the direction of displacement for both the Tr and Hd animals using a random-numbers table containing only integers between 1 and 360 translated to compass bearings with 360 representing due north. We restricted our choice of direction for moving the snakes to avoid moving a snake up to 500 m or more in a single direction if it happened not to move far from the previous week's release point: random numbers were selected and eliminated until a bearing was selected that did not fall within 45° of the previous week's bearing. We measured movement distance using a handheld global positioning system (GPS) unit (Garmin Legend, Garmin) and placed Tr snakes in the nearest suitable cover (often the burrow of a California Ground Squirrel, *Otospermophilus beecheyi*) within 10 m of their relocation site. Tracking, handling, and translocation of snakes was initiated on 23 April 2010. Application of treatments occurred

approximately weekly until 5 June 2010, resulting in six total translocations or handlings per snake. Final capture of snakes occurred between 19 and 24 June 2010. We observed no mortality during the course of the study.

*Hormone Sampling and Radioimmunoassay.*—Immediately after the initial capture, we drew blood from the caudal vein using a heparin-treated syringe to ascertain baseline plasma concentrations of CORT and T. The mean duration of the blood-drawing process was about 4 min and was not correlated with plasma CORT concentration ( $P > 0.05$ ). We then placed the snake in an opaque white 9.5-L plastic bucket for 1 h before the drawing of an additional blood sample. We implemented an identical blood-draw protocol at the end of the study (final capture), resulting in four blood samples for each snake. We were unable to obtain blood from one animal (from the Hd group) during initial capture because it was copulating with a female. Also, signs of imminent transmitter failure resulted in another Hd snake receiving a second surgery halfway through the study period and so data from this snake were excluded from the analysis and results.

We took multiple steps to avoid affecting hormone concentrations during the handling of blood samples. Because transporting blood for several hours at high temperatures is unlikely to affect steroid hormone concentrations (Taylor and Schuett, 2004), we transported blood samples in an opaque bag and kept them out of direct sunlight while in the field. We stored whole blood in a refrigerator at 4°C, centrifuged it to obtain plasma within 48 h of collection, and then stored frozen plasma at –20°C until radioimmunoassay.

We measured plasma concentrations of CORT and T by standard radioimmunoassay techniques following extraction and chromatographic separation (sensu Lind et al. 2010). Mean recoveries (% hormone recovered after extractions) were 63% for T and 61% for CORT. Serial dilutions for the standard curves were performed in triplicate (T curve range = 500–1 pg; CORT curve range = 2000–4 pg). Average intra-assay coefficients of variation (measures of precision within the assay) were 6.0% for T and 8.3% for CORT.

*Snake Movements and Behaviors.*—On average, we located all snakes by radiotelemetry four times weekly between 23 April and 19 June, 2010. Each location was recorded to an accuracy of 10 m with a Garmin Legend GPS unit. We used Arcview Version 3.3 (ESRI, Redlands, California) to analyze the spatial data obtained from each snake. We estimated the activity range (minimum convex polygon, or MCP, at the 100% and 95% levels), total distance moved (the summed distance between all successive locations, minus translocation distances for the Tr group), movement rate (the total distance moved by each snake divided by the total number of days that elapsed between the initiation and termination of tracking), and movement frequency (the percentage of tracking days on which a snake had shown movement from its previous location) for each snake. In calculating movement frequency, we considered successive points greater than 10 m apart to represent a movement to account for GPS error. While radiotracking snakes, we recorded several behaviors potentially associated with risk avoidance as a way to determine if natural behaviors may have been affected by our treatments. As a measure of snake “detectability,” we recorded how frequently a snake was above ground or near the surface. We also recorded the frequencies of observations when a snake was moving vs. stationary, when a snake rattled at the observer before capture, and visually estimated the proportion of the dorsum that was sunlit during each visit.

*Snake Body Condition and Change in Mass.*—We obtained mass and SVL at initial and final capture, and the mass of any food items extracted from the snakes at final capture was excluded when calculating body condition and change in mass. We did not detect, by palpation, any food items during initial capture of the snakes. We regressed  $\log_{10}$  mass vs.  $\log_{10}$  SVL for 68 other male *C. o. oregonus* captured previously at our study site but not part of this study, and the resultant regression equation was used to calculate residuals for the snakes in the current study. We used the residual for each study snake as a body condition measure following Taylor et al. (2005).

