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Livetrapping is not biased by the endocrine stress response: a preliminary study in the degu (*Octodon degus*)

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While livetrapping is a vital field research tool, it is not a completely unbiased method of sampling. Biased trapping arises during field endocrinological studies whenever hormone levels or response influence the probability of capture of a subject. We repeatedly captured wild, free-living adult degus (*Octodon degus*) from the same location over 12 days to determine whether individuals with a certain endocrine stress profile were more likely to be captured repeatedly than others. We measured baseline cortisol (CORT), stress-induced CORT, and negative feedback efficacy via a dexamethasone suppression test in adult male and lactating and nonlactating female degus upon initial capture. We successfully recaptured approximately half of the degus. None of the 3 indices of the stress response at initial capture predicted whether a degu would be recaptured. However, baseline CORT levels at 1st capture had a weak, negative relationship with the number of days between 1st and 2nd capture. Because most animals interpret capture and restraint as an acute stressor, we also analyzed the effect of recapture on the endocrine stress response. Baseline and stress-induced CORT concentrations were measured upon each subsequent recapture for up to 5 total captures. Upon subsequent recaptures, neither stress-induced CORT nor baseline CORT changed significantly. Additionally, individual stress-induced and baseline CORT titers were repeatable within our sample population. These findings suggest that livetrapping does not select for animals with certain endocrine stress profiles, and that degus fail to habituate to repeated capture and restraint stress.

Aunque la captura viva de animales ha sido fundamental en estudios de campo, el método de captura puede incluir algunos sesgos. En estudios conductuales y endocrinológicos estos sesgos se producen cuando la probabilidad de captura de un individuo es afectada por su respuesta o por niveles hormonales. Se capturaron y recapturaron individuos silvestres del roedor *Octodon degus* durante 12 días para determinar si los individuos con un perfil hormonal particular en respuesta a estrés por captura tienen una mayor probabilidad de ser capturados. Se examinaron tres componentes de la respuesta fisiológica de estrés en degus hembras y machos: nivel basal de cortisol, nivel de cortisol inducido por estrés, y eficacia del sistema de retroalimentación negativa (a través de un ensayo con dexametazona). Durante el estudio se recapturaron cerca de la mitad de los individuos marcados originalmente. Ninguno de los tres componentes de la respuesta de estrés examinado fue capaz de predecir la probabilidad de recaptura en los degus examinados. Tampoco se detectó una relación entre los niveles de cortisol medidos en la primera captura y el tiempo transcurrido hasta la primera recaptura. También se examinó un posible efecto del número de recapturas sobre los niveles de cortisol basal y cortisol inducido por estrés. Aunque los niveles de cortisol inducido por estrés no cambiaron con el número de capturas, si se registró una disminución en los niveles basales de cortisol. Por último, detectamos que los niveles de cortisol basal y cortisol inducido por estrés son repetibles en la población estudiada. En conjunto, los resultados indicaron que el trampeo reiterado no selecciona degus con perfiles hormonales particulares y que estos animales no muestran hábito al estrés producto de captura e inmovilización reiteradas.

Key words: baseline CORT, cortisol, hypothalamic–pituitary–adrenal axis, livetrapping, negative feedback, *Octodon degus*, repeatability, stress-induced CORT, trappability

Livetrapping is a necessary research tool for mammalogists in many disciplines. For example, livetrapping has proven vital for estimating numerical indices of small animal population dynamics such as population size, density, abundance, and home range (Nichols and Pollock 1983; Slade and Russell 1998; Slade and Blair 2000). Field studies of ecology, behavior, and physiology generally require livetrapping to monitor and sample populations (Shargal et al. 2000; Upham and Hafner 2013). Livetrapping is also essential for studying the health and reproductive success of wild, small mammals (Flynn et al. 2011; Cudworth and Koprowski 2013; Kelt et al. 2013). Livetrapping also has allowed the endocrine stress response to be linked to individual fitness and survival in many taxa (e.g., Wingfield et al. 1997; Cyr and Romero 2007), and long-term exposure to high levels of glucocorticoids (GCs) can correlate negatively with fitness (e.g., Sapolsky et al. 2000; Romero and Wikelski 2001, 2010). In addition, conservation biologists have begun using livetrapping to measure GCs in the field as an index of wild animal population health in response to human disruption and environmental change (Wingfield et al. 1997; Homan et al. 2003; Wingfield et al. 2011).

