CHARACTERIZING METRICS AND OUTCOMES OF STRESS IN A CLIMATE-SENSITIVE SPECIES, THE AMERICAN PIKA

by

JENNIFER LEE WILKENING

B.A. University of Colorado, Boulder

M. S. University of Nevada, Reno

A thesis submitted to the

Faculty of the Graduate School of the

University of Colorado in partial fulfillment

of the requirement for the degree of

Doctor of Philosophy

Department of Ecology and Evolutionary Biology

2014

This thesis entitled:

Characterizing metrics and outcomes of stress in a climate-sensitive species, the American pika

written by Jennifer Lee Wilkening

has been approved for the Department of Ecology and Evolutionary Biology

_____

Dr. Sharon Collinge

_____

Dr. Chris Ray

Date_____

The final copy of this thesis has been examined by the signatories, and we find that both the content and the form meet acceptable presentation standards of scholarly work in the above mentioned discipline.

Wilkening, Jennifer Lee (Ph.D., Ecology and Evolutionary Biology)

Characterizing metrics and outcomes of stress in a climate-sensitive species, the American pika

Thesis directed by Professor Sharon Collinge and Research Associate Chris Ray

ABSTRACT

The American pika (*Ochotona princeps)* is considered a sentinel species for detecting ecological effects of climate change. Pikas are declining within a large portion of their range, but previous studies have focused only on local pika extirpation as a metric of change. This approach does not take into account the role of behavioral thermoregulation and the pika's use of microhabitats to ameliorate variations in climate. Without evidence of a direct climatic impact on pikas, studies correlating pika habitat occupancy with climate metrics provide relatively weak support for projecting effects of climate change on this species. This dissertation research focuses on the physiological stress response of pikas to differences in microhabitat and microclimate. First, I developed and validated bio-assays to measure physiological stress in pikas. Second, relationships were established between pika stress and habitat characteristics associated with sub-surface ice features, which are an important component of water resources. Third, I examined effects of local climate on stress, while accounting for the influence of environmental characteristics on a frequently used stress metric (fecal CORT). Lastly, annual individual survival was analyzed in relation to two different stress metrics (fecal and plasma CORT). Research efforts resulted in the establishment of non-invasive methods for estimating stress in pikas, and also emphasize the importance of considering environmental influences on stress measurements collected non-invasively across different eco-regions. Additional results suggest that pikas may serve as a sensitive bio-indicator of hydrological change in high elevation

watershed areas. Finally, pikas appear to be experiencing chronic stress, and reduced survival, in some microhabitats regardless of behavioral adaptations.

DEDICATION

This dissertation is dedicated to my daughter, Avi Sandra Rogers.

AKNOWLEDGEMENTS

Funding for this research was provided by the National Park Service George Melendez Wright Climate Change Fellowship, the Indian Peaks Wilderness Alliance David Paddon Memorial Scholarship, CU Boulder Department of Ecology and Evolutionary Biology research grants, the Boulder County Nature Association research grant, the Colorado Mountain Club Neal B. Kindig Fellowship, the American Society of Mammalogists Grant-In-Aid of research, the Joyce Gellhorn grant, and the American Alpine Club grant. In-kind support was provided by the Niwot Ridge Long Term Ecological Research Site (LTER) and the National Science Foundation REU program. I would like to thank the following individuals for their assistance with the collection of field data and physiological samples: Cassandra Crnich, Gerardo Dillehay, Adelaide Lindseth, Dan Luccock, Sara McLaughlin, Taylor Stratton and Riley Stuckey. I also thank Nathan Kleist and Riley Stuckey for their help in the laboratory with sample analysis, Christopher Stamper and Johanna Varner for expert help in laboratory techniques, and Rebecca Safran for the use of laboratory equipment. I am extremely grateful to the many field assistants, US National Park Service staff, and project volunteers who collected samples used in this study, and a special thanks to Jessica Castillo for organizing and sending fecal samples. I thank my co-advisors, Sharon Collinge and Chris Ray, for their patience and guidance. And finally, none of this would have been possible without the love and support of my husband, Louis Rogers, and our family, Indea, Eli and Avi Rogers.

CONTENTS

TABLES

Chapter 1:

Chapter 2:

Chapter 3:

Chapter 4:

Chapter 5:

FIGURES

Chapter 1:

Chapter 2:

Chapter 3:

Chapter 4:

Chapter 5:

Chapter 1

STRESS HORMONE CONCENTRATION IN ROCKY MOUNTAIN POPULATIONS OF THE AMERICAN PIKA (*OCHOTONA PRINCEPS*)

ABSTRACT

The American pika (*Ochotona princeps*) is considered a sentinel species for detecting ecological effects of climate change. Pikas are declining within a large portion of their range, but previous studies have focused only on local pika extinction as a metric of change. We designed a procedure which can provide an earlier warning signal, based on non-invasive sampling and analysis of physiological stress in living pikas. Pikas were sampled at several locations in the Rocky Mountains for the measurement of glucocorticoid metabolites (GCMs) in feces. Using a time series of fecal pellets from 12 individuals, we detected a significant increase in fecal GCM level in response to capture, thus validating the use of a corticosterone enzyme immunoassay. We also established baseline, peak, and post-peak GCM concentrations for pikas in the Rocky Mountains, which varied according to gender and individual. This is the first study to measure stress hormone metabolites in any species of pika. The methods developed and validated in this study can be used to add non-invasive measurements of physiological stress to pika monitoring programs and other research designed to assess pika vulnerability to predicted changes in climate. Pika monitoring programs currently in place use a protocol that relates current site use by pikas with data on local habitat characteristics, such as elevation, to infer potential effects of climate change. Data generated by these monitoring studies can be used to identify the trends in site use by pikas in relationship to habitat covariates. However, this approach does not take into account the role of behavioral thermoregulation and the pika's use of microhabitats to ameliorate variations in climate. Incorporating a stress metric, such as GCM concentration, will provide

relatively direct evidence for or against the hypothesis that pikas can be stressed by climate regardless of behavioral adaptations.

INTRODUCTION

Due to the severity of change in alpine climates, alpine mammals are predicted to be among the species most threatened by climate change (Hughes 2000; Parmesan 2006; Moritz *et al*. 2008). The American pika (*Ochotona princeps)*, which occurs only where it can access cool microclimates, is considered a sentinel species for detecting ecological effects of climate change (McDonald and Brown 1992; Hafner 1993 & 1994; Lawlor 1998; Beever *et al*. 2003; Krajick 2004; Smith *et al*. 2004; Grayson 2005; Morrison & Hik 2007). The lowest recorded occurrence of pika in the Great Basin has risen 150 meters during the past century (Grayson 2005), and climate has been implicated as a driver of recent pika losses in the Great Basin (Beever *et al.* 2010 & 2011; Wilkening *et al*. 2011). Projections suggest that pikas in the US may disappear from 55% (95% CI = 35%-66%) of their current range even under low emission scenarios (Ray *et al*. 2012), and the species has been considered for listing as threatened or endangered at both federal and state levels (USFWS 2010; Osborne & Applebee 2011). The pika's recent, apparently climate-driven range retraction has been highly publicized, and there has been a growing interest in documenting changes in pika distribution. Research efforts have intensified over the past decade, and government agencies and citizen science organizations throughout the western US have initiated programs to monitor pika distribution and assess vulnerability to predicted climate change (Garrett *et al.* 2011).

Studies of the American pika are well suited for testing hypotheses regarding the potential nature of climate-mediated range shifts. This species has a narrow thermal tolerance,

due to its relatively high metabolic rate and low thermal conductance (MacArthur & Wang 1973). Pikas do not hibernate, and most pika species rely on vegetation stored in the form of "haypiles" to supply nutritional needs throughout the winter (Dearing 1993). Collecting this vegetation during the summer is a highly energetic activity, generating body heat that must be dissipated passively (MacArthur & Wang 1974; Smith 1974). Consequently this species is a habitat specialist, occurring in the western USA only in taluses, boulder-fields, lava beds and similar areas of blocky debris which traps cooler air in pockets between the rocks (Smith & Weston 1990; Millar & Westfall 2010).

Most efforts to understand effects of climate change on the pika and other species have relied on establishing relationships between habitat characteristics (such as temperature and elevation) and the distribution of the species (e.g., Beever *et al.* 2003 &2010; Grayson 2005). The major determinants of range for many species are temperature and precipitation, and these two factors also appear to control recent pika dynamics. Several studies have shown that the American pika is more likely to occur and persist at sites with higher precipitation, where surface and sub-surface temperatures are less extreme, and where vegetation communities are dominated by forbs (Ray & Beever 2007; Millar & Westfall 2010; Rodhouse *et al.* 2010; Beever *et al.* 2010 & 2011; Erb *et al.* 2011; Wilkening *et al.* 2011). These results suggest both direct and indirect effects of temperature and precipitation on the species' distribution. Identifying more precisely which habitat characteristics are associated with individual pika fitness will improve our understanding of potential climate change effects. Without evidence of a direct climatic impact on individual pikas, studies correlating pika presence with climate metrics provide relatively weak evidence for projecting effects of climate change on this species. For a species that thermoregulates behaviorally by switching between surface and sub-surface habitats, it may be

especially difficult to use presence/absence data to identify the specific habitat or climatic variables driving range dynamics.

A more direct method for identifying potential climatic stressors is to measure physiological stress in individuals. This approach has the added benefit of being able to identify stressors before local extinctions have occurred. It can also suggest which factors stress individuals directly vs. indirectly; for example, individual stress metrics might be lagged with respect to precipitation, suggesting indirect effects mediated by vegetation quality. Measuring physiological stress may be preferable to other metrics of fitness that require mark-recapture studies which can be disruptive to the animal and resource intensive.

One stress metric that can be measured non-invasively is glucocorticoid (GC) concentration. In response to a stressor, vertebrates release glucocorticoid hormones (such as cortisol or corticosterone), which can then be measured in physiological samples (such as plasma, saliva, or feces). This release of GCs in response to short term stress permits rapid energy mobilization (Sapolsky *et al.*2000) and behavioral changes, that can result in improved survival and fitness (Bonier *et al.* 2009). An evolved stress response is often what enables vertebrates to successfully respond to daily or seasonal environmental variability (Romero 2002). However, continued release of GCs resulting from chronic stress can lead to potentially harmful effects such as the death of nerve cells, hyperglycemia, muscle and bone atrophy, hypertension immunosuppression and reduced reproduction (Riley 1981; McEwen & Sapolsky 1995; von Holst 1998; Wingfield & Sapolsky 2003; Engelmann *et al.* 2004; Romero 2004; Boonstra 2005; Korte *et al.* 2005).

Glucocorticoids travel to their target tissues via the blood, and the concentration of GC in plasma has been traditionally used to assess stress in various species (Wingfield *et al.* 1992 &

1994; von Holst 1998; Sapolsky *et al.* 2000; Mostl & Palme 2002; Romero 2002 &2004; Wingfield & Sapolsky 2003; Korte *et al.* 2005). However, animals must be captured in order to collect blood, and circulating hormone levels can increase rapidly in response to capture and handling stress (Cook *et al.*, 2000; Romero, 2004). Samples collected after 3 minutes of capture are not considered to be baseline measurements, and for many wild animals, blood samples can rarely be obtained within this timeframe using common trapping techniques. Furthermore, the capture and handling of rare or endangered species may not be possible, which underscores the importance of developing alternative methods of GC measurement.

Non-invasive methods to quantify stress have been developed for many different taxa. The measurement of glucocorticoid metabolites (GCMs) found in fecal samples is now one of the most common of these methods (Miller *et al.* 1991; Kirkpatrick *et al.* 1996; Palme *et al.* 1996; Berkeley *et al.* 1997; Jurke *et al.* 1997; Wasser *et al.* 1997; Goymann *et al.* 1999 & 2002; Harper and Austad 2000; Dehnhard *et al.* 2001; Ganswindt *et al.* 2003; Turner *et al.* 2003; Hunt *et al.* 2004; Touma *et al.* 2004; Cyr & Romero 2008; Chinnadurai *et al.* 2009; Sheriff *et al.*2009; Ashley *et al.* 2011; Scarlata *et al.* 2011; Hogan *et al.* 2012; Howell-Stephens *et al.* 2012; Laver *et al.* 2012; Shutt *et al.* 2012; Smith *et al.* 2012). Steroid hormones circulating in the body are metabolized by the liver, and then excreted as metabolites into the gut (Taylor 1971; Palme *et al.* 1996; Mostl & Palme 2002), and GCMs can be detected in the excrement of birds and mammals. This technique offers several advantages. In many cases, fecal samples can be collected relatively easily without disturbing or endangering the animal, which allows for repeated sampling over time (Mostl & Palme 2002; Millspaugh & Washburn 2004). Additionally, GCM measurements obtained from collected fecal samples are not biased by capture-induced stress, and may provide a more precise assessment of the endocrine condition of an animal (Harper &

Austad 2000; Millspaugh *et al.* 2001; Touma *et al.* 2003). Finally, GCM measurements typically reflect an average level of circulating glucocorticoids over time. Unlike a blood sample taken at a single point in time, GCM concentrations appear to be less affected by the pulsatile nature of hormone secretion (Palme *et al.* 1996; Harper & Austad 2000; Palme 2005). Therefore, GCM concentrations may represent the average stress level of an animal more accurately than a plasma sample (Touma & Palme 2005).