*Data Analysis.*—First, we determined whether differences in CORT and T concentrations between initial and final capture were affected by our treatments (Tr, Hd, Cn). We considered the minutes between capture and baseline blood sampling, time of day, SVL, body condition (see below), and presence or absence of stomach contents at final capture as potential covariates in general linear models of both hormones and eliminated each on the basis of nonsignificance (all  $P > 0.1$ ). We used repeated-measures ANOVA to compare the baseline and stressed plasma concentrations of CORT and T during the final capture, with CORT or T as the response variables, and treatment (Tr, Hd, or Cn), time (baseline or stressed), and their interaction included in the model as independent variables. All analyses involving T concentrations required log transformation to meet the assumption of normality. We analyzed body condition using a two-way ANOVA with treatment and the presence/absence of stomach contents at final capture as factors. We compared the area of MCP activity ranges using analysis of covariance with treatment as a factor and individual SVL as a covariate. Change in mass over the course of the study, all movement parameters other than MCP area, and all behaviors were compared using separate one-way ANOVAs with treatment as a main effect.

For a significant  $F$ -test, we used Tukey's post hoc comparisons and all test statistics were considered significant at  $\alpha = 0.05$ . All statistical analyses were performed using Minitab version 16 (Minitab, Inc., State College, Pennsylvania).

## RESULTS

*Effects of Treatment on Corticosterone.*—Handling and translocation did not affect any facet of CORT physiology that we measured. There were no significant differences in: 1) baseline CORT concentration during the final capture ( $F_{2,18} = 0.17$ ,  $P = 0.844$ , Fig. 1), 2) stressed CORT concentration during final capture ( $F_{2,17} = 0.63$ ,  $P = 0.545$ ), 3) the magnitude of the CORT stress response during final capture ( $F_{2,18} = 0.43$ ,  $P = 0.43$ ), 4) the change in baseline CORT concentration from initial to final capture ( $F_{2,17} = 0.94$ ,  $P = 0.411$ ), 5) the change in stressed CORT concentration from initial to final capture ( $F_{2,17} = 0.61$ ,  $P = 0.557$ ), or 6) the change in the magnitude of the CORT stress response from initial to final capture ( $F_{2,17} = 0.42$ ,  $P = 0.666$ ).

Because treatment did not affect plasma CORT concentrations, we pooled data and compared baseline and stressed concentrations of both CORT and T among all snakes using paired  $t$ -tests. Stressed plasma CORT concentrations were significantly greater than baseline values during both the initial capture ( $t = -5.28$ ,  $df = 19$ ,  $P < 0.001$ , Fig. 2) and final capture ( $t = -9.65$ ,  $df = 20$ ,  $P < 0.001$ ). Baseline CORT concentrations did not differ between the initial and final capture periods ( $t = 0.53$ ,  $df = 19$ ,  $P = 0.60$ ), whereas stressed CORT concentrations were significantly higher during the final capture period than the initial capture period ( $t = 3.58$ ,  $df = 19$ ,  $P = 0.002$ ). The

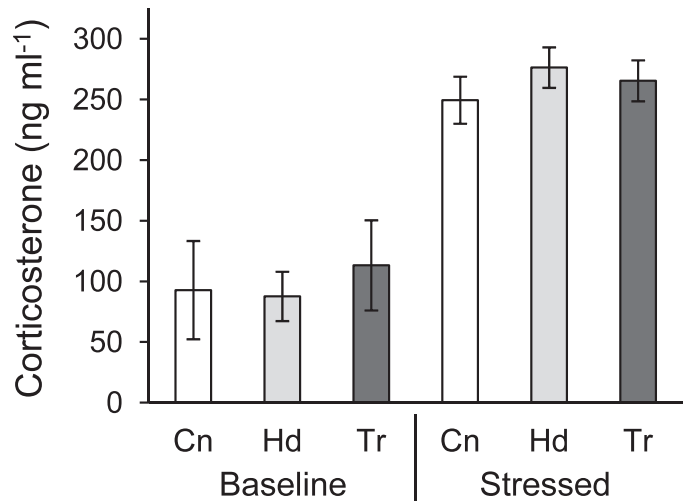


FIG. 1. Baseline and stressed corticosterone (CORT) concentrations in the plasma of translocated (Tr), handled (Hd), and undisturbed control (Cn) male *Crotalus oregonus oregonus* during their final capture, shown as mean  $\pm$  1 SE.

magnitude of the CORT stress response was therefore significantly larger during the final capture period ( $t = -3.67$ ,  $df = 19$ ,  $P = 0.002$ ).