However, livetrapping can be biased. For example, lightweight individuals may fail to trigger traps, thus biasing data toward heavier individuals within a population (Cowan and Forrester 2012). Sex, age, and body condition also bias trapping data (Buskirk and Lindstadt 1989; Dufour et al. 1993; Slough and Mourat 1996). Camera traps can provide a more unbiased "trapping" method for behavioral and demographic data, but they are useful only for large species with distinctive markings (Pesenti and Zimmermann 2013). There is also concern that trapping may select for bold, explorative individuals over shy, reactive individuals (Montiglio et al. 2012), which may reflect differences in animals' physiological responses to stressors (Koolhaas et al. 1999; Carere et al. 2010).

The endocrine stress response is regulated by the hypothalamic–pituitary–adrenal (HPA) axis, an important pathway of the vertebrate stress response (Sapolsky et al. 2000). Within minutes of perceiving a stressor, activation of the HPA axis increases GC secretion from the adrenal gland (Sapolsky et al. 2000). GCs exert numerous physiological effects; baseline levels maintain metabolic processes and permissively enhance the immediate catecholamine response to stressors, whereas stress-induced levels alter immunocompetence, decrease growth and reproductive behavior, and increase hepatic gluconeogenesis, foraging and escape behaviors (Wingfield and Romero 2001; Wingfield 2003). Overall, GCs function to reduce unnecessary energy expenditure, increase energy availability, and promote both short- and long-term survival by preparing the animal for future stressors (Wingfield and Romero 2001; Korte et al. 2005; Wingfield 2005). Accordingly, baseline and stress-induced levels of GCs can vary by sex, life history stage, time of day, and type of stressor (Sapolsky et al. 2000; Wingfield and Romero 2001; Romero et al. 2008). Capture and restraint causes a stress response in wild mammals (Kenagy and Place 2000; Place and Kenagy 2000; Romero et al. 2008), and repeated capture and activation of the HPA axis could disrupt proper functioning of the endocrine stress response (Romero 2004; Rich and Romero 2005; Sheriff et al. 2011).

We analyzed the stress response in wild, free-living common degus (*Octodon degus*) to address 3 hypotheses: 1) livetrapping introduces a sampling bias by favoring recapture of individuals with less reactive stress profiles, and 2) recapture and repeated sampling procedures alters the stress response over a short period of time. To address the 1st hypothesis, we asked if different components of the stress response predicted probability of recapture and the number of days until recapture. For the 2nd hypothesis, we analyzed whether components of the stress response changed over the course of several captures within a 2-week period. And finally, because an individual's stress response must be repeatable for trappability and stress reactivity to be meaningfully correlated, we also tested whether 3) an individual's stress response is repeatable over multiple captures. To assess the 3rd hypothesis, we examined the repeatability of each degu's baseline and stress-induced GC levels. Because GC levels can be affected by many different factors including time of day (Dallman et al. 1993), season (Romero et al. 2008; Ouyang et al. 2011), number of trapping events (Häemäeläeinen et al. 2014), and environmental conditions (Romero et al. 2000), we assessed repeatability among individuals, rather than within individuals (Romero and Reed 2008).

Materials and Methods

Study species.—Degus are semifossorial, diurnal, caviomorph rodents endemic to the semiarid matorral of central Chile (Kenagy et al. 2002; Ebensperger et al. 2004). Adult degu body mass varies with season, ranging from 100 to 300 g (Bauer et al. 2014). With a typical Mediterranean climate, the matorral is characterized by grassy, open land scattered with woody shrubs (primarily *Acacia caven* and *Proustia pungens*—Fulk 1976; Iriarte et al. 1989). Degus live in social groups of 1–12 adults and communally nest in shared burrow systems (Ebensperger et al. 2004; Burger et al. 2009). Plasma cortisol (CORT), the primary GC of degus (Kenagy et al. 1999), has been measured successfully in the wild (Bauer et al. 2013, 2014; Quispe et al. 2014). Range area and activity level of degus during the austral summer are also well understood (Kenagy et al. 2002; Quirici et al. 2010); previous studies at this field site have trapped degus repeatedly (Ebensperger et al. 2009, 2014).

Study site and trapping.—Trapping took place for 12 days during the austral summer (2–13 January 2013) at Estación Experimental Rinconada de Maipú (33°23′S, 70°31′W, elevation 495 m), a Universidad de Chile field station located 15 km outside Santiago, Chile. Animals were livetrapped using Tomahawk traps (48 × 15 × 15 cm; Tomahawk Live Trap Company, Hazelhurst, Wisconsin) baited with plain, rolled oats, which effectively captures adult degus (Burger et al. 2009). We selected the trapping area based on observed abundance of degus on the 1st day in the field. This trapping area within the Rinconada field site had not been used before, so degus could be assumed to be naïve to traps. We set 42 traps near burrow entrances and along runways as suggested by Burger et al.