Here we present the first study to develop and validate methods that can be used to non-invasively measure physiological stress in pikas. Unlike other members of the family Ochotonidae, American pikas are easily stressed in captivity and experience high mortality in laboratory settings (Krear 1965; MacArthur & Wang 1974; Dearing 1995). Several zoos have been unsuccessful in their attempts to maintain viable populations of pikas (http://www.zoochat.com/2/pikas-zoos-301266/; accessed 5/7/13), and there are currently no captive populations of American pikas in zoos or lab settings. Non-invasive methods of assessing stress are particularly useful for studying the stress response of a sensitive species to variation in its natural habitat.

The first objective of our study was to validate an enzyme immunoassay biologically for measuring GCMs in pika feces. Although GCM measurement has been used for over 20 years in numerous wildlife species, GCMs have never been quantified for any species of pika. When applying this technique to a new species for the first time, validation of all protocols related to sample storage, extraction procedure, and assay selection must occur in order for reliable interpretation of results (Touma & Palme, 2005). We collected a time series of fecal pellets from individual pikas that were live-trapped and later released, to measure individual baseline GCM levels followed by the fecal GCM response to capture. Our second objective was to investigate

individual and gender based differences in GCM concentration. Our third objective was to establish baseline GCM measurements for pikas, which could be used as a basis for interpreting GCM levels measured in future samples. At the level of individuals, we used our time series to establish pre-capture (pre-stress) GCM levels. At the level of populations, we compared these baseline stress metrics between pikas occurring at different latitudes, to investigate environmental trends. We focused on pika populations occurring in the Rocky Mountains, where this species has not experienced the magnitude of decline observed in the Great Basin (Erb *et al*. 2011), and may better represent baseline physiology for the species. Finally, we present several conservation applications, and discuss how our established techniques can be used in conjunction with ongoing pika research to develop a greater understanding of climate change impacts for this indicator species.

METHODS

Study area and animal sampling

Pikas were non-lethally sampled in the Rocky Mountains July-August 2011-2012 at Niwot Ridge long-term ecological research site (LTER) and Brainard Lake Recreation Area, both located in Boulder County, (CO, USA), and Emerald Lake, located in Gallatin County (MT, USA). Study sites were located in areas of typical pika habitat, characterized by large regions of broken rock (talus) interspersed with alpine meadows ranging in elevation from 2,500 to 3,700 meters. Pikas were captured using wire mesh traps (Tomahawk Model 201) placed near pika haypiles and provisioned with local vegetation (forbs and grasses native to the Rocky Mountains supplemented with commercial salad greens). Traps were checked within two hours of setting them. Prior to handling, animals were self-transferred to an induction chamber containing an inhalant anesthetic agent (isoflurane). Light anesthesia was maintained throughout the 20-minute

handling process, using repeated induction if necessary. Each summer, approximately 50 pikas were sampled and ear-tagged for a long term study of individual survival. Demographic data (stage and sex) and physiological samples (feces) were collected at point of capture, and most animals were released immediately. Eight adult males, one juvenile male, six adult females and one juvenile female were held on site for approximately 24 hours in a holding chamber specifically designed for collection of fecal samples. The holding chamber consisted of a plastic bin (length x width x height = 60 x 30 x 16 cm) with a detachable lid, modified to include an internal air thermometer, air vents and a mesh screen floor with pull-out drawer for periodic fecal sample removal. The chamber was provisioned with surplus vegetation (as described for traps) pre-moistened with water using a mister. Individuals were retained inside the chamber one at a time, and researchers monitored the holding chamber continually. Each pika was released at point of capture at the end of the holding period. Trapping and sampling procedures were reviewed and authorized by Colorado Parks and Wildlife (license no. TR2014) and the University of Colorado-Boulder Institutional Animal Care and Use Committee (protocol 1104.06).

Like all lagomorphs, pikas are coprophagic and excrete two different types of feces. Cecal feces contain partially digested vegetation and are often re-ingested in order to obtain additional nutrients. Fecal pellets, which are not re-ingested, are the more common type of fecal material found in natural environments. Given that non-invasive studies of pika stress hormone levels will be likely to involve this more abundant type of feces, only fecal pellets were analyzed for this study. To further ensure that our fecal samples were similar to what would be available for non-invasive studies, we analyzed only fecal pellets that had been urinated on. Pikas maintain established latrines in the wild, and habitually urinate on their fecal droppings. Pikas in the

holding chamber were watched constantly and researchers collected most fecal samples within minutes of excretion. For the majority of individuals, fecal samples were collected regularly every 2-4 hours; however, some animals (n =3) did not defecate until 6-8 hours after time of capture. The time of sample collection (0-23 hours) was recorded for each sample. Fecal samples were held on ice in the field, and were transferred within 48 hours to -20°C freezer at the University of Colorado, Boulder (CO, USA).

Extraction of hormone metabolites and enzyme immunoassays (EIA)

Each entire sample was lyophilized and ground into powder using a mortar and pestle, then subjected to the steroid solid extraction protocol given by Arbor Assay Design, Inc. (Ann Arbor, MI, USA). Briefly, 0.100 g of dried fecal material was blended with 1.000 ml of 90% aqueous ethanol using a vortex shaker (Vortex Genie 2; Scientific Industries, Inc.).Samples were shaken for one hour at 350 r.p.m. at room temperature to ensure thorough extraction of the hormone metabolite. They were then centrifuged at 5000 r.p.m. for 15 minutes, and the resulting supernatant was drawn off and transferred to a clean tube for evaporation. The supernatant solution was evaporated to dryness in a vacuum centrifuge, and extracts were stored at -20°C. Immediately prior to enzyme immunoassay analysis, extracts were reconstituted with a mixture of ethanol and assay buffer (1:20 ratio v/v) and were mixed completely using the above vortex shaker.

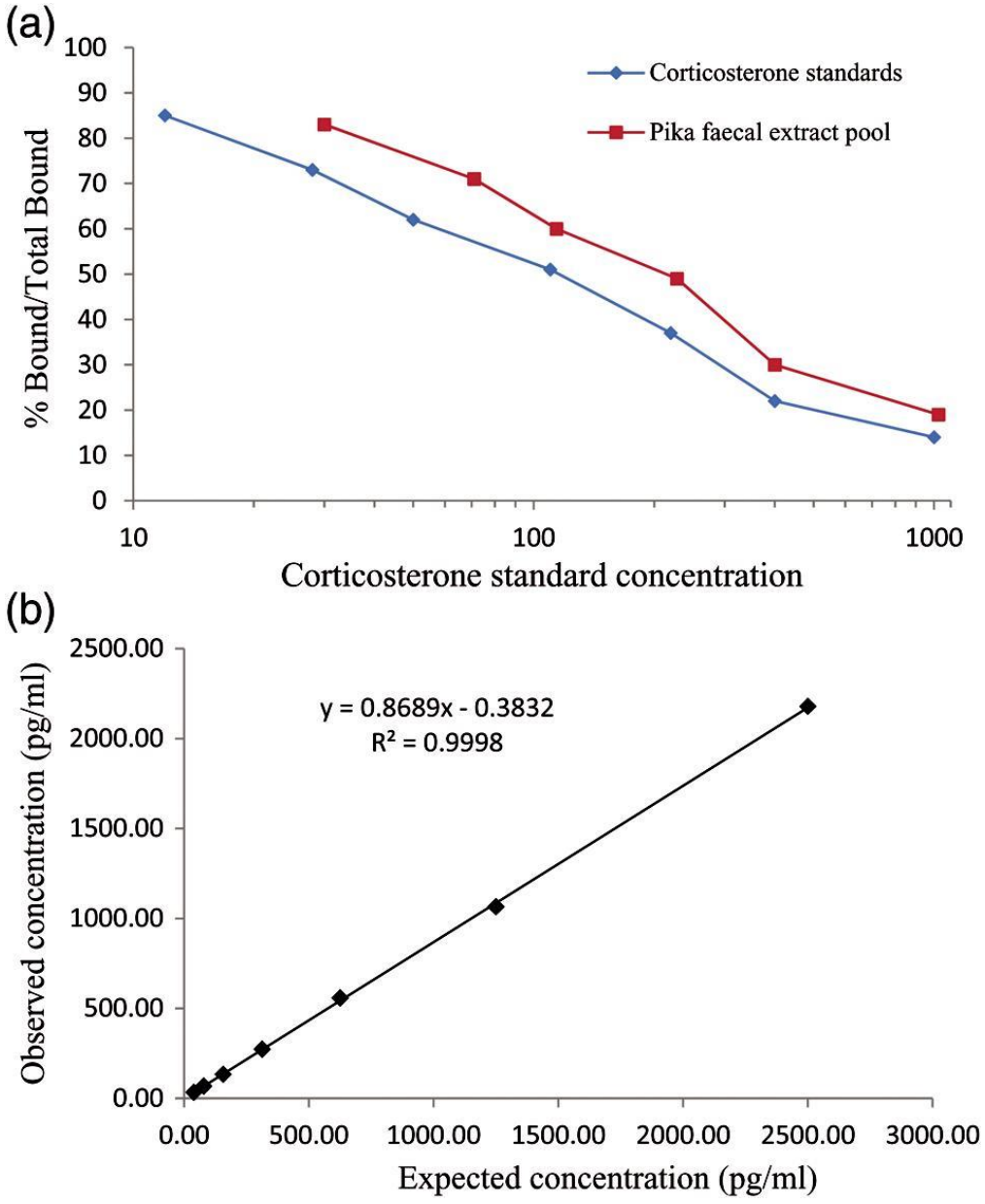
Comparative analysis of GCM levels in samples was conducted using a commercially available Corticosterone Enzyme Immunoassay Kit (Arbor Assay Design, Inc.; catalogue no. K014-H1). The enzyme immunoassay used a sheep polyclonal antibody specific for corticosterone, and was designed to measure total corticosterone in numerous physiological samples, including extracted fecal samples. Corticosterone, rather than cortisol, was measured in

pikas because corticosterone was found to be the major GC in other lagomorphs (Teskey-Gerstl *et al.* 2000; Monclus *et al.* 2006; Rehnus *et al.* 2009; Sheriff *et al.* 2009; Scarlata *et al.* 2011). During each assay, we ran extracted samples in triplicate alongside a standard curve of seven known concentrations of corticosterone (5000, 2500, 1250, 625, 312.50, 156.25, 78.125 pg/ml). A standard curve was constructed using results from known corticosterone concentrations. Values for each extracted sample were generated using a micro plate reader (BioTek Microplate Reader Synergy HT; 2005 Biotek Industries, Inc.) and Gen 5 1.11 Data Analysis software. Intra-assay coefficients of variation were 5.2 and 3.1% for the low and high binding controls, and inter-assay coefficients of variation were 12.6 and 11.4%, respectively. Final concentrations of fecal GCM were expressed as nanograms per gram of dry feces. To validate the use of the corticosterone enzyme immunoassay kit, we demonstrated the following characteristics: (1) parallelism between the standard curve and serial dilutions of pooled fecal samples, and (2) significant recovery (86%) of corticosterone standard added to fecal extracts prior to analysis (Figure 1a and b; Touma & Palme 2005). The appropriate dilution (1:20) was calculated from the parallelism results.

Data analysis

Data were analyzed separately by sex (male and female) and age (adult and juvenile). Given that fecal sample production was inconstant over time, we binned samples by time elapsed since capture to better characterize the timing of any GCM response. Samples were binned into six periods of five hours each (0:00-5:00, 5:01-10:00, 10:01-15:00, 15:01-20:00, 20:01-25:00 and 25:01-30:00 hours:minutes after capture). GCM levels were averaged within time periods for each individual. Thus, the timing of "peak" (highest) GCM level for each individual was

Figure 1. Binding displacement curves of serially diluted pika fecal extracts (**a**) and recovery of exogenous corticosterone standard added to pika fecal extracts (**b**).



determined to within 2.5 hours. Pre-peak "baseline" GCM level was calculated for each individual by averaging all samples within all time periods prior to the individual's peak GCM level. Likewise, post-peak GCM level was calculated by averaging all samples within all time periods after the individual's peak GCM level. One-way ANOVA was used to test for the following attributes: (1) differences among individuals in baseline GCM levels and (2) differences between males and females in baseline GCM levels and (3) differences over time in post-stress GCM levels. Normal probability plots were examined, and a Shapiro-Wilk statistic was calculated to test for normality. Tukey's Honest Significant Difference test was used to identify differences between peak and non-peak GCM levels for adult pikas. All statistical analyses were conducted using R 3.0.1 (R Core Team, 2013), and significance was assessed at the alpha=0.05 level.