*Effects of Treatment on Testosterone.*—As with CORT, treatment did not affect plasma T concentrations, but a temporal effect was detected. During the final capture of the snakes, treatment did not significantly affect: 1) baseline T concentrations ( $F_{2,18} = 0.49$ ,  $P = 0.620$ ), 2) stressed T concentrations ( $F_{2,18} = 0.24$ ,  $P = 0.789$ ), or 3) the magnitude of the change in T experienced during the acute stressor ( $F_{2,18} = 0.24$ ,  $P = 0.788$ ). The change in T concentrations from initial to final capture was not affected by treatment: 1) change in baseline T concentration ( $F_{2,17} = 2.04$ ,  $P = 0.161$ ); 2) change in stressed T concentration ( $F_{2,17} = 0.60$ ,  $P = 0.563$ ); or 3) change in the magnitude of the T stress response ( $F_{2,17} = 0.41$ ,  $P = 0.668$ ).

We pooled the treatment groups to examine T concentrations at initial and final capture for comparisons using paired  $t$ -tests. Baseline T concentration decreased significantly from initial to final capture ( $t = 11.02$ ,  $df = 20$ ,  $P < 0.001$ , Fig. 3). Acute stress

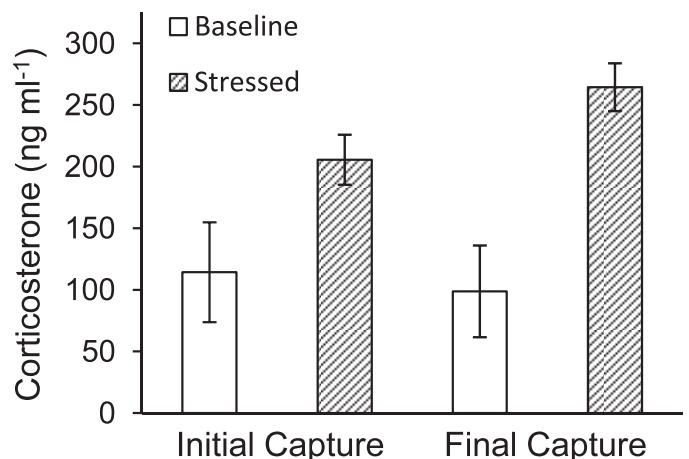


FIG. 2. Corticosterone concentrations (CORT, mean  $\pm$  1 SE) in the plasma of male *Crotalus oregonus oregonus* during both initial and final capture (all treatment groups pooled).

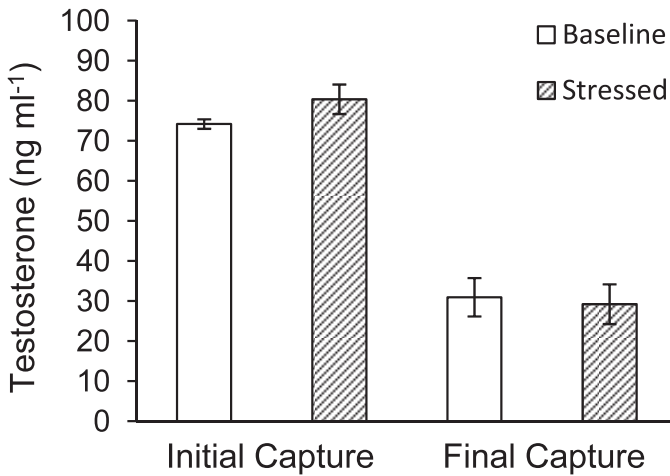


FIG. 3. Baseline and stressed testosterone (T, mean  $\pm$  1 SE) concentrations in the plasma of male *Crotalus oreganus oreganus* at initial and final capture (all treatment groups pooled). Note that this was a paired test, allowing detection of a small change.

did not significantly affect T concentrations during initial capture ( $t = -1.90$ ,  $df = 19$ ,  $P = 0.07$ ), but there was a significant acute decrease in T concentrations during final capture ( $t = 2.13$ ,  $df = 20$ ,  $P = 0.046$ ). We did not detect relationships between the plasma concentrations of CORT and T at baseline, stressed CORT and T, and the magnitude of the CORT and T stress responses ( $P > 0.05$  in all cases).

