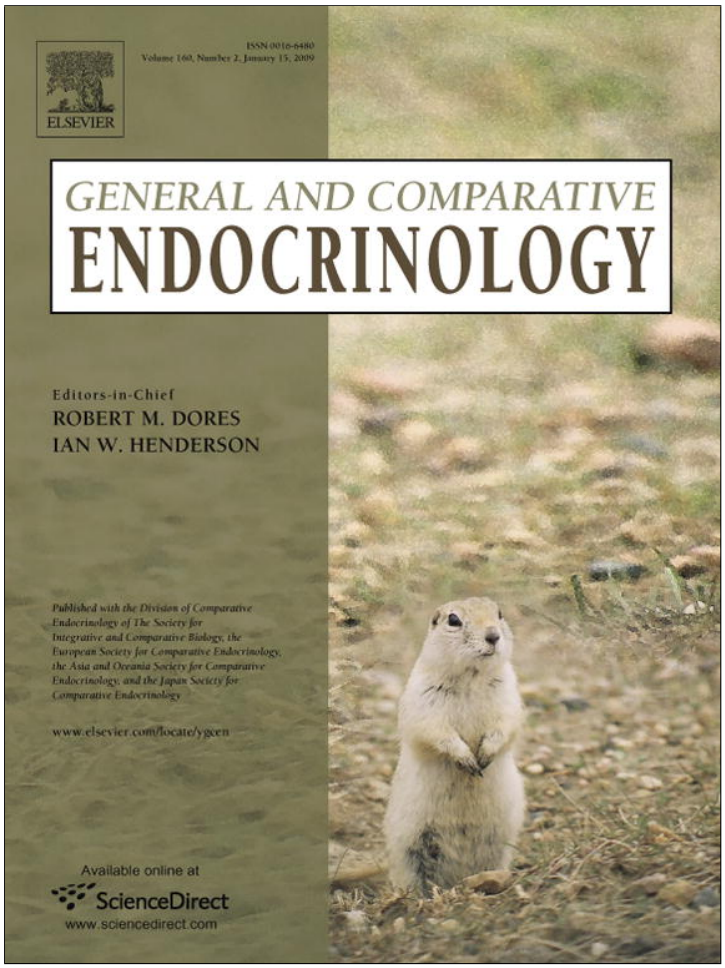

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Impact of live trapping on stress profiles of Richardson's ground squirrel (*Spermophilus richardsonii*)

Brendan Delehanty *, Rudy Boonstra

Department of Biological Sciences, Centre for the Neurobiology of Stress, University of Toronto at Scarborough, Toronto, Ont., Canada M1C 1A4

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ABSTRACT

Researching the physiological ecology of natural populations requires an understanding of the impact of capture-induced stress because of its numerous effects on physiological processes. In many cases, initial blood samples to which comparisons are made are obtained well after capture and may differ markedly from free-ranging conditions. We examined the extent to which stress profiles of male Richardson's ground squirrels (*Spermophilus richardsonii*) were affected by short-term responses to live trapping. We compared stress profiles of true base animals (blood samples obtained <3 min of capture) with those of nominal base animals (blood samples obtained >1 h after capture). Total cortisol increased almost 40% whereas our measure of corticosteroid binding globulin (CBG) decreased by 21%, resulting in a two-fold increase in free cortisol levels in nominal base animals compared with true base animals. Capture caused androgen concentrations to fall to almost half of those of true base animals. Energy mobilization increased markedly (22% in glucose and 221% in free fatty acids). Although white blood cell counts did not change, the number of neutrophils was 48% higher in true base animals. There were no changes in hematocrit or lymphocyte counts. Although most of the changes were predictable, the changes in CBG and androgens were unexpected based on previous work on closely related Arctic ground squirrels (*Spermophilus parryii*). Our results emphasize the value of obtaining true base measurements whenever possible in order to assess the directions and degree of bias introduced by trapping.

1. Introduction

The hypothalamic–pituitary–adrenal axis (HPA axis or stress axis) is a critical neuroendocrine system that allows vertebrates to respond adaptively to diverse physical, social, and environmental stressors. Because the stress axis is closely tied to the physiological controls of reproduction, aging, and immunity, the stress axis is of particular interest to researchers studying the physiological ecology of wild populations and the evolution of physiological traits (Boonstra, 2005; Mateo, 2007; Reeder and Kramer, 2005).