RESULTS

Characterization of baseline, peak, and post-peak GCM levels

We collected 283 samples from 16 different individuals at multiple times from 0 to 28 hours after capture. However, two individuals were released < 8 hours after capture due to unsafe weather conditions at the field site. Results from these individuals were not included in final analyses, resulting in 247 samples from 14 individuals. Baseline GCM levels (Figure 2) varied between individuals ($F = 9.196$, $P < 0.001$) and also between males and females ($F = 22.226$, $p < 0.001$), but no effect of study site location was detectable at this samples size ($t = -0.979$ for a fixed effect of location given $n = 60$ samples from six males).

Baseline, peak, and post-peak GCM levels were higher for adult males than for females and juveniles (Table 1). Glucocorticoid metabolite levels peaked for all adult pikas 10-15 hours after initiation of the stressor (capture), with the exception of one individual, in which GCM

Figure 2. Baseline (pre-stress) levels of glucocorticoid metabolites (GCMs) measured in 11 adult American pikas (*Ochotona princeps*), including four males from Montana, two males from Colorado, and five females from Colorado. A sixth adult female sampled in Colorado did not produce fecal pellets during the pre-stress period. Samples sizes from males and females ranged from six to 18 and from one to five, respectively.

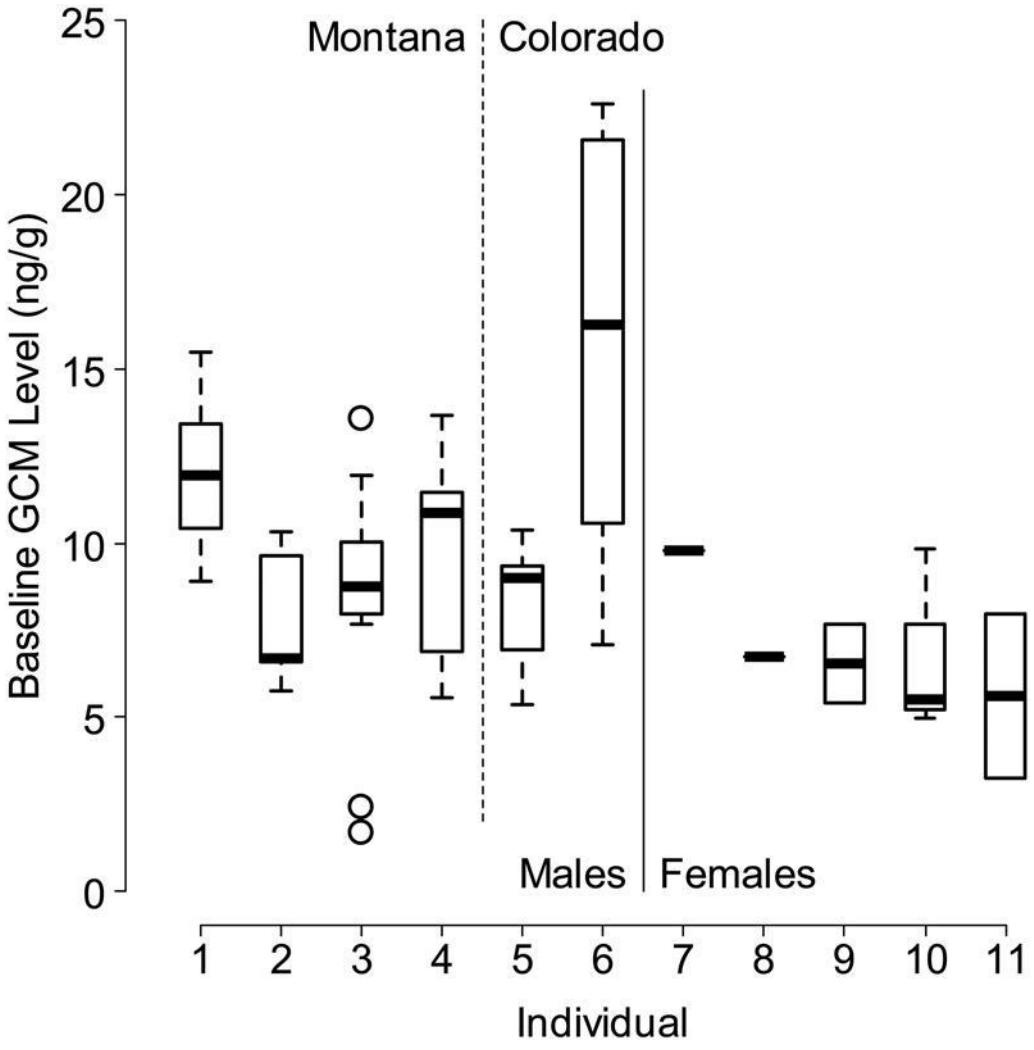


Table 1. Fecal glucocorticoid metabolites (expressed as nanograms per gram of dry feces) measured in male and female adult and juvenile American pikas (*Ochotona princeps*). Individuals are identified and named by color coded ear tags (e.g. BYYB), and are classified as male or female (M or F) and adult or juvenile (A or J). Values are means ±SEM. Abbreviations: GCM, glucocorticoid metabolite; and NA, not assessed.

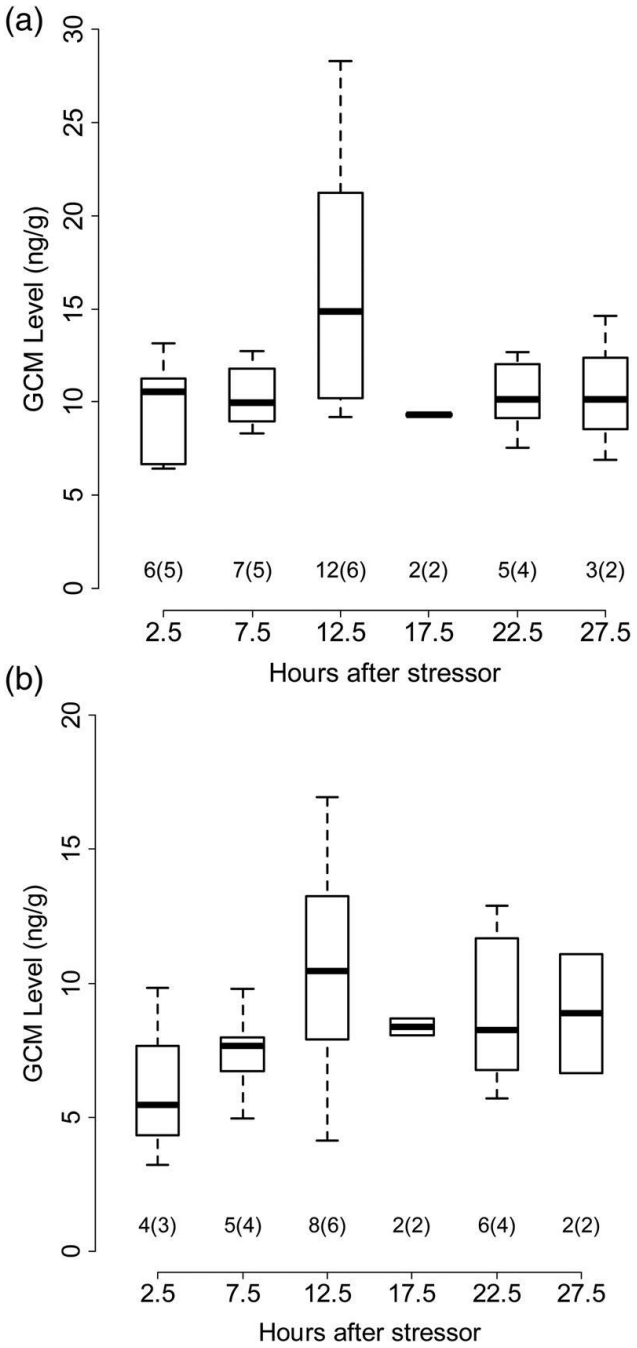
Individual	**Sex**	**Status**	**Baseline GCM**	**Mean Peak GCM**	**Post Peak GCM**
BYYB	M	A	11.93 ± 0.41	21.00 ± 1.46	11.95 ± 0.85
GRRY	M	A	7.62 ± 0.76	15.28 ± 0.94	13.09 ± 0.61
Mo RYYR	M	A	8.33 ± 1.07	14.33 ± 1.13	9.15 ± 0.39
BGGG	M	A	9.53 ± 1.17	22.10 ± 0.67	7.72 ± 0.58
CO RYYR	M	A	8.39 ± 0.53	15.24 ± 0.79	9.86 ± 0.41
WBWB	M	A	15.80 ± 2.23	28.27 ± 1.28	14.46 ± 0.28
YWGY	M	J	5.38 ± 0.35	7.45 ± 0.87	4.81 ± 0.31
GGBY	F	A	9.78 ± 2.65	16.92 ± 0.67	NA
YBBG	F	A	6.72 ± 0.45	11.48 ± 0.69	7.29 ± 1.08
RYYB	F	A	6.55 ± 0.58	9.42 ± 0.73	8.22 ± 0.25
BGBG	F	A	7.67 ± 1.01	13.26 ± 0.65	NA
BBGG	F	A	4.67 ± 0.23	8.76 ± 0.60	10.93 ± 0.57
GBYG	F	A	5.60 ± 1.07	13.20 ± 0.31	6.70 ± 0.25
RBGB	F	J	5.26 ± 0.11	8.39 ± 0.18	4.68 ± 0.20

levels continued to increase after 15 hours. For adults, GCM levels during the "peak" period (10-15 hours after capture) were 185% (±7% SEM) higher than baseline GCM levels (Table 1). Relative to samples from adults, GCM levels peaked slightly sooner (8-10 hours after capture) in samples from two juveniles (one male and one female). In most cases, GCM concentration declined to nearly pre-stress levels (baseline) by the time of release, although for some individuals (n = 3) the GCM levels remained high (Table 1). Adult males displayed a mean baseline GCM level of 10.47 ± 1.16 ng/g dry feces and a mean peak GCM level of 19.37 ± 2.03 ng/g dry feces. Adult females exhibited a mean GCM baseline level of 6.83 ± 0.66 ng/g dry feces and a mean peak GCM level of 12.17 ± 1.11 ng/g dry feces.

Biological validation of fecal glucocorticoid metabolite measurements

As predicted, stress related to capture and handling triggered an increase in fecal GCM level in adult male and female pikas (Figure 3a and b). Consistent within-individual responses (Table 1) allowed us to detect an increase in GCM level subsequent to capture and restraint. Student's paired *t*-tests comparing results from n=12 pikas with complete data (Table 1) revealed a significant rise in GCM level between baseline and peak periods (t = -6.52, P < <0.001), a significant fall in GCM level between peak and post-peak levels (t = 3.90, P = 0.001), and no significant difference between baseline and post-peak GCM levels (t = -1.52, P = 0.07). Glucocorticoid metabolite levels clearly peaked in samples from males at 12.5 hours, and peaked weakly in the same period for females (Figure 2). Analysis of variance revealed a significant effect of time on GCM response in males only (F = 3.056, P = 0.025 for males; F = 1.363, P = 0.278 for females). However, after standardizing the response within groups (sexes) as $y\hat{}_{is} = y\hat{}_{is} - \mu_s / \sigma_s$ (where i = individual, s = sex, and μ and σ = mean and standard deviation in baseline GCM), we found a highly significant effect of time using combined data from males and females

Figure 3. Glucocorticoid metabolite levels measured in male (**a**) and female American pikas (*Ochotona princeps*; **b**). Samples were binned into six time periods to generate summary statistics. Each box plot summarizes a median (bold line), interquartile range (box) and full range (whiskers). Sample sizes are reported as *i*(*j*), where *i* = number of fecal samples, and *j* = number of individuals producing those samples.



($F = 4.337$, $P = 0.002$, n = 62). Tukey's Honest Significant Difference test confirmed that samples collected 10–15 h after capture (period 3 in Figure 4) showed significantly higher levels of GCM than samples collected earlier.

Timing of peak glucocorticoid metabolite level

The timing of the initiation of the stressor varied among individuals, because animals entered traps at different times in the morning. All individuals were trapped between the hours of 6:30-11:30 AM, and all pikas (except for one individual where GCM levels continued to climb) displayed maximum GCM levels 10-15 hours after first being trapped. Individuals that were trapped later in the day had GCM levels that peaked later that evening, or early the next morning. Individuals were not synchronous in the timing of their peak GCM level; the displayed peak in GCM level was explained well by the time elapsed since the initiation of the stressor (Figure 5) rather than circadian rhythm.

DISCUSSION

This study was the first to use fecal glucocorticoid metabolite analysis to assess stress in pikas. Here we recorded a stress induced peak in GCM level that represented a 2-fold increase over baseline. A 2-fold response in GCM is relatively small in comparison to responses recorded for some species resulting from pharmacological stimulation of the stress axis through adrenocorticotropic hormone (ACTH) challenge (Touma & Palme 2005). However, a similar magnitude of response to a biological stressor has been documented for several other lagomorphs (Monclus *et al.* 2006; Sheriff *et al.* 2009; Scarlata *et al.* 2011), and other small mammals (Fletcher & Boonstra 2006; Chelini *et al.* 2010).