(2009). The trapping area was spread over approximately 1,500 m^2, which did not exceed the maximum range area of degus (Quirici et al. 2010). Therefore, trapping effort was distributed equally, and all animals could theoretically be trapped by any 1 of 42 traps. We opened traps every morning prior to the degus' emergence from their burrows (approximately 1 h after sunrise) and closed them 4–6 h later. After this time, degu aboveground activity declines rapidly and remains low throughout the afternoon due to persistently high summer temperatures (Kenagy et al. 2002).

Because our study system was not closed, there was a possibility that we never captured some individuals within our study area. However, by extensively surveying with binoculars, previous studies at Rinconada confirmed that all individuals living within a targeted area can be captured within 1 week of setting traps (C. Bauer, pers. obs.). Additionally, the study on which we based the trapping layout (Burger et al. 2009) found this protocol produces unbiased demographic data. Therefore, it is unlikely that the study design was biased significantly by "untrappable" individuals.

Overall, we captured 37 degus during the trapping period. Of those 37, we excluded 4 animals from this analysis because their initial capture occurred on the last field day, making them impossible to recapture. Therefore, we analyzed data from 33 degus. These animals fit into 1 of 3 categories: nonlactating females (approximately 4 months old, $n = 12$), lactating females (> 1 year old, $n = 8$), or males ($n = 13$). Females were categorized as lactating if they had elongated teats; otherwise they were classified as nonlactating. While we captured more females than males in this study, a trapping sex bias is unlikely because a previous study at this field site verified that Tomahawk traps capture the sexes in proportion to abundance, and that females usually outnumber males in this population (Burger et al. 2009).

Blood sampling and assay.—All blood samples were taken from the saphenous vein and collected in heparinized, microhematocrit capillary tubes. While traps were open, 1–3 observers surveyed with binoculars to determine the exact time of capture. Baseline blood samples (~ 60 µl) were taken within 3 min of the trap door closing to control for the increase in CORT that occurs starting 3 min after disturbance (Romero and Reed 2005). Any degus caught without detection time recorded or bled after 3 min were excluded from baseline CORT analyses, but were included in stress-induced CORT and negative feedback analyses since we knew the approximate time of capture within a few minutes.

After this initial sampling, degus were returned to their traps and brought to a shaded area for further processing. Degus were sexed, weighed to the nearest 0.1 g, and ear-tagged for further identification. Stress-induced blood samples (~ 30 µl) were collected 30 min after capture. Degus were then given an intraperitoneal injection of dexamethasone (DEX) (Vedco, St. Joseph, Missouri) at a 1 mg/kg of body weight dose before being placed back into their shaded traps. DEX is a potent GC agonist used as an indicator of negative feedback efficacy (Carroll et al. 1981; Reeder and Kramer 2005). Post-DEX blood samples (~ 60 µl) were taken 90 min after injection (Bauer et al. 2013) based on time frames used in other studies (e.g., Sapolsky 1983; Sapolsky and Altmann 1991; Boonstra and Singleton 1993). All degus were given oats during later stages of processing.

Of the 33 total degus analyzed in this study, 18 were recaptured at least once. We collected baseline and stress-induced samples with each recapture event, up to a maximum of 5 total captures. We also weighed degus after each recapture; however, DEX sampling was not repeated on any animal. Degus caught more than 5 times were kept in the shade until processing was complete and they could be released with the other captured animals (up to 4 h total). This experimental protocol was approved by the Institutional Animal Care and Use Committee at Tufts University and carried out according to guidelines of the Association for Assessment of Laboratory Animal Care. All protocols and procedures also adhered to the Guidelines of the American Society of Mammalogists for the use of wild animals in research (Sikes et al. 2011).

All blood samples were immediately placed in a cooler with cold packs after collection. No more than 9 h passed between sample collection and centrifugation. Samples were centrifuged at approximately 230 gravity (G) for 3 min. The plasma was then drawn off and stored at −20°C until further processing. Plasma samples were measured for CORT with I^{125} radioimmunoassay kits (Corti-Cote Solid Phase Component System, MP Biomedicals LLC, Irvine, California). Samples were assayed in duplicate. Distilled water was added to plasma to bring the total volume up to 25 µl. Assay sensitivity was 0.7 ng/ml, and intra- and inter-assay variations were 4.6% and 7.4%, respectively.