Because the term "stress" is often used vaguely, it is important to clearly define both the conception of stress being used and the measures used to quantify and characterize stress. In our research, we define "stress" simply as a state of physiological challenge to homeostasis that has activated the HPA axis, resulting in the release of glucocorticoids (GCs). Because of the diverse range of targets and effects of GCs, stress is most informatively quantified and described using an array of physiological measures that, taken together, provide a comprehensive understanding of an animal's physiological response to stressors that it is or has been experiencing. We therefore define a "stress profile" as a suite of physiological measurements that describe both the current state of HPA axis functioning and the impact of HPA activation on GC targets. Specifically, our direct measures of HPA activation are total cortisol levels and corticosteroid binding globulin (CBG) concentration (our measure being maximum corticosteroid binding capacity—MCBC) from which we calculate the biologically active free cortisol level. Our measurements of glucocorticoid targets include energy mobilization (blood glucose and free fatty acid levels), immune function (white blood cell counts and neutrophil to lymphocyte ratio) and general health (hematocrit).

A common problem for studies of stress in wild animals is that the stress of capture and handling often prevents researchers from obtaining a "true base" stress profile. There are now numerous wildlife studies showing that plasma GC levels increase significantly after 2–5 min from capture (Boonstra et al., 1998, 2001a; Boonstra and Singleton, 1993; Cash et al., 1997; Mueller et al., 2006; Place and Kenagy, 2000; Romero et al., 2008; Romero and Reed, 2005; Romero and Romero, 2002; Wada et al., 2007). However, it is often impractical or impossible to obtain blood samples from wildlife this quickly under field conditions, especially where cage-type traps are used for capture (e.g. Lynn and Porter, 2008). Instead, researchers are often limited to obtaining "nominal base" blood samples collected after an animal has been trapped for periods often measured in hours. Ultimately, though, we are interested

* Corresponding author. Fax: +1 416 287 7676.
E-mail address: brendan.delehanty@utoronto.ca (B. Delehanty).

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in how the animal was functioning under natural conditions so it is important to know how much capture and handling have shifted the stress profile of the animal and whether the stress profile has shifted in a way that would compromise the interpretation of the results (e.g. if the response to capture maximized the capacity of the animals to respond, thereby masking differences between animals in their natural state).

In a larger study looking at the stress response of breeding male ground squirrels (*Spermophilus* spp.), we were interested in comparing how the stress profiles of males changed between the pre-breeding period (prior to females emerging from hibernation) and the end of the intense, competitive breeding season. In that larger study, we used a hormone challenge protocol in which hormone injections are used to override the stress of capture and probe the responsiveness of the animal's stress axis (Boonstra, 2005). However, even when using a hormone challenge protocol, it is often valuable to look at the measurements made after capture but before the administration of hormones (the nominal base measurement). In order to properly interpret the nominal base results, it is helpful to have some data on true base values because some, but not all, parameters are likely to change significantly in response to capture and handling. The goals of this study were to examine what components of the stress profile of Richardson's ground squirrels (*Spermophilus richardsonii*) were affected by our capture protocol, to quantify those effects, and to understand how those effects would impact our interpretation of our base and hormone challenge results. We were also interested in comparing how Richardson's ground squirrels differed in their response to capture stress from the closely related Arctic ground squirrel (*Spermophilus parryii*) studied by Boonstra et al. (2001a).

There is some evidence that nominal base GC levels are still capable of revealing seasonal or inter-annual changes (Boonstra et al., 1998; Place and Kenagy, 2000) or experimental treatment effects (Boonstra and Singleton, 1993). That is, although capture raises GC levels above true base levels, the seasonal, annual or experimental changes in GC levels in these studies were sufficiently large that they were not masked by the fact that the nominal base samples were elevated compared to true base values. However, there are no studies that have looked at how capture and handling affect the full suite of parameters that we include in our stress profile in a single species.

2. Methods and materials

2.1. Study area

The study was conducted at the University of Alberta's Kinsella Ranch (53°N 111°33′W), 150 km southeast of Edmonton, Alberta. We trapped in rolling elk and cattle pastures of mixed grasses and forbs, low shrubs and occasional stands of aspen poplar. All procedures were carried out under University of Toronto animal use protocol 200006524 and as approved by the University of Alberta Faculty Animal Policy and Welfare Committee.