**Snake Movement and Behavior.**— Short-distance-translocated *C. o. oreganus* tended to return to the original site of capture. On seven occasions, snakes returned to the capture site within 1 day of translocation, and each of these instances involved a male that was moved away from a female with which it had been associated. Treatment significantly affected the area of the MCP activity ranges of the snakes (100% MCP:  $F_{2,15} = 10.04$ ,  $P = 0.002$ ; 95% MCP:  $F_{2,15} = 9.10$ ,  $P = 0.003$ , Table 1): Tr snakes had larger 100% MCPs than did Cn snakes ( $P = 0.02$ ), but Hd snakes did not differ significantly from either Tr ( $P = 0.06$ ) or Cn snakes ( $P = 0.88$ ). At the 95% MCP level, the relationship remained the same: Tr snakes had larger MCPs than Cn snakes ( $P = 0.01$ ), whereas Hd snakes did not differ significantly from either Tr ( $P = 0.05$ ) or Cn snakes ( $P = 0.78$ ). There was no effect of treatment on total distance moved (ANOVA;  $F_{2,18} = 0.60$ ,  $P = 0.557$ ), movement rate ( $F_{2,18} = 0.70$ ,  $P = 0.509$ ), or movement frequency ( $F_{2,18} = 0.52$ ,  $P = 0.596$ , Table 1).

Translocation and handling did not affect any of the behaviors that we recorded (Table 2). The ANOVAs with the treatment effect that included control, translocation, and handling levels were not significant: 1) snake detectability ( $F_{2,18} = 0.12$ ,  $P = 0.89$ ), 2) percentage of the snake's dorsum sunlit ( $F_{2,18} = 0.14$ ,  $P = 0.87$ ), 3) proportion of visits during

which a snake was found on the move ( $F_{2,18} = 0.41$ ,  $P = 0.67$ ), or 4) proportion of visits during which the snake rattled ( $F_{2,18} = 0.46$ ,  $P = 0.64$ ).

**Body Condition and Change in Mass.**— Health, as evidenced by body condition and changes in body mass, of *C. o. oreganus* was unaffected by the handling and translocation. Treatment did not affect body condition ( $F_{2,17} = 1.53$ ,  $P = 0.25$ ), but body condition was higher when the snake had a prey item in its stomach at final capture ( $F_{1,17} = 22.35$ ,  $P < 0.001$ ), even though the prey mass was removed from the calculations. Change in mass was also not affected by treatment ( $F_{2,16} = 0.26$ ,  $P = 0.77$ ) and was not related to initial mass ( $P = 0.3$ ).

## DISCUSSION

*Crotalus oreganus oreganus* appear robust to the physiological impacts of a fairly frequent application of handling and translocation. Despite the fact that snakes were handled weekly and Tr snakes had elevated activity ranges, we found no evidence of changes in hormone concentrations, stress reactivity, behaviors, or body condition. Baseline CORT and stress reactivity during final capture, and the magnitude of the change in baseline CORT from initial to final capture, were unaffected by treatment. Accordingly, our hypothesis, that repeated handling and translocation represent chronic stressors to reptiles, was not supported.

Higher baseline CORT is often the central measure for diagnosing chronic stress, as was the case in our study. Chronic captivity stress has raised baseline CORT in several reptiles (Moore et al., 1991; Jones and Bell, 2004; Sykes and Klukowski, 2009). Additionally, the effects of chronic disturbance by humans on baseline CORT levels of a free-ranging reptile have been documented in Tree Lizards (*Urosaurus ornatus*), where CORT was lower in animals in urban environments compared with more rural areas (French et al., 2008). However, we did not detect an effect of repeated translocation or handling on baseline CORT concentrations in *C. o. oreganus*, which means that this procedure does not induce chronic stress in rattlesnakes. It is possible that each translocation, handling event, or both induced acute stress but failed to lead to chronically elevated plasma CORT concentrations. Therefore, our study agrees with that of Kahn (2006) in suggesting that any potential stress of translocation has few physiological implications for reptiles beyond the acute stressor of capture and movement to the new site.

Although we did not detect differences in baseline CORT concentrations between initial and final capture, the magnitude of the CORT stress response (rise in CORT following capture and confinement) was larger during final capture. Since all snakes underwent some captures and surgery followed by repeated observation, it is possible that all snakes, regardless of treatment, showed a larger stress response at the end of the study due to experience. Conversely, the stress response may

TABLE 1. Minimum convex polygon (MCP) activity ranges, total distance moved, movement rate, and frequency of movement (proportion of days where movement occurred) for male *Crotalus oreganus oreganus* in each treatment group (means  $\pm$  1 SE). Different lowercase letters within a column indicate significant differences between groups for that variable.