Confinement and trapping has been shown repeatedly to induce a significant increase in GCM level in many mammal species (Harper & Austad 2001; Fletcher & Boonstra 2006; Bosson

Figure 4. Evidence of a peak in GCM concentration during hours 10– 15 (period 3 in Figure 2) following the stress of capture and handling. Tukey's Honest Significant Difference test reveals a higher GCM level in samples from period 3 compared with periods 1 and 2 [confidence intervals (CIs) do not overlap zero], and suggests a lower GCM level in samples from periods 4–6 compared with period 3 (CIs sparely overlap zero). Standardized samples from males and females were pooled for this test (n = 62).

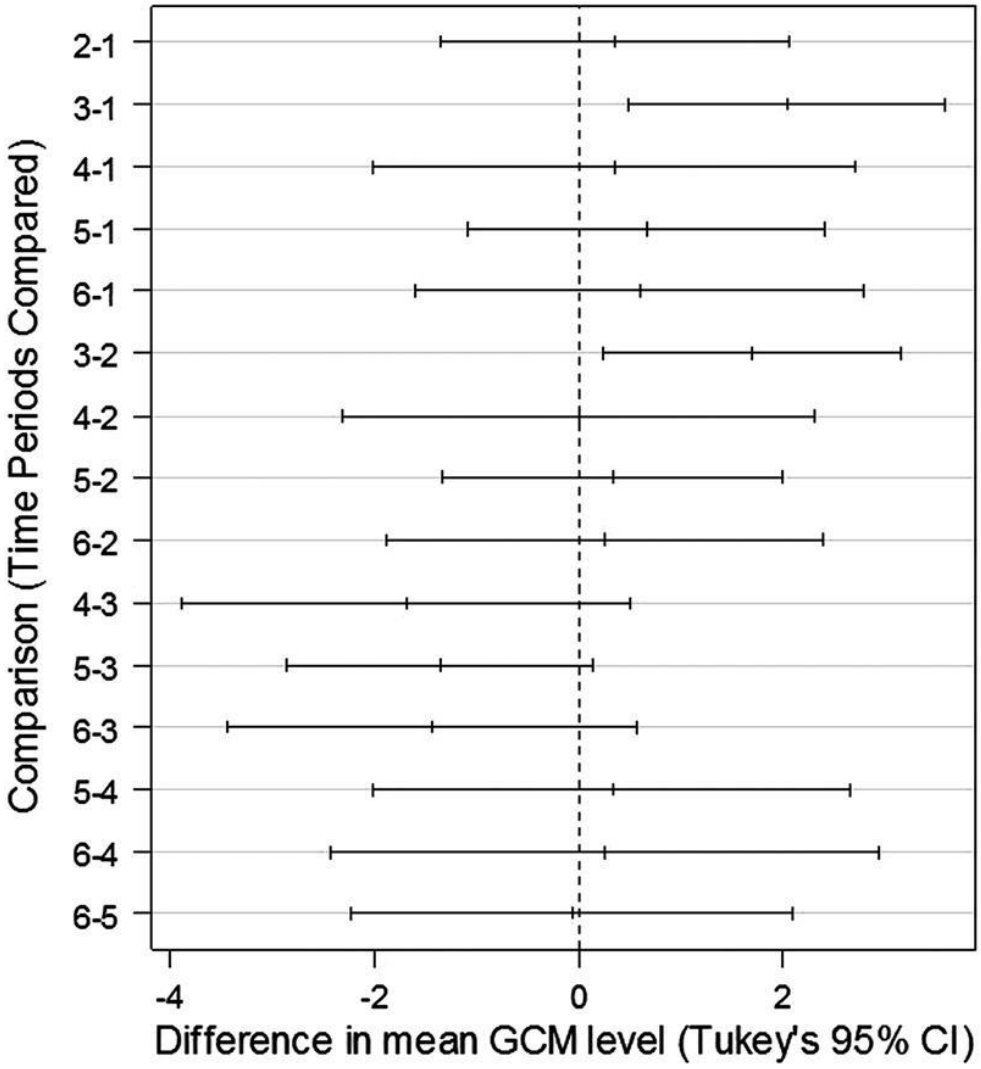
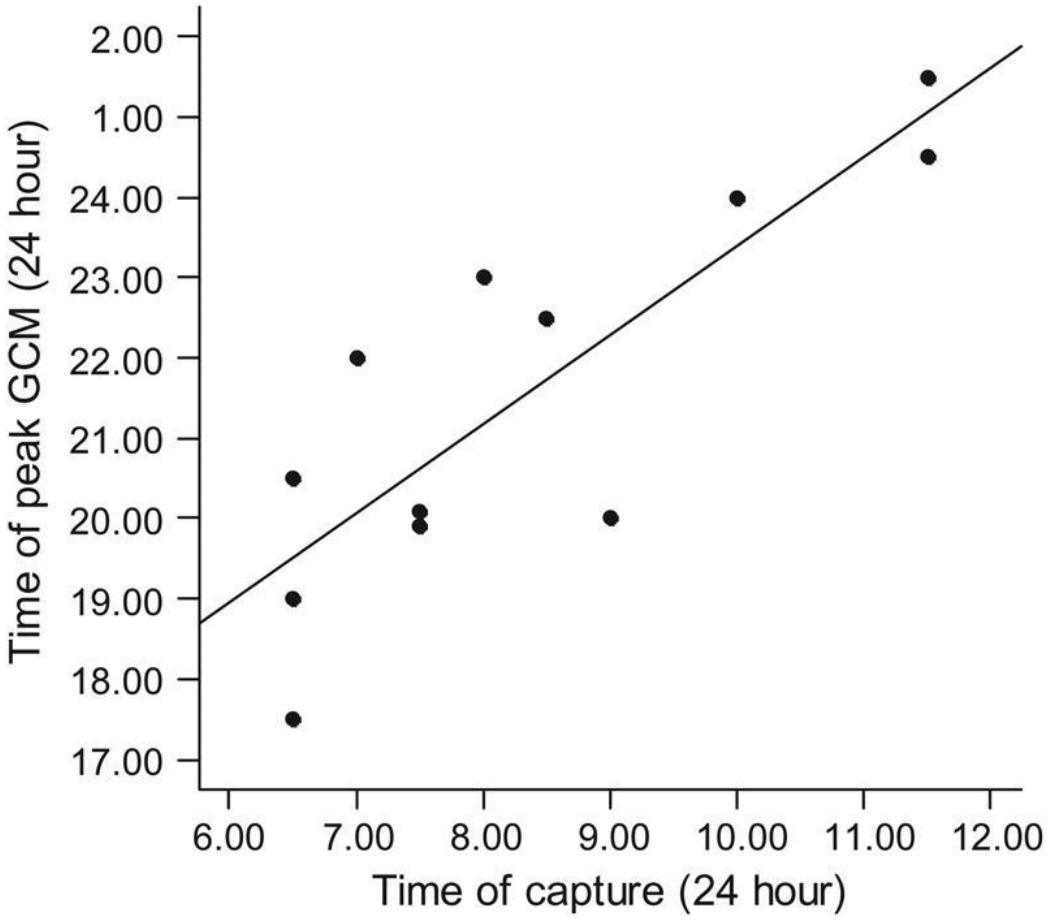


Figure 5. Time of peak GCM as a linear function of capture time for data from 12 American pikas (*Ochotona princeps*; adjusted $R^2 = 0.6633$, $F = 22.67$ on 1 and 10 degrees of freedom, $P < 0.001$). Data were jittered slightly to differentiate points.



et al. 2009), so we expected the stress response elicited by our methods likewise to increase GCM concentrations. Naturally occurring events that may elicit a stress response have been used for a variety of vertebrate species to validate fecal GCM analysis. Measurement of GCM levels before and after a translocation event has been used to biologically validate fecal metabolite analysis in spotted hyenas (*Crocuta crocuta*; Goymann *et al.* 1999), dairy cattle (*Bos taurus*; Morrow *et al.* 2002) and pygmy rabbits (*Brachylagus idahoensis*; Scarlata *et al.* 2011). Other stressful events such as repeated disturbance, exposure to a predator, or immobilization have been utilized in biological validations for European hares (*Lepus europaeus*; Teskey-Gerstl *et al.* 2000), snowshoe hares (*Lepus americanus*; Sheriff *et al.* 2009), and cheetahs (*Acinonyx jubatus;* Terio *et al.* 1999) and roe deer (*Capreolus capreolu;* Dehnhard *et al.* 2001) respectively.

The time delay between exposure to a known stressor and detection of elevated GCMs in fecal material is believed to approximate the time required for food to pass through the digestive system (Palme *et al.*1996) for a particular species. The time delay to detection recorded here (11-15 hours) is very similar to the gut passage time reported for other lagomorphs (Piekarz *et al.* 1963; Monclus *et al.* 2006; Rehnus *et al.* 2009; Sheriff *et al.* 2009). Thus, approximately 12.5 hours may be the gut passage time for American pika adults, a statistic previously unreported. We observed shorter response times for juvenile pikas, as expected if juveniles have shorter gut passage times.

As expected, measured baseline and peak GCM levels varied significantly between individuals within both male and female pikas. Numerous studies assessing adrenocortical response in vertebrate species document a large degree of variation between individuals, and this variation most likely reflects an individual's ability to cope with environmental change (Moberg 2000; Koolhaas *et al.* 2000). Multiple factors come into play when determining how an

individual will physiologically respond to a stressor, such as previous exposure, genetics, temperament, age, and physical state (Moberg 2000; Owen *et al.* 2004). Additionally, sex hormones may alter the release of glucocorticoids (von der Ohe & Servheen 2002), and this may explain the higher overall GCM levels in males observed here. Breeding is highly seasonal in pikas, and increases in glucocorticoid level during the breeding season have been displayed in other alpine species with similarly short breeding seasons (Boonstra *et al.* 2001). Testosterone, linked to increased aggression and conflict in males during the breeding season, has been shown to increase the release of glucocorticoids. In contrast, progesterone which is released in greater amounts in females during estrus, pregnancy, and lactation, appears to decrease the GC response (Kenagy & Place 2000; Owen *et al.* 2004; Reeder *et al.* 2004; Dantzer *et al.* 2010). Two of the adult females used in this study were pregnant, and the remaining adult females had all recently given birth and completed lactation within the season (0-3 months prior to sampling). It is interesting to note that GCM levels in female pikas tended to remain high even after the peak GCM period, and in most cases did not decline to baseline (Table 1 and Figure 2b). Based on our behavioral observations, adult females appeared to be more distressed in captivity, and were less active in comparison to adult males. This observation might be attributed to the physiological demands on adult females during pregnancy and lactation.

Although the secretion of glucocorticoids into the bloodstream follows a circadian rhythm (Thun *et al.* 1981), the pattern related to GCM secretion in fecal material is less clear. Many everyday behaviors, such as arousal from sleep, are cued by the release of GCs and this event usually represents the largest release within a 24 hour period. Thus, GC secretion typically peaks early in the morning for diurnal animals, and early in the evening for nocturnal animals. Depending on the species specific lag time required for GC metabolites to appear in feces, one

would expect a peak in GCM level in the afternoon for diurnal mammals and late at night or early the next morning for nocturnal mammals. Given that pikas are diurnal, we would have expected a peak GCM level recorded in the afternoon/early evening, if a circadian rhythm could be detected. However, our peak GCM levels occurred in samples collected from 5:30 pm to 1:30 am, and varied according to when the animal was trapped (Figure 5). Fluctuations in daily patterns of hormone secretion are not known to vary widely among individuals within the same species (Goldman 1999). Although some studies have detected a diurnal fluctuation in fecal GCM concentration (Bamberg *et al.* 2001; Pihl & Hau 2003; Bosson *et al.* 2009), other research has documented the absence of a rhythm in GCM levels (Bauer *et al.* 2008; Chelini *et al.* 2010). Fecal GCM concentrations reflect pooled quantities of glucocorticoids released over time and even with frequent sampling, brief or small increases in circulating glucocorticoids could be masked by the pooling of metabolites in feces. It may not be possible to detect diurnal changes of circulating GC levels in fecal samples for some species (Touma & Palme 2005), especially for those that defecate infrequently. In this study, we did not detect a circadian rhythm of GC secretion, even though we used a time series of pellets, indicating that a circadian rhythm in GCM level may not be discernible for pikas.

Pikas habitually urinate on their fecal droppings in natural environments. Current genetic and hormone research on pikas requires fresh fecal samples, and existing collection protocols specifically instruct that fecal samples can be identified as fresh only if they are still cemented to rocks with urine. Therefore, only fecal pellets coated with urine were included in our analysis, because these are the type of pellet that will be used for future studies based on non-invasive sampling.