2.2. Trapping and field sampling

Two groups of Richardson's ground squirrels were live-trapped in home made burrow traps (Wobeser and Leighton, 1979) to measure blood parameters, the first to provide true base estimates and the second to provide nominal base estimates. In all cases, we arrived at the trapping site prior to the majority of animals emerging for the day and placed traps in burrows that animals were seen to have entered, as well as in any nearby burrows that may have been connected to the first burrow.

Nominal base animals were trapped between March 25, 2007 and March 27, 2007. Traps were set and checked frequently (<30 min). When an animal was found in a burrow trap, it was weighed and transferred to a Tomahawk live trap (Tomahawk Live Trap Company, Tomahawk, WI, USA), covered with a pillowcase, and placed in a quiet central holding area. Once a sufficient number of animals were captured, the caged squirrels were brought back to a mobile laboratory and placed in a cool, quiet location, with each trap still covered by a pillowcase. After 1 h, the first animal was retrieved, released from the trap into a pillowcase, and anesthetized using isoflurane (IsoFlo, Abbott Laboratories, Saint-Laurent, Que., Canada). A 0.6 ml blood sample was taken from either the suborbital sinus or by cardiac puncture (see Section 3). Because these animals were to be used in another study, they were placed back in the Tomahawk trap and returned to the holding area. All animals captured in the same day were sampled within 30 min of each other. The maximum time in a trap before the nominal base sample was taken was 4 h.

True base animals were trapped on March 28, 2007. Traps were set in the same manner as for nominal base animals, except fewer traps were set and the traps were monitored continuously. As soon as an animal was in the trap, we immediately approached the trap, released the animal from the trap into a pillowcase, and anesthetized the animal with isoflurane using a nose cone method. A 0.6 ml sample of blood was taken by cardiac puncture. The time from when the animal was seen to be trapped to the time the blood sample was obtained ranged from 105 s to 153 s.

In all cases, blood samples were placed in 1.3 ml lavender tip microtube vials (Sarstedt, Germany) once collected. Glucose levels (mg/dl) were immediately measured using a FreeStyle glucose meter (Abbott Laboratories, Alameda, CA, USA) using residual blood in the syringe.

2.3. Necropsy

After the completion of the blood sampling (for the true base animals) or after the hormone challenge protocol (for the nominal base animals), the animals were euthanized by halothane overdose and necropsied. Adrenal weights were measured to the nearest milligram, and testes were weighed to the nearest 0.01 g.

2.4. Laboratory analyses

After all blood samples were collected, two microhematocrit tubes were filled (approximately 75 μl each) from the lavender tip vials and spun at 13460*g* for 5 min in an IEC MB microhematocrit centrifuge (International Equipment Company, Needham Heights, MA, USA). The packed cell volume was recorded. Of the blood remaining in the lavender tip vial, a sample of approximately 0.15 ml was placed in a 0.6 ml microcentrifuge tube and spun at 8800*g* for 8 min. The plasma from the microhematocrit tubes and the microcentrifuge vial was placed in a clean vial and stored at −20 °C until returning to the laboratory where it was stored at −80 °C. The final 0.3 ml portion of each blood sample was retained in the lavender tip vial and sent away for a complete blood count (Vita-tech Veterinary Laboratory Services, Markham, ON, Canada). Upon return to the laboratory, plasma samples were analyzed for cortisol, maximum corticosteroid binding capacity, androgens, and free fatty acids.

We measured total cortisol by radioimmunoassay following the methods of Etches (1976) as modified by Boonstra et al. (2001b), and as further modified here. Briefly, 40 μl of ultrapure water and 20 μl of NH_4OH were added (to saponify triglycerides) to duplicate 10 μl samples of plasma in 12 × 75 mm polypropylene test tubes. We added 2 ml dichloromethane (Fisher) and vortexed for 4 min. Samples were then centrifuged at 1000 rpm for 5 min, and the aqueous layer was aspirated. One 600 μl aliquot of the dichloromethane extraction was removed to a fresh test tube (un-