Some studies suggest that both "free" and "bound" GC concentrations should be quantified, as only unbound GCs are available to bind to receptors (Breuner et al. 2013). However, this assertion is under debate, as corticosteroid binding globulin (CBG) facilitates transportation of CORT through the bloodstream, and it may act as a "CORT reservoir" (Schoech et al. 2013). Therefore, we did not measure CBG concentrations in our study. Additionally, we analyzed stress-induced levels of CORT at their absolute levels rather than relative levels (stress-induced CORT – baseline CORT), because absolute levels may have more biological relevance (Romero 2004).

Statistical analyses.—We conducted all analyses with SPSS version 20.0 (IBM Corp., 2011) at a significance level of $P < 0.05$. Integrated CORT after DEX injection was calculated as a measure of negative feedback efficacy in accordance with Bauer et al. (2013) and Dickens et al. (2009). Sample sizes varied for each stress response variable due to sample loss and unnoticed captures. We used binomial logistic regression analysis to determine if baseline CORT, stress-induced CORT, negative feedback efficacy, and status (nonlactating female, lactating female, or male) significantly predicted whether an animal would be recaptured. The same data were used to both build and evaluate the model.

We used Poisson regression to determine if an individual's stress profile at 1st capture was related to the total number of capture events (dependent variable). Status, baseline CORT, stress-induced CORT, and integrated CORT after DEX injection

at 1st capture were used as predictor variables. We also used Poisson regression to determine if an individual's stress profiles at 1st and 2nd capture were related to the number of days between 1st and 2nd capture (dependent variable). Status, baseline CORT at 1st capture, stress-induced CORT at 1st capture, integrated CORT after DEX injection at 1st capture, baseline CORT at 2nd capture, and stress-induced CORT at 2nd capture were used as predictor variables. We confirmed that data met collinearity and independent error assumptions by checking variance inflation factor values and running a Durbin-Watson test, respectively. After analysis, standardized residuals were plotted against predicted values to check for constant variance.

To analyze the effect of recapture on baseline and stress-induced CORT, we used 1-way repeated measures analyses of variance (ANOVAs) for all animals captured at least 4 times (n = 7). Stress-induced CORT concentrations met normality assumptions; baseline CORT concentrations were log-transformed. If sphericity assumptions were not met, we used the Greenhouse-Geisser adjustment (Bathke et al. 2009). Significant time effects were analyzed with Fisher's least significant difference post-hoc tests. Because sample sizes within each sex and reproductive status category were low for animals caught at least 4 times, we also performed 2-way repeated measures ANOVAs on all degus caught at least 2 times (n = 18) to see if baseline and stress-induced CORT changed significantly over time and within sex and reproductive status category.

To determine whether baseline and stress-induced CORT concentrations were consistent over time across individuals, we analyzed all degus caught at least 4 times (n = 7). Following statistical procedures outlined in Romero and Reed (2008), we ranked individuals from the lowest to highest CORT concentrations for each of the 4 time measures. Because baseline CORT can vary with daily conditions such as temperature (Romero et al. 2000) and could also change each time animals are captured, we felt that testing repeatability among individuals was more appropriate than testing repeatability within individuals. In this way, we were able to test whether a "low-responding" individual was always a low responder. We then performed a Kruskal–Wallis test to determine whether mean rank was consistent for each individual. Finally, we calculated the repeatability statistic (r):

$$r = \frac{s_A^2}{s^2 + s_A^2}$$

where s^2 = error mean squares, s^2_A = (model mean squares – s^2)/n_0, and n_0 = number of samples per individual (Lessells and Boag 1987).

Results

Relationship between stress response and recapture.—Overall, we recaptured 18 of 33 degus (55%) at least once. We used logistic regression to predict the probability that a degu would be recaptured. When compared with a model including only the intercept, the full model was not statistically significant (χ_5^2 = 6.37, n = 31, P = 0.27). Holding all other variables constant, none of the predictor variables significantly affected recapture rate (baseline CORT: Wald χ_1^2 = 0.01, P = 0.75; stress-induced CORT: Wald χ_1^2 = 0.23, P = 0.63; DEX: Wald χ_1^2 = 0.72, P = 0.40), except that lactating females (n = 8) were significantly more likely to be recaptured compared to males (n = 13) (Wald χ_1^2 = 4.11, P = 0.04). Lactating females and males did not differ significantly in recapture rates compared to nonlactating females (n = 12; Wald χ_1^2 = 1.78, P = 0.18 and Wald χ_1^2 = 1.34, P = 0.25, respectively).