One drawback associated with this approach is that the proportion of GCMs excreted in

urine or feces can vary based on the species. For example, research conducted on marmosets, macaques, and chimpanzees has shown that 82–91% of GCMs are excreted via the urine, while only 9–18% are excreted in feces (Bahr *et al*. 2000). However, for other species the opposite pattern has been displayed, with a much higher proportion of GCMs being excreted in the feces. Following injection of radioactive corticosterone, 20% of metabolites recovered were found in the urine and 80% in the feces of male Sprague–Dawley rats (Bamberg *et al.* 2001). Touma *et al.* 2003 also found that radioactive metabolites were recovered predominantly in the feces rather than in urine in mice (*Mus musculus*), and that males excreted significantly higher proportions via the feces than females. This suggests that gender may also play a role in determining excretion patterns of metabolites, even within the same species. Given our lack of knowledge concerning the excretion route of GCMs for pikas, we cannot be certain whether our measurements more accurately reflect the GCM concentration found in urine or in feces. However, with any particular assay, only a proportion of the total GCMs are measured, and the assay is still useful if comparisons are made using the same measured relative proportion of GCM concentration (Millspaugh & Washburn 2004). The samples we analyzed were completely saturated with urine; thus, the proportion of urine per sample did not differ among samples, eliminating this as a potential source of sample variability. By analyzing fecal and urine portions together, we were able to obtain a more comprehensive estimate of total GCMs.

In conclusion, our results confirm that fecal hormone analysis can be used to assess physiological stress in the American pika. However, our approach has some limitations. Due to field sampling constraints and the sensitivity of the species, we conducted only a biological validation of our techniques. Given the high mortality of these animals in captivity (Krear 1965; MacArthur & Wang 1974; Dearing 1995), information regarding optimal holding conditions is

needed prior to additional laboratory studies. Future studies could incorporate an adrenocorticotrophic challenge test or similar physiological validation, if possible (Touma & Palme 2005). High-performance liquid chromatography could also be performed to determine the exact fecal GCMs being excreted (Rose & Jusko 1979). Furthermore, a radio-labeled metabolism study could be carried out to determine the major route and the exact rate of excretion of GCMs in pikas (Palme *et al.* 2005). Results from these suggested research areas would clarify information related to adrenal activity, and contribute to a better understanding of the stress response for this species.

However, current methods do provide a foundation for further research into how changes in the environment directly affect adrenocortical activity in the American pika. The methods developed and validated in this study can be used to add non-invasive measurements of physiological stress to pika monitoring programs and other research designed to assess pika vulnerability to predicted changes in climate. For example, by measuring GCM concentration in fecal samples collected from pikas living in different habitats, we can determine environmental correlates of GCM measurements. We caution, however, that environmental conditions (e.g. precipitation) may influence the GCM concentration measured in samples collected non-invasively (Kahn *et al.* 2002; Terio *et al.* 2002; Millspaugh *et al.* 2003; Palme 2005), so that it is necessary to account for direct effects of environment on GCM levels. For example, measured GCM concentration was higher in white-tailed deer (*Odocoileus virginianus*) feces that had been exposed to artificial precipitation, most probably due to increased microbial activity in response to elevated moisture levels (Washburn & Millspaugh 2002). Likewise, elevated temperatures have been shown both to increase (Millspaugh *et al.* 2003) and to decrease (Terio *et al.* 2002) the GCM concentration measured in fecal samples from different species. Environmental conditions

may alter the GCM concentration in pika feces, and one proposed method to test this would be to make comparisons between control samples and those exposed to different temperature and moisture levels.

Measurements of stress response have many applications in conservation. For example, Creel *et al.* (2002) compared GCM levels measured in samples collected from several national parks, in order to assess the impact of human disturbance on North American elk and wolf populations. Higher GCM levels were measured in response to increased snowmobile activity for both species (Creel *et al.* 2002). A similar study involving maned wolves *(Chrysocyon brachyurus)* in South America measured higher GCM levels from wolves living in areas outside of national parks and other protected regions (Spercoski *et al.* 2012). When using measurements of stress response to guide wildlife management decisions, it can often be difficult to separate effects of ecological and anthropogenic disturbance on the stress response. Recent research conducted on the endangered southern resident killer whale *(Orcinus orca)* used a combination of approaches to determine the relative importance of factors affecting the population (Ayres *et al.* 2012). Fecal thyroid and GCM measurements were used to assess the impacts of reduced prey (Chinook salmon) and increased tourist boat traffic on whales, respectively. Effects associated with boat traffic were overshadowed by a reduction in prey items, indicating that restoration of Chinook salmon runs is most important for population recovery (Ayres *et al.* 2012). These are just a few examples of how noninvasive endocrine monitoring can help resolve major conservation questions.

Developing stress metrics as bio-indicators is another possible conservation application. Stress in the American pika might indicate a decline in water resources key to downstream ecosystems. Pikas appear to persist primarily in locations that contain sub-surface water features

such as seasonal ice or permafrost (Millar & Westfall 2010), and in sites with more precipitation and better winter snow cover (Beever *et al.* 2010; Erb *et al.* 2011), which would help maintain sub-surface water features. These features are key to water storage and production from alpine habitats (Molotch *et al.* 2008),and should represent an increasingly important component of water resources as surface water-storage features (glaciers and snowpacks) diminish in a warming climate (Clark *et al.* 1994; Schrott 1996; Millar & Westfall 2008). Sub-surface water features also moderate the sub-surface microclimates pikas need to survive the physiological demands of both summer and winter (MacArthur & Wang 1973 & 1974; Millar & Westfall 2010). Thus, pikas should be stressed by a decline in the extent or quality of sub-surface water features. Such a response could serve as a bio-indicator, allowing managers to track the spatial and temporal extent of subsurface ice features to better characterize potential watershed productivity and ecosystem resilience. This use of stress metrics as bio-indicators of hydrological change could have several advantages over direct methods of monitoring. Monitoring a stress response may provide an early-warning of ecosystem change. Using a mammalian stress response as a bio-indicator is attractive because the physical condition of a mammal represents a relatively complex set of inputs integrated over space and time. A less integrative bio-indicator might not be as effective for estimating the condition of a system that is complex and spatiotemporally extended (e.g. a watershed).

Chapter 2

RELATING SUB-SURFACE WATER RESOURCES TO PHYSIOLOGICAL STRESS IN AN INDICATOR SPECIES: IMPLICATIONS FOR TRACKING EFFECTS OF CLIIMATE CHANGE IN MONTANE WATERSHEDS

ABSTRACT

The American pika (*Ochotona princeps)* is considered a sentinel species for detecting ecological effects of climate change. Pikas are declining within a large portion of their range, and ongoing research suggests loss of sub-surface ice as a mechanism. However, no studies have demonstrated physiological responses of pikas to sub-surface ice features. Here we present the first analysis of physiological stress in pikas living in and adjacent to habitats underlain by ice. Fresh fecal samples were collected non-invasively from two adjacent sites in the Rocky Mountains (one with sub-surface ice and one without) and analyzed for glucocorticoid metabolites (GCM). We also measured pika-relevant microclimates in each habitat. Results indicate lower GCM concentration in sites with sub-surface ice, suggesting that pikas are less stressed in favorable microclimates resulting from sub-surface ice features. GCM response was well predicted by habitat characteristics commonly associated with sub-surface ice features, such as lower mean summer temperatures. These results suggest that pikas inhabiting areas without sub-surface ice features are experiencing higher levels of physiological stress and may be more susceptible to changing climates. In addition, pika presence and GCM levels may be useful indicators of sub-surface ice features. Sub-surface ice features are key to water cycling and storage and will likely represent an increasingly important component of water resources in a warming climate. By establishing a relationship between these features and a metric of physiological stress in pikas, our results suggest that pikas may serve as a sensitive bio-indicator

of hydrological change in alpine ecosystems that may not be detected by monitoring physical features alone. This novel approach complements other efforts to understand how landscape change will affect water resource availability.

INTRODUCTION

Climate change in the form of rising temperatures, changing precipitation patterns and increased frequency of extreme weather events is occurring at an unprecedented rate (IPCC 2014). Shifting climates are affecting species distributions, phenology, and physiology worldwide (Walther *et al.* 2002; Parmesan 2003; Root *et al.* 2003). Increased atmospheric CO_2 has also resulted in changes in plant productivity and plant-herbivore interactions (Watt *et al.* 1995; Rusterholtz *et al.* 1998).

Climate change is also likely to have profound impacts on ecological systems and the services they provide. For example, much of the world's human population depends on water resources that originate in alpine ecosystems, and up to 80% of the planet's fresh surface water comes from high elevation watershed areas (Viviroli *et al.* 2003). These mountainous and highland regions are often referred to as the world's natural "water towers", and evidence suggests that warming may occur more rapidly at these higher elevation locations (Diaz & Bradley 1997; Hughes 2000; Naftz *et al.* 2002; Beniston 2003).Climate warming has already forced a global retreat of glaciers (Paul *et al.* 2004) as well as declines in the number of winter days with frost (Walther 2002) and the duration of snowpack at low and middle elevations (Laternser & Schneebli 2003). As a result, water derived from snowmelt in the US Pacific Northwest has decreased by 20% since 1950 (Clark *et al.*1994). Similar declines around the world will have far reaching consequences for both humans and wildlife (IPCC 2014).

One important contributor to these alpine water resources is rock glaciers, or permafrost insulated by a rocky covering. Rock glaciers and associated rock-ice features (RIFs) typically occur in arctic and alpine landscapes characterized by cold temperatures, low humidity, and the presence of talus or broken rock (Millar & Westfall 2008). Most studies documenting the decline of glaciers worldwide have measured retreats of surface ice, which tends to melt faster than rock glaciers. As climate change depletes surface water-storage features (e.g., glaciers and snowpack; Clark *et al.* 1994; Schrott 1996; Millar & Westfall 2008), RIFs will likely become key components of water cycling and storage (Molotch *et al.* 2008). Located beneath an insulating layer of broken rock (talus), rock glaciers are less affected by rising air temperatures, and overall melting rates of rock glaciers appear to be slower than those of ice glaciers (Clark *et al.* 1994). As a result, rock glaciers and related RIFs may become critical water reservoirs in alpine areas, especially under warmer temperature regimes (Schrott 1996).

Although the hydrologic contribution of RIFs in the context of climate change remains understudied (Burger *et al.* 1999), the role of RIFs and their implications for watersheds will likely become more important as precipitation patterns in mountainous areas continue to change (Service 2004). However, one problem with studying RIFs in alpine areas is that they can be extremely difficult to detect and are often overlooked in the environment (Burger *et al.* 1999).

Due to their important hydrological implications, additional tools for identifying RIFs would have useful watershed management applications. Here, we present a practical application of previously validated procedures, based on non-invasive sampling and analysis of physiological stress in an indicator species, the American pika (*Ochotona princeps*). Pikas are cold-adapted members of the rabbit order that require cool microclimates and talus habitat [21]. As a result of recent climate-mediated population declines, pikas have become widely considered

a sentinel species for detecting ecological effects of climate change (Hafner 1993 & 1994; McDonald & Brown 1992; Lawlor 1998; Beever *et al.* 2003; Krajick 2004; Smith *et al.* 2004; Grayson 2005; Morrison & Hik 2007). Due to its unique topography, talus associated with RIFs tends to be cooler in the summer and warmer in the winter (Millar & Westfall 2010), climatic conditions that have been positively related to pika persistence (Beever *et al.* 2010 & 2011; Wilkening *et al.* 2011).

As a result of these microclimate features, we hypothesized that the persistence of pikas in alpine areas could potentially aid in identifying RIFs. Furthermore, the health and fitness of individual pikas within a watershed area may be an even more sensitive proxy for watershed condition. Analyses of stress hormone metabolites in fecal material are increasingly employed to evaluate the health of sensitive animal populations (Harper & Austad 2000; Millspaugh & Washburn 2004; Cyr & Romero 2008), and these techniques have recently been validated for pikas (Wilkening *et al.* 2013). Because samples can be collected without disturbing or endangering the animal, these measurements are not biased by capture-induced stress and reflect an average level of circulating stress hormones. Thus, analysis of fecal samples can provide the most precise estimate of the current endocrine condition of an animal (Harper & Austad 2000, Millspaugh *et al.* 2001; Touma *et al.* 2003). Although fecal stress hormone metabolites have been increasingly used as proxies of animal health and fitness, few studies account for the potential influence of environmental factors when using fecal samples collected non-invasively. Environmental conditions such as temperature or humidity can alter microbial activity, which can result in decomposition of steroid metabolites and biased measurements of metabolite concentration (Khan *et al.*2002; Terio 2002; Millspaugh & Washburn 2004; Palme 2005).

In this study, our first objective was to examine the effects of varying environmental conditions on stress hormone metabolite concentration (glucocorticoid metabolite; GCM) in pika fecal pellets. By comparing GCM concentration measured in pika fecal pellets that were placed outside in timed exposure trials, we tested whether environmental conditions directly alter GCM concentrations measured in samples. Our second objective was to compare GCM concentration measured non-invasively in pikas living in habitats with and without RIFs. If habitats underlain by ice promote pika health, then we hypothesized that GCM concentration should be lower in samples collected from within RIFs compared to samples collected outside RIFs. As part of this objective, we also tested the hypothesis that GCM concentrations in pika scat could be used to identify specific microclimates associated with RIFs to further define the role of pikas as indicators of climatic or hydrological change.