less there was insufficient plasma to run the sample in duplicate, in which case two 600 μl aliquots were removed to separate test tubes and treated as duplicates from this point onward) and dried under filtered air. Next, 300 μl of phosphate buffer (pH 7) was added to each tube and allowed to equilibrate at room temperature for 1 h, at which point 100 μl of diluted [1,2,6,7-^{3}H] cortisol (Amersham Biosciences, USA) and 100 μl of diluted anti-cortisol antibody A-155 obtained from Western Chemical (Fort Collins, CO) were added to each tube. Cross reactivities of this antibody are provided in Boonstra et al. (2001b). Samples were left overnight at 4 °C. The following morning, 200 μl of dextran-coated charcoal was used to separate bound and free hormone. After a 30 min incubation at 4 °C, the samples were centrifuged at 2800 rpm for 12 min and 500 μl of supernatant was added to 2 ml scintillation fluid (ACS, Amersham Biosciences, USA) and left in the dark at room temperature for at least 7 h before being read in a scintillation counter (Packard Tri-Carb 2900TR, Boston, MA, USA). The intra- and inter-assay coefficients of variation were 4% and 8%, respectively. This method has a reported mean recovery of 105% (SE = 1.2%, range 100–107%) and a detection limit of 10 pg/10 μl (Boonstra and McColl, 2000).

To estimate the amount of protein-bound cortisol, we calculated the maximum corticosteroid binding capacity (MCBC) in duplicate 10 μl samples of plasma using the techniques described in Boonstra et al. (2001b). This technique requires knowing the dissociation constant of CBG, which we calculated by performing a saturation analysis following Hammond and Lähteenmäki (1983). Using the saturation curve (data not shown), we calculated the dissociation constant using nonlinear regression (SAS PROC NLIN) to fit the equation $y = B_{max}(x/(x + K_d))$, where y equals the amount of hormone bound, x equals the amount of tritiated hormone added, B_{max} equals the maximum binding capacity, and K_d equals the dissociation constant. The K_d for Richardson's ground was calculated to be 20.9 nM (the K_d for Arctic ground squirrels reported by Boonstra et al. (2001b) was 22.2 nM). We assumed that the albumin concentrations and ratio of albumin-bound to free cortisol were comparable to those calculated for Arctic ground squirrels in Boonstra et al. (2001b). Briefly, we added a known specific activity of [1,2,6,7-^{3}H] cortisol, non-tritiated cortisol, and 400 μl of phosphate buffer (pH 7) to duplicate 10 μl plasma samples in 12 × 75 mm polypropylene test tubes. Samples were vortexed and equilibrated overnight at 4 °C. The next morning, 200 μl of dextran-coated charcoal was added to the tubes, which were centrifuged at 2800 rpm for 12 min after a 30 min incubation time at 4 °C. A 500 μl aliquot of the supernatant was removed to 2 ml of scintillation fluid and left in the dark at room temperature for at least 7 h before being read in a scintillation counter. This assay was run concurrently with the cortisol assay for the same samples.

The antibody used in our testosterone assay (P43/11, from Croze and Etches, 1980) has a 62% cross reactivity with dihydrotestosterone, so we refer to "androgen levels" rather than testosterone levels in this paper. We used the radioimmunoassay procedure described in Boonstra et al. (2001b). Our intra- and inter-assay coefficients of variation were 5% and 5.5%, respectively. Recovery rates for this assay is reported as 96.5% (SE = 0.7%, range 92–102%) with a detection limit of 10 pg/25 μl (Boonstra et al., 2001a).

Free fatty acids were assayed using a commercially available kit (NEFA-C, Wako Chemicals USA Inc., Richmond VA, USA), modified to be used in a 96 well microtitre plate (Johnson and Peters, 1993). Reagents were made up according to kit instructions, but Reagent A was then diluted with 13.3 ml of 0.5 M phosphate buffer and Reagent B was diluted with 33.3 ml buffer. Because an initial trial using 5 μl of plasma were found to have higher concentrations than the supplied standard solution, we used 2.5 μl of plasma per well. After adding plasma or standard solutions to wells, 95 μl of Reagent A was added to each well, shaken for 1 min and then incubated at room temperature for 30 min. Near the end of the incubation period, the plate was pre-read (absorbance at 550 nm) in a spectrophotometer in order to compensate for absorbance by hemolysed plasma samples. At the end of the 30 min incubation, 195 μl of Reagent B was added to each well. The plate was shaken for 1 min and then incubated at room temperature for 30 min before being reading the absorbance at 550 nm in the spectrophotometer. Intra- and inter-assay coefficients of variation were 6.6% and 10.0%, respectively. The detection limit was considered to be the lowest concentration on the standard curve, 0.06 mM.