The mean number of times an individual degu was captured was 2.3 ± 0.3 (*SE*, n = 33), with a maximum number of 8 captures for an individual. Status and stress profile at 1st capture were not significantly related to the total number of times an individual degu was captured ($\chi_{5,25}^2$ = 2.67, P = 0.75, McFadden's pseudo R^2 = 0.03). For individuals captured more than once, the mean time between 1st and 2nd capture was 3.8 ± 0.8 days (n = 18 degus). The longest interval between 1st and 2nd capture for an individual was 11 days. Status and stress profile at 1st capture and 2nd capture were significantly related to the number of days between 1st and 2nd capture ($\chi_{7,16}^2$ = 15.97, P = 0.03, McFadden's pseudo R^2 = 0.22). For degus caught at least twice, the number of days between 1st and 2nd capture was significantly shorter for males (n = 4) compared to both lactating (n = 6) and nonlactating females (n = 6; Wald χ_1^2 = 8.19, P < 0.01 and Wald χ_1^2 = 9.42, P < 0.01, respectively). Lactating and nonlactating females did not significantly differ in the number of days between 1st and 2nd capture (Wald χ_1^2 = 0.05, P = 0.83). Baseline CORT levels at 1st capture had a weak, negative relationship with the number of days between 1st and 2nd capture (Wald χ_1^2 = 4.93, B = –0.003, P = 0.03). The number of days between 1st and 2nd capture was not significantly related to stress-induced CORT levels at 1st capture, integrated CORT after DEX injection at 1st capture, baseline CORT levels at 2nd capture, or stress-induced CORT levels at 2nd capture (Wald χ_1^2 = 0.03, P = 0.86; Wald χ_1^2 = 0.22,

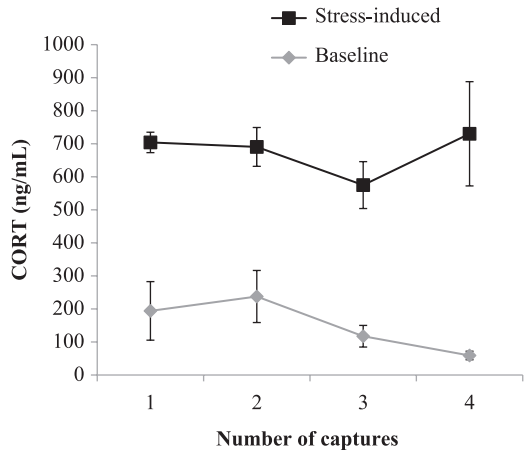

Fig. 1.—Mean (± *SE*), baseline (n = 6), and stress-induced (n = 7) cortisol (CORT) at 1st, 2nd, 3rd, and 4th capture for degus (*Octodon degus*) trapped at Rinconada de Maipú, Chile, between 2 and 13 January 2013. Only degus captured at least 4 times were included in this analysis. Neither baseline nor stress-induced CORT changed significantly with repeated captures.

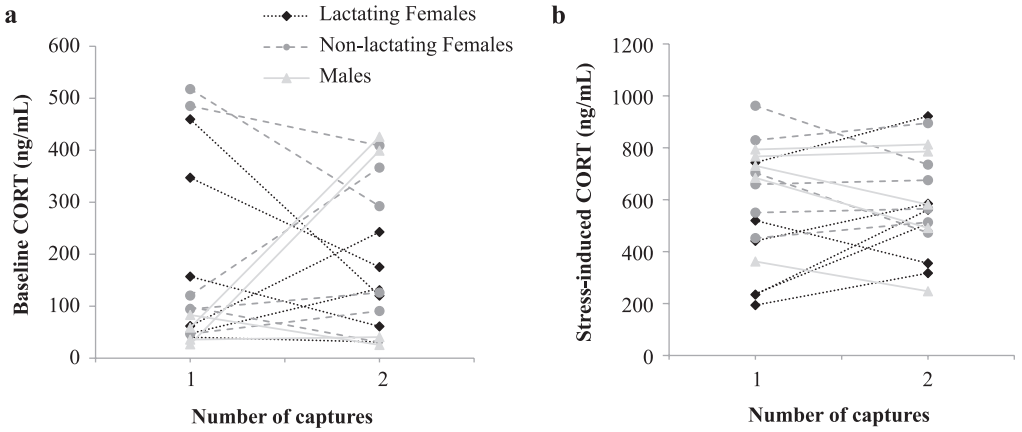

Fig. 2.—Individual a) baseline (n = 16) and b) stress-induced (n = 17) cortisol (CORT) levels at 1st and 2nd capture from lactating female (n = 6 and 6), nonlactating female (n = 6 and 6), and male (n = 4 and 5, respectively) degus (*Octodon degus*) trapped at Rinconada de Maipú, Chile, between 2 and 13 January 2013. Only degus captured at least 2 times were included in this analysis. Neither baseline nor stress-induced CORT changed significantly from 1st to 2nd capture across all individuals or within any sex and reproductive status group.