METHODS

Study area

This research was conducted in the US Rocky Mountains at five study sites: Niwot Ridge Long Term Ecological Research Site (NWT), Green Lakes Valley Watershed (GLVW; the only site with RIFs), Brainard Lake Recreation Area (BLRA), and Rocky Mountain National Park (RMNP) in Colorado, and Emerald Lake (EL) in Montana (Table 1). All sites comprised numerous patches of typical pika habitat, characterized by large regions of broken rock (talus) interspersed with alpine meadows ranging in elevation from approximately 2500 to 3700 m.

Objective 1: Exposure trials

To obtain fecal samples for the exposure trials in objective 1, pikas at NWT and BLRA were live trapped and fresh fecal pellets were collected only from adult female pikas (to control for observed differences in GCM concentration resulting from gender and age) during summer 2012.

Table 1. Site name and abbreviation as used throughout the study. Mean latitude and longitude refer to the average location within each site where samples were collected or exposed. Elevation range gives the range of elevations of locations within each site where samples were collected or exposed.

Site Name	**Site Abbreviation**	**Mean Latitude**	**Mean Longitude**	**Elevation range (m)**
Niwot Ridge LTER	NWT	40.06	105.60	3587-3625
Green Lakes Valley Watershed	GLVW (RIFs)	40.05	105.62	3458-3779
Brainard Lake Recreation Area	BLRA	40.07	105.59	3300-3326
Rocky Mountain National Park	RMNP	40.40	105.67	3246-3313
Emerald Lake	EL	45.41	110.93	2748-2846

Light anesthesia (inhalant anesthetic, Isoflurane) was maintained throughout the 20-minute handling process and all efforts were made to minimize suffering. After handling, pikas were released back into their home territories. Trapping and sampling procedures were reviewed and authorized by Colorado Parks and Wildlife (license no. TR2014) and procedures followed those approved by the University of Colorado-Boulder Institutional Animal Care and Use Committee (protocol 1104.06). Fecal samples were kept on ice in the field and transferred within 12 h to a −20°C freezer. Several hundred pellets were needed for exposure trials, and captured pikas defecated infrequently. Therefore, pellets collected from 11 different individuals were pooled and divided at random into 3 controls plus 12 samples slated for exposure. Each exposure sample consisted of approximately 15 pellets placed inside a modified plastic food-storage container (9 cm × 9 cm × 12 cm) with a detachable lid and mesh sides. These "exposure boxes" were then semi-buried within the talus environment during August 2012 at 3 different locations within each of four sites: NWT, BLRA, RMNP and EL. Placement of exposure boxes shielded pellets from direct sunlight and rain and mimicked the location of naturally occurring pika latrines. Boxes were placed only in currently occupied pika territories, adjacent to fresh fecal piles. After two weeks, exposure boxes were collected from all sites, and fecal samples were transferred to the lab for analysis. Control samples were maintained in the -20°C freezer during the entire exposure period and GCM analysis was performed on all samples at the same time.

GCM extraction and analysis proceeded according to protocols previously validated for pikas (Wilkening *et al.* 2013). Briefly, fecal samples were lyophilized and ground into powder using a mortar and pestle. We then followed the steroid solid extraction protocol provided by Arbor Assay Design, Inc. Comparative analysis of GCM levels in samples was conducted using a commercially available Corticosterone Enzyme Immunoassay Kit (Arbor Assay Design, Inc.,

Ann Arbor, MI; cat. no. K014-H1). During each assay, we ran extracted samples in triplicate alongside a standard curve of seven known concentrations of corticosterone (5000, 2500, 1250, 625, 312.50, 156.25, 78.125 pg/ml.) Values for each extracted sample were generated using a micro plate reader (BioTek Microplate Reader Synergy HT; 2005 Biotek Industries, Inc.) and Gen 5 1.11 Data Analysis software. Intra-assay coefficients of variation were less than 10% and inter-assay coefficients of variation were less than 15%. Final concentrations of fecal GCM were expressed as ng GCM/g dry feces.

One-way ANOVA was used to test for differences in GCM concentration among control samples and exposed samples at each site. Prior to analysis, data were checked for outliers, normal probability plots were examined, and a Shapiro-Wilks statistic was calculated to test for normality. All statistical analyses were conducted using R 3.0.1 (R Core Team 2013) and significance was assessed at the $\alpha=0.05$ level.

Objective 2: Sample and temperature data collection

To compare GCM concentration across habitat types in objective 2, we collected feces from two adjacent sites: one with RIFs (GLVW) and one without RIFs (NWT). These areas are situated at the northern edge of the Front Range, near Boulder, Colorado and comprise an elevation range of 3200-4000 meters (Appendix I). The region is characterized by low temperatures throughout the year and receives most of its precipitation in the form of winter and spring snowfall. The GLVW consists of two alpine catchments, and provides approximately 40% of the water supply for the City of Boulder. It also has persistent rock glaciers and other sub-surface ice features (Williams *et al.* 2006). NWT has been the location of hydrological and snow studies since 1971 (Ives & Fahey 1971; Ives 1973). In contrast to GLVW, permafrost and similar RIFs have not been found at NWT since 2008. Recent research documents the absence of permafrost at NWT, suggesting

that previously designated permafrost areas have either melted over time or were initially overestimated (Leopold *et al.* 2008; Leopold *et al.* 2010; Matthias *et al.* 2014).

Pika scat is easy to identify and was abundant at both GLVW and NWT. Fresh fecal samples were collected from territories currently occupied by adult pikas during summer 2011-2013 at both sites (N = 34 GLVW: 30 NWT). Pikas maintain established latrines within individual territories, and fecal samples were identified as fresh by color, consistency, and relative position. Samples were immediately put on ice after collection, and transferred to deep freeze prior to analysis. Care was taken at both sites to collect samples from locations of varying aspect and elevation. Extraction and estimation of GCM concentration from fecal samples followed procedures previously described above.

We also characterized microclimates at each site, which might contribute to observed patterns of physiological stress. In models designed to predict patterns of recent pika declines, the relative importance of climatic factors has risen dramatically over the past decade (Beever *et al.* 2011; Braganza *et al.* 2004). Pikas have a narrow thermal tolerance; when ambient temperatures increase, they reduce their activity levels and shed heat passively by retiring to cooler microclimates (MacArthur & Wang 1973; Smith 1974). In addition, pikas also appear to be sensitive to cold stress during winter, due to reduced snowpack and consequent loss of insulation from sub-freezing temperatures (Morrison & Hik 2007; Beever *et al.* 2010 & 2011; Ray *et al.* 2012). At each site, we placed 18-20 temperature data loggers (DS1921G, Thermochron iButtons, Maxim Integrated Products, Sunnyvale, CA) inside the talus habitat, approximately 0.5-1.0 meters below the surface. Within GLVW, data loggers were placed and fecal samples were collected, in areas known to contain RIFs (Davinroy 2000). Data loggers recorded temperature every four hours from June 2011-December 2013.

Data analysis, response and predictor variables

We applied F-tests to assess the equality of variance between GCM samples, and Welch's t-test to identify overall differences in GCM concentration between samples collected at GLVW and NWT. Prior to analysis, data were checked for outliers, normal probability plots were examined, and a Shapiro-Wilks statistic was calculated to test for normality. All statistical analyses were conducted using R 3.0.1 (R Core Team 2013) and significance was assessed at the $\alpha=0.05$ level.

For models of GCM concentration, candidate predictor variables represented potential indicators of chronic and acute stress identified in previous studies of the American pika (Beever *et al.* 2010 & 2011; Wilkening *et al.* 2011; Erb *et al.* 2011; Jeffress *et al.* 2013). These included: elevation, average summer temperature, numbers of days below negative 10°C, potential solar gain, and summer diurnal temperature range. Predictors were abbreviated as:

ELEV = elevation in meters

AST = average summer temperature June-September

DB-10 = number of days below negative 10°C

PSG = potential solar gain

SUMDTR = average summer diurnal temperature range June-September

Elevation (ELEV) was recorded in meters at each fecal sampling location as a proxy for both chronic heat and cold stress; pika occurrence has been shown to increase with increasing elevation (Hafner 1993), and we hypothesized that heat and cold stress would decrease with elevation. Average summer temperature (AST) represents a measure of chronic heat stress, and was calculated by averaging all sub-surface temperature measurements during June-September. Number of days below 10°C (DB-10) is an indicator of acute cold stress, and this was measured by summing the total number of days throughout the year that experienced sub-surface

temperatures below this threshold. Potential solar gain (PSG) exemplifies how slope and aspect interact to create individualized micro-climatic conditions leading to chronic heat or cold stress. This variable was estimated as sine(slope) x cosine(aspect); values ranged from -1 to 1 with steeper north facing slopes having larger positive values, and steeper south facing slopes having larger negative values (Jeffress *et al.* 2013). Aspect and slope were recorded at each fecal sampling location using a compass and clinometer. Summer diurnal temperature range (SUMDTR) was considered an indicator of both acute and chronic heat stress. This variable has not been previously considered in analyses related to pikas and climate change; however, climate change is affecting diurnal temperature range, and other alpine species appear to be responding to these increased fluctuations (Walther *et al.* 2002). Diurnal temperature range measurements may differ between seasons (Karl *et al.* 1991; Braganza *et al.* 2004), and we used only a summer value to test for physiological constraints pikas may experience during the summer foraging season. Summer diurnal temperature range was obtained by averaging the daily difference between sub-surface temperature maxima and minima measured during June-September.

Timing of fecal sample and temperature data collection

GCM response was measured in fecal samples collected from GLVW during 2011-2013 and from NWT during 2012-2013. Time averaged temperature metrics were obtained from data loggers continuously in place from June 2011- December 2013. Fecal samples were not always available within the immediate vicinity of data loggers, so a distance matrix was used to associate fecal samples with distance-weighted average temperature metrics. For example, the distance-weighted average summer temperature over 2011-2013 at sampling location *i* was calculated as $AST_i = \sum_j AST_j \, (1/d_j \, / \, [\sum_j 1/d_j])$, for $j = 1$ to n sensors at the study site.

Linear mixed effects models

Fecal GCM concentration, a validated indicator of physiological stress (Wilkening *et al.* 2013) was used as a response variable in linear mixed-effects models. Mixed-effects models were used to address repeated sampling from similar locations (Buckley *et al.* 2003). Models were developed and then compared using an information-theoretic framework. We evaluated the relative support for each predictor variable within and between sampling areas using 34 candidate models (Table 2). This model set represents alternative stressors (i.e., predictor variables), as well as stressors working in combination. Several predictor variables were highly correlated (Spearman's $r > 0.5$), some within sites and some across the two-site dataset. To maximize the number of models considered while avoiding collinearity, we examined GCM response in three separate analyses: 1) within GLVW, 2) within NWT, and 3) across both sites. Within each analysis, highly correlated predictors appeared only in separate models (Appendix II).

The relative support for each model was calculated using an information criterion (AIC; Burnham & Anderson 2002). A lower AIC score indicated that the model was better supported by the data, and the best models had the lowest AIC scores within each of the 3 analyses (i.e., each site alone or both sites together). We considered models with an AIC score 0-2 units higher than the lowest score to have support similar to the best model, while models with a score 4 or more units higher had little support. Model averaging and the ranking of predictor variables were performed using the R package MuMIn (Multi-Model Inference; Barton 2014) for the analysis of a global model including all 5 predictors and no interaction effects.

Models were fit using *lme4* (Bates *et al.* 2012) in R 3.1.0 (R Development Core Team 2014). Models contained one or more predictor variables (ELEV, AST, DB-10, PSG, SUMDTR) as fixed effects. Site (analysis 3) and year were treated as random effects on model intercept and

Table 2. Candidate models. Predictors in each candidate model include elevation (ELEV), average summer temperature (AST), number of days below negative 10°C (DB-10), potential solar gain (PSG), average diurnal temperature range for summer (SUMDTR).

Analysis 1: GLVW alone	
Model	**Predictors**
1	ELEV
2	ELEV,AST
3	ELEV,AST, DB-10
4	ELEV, DB-10
5	ELEV, DB-10, SUMDTR
6	ELEV, SUMDTR
7	AST
8	AST, DB-10
9	AST, DB-10, PSG
10	AST, PSG
11	DB-10
12	DB-10, PSG
13	DB-10, PSG, SUMDTR
14	DB-10, SUMDTR
15	PSG
16	PSG, SUMDTR
17	SUMDTR
Analysis 2: NWT alone	
Model	**Predictors**
18	ELEV
19	ELEV, AST
20	AST
21	AST, DB-10
22	AST, PSG
23	DB-10
24	PSG
25	SUMDTR
Analysis 3: Both Sites	
Model	**Predictors**
26	ELEV

27	ELEV, AST
28	ELEV,SUMDTR
29	AST
30	DB-10
31	DB-10, PSG
32	PSG
33	PSG, SUMDTR
34	SUMDTR

slope(s). Fixed effects differed by model, but random effects were the same for every model within each analysis. Since only fixed effects differed among models, maximum likelihood (rather than restricted maximum likelihood) was used to estimate coefficients and compare these nested models (Pinheiro & Bates 2000). Interaction terms were omitted if not significant (p-value > 0.10).