2.5. Statistics

All data are expressed as means ± SE unless otherwise stated. All data were analyzed using SAS 9.1.3 (SAS Institute Inc., Cary, NC, USA). Data were examined for normality using PROC UNIVARIATE NORMAL. The raw white blood cell count data was non-normal but were normalized with a natural log transformation. The neutrophil and lymphocyte counts were not normal and transformations did not make them normal, we therefore used the Wilcoxon–Mann–Whitney non-parametric two sample test using the EXACT option that generates a Monte Carlo-based exact p-value. All other variables were compared with PROC TTEST. In the case of free fatty acids, the variance of nominal base samples was significantly greater than the variance of the true base samples so the Satterthwaite adjustment for unequal variances was used. Variances of all other variables did not differ between true and nominal base samples, so we used the standard pooled variance t-tests for those data.

3. Results

There were no differences between true and nominal base animals in body mass, adrenal mass or mass of testes (Table 1). We collected blood from the first six nominal base animals from the suborbital sinus, but for the next nine nominal base animals we collected blood by cardiac puncture. Therefore, before comparing true and nominal base animals, we compared the blood parameters of nominal base animals from suborbital versus heart bleeds. The only parameter that showed a significant difference was MCBC (suborbital: $N = 6$, 112.0 ± 8.5 ng/ml; heart: $N = 9$, 82.2 ± 4.9 ng/ml; $t = -3.27$, $p = 0.006$). Accordingly, we excluded the suborbital bleeds from comparisons with the true base animals for the MCBC and free cortisol comparisons. For consistency and to make the total cortisol values directly comparable to the free cortisol values, we also excluded the suborbital bleeds from the comparison of total cortisol levels in true and nominal base animals.

The results are summarized in Table 1. Total cortisol levels were 39.6% higher in nominal base animals than in true base animals. However, because nominal base animals had lower MCBC than true base animals (nominal base levels were 78.5% those of true base) free cortisol levels in nominal base animals were 208% those of true base animals. Androgen levels showed signs of rapid suppression: nominal base animals had androgen levels that were just 57.4% those of true base animals.

In terms of energy mobilization, our standard handling protocol of keeping animals in traps for at least an hour prior to taking the nominal base blood sample resulted in significant increases in glucose and free fatty acid levels. Glucose levels in nominal base animals were 22.2% higher than in true base animals and free fatty acid levels were 221.0% higher.

In contrast to the hormone and energy parameters, there were no dramatic differences in the other blood parameters. Packed cell volume in true base animals was slightly higher than in nominal base animals, but the difference was not statistically significant ($p = 0.087$). Cell counts also showed no difference between true

Table 1
Comparison of physiological parameters measured in true base (blood taken in <3 min from time of capture) and nominal base animals (blood taken up to several hours after capture), with significant differences highlighted in bold. All comparisons are two-tailed *t*-tests with pooled variances except *Satterthwaite adjustment for unequal variances and †Wilcoxon–Mann–Whitney non-parametric two sample test calculated in SAS PROC NPAR1WAY with the EXACT option to produce exact *p*-values. Abbreviations: MCBC, maximum corticosteroid binding capacity; FFA, free fatty acids; WBC, white blood cells.