Treatment	100% MCP (ha)	95% MCP (ha)	Total distance moved (m)	Movement rate (m/day)	Movement frequency
Control	12.16 $\pm$ 4.65 a	10.55 $\pm$ 3.93 a	2,016 $\pm$ 294 a	35.36 $\pm$ 5.15 a	0.692 $\pm$ 0.04 a
Handled	14.51 $\pm$ 4.28 ab	13.41 $\pm$ 4.28 a	2,175 $\pm$ 370 a	38.16 $\pm$ 6.50 a	0.732 $\pm$ 0.04 a
Translocated	27.33 $\pm$ 5.74 b	25.56 $\pm$ 5.40 b	2,537 $\pm$ 350 a	45.29 $\pm$ 6.35 a	0.668 $\pm$ 0.07 a

TABLE 2. Percentage of tracking episodes during which a snake was detected above ground, percentage of the snake's dorsum that was sunlit, percentage of tracking episodes in which snakes were moving, and percentage of tracking episodes during which a rattling response to approach was elicited from male *Crotalus oreganus oreganus* in each treatment group (means  $\pm$  1 SE).

Treatment	Detectability	% Sunlit	% Moving	% Rattling
Control	71.34 $\pm$ 6.33	37.67 $\pm$ 3.83	8.80 $\pm$ 2.14	12.13 $\pm$ 4.87
Handled	73.38 $\pm$ 3.77	34.84 $\pm$ 4.46	5.86 $\pm$ 1.11	11.38 $\pm$ 4.12
Translocated	70.93 $\pm$ 1.34	35.73 $\pm$ 2.79	6.42 $\pm$ 1.91	16.62 $\pm$ 4.11

vary seasonally in this population of *C. o. oreganus*. Seasonal modulation of the stress response is well documented in reptiles, especially in accordance with reproductive activities in males. Suppression of the CORT stress response during the mating season, demonstrated in several male reptiles (Dunlap and Wingfield, 1995; Cree et al., 2000; Moore et al., 2001; Cartledge and Jones, 2007; Lutterschmidt et al., 2009), may prevent males' sex hormone concentrations from declining due to the effects of high plasma CORT. In our study, rattlesnakes mounted significant CORT stress responses to capture and confinement during both spring and summer, but the lower stress reactivity during spring, when T levels are high and peak mating activity occurs (Lind et al., 2010), may reflect modulation of the adrenocortical axis to shield reproductive activities from the negative effects of stress.

Plasma T concentrations are important for reproductive success, especially in male snakes, and are often tightly linked to stress and CORT concentrations (Tokarz and Summers, 2011). The drop in baseline plasma T we observed from initial to final capture is congruent with previous studies of seasonal hormonal variation in this population of *C. o. oreganus* (Lind et al., 2010), in which androgen concentrations are elevated during the spring mating season and decline during summer. Neither baseline T concentrations during final capture nor the magnitude of the drop in baseline T from initial to final capture were affected by our handling and translocation treatments. However, we did detect a difference in the T response to acute stress between our initial and final captures. During initial capture in spring, T concentrations did not show significant change after 1 h of acute stress and the T concentrations increased on average, whereas at final capture in summer, T concentrations decreased significantly. Seasonality in the response of T concentrations to stress is further evidence that *C. o. oreganus* may modulate their gonadal response to acute stress such that T does not decrease during the spring mating period when a decrease in T may negatively affect physiological or behavioral processes important in reproduction (reviewed in Greenberg and Wingfield, 1987; Tokarz and Summers, 2011). Similarly, the plasma T concentrations of Red-Sided Garter Snakes (*Thamnophis sirtalis parietalis*) do not change in response to acute stress during the spring mating season, but do decrease during summer and fall (Moore et al., 2001). In Timber Rattlesnakes (*Crotalus horridus*), T concentrations actually increase during stress in the spring and fall, but not during summer (Lutterschmidt et al., 2009). The marked seasonal and interspecific differences in how sex hormone release is modulated by acute stress remain a poorly understood aspect of reptile field endocrinology.