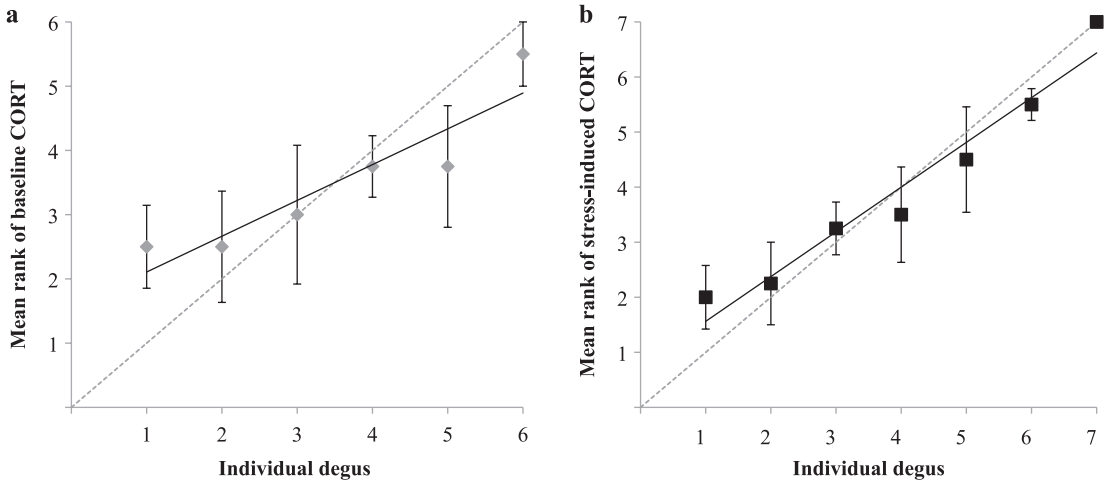

Fig. 3.—Mean rank (± *SE*) of a) baseline (n = 6, filled diamonds) and b) stress-induced (n = 7 , filled squares) cortisol (CORT) titers over 4 captures for degus (*Octodon degus*) at Rinconada de Maipú, Chile, between 2 and 13 January 2013. Data for individual degus were placed on the x-axis in ascending order of mean rank from the 4 bleeds. Equation for the line of best fit is: $y = 0.557x + 1.55$ for baseline CORT ($R^2 = 0.441$) and $y = 0.813x + 0.75$ for stress-induced CORT ($R^2 = 0.621$). A perfect repeatability relationship would have a best-fit line with a slope of 1 (dashed lines).

$P = 0.64$; Wald $\chi_1^2 = 0.60$, $P = 0.44$; Wald $\chi_1^2 = 0.28$, $P = 0.60$, respectively).

Effect of repeated trapping on the stress response.—Baseline CORT did not change significantly over the course of 4 captures (1-way repeated measures ANOVA: $F_{3,15} = 1.44$, $P = 0.27$, partial $\eta^2 = 0.22$; Fig. 1). Furthermore, stress-induced CORT did not change significantly over the course of 4 captures (1-way repeated measures ANOVA: $F_{1.328,7.970} = 1.016$, $P = 0.37$, partial $\eta^2 = 0.15$; Fig. 1).

Time, status, and the interaction between time and status did not affect baseline CORT levels significantly from 1st to 2nd capture (2-way repeated measures ANOVA, effect of time, $F_{1,1} = 0.75$, $P = 0.40$, partial $\eta^2 = 0.06$; effect of status, $F_{2,13} = 1.11$, $P = 0.36$, partial $\eta^2 = 0.15$; effect of time * status, $F_{2,2} = 0.98$, $P = 0.40$, partial $\eta^2 = 0.13$; Fig. 2a). Similarly, time and status did not affect stress-induced CORT levels from 1st to 2nd capture (2-way repeated measures ANOVA, effect of time, $F_{1,1} = 0.01$, $P = 0.91$, partial $\eta^2 = 0.001$; effect of status, $F_{2,14} = 1.92$, $P = 0.18$, partial $\eta^2 = 0.27$). However, the interaction between time and status was significant ($F_{2,2} = 4.38$, $P = 0.03$, partial $\eta^2 = 0.39$; Fig. 2b). Subsequent examination showed that nonlactating females and males had nonsignificant decreases in stress-induced CORT from 1st to 2nd capture (1-way repeated measures ANOVA, nonlactating females: $F_{1,5} = 0.77$, $P = 0.42$, partial $\eta^2 = 0.13$; males: $F_{1,4} = 3.65$, $P = 0.13$, partial $\eta^2 = 0.48$), whereas lactating females appeared to increase stress-induced CORT from 1st to 2nd capture, but this result was not statistically significant ($F_{1,5} = 4.39$, $P = 0.09$, partial $\eta^2 = 0.47$).