Parameter	True base (mean ± SE)	Nominal base (mean ± SE)	*t*	df	*p*
Weight (g)	372 ± 14.6	370 ± 9.9	0.10	18	0.918
Adrenal weight (mg)	73.2 ± 8.2	77.3 ± 5.3	−0.40	18	0.695
Testes weight (g)	2.16 ± 0.19	2.051 ± 0.08	0.62	18	0.546
Total cortisol (ng/ml)	**126.24 ± 19.3**	**176.22 ± 7.5**	**−2.89**	**12**	**0.014**
MCBC (ng/ml)	**104.7 ± 6.5**	**82.2 ± 4.9**	**2.76**	**12**	**0.017**
Free cortisol (ng/ml)	**32.7 ± 8.6**	**69.2 ± 3.2**	**−4.81**	**12**	**<0.001**
Androgen (ng/ml)	**9.42 ± 1.23**	**5.41 ± 0.67**	**2.96**	**18**	**0.008**
Glucose (mg/dl)	**103.2 ± 4.1**	**126.13 ± 5.4**	**−2.34**	**18**	**0.031**
FFA (mM)	**0.2458 ± 0.021**	**0.5432 ± 0.067**	**−4.27***	**16.3***	**<0.001**
Hematocrit (%)	49.7 ± 0.64	46.7 ± 0.91	1.81	18	0.087
Neutrophils ($\times 10^9$/l)	**3.64 ± 0.43**	**2.46 ± 0.41**	***S* = 63.5†**		**0.047**
Lymphocytes ($\times 10^9$/l)	1.32 ± 0.36	1.29 ± 0.17	*S* = 47.0†		0.859
WBC ($\times 10^9$/l)	5.44 ± 0.89	4.10 ± 0.57	1.41	15	0.178
N:L ratio	**3.5236**	**2.067**	**2.25**	**18**	**0.040**

and nominal base animals, but the N:L ratio in nominal base animals was 58.7% that of true base values.

4. Discussion

4.1. The importance of measuring free cortisol

As expected, total cortisol levels were affected significantly by our standard capture and handling protocol. However, the magnitude of the effect varies considerably depending on whether or not one assesses the effect of corticosteroid binding globulin (CBG). Under the "free hormone hypothesis", only those GCs not bound to carrier proteins like albumin or CBG are biologically active (Rosner, 1990; Siiteri et al., 1982). It has been clear for some time that this understanding is not strictly true because CBG acts in some cases as a carrier protein, delivering GCs to specific tissues, and there is evidence that the CBG–GC complex can interact with specific cell membrane receptors (see reviews in Hammond, 1995 and Breuner and Orchinik, 2002). However, in terms of the core GC functions that are typically of interest to physiological ecologists (e.g. its role in maintaining allostasis (McEwen and Wingfield, 2003) and playing a central role in the "flight or fight" response), the evidence suggests that circulating free GC levels are the critical measure.

In our study, total cortisol levels increased by 39.6% in response to capture stress, but free cortisol increased by 212% due to a rapid drop in MCBC. The interaction between cortisol and binding capacity allow Richardson's ground squirrels to mount an effective cortisol response that is five times greater than that suggested by the total cortisol levels. In contrast to these results, in a comparison of Arctic ground squirrel males that were either shot (true base) or had been trapped for 20–90 min prior to having blood taken (nominal base), Boonstra et al. (2001a) found a three- to fourfold increase in free cortisol as a result of capture stress but the increase was strictly a result of increased total cortisol production as there was no change in MCBC levels. Boonstra and McColl (2000) found a 4% drop in Arctic ground squirrel binding capacity after 4 h, whereas we observed a 21% drop in Richardson's binding capacity in the same time frame. Rapid (<1 h) changes in GC binding capacity were observed in five of nine species of birds studied by Breuner et al. (2006) and in snowshoe hares (*Lepus americanus*) (Boonstra and Tinnikov, 1998). However, CBG levels in other species only respond to stress after a period of 6–24 h (rats: Fleshner et al., 1995; Marti et al., 1997) or appear to be resistant to chronic stress (starlings, *Sturnus vulgaris*: Cyr et al., 2007). Thus, while it is known that species differ in how quickly their CBG levels respond to stress, it is somewhat surprising that two species as closely related as Richardson's and Arctic ground squirrels differ so much in this respect.