Our results are comparable with those of other translocation studies in that short-distance translocation altered a spatial parameter (activity range area) but produced no adverse impacts on body condition, mortality, or behaviors. At the northern extent of the range of *C. o. oreganus*, 500-m

translocations increased total distance moved but not activity range size (Brown et al., 2009). In the present study, activity range was the only movement parameter affected, showing that even short translocations can cause unnatural expansion of the range over which a snake may roam. Considering our sample size, the difference between Tr and Hd snakes in MCP area was likely biologically significant, whereas no other movement parameters (total distance moved, rate, frequency) were affected. It is therefore likely that the increase in activity range size was driven only by the distance of translocation itself. Yet we did not observe an effect of translocation or handling on body condition, suggesting that the increase in activity range of the snakes did not increase energy expenditure or decrease energy acquisition to the point where it could affect body condition negatively. Similarly, short-distance translocation did not affect the body condition of *C. o. oreganus* at the northern extent of their range (Brown et al., 2009), and long-distance translocation did not affect body condition in Western Diamond-Backed Rattlesnakes (Nowak et al., 2002) or Timber Rattlesnakes (Reinert and Rupert, 1999). Rattlesnakes have extremely low energy expenditure requirements relative to many other reptiles (Beaupre and Duvall, 1998), and it is possible that the increased movement, activity range size, and potential stress induced by translocation in our study and the aforementioned studies were not sufficient to exact a decline in body condition. Low energy specialization may therefore be one feature that protects rattlesnakes from the negative impacts of translocation.

Although we predicted that repeated handling and translocation might increase frequency of risk-averse behaviors, such as rattling upon approach, and might induce snakes to coil in less exposed areas, it appears that *C. o. oreganus* do not alter normal behavior in response to repeated interactions of this nature. Cottonmouths (*Agkistrodon piscivorus*) habituated to human encounters over a 5-day period by showing fewer aggressive behaviors, but this effect disappeared when snakes were handled again 9 days later (Glaudas, 2004). The *C. o. oreganus* in our study went at least 1 week between successive handling or translocation events, with only observation periods in between. If the snakes we studied are similar in their habituation regime to the Cottonmouths studied by Glaudas (2004), then our stress regime may not have been vigorous or frequent enough to alter behavior. For practical purposes, however, our handling regime was likely similar to, or more stressful than, what is enacted during most studies of free-ranging reptiles or applications of translocation. Our results are encouraging in this light because they suggest that a researcher imposing frequent captures in a study is unlikely to detrimentally alter the behaviors of their focal snakes.

The importance of physiological ecology to conservation biology has been highlighted previously (Tracy et al., 2006; Dickens et al., 2010) and physiological evaluations of translocation may be key in elucidating the causes of repatriation

failures and translocation-induced mortality. We assayed quantifiable measures of stress—CORT concentrations at both acute and chronic timescales—and showed that translocation and handling did not affect these variables. Furthermore, we observed no effect on other variables that may relate to the organism's fitness, including T concentrations, movement, and body condition. It may be of interest to study the impacts of long-distance translocation on stress reactivity in translocated rattlesnakes, as these translocations seem to cause aberrant behavior and result in increased mortality (Reinert and Rupert, 1999; Nowak et al., 2002; Butler et al., 2005). Understanding how these causes of translocation failure may be exacerbated by stress could lead to more successful animal translocation programs (Dickens et al., 2010). Our study supports the notion that short-distance translocation is an acceptable mitigation strategy in terms of impacts on the snake, but it is clear that additional research is necessary to continue to inform conservation biologists and wildlife managers in their translocation and repatriation efforts.

*Acknowledgements.*—We thank C. Strand, J. Blank, and two anonymous reviewers for comments on the manuscript; S. Frame, J. Walker, and D. LeBlanc for help with statistics; and B. Stafford and the California Department of Fish and Game for access to the Chimineas Ranch. The study was funded by a National Science Foundation Graduate Research Fellowship awarded to MLH, a National Science Foundation grant to ITM (IOS 0545735), a Herpetologists' League's E.E. Williams Research Grant to MLH, and the California Polytechnic State University Biological Sciences Department. These funding sources were not involved in the design or execution of the study. The collection of snakes for research was carried out under the California Department of Fish and Game California Scientific Collection Permit # 801072-05. The use and treatment of snakes was approved by the California Polytechnic State University Institutional Animal Care and Use Committee (protocol # 910), and all research was conducted in accordance with California state and United States federal law.