Repeatability of the stress response.—Relative baseline CORT concentrations were consistent across individuals from the 1st to 4th capture (Kruskal–Wallis: $H_5 = 12.32$, $P = 0.03$; repeatability statistic: $r = 0.44$; Fig. 3a). Relative stress-induced CORT concentrations were also consistent across individuals from the 1st to 4th capture (Kruskal–Wallis: $H_6 = 18.44$, $P < 0.01$; repeatability statistic: $r = 0.62$; Fig. 3b).

Discussion

Probability of recapture could not be predicted by any stress response components examined; initial baseline CORT, stress-induced CORT, and negative feedback efficacy did not differ between recaptured and nonrecaptured animals. An individual's stress response components also were not related to the total number of times it was captured. While there was a weak relationship between baseline CORT at 1st capture and the number of days between 1st and 2nd capture, no other stress response components were significantly related with the duration of time between 1st and 2nd capture. These results indicate no trapping bias toward certain stress profiles when capturing wild, free-living degus, at least during initial capture.

Although recapture probability was not related to stress reactivity, recapture probability may have been linked with an individual's "motivation." Rather than repeatedly capturing individuals with certain stress profiles, we may have recaptured the hungriest or most food-motivated degus. Our finding that lactating females were significantly more likely to be recaptured than were males supports this hypothesis. Lactation is typically the most energetically challenging life history stage for female mammals (Speakman 2008), and individuals nursing litters may have been more motivated to seek out and consume the oats we used for trap bait. However, lactating females were not significantly more likely to be recaptured than were nonlactating females ($P = 0.18$). This finding also supports the hypothesis that age does not influence trappability, because lactating females were 1 year older than nonlactating females, who were 4 months old. In our population, 97% of adult females exhibited a 2nd pregnancy and lactation during late spring and early summer of 2012–2012 (L. Ebensperger, pers. obs.). In contrast, all younger individuals were nonlactating because they could not have reached sexual maturity and given birth within 4 months. Trappability also could be influenced by sex, but recapture rates did not differ between nonlactating females and males. Furthermore, Burger et al. (2009) did not find a sex bias in the trapping techniques used at this field site. Whereas we demonstrated that stress response components did not differ between degus caught once and degus caught more than once, we cannot assert that captured animals had similar stress profiles to noncaptured animals. However, as we mentioned earlier, it was unlikely that we had more than a few uncaptured individuals in the trapping area.

Edge effects also may have a potential confounding influence on this study. Because the study site was not closed, individuals living on the periphery of the trapping area may have had a lower chance of recapture. However, because individuals were almost always recaptured in the same section of the trapping grid where they were first captured, it is unlikely that edge effects significantly influenced this study's findings. Future studies could control for this factor by either increasing sample size or by radiocollaring individuals to determine burrow locations and home ranges.

Our finding that individual stress profiles and recapture probabilities were not related could be due to low sample size. Several factors, such as age, sex, and reproductive status, may have increased the noise within the data. Lengthening the duration of the study, or carrying out the same multi-day trapping regime at multiple sites, would effectively increase sample size. Increasing the trapping area would not be as effective, because large areas would be more difficult to monitor and would require more manpower to prevent a "processing backlog." Our finding that the number of days between 1st and 2nd capture was influenced by sex and baseline CORT at 1st capture may have implications for long-term studies with wild degu populations, as individuals with low baseline CORT at 1st capture may be trapped somewhat less frequently but males may be trapped more frequently. Whether this trend would hold true for subsequent recaptures remains unanswered, as we had a low sample size for degus captured more than twice. Additionally, while the relationship between baseline CORT and number of days between 1st and 2nd capture was statistically significant, the relatively low slope of the relationship ($B = –0.003$) implies a very small biological effect.