Richardson's and Arctic ground squirrels are closely related, belonging to the same clade, having diverged an estimated 1.3 million years ago (Harrison et al., 2003). They are both obligate hibernators living in highly seasonal environments with long, cold winters. The males of both species emerge prior to the females at times when there is no new food available and the animals subsist on food cached in their burrows the previous year (Buck and Barnes, 1999; Gillis et al., 2005; Michener, 1992). Males of both species are short-lived; the annual disappearance rates of males for both species is about 80% (Gillis, 2003; Michener, 1998). Only about 20% of males in a Richardson's population were 2 years old and fewer than 5% were 3 or 4 years old (Michener, 1998). Thus, the majority of males in both species will only live long enough to participate in one breeding season. The difference in how these two species respond to capture stress has implications for interspecific comparisons of stress profiles. For example, Richardson's and Arctic ground squirrels differ in how their maximum corticosteroid binding capacity is affected by capture stress: the GC binding capacity of Richardson's ground squirrels drops whereas that of Arctic ground squirrels stays constant. In the wild, then, an Arctic ground squirrel's free cortisol response to a natural stressor will be a direct function of its total cortisol production whereas the free cortisol response of a Richardson's ground squirrel will depend not only on total cortisol production, but also on how quickly binding capacity falls. If binding capacity falls more slowly than total cortisol production rises, then the higher baseline binding capacity in Richardson's ground squirrels will buffer them from the effects of acute stressors in comparison to Arctic ground squirrels initially. Furthermore, our standard capture protocol results in squirrels being stressed for at least an hour, but if natural stressors are typically more fleeting rather than that, it is possible that binding capacity in Richardson's does not fall and that their stress response is more muted than that of Arctic ground squirrels. These results and those of Boonstra et al. (1998), Boonstra and Tinnikov (1998) and Breuner et al. (2006) highlight the importance of measuring GC binding capacity and not just relying on total GC levels.

4.2. Rapid suppression of androgens

The HPA axis is intricately tied to the hypothalamic–pituitary–gonadal (HPG) axis (Wingfield and Sapolsky, 2003) and because we are interested in how the HPA axis is adapted to support alternative reproductive strategies, it is informative to include androgen

levels as part of the stress profile. Surprisingly few studies have measured GC and androgen levels of wild animals simultaneously, and in those, the results are variable. Boonstra et al. (2001a) found that shot Arctic ground squirrel males (true base) had significantly lower androgen levels than males subject to capture stress. Place and Kenagy (2000) found a similar pattern of increased testosterone in male yellow-pine chipmunks (*Tamias amoenus*) subject to capture stress, but only in the spring; in the fall, they found no difference between true base and stressed animals (although testosterone levels were very low at this time of year, being <0.5 ng/ml). In contrast, Boonstra and Singleton (1993) found that androgen levels were lower in snowshoe hares subject to capture stress compared with true base animals, as did Lance et al. (2004) in their study of American alligators (*Alligator mississippiensis*), and Moore et al. (1991) in their study of the tree lizard, *Urosaurus ornatus*. Moore and Mason (2001) and Cease et al. (2007) found no effect of exogenous GCs and no effect of capture stress, respectively, on testosterone in red-sided garter snakes (*Thamnophis sirtalis parietalis*), but Moore et al. (2000) found an increase in corticosterone and decrease in testosterone in red-sided garter snakes in response to a 4 h capture stress protocol.

Given the similarities between Richardson's and Arctic ground squirrels, we had expected that Richardson's would respond to the stress of capture by increasing androgen levels as Boonstra et al. (2001a) found in Arctic ground squirrels. Instead, we found a dramatic decrease from 9.4 ng/ml to 5.4 ng/ml. This raises interesting questions about the adaptive significance of how androgen levels responds to stress. Boonstra and Boag (1992) and Wingfield and Sapolsky (2003) have predicted that species with compressed breeding seasons and those that may only be able to breed once in their lifetime should show reproductive resistance to stress. One mechanism for preventing the decline of sex hormones in response to stress is compensatory stimulation of the gonadal axis in times of stress (Wingfield and Sapolsky, 2003), and it appears that some form of compensatory androgen production is used by Arctic ground squirrels (Boonstra et al., 2001b). Clearly, however, Richardson's ground squirrels are either not resistant to stress or they have a different mechanism for resisting stress; further study is required to understand this unexpected observation.

4.3. Mobilization of energy

We measured blood glucose levels and plasma free fatty acid levels as measures of energy mobilization. Stress-induced levels of glucocorticoids stimulate gluconeogenesis in the liver, inhibit insulin activity, decrease peripheral tissue uptake of glucose, and ultimately promote the breakdown of protein and lipids to produce substrates for gluconeogenesis in the liver (Sapolsky et al., 2000). We therefore expected to see an increase in glucose levels in nominal base animals compared with true base, as indeed we did (Table 1). Our findings are consistent with those of other studies that have looked at the effect of capture stress on glucose levels (American alligator, Lance et al., 2004; snowshoe hares, Boonstra and Singleton, 1993; and meadow voles (*Microtus pennsylvanicus*), Fletcher and Boonstra, 2006).