#### LITERATURE CITED

- BEAUPRE, S. J., AND D. J. DUVALL. 1998. Integrative biology of rattlesnakes: contributions to biology and evolution. *Bioscience* 48:531–538.
- BROWN, J. R., C. A. BISHOP, AND R. J. BROOKS. 2009. Effectiveness of short-distance translocation and its effects on Western Rattlesnakes. *Journal of Wildlife Management* 73:419–425.
- BROWN, T. K., J. M. LEMM, J. MONTAGNE, J. A. TRACEY, AND A. C. ALBERTS. 2008. Spatial ecology, habitat use, and survivorship of resident and translocated Red Diamond Rattlesnakes (*Crotalus ruber*). In W. K. Hayes, K. R. Beaman, M. D. Cardwell, and S. P. Bush (eds.), *The Biology of the Rattlesnakes*, pp. 377–394. Loma Linda University Press, Loma Linda, CA.
- BUTLER, H., B. MALONE, AND N. CLEMANN. 2005. The effects of translocation on the spatial ecology of Tiger Snakes (*Notechis scitatus*) in a suburban landscape. *Wildlife Research* 32:165–171.
- CARTLEDGE, V. A., AND S. JONES. 2007. Does adrenal responsiveness vary with sex and reproductive status in *Egernia whitii*, a viviparous skink? *General and Comparative Endocrinology* 150:132–139.
- CREE, A., A. P. AMEY, AND J. M. WHITTIER. 2000. Lack of consistent hormonal response to capture during the breeding season of the bearded dragon, *Pogona barbata*. *Comparative Biochemistry and Physiology* 126:275–285.
- DICKENS, M. J., D. J. DELEHANTY, AND L. M. ROMERO. 2010. Stress: an inevitable component of animal translocation. *Biological Conservation* 143:1329–1341.
- DODD, C. K., AND R. A. SEIGEL. 1991. Relocation, repatriation, and translocation of amphibians and reptiles: are they conservation strategies that work? *Herpetologica* 47:336–350.
- DUNLAP, K. D., AND J. C. WINGFIELD. 1995. External and internal influences on indices of physiological stress I. Seasonal and population variation in adrenocortical secretion of free-living lizards, *Sceloporus occidentalis*. *Journal of Experimental Biology* 271:36–46.
- FRENCH, S. S., H. B. FOKIDIS, AND M. C. MOORE. 2008. Variation in stress and innate immunity in the Tree Lizard (*Urosaurus ornatus*) across an urban–rural gradient. *Journal of Comparative Physiology* 178:997–1005.
- GLAUDAS, X. 2004. Do Cottonmouths (*Agkistrodon piscivorus*) habituate to human confrontation? *Southeastern Naturalist* 3:129–138.
- GREENBERG, N., AND J. C. WINGFIELD. 1987. Stress and reproduction. In D. O. Norris and R. E. Jones (eds.), *Hormones and Reproduction in Fishes, Amphibians and Reptiles*, pp. 461–503. Plenum, New York.
- GUILLETTE, L. J., A. CREE, AND A. A. ROONEY. 1995. Biology of stress: interactions with reproduction, immunology, and intermediary metabolism. In C. Warwick, F. L. Frye, J. B. Murphy (eds.), *Health and Welfare of Captive Reptiles*, pp. 32–81. Chapman and Hall, United Kingdom.
- HARDY, D. L., H. W. GREENE, B. TOMBERLIN, AND M. WEBSTER. 2001. Relocation of nuisance rattlesnakes: problems using short-distance translocation in a small rural community. *Sonoran Herpetologist* 10: 26–31.
- JONES, S. M., AND K. BELL. 2004. Plasma corticosterone concentrations in males of the skink *Egernia whitii* during acute and chronic confinement and over a diel period. *Comparative Biochemistry and Physiology* 137:105–113.
- KAHN, P. F. 2006. *The Physiological Effects of Relocation on Gopher Tortoises (Gopherus polyphemus)*. Unpubl. Ph.D. diss., Auburn University, Auburn, AL.
- LANGKILDE, T., AND R. SHINE. 2006. How much stress do researchers inflict on their study animals? A case study using a scincid lizard, *Eulamprus heatwolei*. *Journal of Experimental Biology* 209:1035–1043.
- LIND, C. M., J. F. HUSAK, C. EIKENAAR, I. T. MOORE, AND E. N. TAYLOR. 2010. The relationship between plasma steroid hormone concentrations and the reproductive cycle in the Northern Pacific Rattlesnake, *Crotalus oreganus*. *General and Comparative Endocrinology* 166:590–599.
- LUTTERSCHMIDT, W. I., D. I. LUTTERSCHMIDT, R. T. MASON, AND H. K. REINERT. 2009. Seasonal variation in hormonal responses of Timber Rattlesnakes (*Crotalus horridus*) to reproductive and environmental stressors. *Journal of Comparative Physiology B* 179:747–757.
- MOORE, I. T., M. P. LEMASTER, AND R. T. MASON. 2000. Behavioural and hormonal responses to capture stress in the male Red-sided Garter Snakes (*Thamnophis sirtalis parietalis*). *Animal Behaviour* 59:529–534.
- MOORE, I. T., M. J. GREENE, AND R. T. MASON. 2001. Environmental and seasonal adaptations of the adrenocortical and gonadal responses to stress in two populations of the male garter snake, *Thamnophis sirtalis*. *Journal of Experimental Zoology* 289:90–108.
- MOORE, M. C., C. W. THOMPSON, AND C. A. MARLER. 1991. Reciprocal changes in corticosterone and testosterone levels following acute and chronic handling stress in the Tree Lizard, *Urosaurus ornatus*. *General and Comparative Endocrinology* 81:217–226.
- MOYNIHAN, J. A. 2003. Mechanisms of stress-induced modulation of immunity. *Brain, Behavior, and Immunity* 17:11–16.
- NOWAK, E. M., T. HARE, AND J. McNALLY. 2002. Management of “nuisance” vipers: effects of translocation on Western Diamond-backed Rattlesnakes (*Crotalus atrox*). In G. W. Schuett, M. Hoggren, M. E. Douglas, and H. W. Greene (eds.), *Biology of the Vipers*, pp. 533–560. Eagle Mountain Publishing, USA.
- PARENT, C., AND P. J. WEATHERHEAD. 2000. Behavioral and life history responses of Eastern Massasauga Rattlesnakes (*Sistrurus catenatus catenatus*) to human disturbance. *Oecologia* 125:170–178.
- PLUMMER, M. V., AND N. E. MILLS. 2000. Spatial ecology and survivorship of resident and translocated hognose snakes (*Heterodon platirhinos*). *Journal of Herpetology* 34:565–575.
- REINERT, H. K. 1991. Translocation as a conservation strategy for amphibians and reptiles: some comments, concerns, and observations. *Herpetologica* 47:357–363.
- REINERT, H. K., AND D. CUNDALL. 1982. An improved surgical implantation method for radio-tracking snakes. *Copeia*:702–705.
- REINERT, H. K., AND R. R. RUPERT, JR. 1999. Impacts of translocation on behavior and survival of Timber Rattlesnakes, *Crotalus horridus*. *Journal of Herpetology* 33:45–61.