We examined baseline and stress-induced CORT over multiple captures to determine whether repeated captures affected the HPA axis. Habituation is commonly cited as an explanation for changes in the stress response over time (Cyr and Romero 2009). Habituation is defined as a decrease in stress-induced CORT that results when an animal no longer perceives a stressor as stressful (Cyr and Romero 2009). Lynn et al. (2010) speculated that wild, free-living female eastern bluebirds (*Sialia sialis*) habituated to handling because birds sampled twice had lower stress-induced CORT than did control birds. Captive, adult male American kestrels (*Falco sparverius*) showed a similar decrease in HPA reactivity after multiple sampling events, and Love et al. (2003) also cited habituation as the likely cause. However, our results indicate that degus did not habituate to capture and handling as stress-induced CORT did not decrease over time. When we examined each of the 3 sex and reproductive status categories separately, we found nonsignificant decreases from 1st to 2nd capture for males and nonlactating females, but only nonlactating females had a large effect size. Lactating females had an opposite trend of a nonsignificant increase from 1st to 2nd capture, with a large effect size as well. This result suggests that reproductive status and sex may affect habituation to capture, but the sample size was not large enough to demonstrate this difference, especially given large effect sizes. Similarly, Häemäeläeinen et al. (2014) found that fecal GC metabolites increased in response to capture, and the strength of this response was not affected significantly by previous captures.

For degus caught at least 2 times, and for those caught at least 4 times, baseline CORT levels did not change significantly with recapture. Häemäeläeinen et al. (2014) also failed to find an effect of repeated trapping and handling on the endocrine stress response in gray mouse lemurs (*Microcebus murinus*); however, their study used more infrequent, monthly trapping intervals. Additionally, we observed a large amount of individual variability in baseline CORT responses from 1st to 2nd capture. Differences among individuals in stress response to the novelty of the trap could account for such variation in baseline

CORT. However, variation in baseline CORT levels may not be totally attributable to individual differences, but rather to conditions right before capture. If degus encountered other stressors before being trapped, their baseline CORT levels may have been higher than normal.

Although baseline and stress-induced CORT did not change significantly over multiple recapture events, we only examined individuals over 4 captures, whereas other studies at the field site captured individual degus almost daily for several months (Ebensperger et al. 2004; Quirici et al. 2011). Whereas our results do not indicate that the degu stress response habituated over 4 captures, substantial attenuation of stress-induced CORT likely could occur in individuals repeatedly captured over long time periods. Long-term trapping regimes also could influence the stress response by increasing food availability. Indeed, past studies in wild animal populations have found that long-term food supplementation can cause an increase (elk, *Cervus canadensis*—Forristal et al. 2012), decrease (juvenile white ibis, *Eudocimus albus*—Herring et al. 2011), or no change (Florida scrub-jays, *Aphelocoma coerulescens*—Schoech et al. 2007) in stress hormone levels. Future studies could determine the relative influence of food supplementation on stress hormone levels by varying the quality or quantity of food used as bait.

The potential relationship between stress reactivity and trappability requires the implicit assumption that the individual stress response is repeatable. Repeatability is commonly measured using the Lessells and Boag (1987) method, where repeatability, r, can range from 0 to 1, with 1 being perfect repeatability (i.e., the trait of interest has the same value every time it is measured). Among individuals, baseline and stress-induced CORT were highly repeatable in degus captured at least 4 times (r = 0.4 and 0.6, respectively), which is consistent with past analyses of stress hormone levels in free-living amphibians (r = 0.6–0.9—Narayan et al. 2012, 2013) and birds (r = 0.1–0.8—Cockrem and Silverin 2002; Kralj-Fišer et al. 2007; Rensel and Schoech 2011), but it is higher than the only other study in a wild, free-living mammal species (r = 0.15—Boonstra and Boag 1992). However, Boonstra and Boag (1992) analyzed repeatability within rather than among male meadow vole (*Microtus pennsylvanicus*) individuals, which may explain why their repeatability calculation is lower than ours. Additionally, repeatability tends to be higher when time intervals between sampling are short (Bell et al. 2009); whereas our animals were sampled every few days, the average time interval between captures was 2 weeks for Boonstra and Boag (1992). Future studies also should determine whether repeatability of the stress response differs with sex and age, as these factors can influence repeatability of other behaviors (Bell et al. 2009).

Overall, our failure to identify a trapping bias is encouraging and should help to relieve the concern that trapping wild animals confounds stress hormone data; however, studies are needed to determine whether captured animals differ from noncaptured animals. These studies could be achieved using noninvasive methods such as fecal sampling, although studies should control for multiple potential confounding variables surrounding GC metabolism (Huber et al. 2003; Goymann 2012). Future studies also could compare stress profiles from animals caught using different capture techniques to determine if particular methods yield more biased sampling from the population than others. Finally, future studies should examine whether long-term, repeated sampling affects the stress response.

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