Both ACTH and GCs have lipolytic actions (Boonstra and Tinnikov, 1998; Sapolsky et al., 2000) so we would expect to see an increase in FFA levels in response to capture stress. The only study that has looked at the effect of capture stress on FFA levels is Handasyde et al. (2003). In that study, five female and two male platypuses (*Ornithorhynchus anatinus*) in the non-breeding season showed increased FFA levels in the stressed sample compared with true base values. We found that nominal base animals had FFA levels that were an average of 21% higher than those of the true base samples.

Both of our measures of energy mobilizations indicate that by the time we take our nominal base samples under our standard protocol, the animals have greatly increased their glucose and FFA levels. Although fewer studies have looked at energy mobilization than cortisol response to capture stress, all studies show a consistent pattern of energy mobilization as one would expect given the central role of rapid energy mobilization in the stress response.

4.4. Blood parameters

Hematocrit (the proportion of blood volume comprised of red blood cells) is sometimes used as a measure of anemia and a general indicator of health (Boonstra et al., 2001b; Clinchy et al., 2004; Kim et al., 2005), the idea being that a low packed cell volume is indicative of anemia, possibly caused by blood loss due to gastric ulcers. However, hematocrit can also be affected by short-term fluctuations in plasma volume unrelated to anemia (e.g. Dawson and Bortolotti, 1997) as well as the release of red blood cells from the spleen (Guntheroth and Mullins, 1963; Opdyke, 1970). Therefore, hematocrit readings need to be interpreted with some caution. In the present study, for example, any change in hematocrit would more likely be the result of a change in plasma volume rather than being evidence of blood loss.

Our nominal base animals had slightly lower mean hematocrits compared with true base animals, but the difference was not statistically significant. Previous studies that have examined the effect of acute capture stress on hematocrit have found increases of approximately 10% (snowshoe hares, Boonstra et al., 1998; meadow voles, Fletcher and Boonstra, 2006) or no effect (Atlantic sharpnose shark (*Rhizoprionodon terraenovae*), Hoffmayer and Parsons, 2001).

The second hematological indicator of stress that we looked for was a stress leukogram: an increase in neutrophils with a concomitant decrease in lymphocytes (Cattet et al., 2003) often reported as an increased neutrophil:lymphocyte (N:L) ratio (e.g. Kim et al., 2005) or, in birds, an increase in the heterophil:lymphocyte (H:L) ratio (e.g. Kilgas et al., 2006). The rapid increase in circulating neutrophils is thought to result from decreased adherence of neutrophils to vascular walls (Cronstein et al., 1992), whereas the decrease in lymphocytes is due to the redistribution of lymphocytes out of circulation and into tissue (Dhabhar and McEwen, 1997). Thus, the N:L ratio can potentially be altered quite quickly. To our surprise, we found a statistically significant decrease in neutrophils, but no statistically significant change in lymphocytes (Table 1). The significant decrease in the N:L ratio therefore appears to be due to the decrease in neutrophils. However, the *p*-values for the neutrophils and N:L ratio are both approximately 0.04. Because we did not apply any Bonferroni corrections, and because we did not see a change in lymphocytes, we consider this increase in N:L ratio to be a preliminary finding that should be interpreted cautiously.

Davis (2005) found a significant decrease in house finch (*Carpodacus mexicanus*) leukocyte numbers between base blood samples and samples taken at 30 or 60 min post-capture. He also found an increase in H:L ratio, but it appeared to be an artifact of repeat blood sampling rather than the stress of capture. We found no such effect on white blood cell counts (Table 1).

5. Conclusions

The two most striking findings in this study are how different the Richardson's ground squirrels are from the closely related Arctic ground squirrels in Boonstra et al. (2001a), and the fact that 8 out of 11 measures in our stress profiles changed significantly in response to capture stress within a period of 4 h. Of those param-

eters that changed in response to capture stress, the majority changed in predictable ways (e.g. total cortisol, glucose, free fatty acids all increased as one would expect), but the sudden decline in MCBC and testosterone were unexpected based on what we knew about the response of Arctic ground squirrels to capture stress. Although it may not be practical to alter standard trapping protocols in order to obtain true base measurements from wild study organisms, our results suggest that in cases where researchers are interested in more than just total cortisol levels, having at least a small sample of true base readings may be valuable when it comes to interpreting nominal base results.

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