- SYKES, K. L., AND M. KLUKOWSKI. 2009. Effects of acute temperature change, confinement, and housing on plasma corticosterone in water snakes, *Nerodia sipedon* (Colubridae: Natricinae). *Journal of Experimental Zoology* 311:172–181.
- TAYLOR, E. N., M. A. MALAWY, D. M. BROWNING, S. V. LEMAR, AND D. F. DENARDO. 2005. Effects of food supplementation on the physiological ecology of female Western Diamond-backed Rattlesnakes (*Crotalus atrox*). *Oecologia* 144:206–213.
- TAYLOR, E. N., AND G. W. SCHUETT. 2004. Effect of temperature and storage duration on the stability of steroid hormones in blood samples from Western Diamond-backed Rattlesnakes (*Crotalus oreganus*). *Herpetological Review* 35:14–17.
- TOKARZ, R. R., AND C. H. SUMMERS. 2011. Stress and reproduction in reptiles. *In* D. O. Norris and K. H. Lopez (eds.), *Hormones and Reproduction in Vertebrates*, Vol. 3, Reptiles, pp. 169–214. Academic Press, USA.
- TRACY, C. R., K. E. NUSSEAR, T. C. ESQUE, K. DEAN-BRADLEY, C. R. TRACY, L. A. DEFALCO, K. T. CASTLE, L. C. ZIMMERMAN, R. E. ESPINOZA, AND A. M. BARBER. 2006. The importance of physiological ecology in conservation biology. *Integrative and Comparative Biology* 46:1191–1205.

Accepted: 3 January 2013.