In addition to model comparison using AIC, pseudo-R^2 values were calculated for each model as a metric of goodness-of-fit. Using methods specified for linear mixed-effects models (Nakagawa & Schielzeth 2013), we computed both a marginal R^2 (i.e., amount of variance explained by the fixed effects) and a conditional R^2 (i.e., amount of variance explained by the fixed and random effects in combination). Models were ranked according to ΔAIC value, rather than R^2, to ensure an appropriate penalty for each predictor variable.

We examined residuals of the best model in each analysis in two ways. Residual plots were visually inspected for deviations from homoscedasticity and normality. To check for spatial auto-correlation in model residuals, we used the *ape* library (v. 3.0–11), (Paradis *et al.* 2004) to calculate Moran's I coefficients.

RESULTS

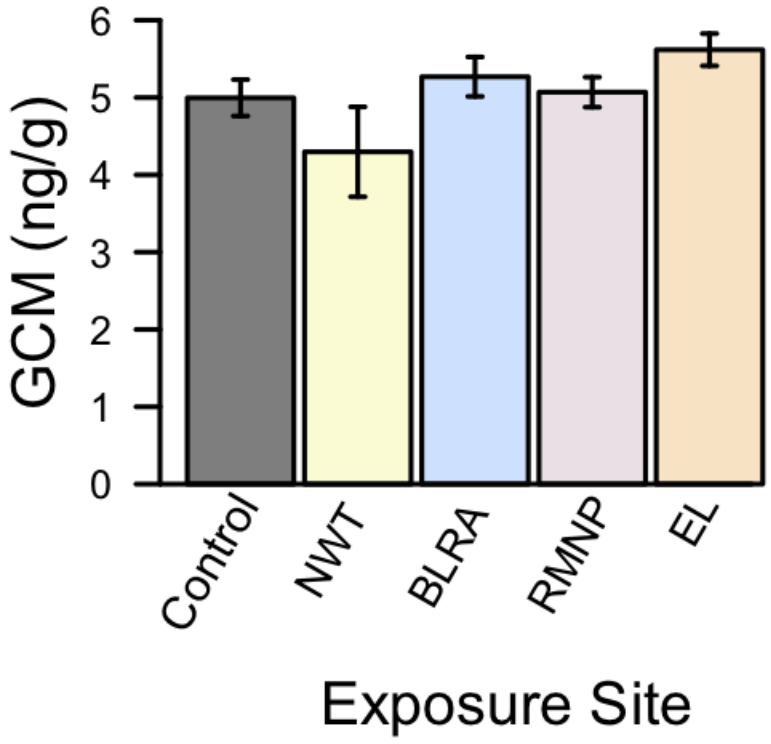
Exposure trials

GCM concentration did not differ significantly, either between control and exposure samples or between samples exposed at different sites $F_{(4,10)} = 0.73$, p-value = 0.591 (Figure 1).

Temperature comparison between GLVW and NWT

The average minimum distance between samples and data loggers was 33.23 meters (SD = 25.10) at NWT and 233.41 meters (SD = 193.31) at GLVW. This difference reflects the smaller size of the NWT study area and higher density of *in situ* temperature data loggers available at

Figure 1. Measured GCM concentration after environmental exposure at sites in the Rocky Mountains. GCM concentration is expressed in nanograms/gram. Site abbreviations are as follows: Niwot Ridge LTER (NWT), Brainard Lake Recreation Area (BLRA), Rocky Mountain National Park (RMNP), Emerald Lake (EL).



that site (placed for other studies). Although GLVW encompassed a larger area and sensor density was lower at that site, physiographic variables (such as elevation) varied little between sampling locations within GLVW. Thermal summaries revealed sub-surface temperature differences overall between GLVW (RIFs present) and NWT (RIFs absent). For temperature variables related to heat stress, AST (average summer temperature) was lower at GLVW than at NWT during 2012 and similar during 2013 (Figure 2A). Additionally, SUMDTR (average summer diurnal temperature range) was also lower at GLVW than at NWT during both years (Figure 2B). For temperature variables related to cold stress, DB-10 (number of days below negative 10°C) was lower at GLVW than at NWT during both seasons (Figure 3).

GCM comparison

An F test did reveal a significant difference in the variances between GLVW and NWT ($F(33,29) = 2.95$, p-value < 0.000). GCM concentration was significantly lower in samples from GLVW than at NWT (Figure 4; Welch's t-test; p-value < 0.001, df 54, N = 34 GLVW: 30 NWT). Average GCM concentration for samples collected during 2011-2013 was 4.27 ng/g from GLVW, 6.71 ng/g from NWT.

Mixed effects models

In the first analysis (GLVW alone), AIC indicated that the four best models included one or several of the following predictors: ELEV, AST, DB-10, SUMDTR (Table 3). AIC was lowest for model 3 (ELEV, AST, DB-10) but differed by <2 for the other three models. Two of the models, (ELEV, AST, DB-10) and (ELEV, DB-10, SUMDTR), included an interaction term (ELEV:DB-10). All other interaction terms were not significant, and were omitted from the models.

Figure 2. Thermal summaries of temperature variables related to heat stress. Values obtained from sub-surface sensors placed at Green Lakes Valley Watershed (GLVW) and Niwot Ridge LTER (NWT) during 2011-2013. Boxes depict medians and 25% and 75% quartiles. Whiskers extend through the 95% interquartile range. AST (Average Summer Temperature; Figure A) corresponds to average temperature during June-September; SUMDTR (Summer Diurnal Temperature Range; Figure B) corresponds to average diurnal temperature range June-September.

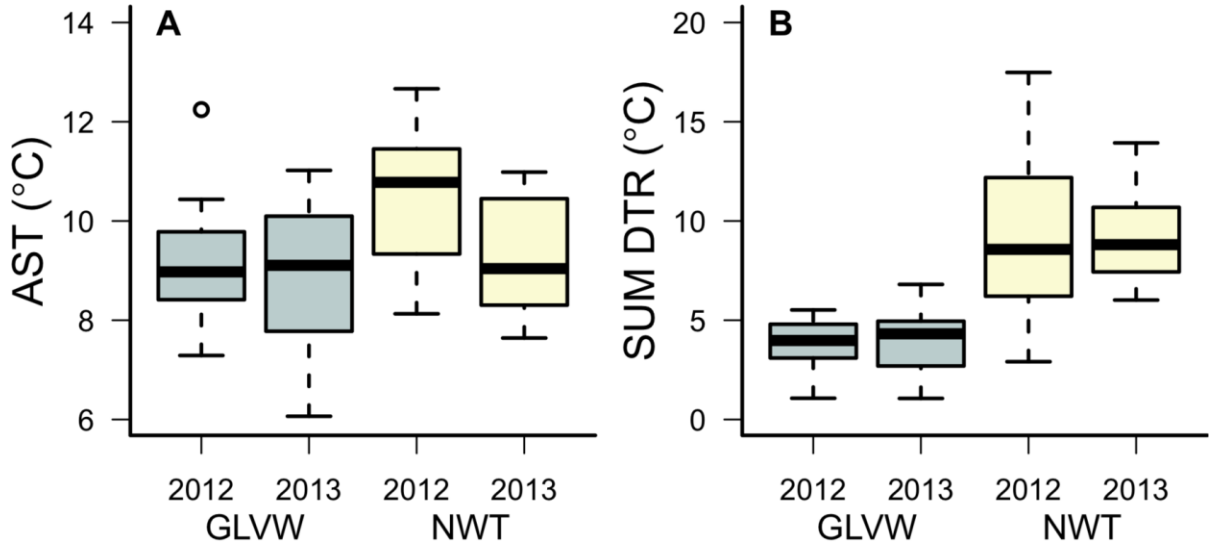


Figure 3. Thermal summaries of a temperature variable related to cold stress. Values (DB-10; days below -10°C) obtained from sub-surface sensors placed at Green Lakes Valley Watershed (GLVW) and Niwot Ridge LTER (NWT) during 2011-2013. Boxes depict medians and 25% and 75% quartiles. Whiskers extend through the 95% interquartile range.

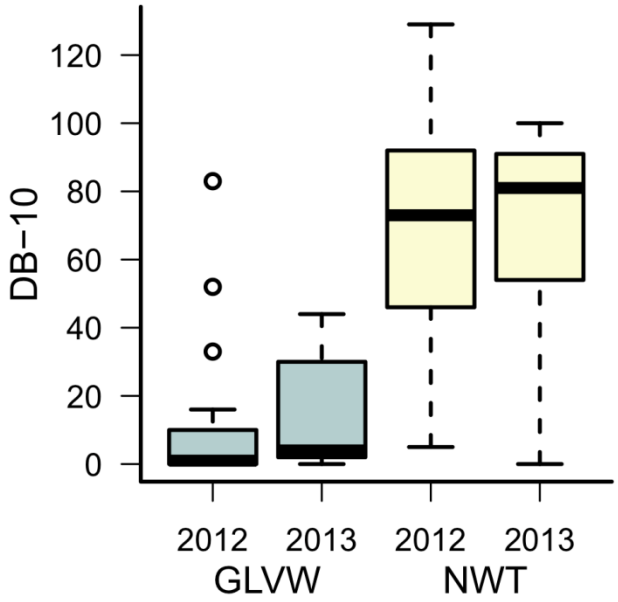


Figure 4. Measured GCM concentration at sites with and without sub-surface ice. Fecal samples were collected during 2011-2013 from a site with RIFs (Green Lakes Valley Watershed, GLVW) and a site without RIFs (Niwot Ridge LTER, NWT). Sample size = 34 GLVW: 30 NWT. GCM concentration is expressed in nanograms/gram.

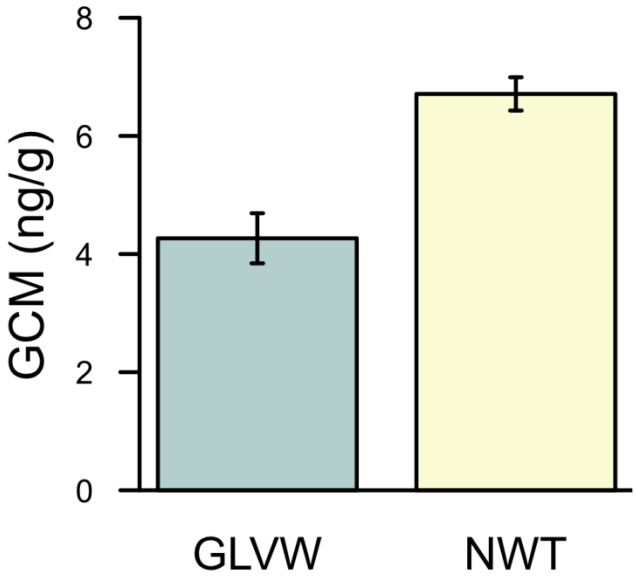


Table 3. Relative support for models of pika stress (GCM concentration) in the Green Lakes Valley Watershed. Models are ranked in order of increasing AIC values (Akaike's information criterion). L denotes likelihood. Delta AIC is the difference between the indicated model and the best model (the model with lowest AIC). Models with Delta AIC > 4 are not shown. For each model, the marginal R^2 represents the amount of variance explained by the fixed effects, while the conditional R^2 represents the amount of variance explained by the fixed and random effects in combination.

Number	**Model**	**AIC**	**Δ AIC**	**Log(L)**	**Marginal R^2**	**Conditional R^2**
3	ELEV, AST, DB10	156.80	-----	-71.40	0.24	0.40
7	AST	157.99	1.19	-74.99	0.09	0.28
5	ELEV, DB10, SUMDTR	158.05	1.25	-72.03	0.21	0.42
8	AST, DB10	158.67	1.87	-74.33	0.12	0.29
2	ELEV, AST	159.08	2.28	-74.54	0.12	0.29
Null	Null	159.92	3.12	-76.96	-----	-----
10	AST, PSG	159.96	3.16	-74.98	0.09	0.28
9	AST, DB10, PSG	160.39	3.59	-74.19	0.13	0.31
1	ELEV	160.55	3.75	-76.27	0.03	0.23
11	DB10	160.78	3.98	-76.39	0.03	0.22

In the second analysis (NWT alone), AST and DB-10 again appeared in the two models with highest support (Table 4). No interaction of AST and DB-10 was supported. The highest marginal and conditional R^2 values were found in models 21 and 23.

In analysis 3 (including data from both sites), the best models were based on AST and ELEV (Table 5). No interaction of AST and ELEV was supported. DB-10, PSG and SUMDTER appeared in models with weak support but highest marginal and conditional R^2 values.

Across all three analyses ELEV was negatively correlated with GCM concentration, while AST, SUMDTR and DB-10 were all positively correlated with GCM concentration. Moran's I statistic indicated no spatial autocorrelation in residuals from the best models of data from GLVW (p-value = 0.441), NWT (p-value = 0.962) or both sites combined (p-value = 0.313). Akaike weights indicated that AST was the strongest predictor, followed by DB10 (Table 6).

DISCUSSION

Although fecal stress hormone metabolites are commonly used to assess animal health and fitness, few studies account for the influence of environmental exposure on GCM measurements of samples collected non-invasively (i.e., after deposition). We found that exposure of samples to microclimatic conditions relevant to pikas across our study region did not alter GCM concentration in fecal samples. Major factors known to increase or decrease GCM concentration include fluctuations in temperature and precipitation conditions (Khan *et al.* 2002; Terio 2002; Millspaugh & Washburn 2004; Palme 2005). In our study, all samples were exposed during the same month (August), and microclimatic conditions during this time may not have varied considerably across our study sites. Importantly, this result suggests that non-invasive sample collection can be used to reliably compare relative stress hormone metabolite concentration in

Table 4. Relative support for models of pika stress (GCM concentration) at the Niwot Ridge LTER site (NWT). Models are ranked in order of increasing AIC values (Akaike's information criterion). L denotes likelihood. Delta AIC is the difference between the indicated model and the best model (the model with lowest AIC). Except for the null model, models with Delta AIC > 4 are not shown. For each model, the marginal R^2 represents the amount of variance explained by the fixed effects, while the conditional R^2 represents the amount of variance explained by the fixed and random effects in combination.

Number	**Model**	**AIC**	**ΔAIC**	**Log (L)**	**Marginal R^2**	**Conditional R^2**
23	DB10	101.45	-----	-46.72	0.35	0.35
21	AST, DB10	102.06	0.61	-46.03	0.38	0.38
22	AST, PSG	104.23	2.78	-46.12	0.38	0.38
Null	Null	111.99	10.54	-53.00	-----	-----

Table 5. Relative support for models of pika stress (GCM concentration) across both study sites (Green Lakes Valley Watershed and Niwot Ridge LTER). Models are ranked in order of increasing AIC values (Akaike's information criterion). L denotes likelihood. Delta AIC is the difference between the indicated model and the best model (the model with lowest AIC). Models with Delta AIC > 4 are not shown. For each model, the marginal R^2 represents the amount of variance explained by the fixed effects, while the conditional R^2 represents the amount of variance explained by the fixed and random effects in combination.

Number	**Model**	**AIC**	**ΔAIC**	**Log (L)**	**Marginal R^2**	**Conditional R^2**
29	AST	275.91	-----	-132.95	0.08	0.33
27	ELEV, AST	277.26	1.35	-132.63	0.09	0.34
30	DB10	278.26	2.35	-134.13	0.08	0.58
Null	Null	279.28	3.37	-135.64	-----	-----
33	PSG, SUMDTR	279.41	3.50	-132.70	0.18	0.33

Table 6. Relative support for predictors of pika stress calculated across all possible linear models based on 1-5 of the 5 predictor variables and excluding interactive effects. The generalized Akaike weight of each predictor indicates the strength of evidence for that particular predictor.

Predictor	Akaike weight
AST	0.85
DB10	0.69
ELEV	0.37
PSG	0.37
SUMDTR	0.26

pika scat across this region within a short sampling period. However, we caution that this result can be applied only to samples collected within a single eco-region during the same season. The effect of environment on GCM concentration in exposed fecal samples requires further investigation within and between more climatically variable regions, before we can conclude that environmental factors do not measurably influence GCM concentration.

Having established that GCM concentrations were not affected by exposure within our region and sampling period, we then compared physiological stress in pikas (as indicated by fecal GCM concentration) and found that stress was significantly predicted by climate variables at two sites. Specifically, GCM concentration was significantly higher in areas with higher summer temperatures (AST, SUMDTR) and a higher frequency of extremely cold days in winter (DB-10; Figures 2,3; Tables 3-5), which are most likely due to recent reductions in snow cover (Mote *et al.* 2005). These results are consistent with other studies of pika population occupancy and persistence (Beever *et al.* 2003; Beever *et al.* 2010; Rodhouse *et al.* 2010; Beever *et al.* 2011; Erb *et al.* 2011; Wilkening *et al.* 2011; Jeffress *et al.* 2013), adding strong support for the hypothesis that recent pika population declines are due to individuals being physiologically impacted by heat and/or cold stress. Although the relationship between individual fitness and physiological stress in pikas is still under investigation, analyses have identified negative correlations between fecal GCM concentration and annual survival in other species (Romero & Wikelski 2001; Rogovin *et al.* 2003; Brown *et al.* 2005). Thus, it is likely that the detection of high GCM concentration can be used to identify populations in the very early stages of decline. By establishing a link between specific climatic variables and physiological stress in pikas, our results also provide further evidence in support of pikas as an indicator species of climatic change.

Additionally, our results support the importance of RIFs in maintaining suitable microclimates for pikas. Overall GCM concentration was significantly lower in pikas living in areas with RIFs (i.e., GLVW) than those without RIFs (NWT, Figure 4). Other studies have previously indicated that the presence of RIFs appears to improve the suitability of pika habitat. For example, in an assessment of 421 pika sites in mountainous regions of the southwestern USA, 83% of pika-occurrence sites occurred in RIF landforms (Millar & Westfall 2010), suggesting that individuals preferentially select these areas or fail to persist outside of these areas. Similar to other studies (Millar & Westfall 2010), our data suggest that microclimates associated with RIFs are more moderate across the year (i.e., cooler in the summer and warmer in the winter; Figure 2,3). We recognize the limitations of this study, as our results derive from only two locations within one watershed. This study provides a basis for replication in additional watersheds.

In addition to microclimate, RIFs may also mediate pika habitat suitability through vegetation quantity and quality. Specifically, dense wetland vegetation often occurs adjacent to RIF areas, and plant diversity is higher in these areas than in non-wetland alpine habitats (Millar & Westfall 2010). Water availability is also higher near RIFs; thus, vegetation growing near RIFs could have higher water content, a feature that pikas select for when foraging (Smith & Erb 2013). Metrics of forb cover and similar vegetation related characteristics have already been shown to predict pika population persistence (Rodhouse *et al.* 2010; Wilkening *et al.* 2011. These RIF-associated meadows could therefore potentially have higher forage value for pikas.

Although pikas are generalist herbivores, they tend to consume plant species according to relative abundance and nutritional content (Huntly *et al.* 1986; Dearing 1996, 1997). Variation in GCM concentration has been explained by differences in diet in some species, since dietary fiber

may affect fecal mass and gut passage time which could influence final GCM concentration (MacDonald *et al.* 1983; von Der Ohe & Serveen 2002). Specifically, in herbivores, the level of fiber in the diet directly affects fecal bulk, and can therefore dilute GCM concentration in fecal samples (Goymann 2005). For example, in baboons (*Papio cynocephalus)*, an increase in dietary fiber led to a decrease in excreted progesterone metabolites (Wasser *et al.* 1993), and adding fiber to food decreased testosterone and corticosterone (GCM) in feces from European stonechats (Goymann 2005). It is possible that differences in GCM concentration could result from differences in forage between RIF and non-RIF habitats. However, our research related to a multi-regional assessment of fecal GCM revealed no significant influence of vegetation type on GCM concentration measured in pika feces (Wilkening *et al., in review*). Although there were considerable differences in regional vegetation communities across our sample collection area in this study, vegetation factors were not predictive when explaining GCM concentration (Wilkening *et al.,in review*). However, the influence of diet on GCM concentration in pikas requires further investigation, and future studies should incorporate vegetation surveys that relate GCM concentration to plant fiber content or dominant vegetation type. The adjacent sites used in this study (GLVW and NWT) appeared to support similar vegetation communities, and we did not quantify vegetation at sampling sites.

Other factors known to influence GCM concentration include predation rates and population density. Predator abundance has been shown to positively correlate with GCM measurements, and the GC response to predators appears to be fairly similar across species. Individuals can experience chronic stress in response to sustained high levels of predation (Boonstra *et al.* 1998; Scheuerlein *et al.* 2001; Clinchy *et al.* 2004), and also rely on an elevated acute stress response as a means of escape (Mateo 2007). Pikas are preyed upon by weasels

(Smith & Weston 1990) and it is possible that weasel abundance could differ between sites with RIFs (GLVW) and those without (NWT). Although we did not directly measure pika population density, this variable appeared to be higher at sites without RIFs (NWT), most likely as a result of the higher density of "edge" habitats (talus-meadow interface) preferred by pikas; taluses in GLVW are larger and pikas do not occupy the center of such large patches (Erb *et al.* 2014). High population densities increase antagonistic interactions and competition, and could therefore lead to an increase in individual stress (Christian 1980). Additionally, annual survival at NWT is relatively low, and GLVW could be serving as a source population for recolonization events occurring every year at NWT. Therefore, resident individuals in GLVW could be experiencing lower levels of stress than immigrant individuals at NWT. All of these factors could contribute to higher GCM concentration in samples collected from NWT, and future studies should incorporate measurements estimating predator abundance, population density, and individual status.

Most efforts to identify the effects of climate change on pikas and other species have relied on the establishment of relationships between climatic characteristics and species distribution. Many species expand or contract their ranges in response to changing temperatures, and significant global bioindicators have been developed based upon past and current observations. For example, the presence of palms has traditionally been used in the paleo-botanical literature to identify warmer climates (Mai 1995). Due to shorter and warmer winters, palms have recently spread northward as far as southern Switzerland, and have been identified as a bioindicator of current climate change in Europe (Walther *et al*. 2007). Similarly, a rapid increase in vascular plant abundance in response to warmer temperatures has been interpreted as a bioindicator of climate change in Antarctica (Smith 1994). Bioindicators can also identify

shifting precipitation patterns, another impact of climate change. For example, the larvae of some invertebrates, such as *Diptera,* decline in abundance with decreased levels of soil moisture (Briones *et al.* 1997; Convey *et al.* 2003), and the absence of these organisms has been used to indicate climate change.

These examples rely upon distributional shifts at the population level to indicate the extent of climate change. In contrast, studies identifying indicators of change based upon physiological response of individual organisms are far less common, though they could be extremely valuable as earlier and more sensitive indicators of change. These studies are becoming more feasible as the relationship between physiological stress and climatic characteristics is documented in more species. For example, higher GCM concentrations have been measured in several species in response to reduced precipitation (e.g. Koala bears; Davies *et al.* 2013, African elephants; Foley *et al.* 2001, and spider monkeys; Rangel-Negrin *et al.* 2009). Similarly, temperature can significantly influence physiological stress levels, with most examples documenting increased GCM concentrations in response to higher temperatures (Foley *et al.* 2001; Davies *et al.* 2013).

Taken together, these studies support the utility of using the physiological stress response of organisms such as the pika as bioindicators of climate change. Given the relative longevity, mobility and trophic position of most mammals, their physical condition represents a relatively complex set of inputs integrated over space and time. These features make the mammalian stress response an attractive bioindicator, because a less integrative bioindicator would not be effective for estimating the condition of a system as complex and spatially variable as a watershed. Monitoring pikas as an indicator of watershed condition therefore provides information on attributes of change that cannot be discerned by monitoring other abiotic factors. However, our

results are derived from only one watershed. Our techniques can serve as a template for further examination of the role of RIFs in reducing pika stress.

Alpine and other high elevation watershed areas are excellent regions to develop climate change bioindicators because they are relatively isolated (and therefore less susceptible to other anthropogenic disturbances) and because changes in climate are expected be more pronounced in these areas (Diaz & Bradley 1997; Hughes 2000; Naftz *et al.* 2002; Beniston 2003). Pikas are highly detectable, diurnal and charismatic, making them ideal candidates for monitoring as a bioindicator. The novel, practical application of small-mammal physiology presented here complements other efforts to model and monitor landscape change in high elevation areas. Watershed monitoring programs, such as Watershed Watch, have been started across the western US where many cities depend on water resources originating in mountain watersheds. In order to mitigate impacts of shifting temperature and precipitation regimes, it is important to be able to predict how and where changes may occur. Our results offer a sensitive early-warning system for identifying subtle changes in water resource distribution, an important consideration in the face of continued climate change.

Chapter 3

PARKS, PIKAS AND PHYSIOLOGICAL STRESS: IMPLICATIONS FOR LONG TERM MONITORING OF AN NPS CLIMATE SENSITIVE SENTINEL SPECIES

ABSTRACT

American pikas (*Ochotona princeps)* are widely considered a sentinel species for detecting ecological effects of climate change. Pikas are declining within a large portion of their range, and the National Park Service (NPS) recently initiated a research program (Pikas in Peril) designed to assess pika vulnerability to predicted changes in climate. As part of the Pikas in Peril (PIP) program, fresh fecal samples were collected from eight western national parks. We measured physiological stress (glucocorticoid metabolites, GCM) in pikas using these samples collected non-invasively. We also tested fecal sample storage techniques, thus validating storage protocols established by NPS surveyors. Our results display considerable variation in baseline values of GCM concentration for pikas between different parks, and these baseline values can be used to identify changes in physiological stress levels for this climate-sensitive species within each park. This research contributes to the understanding of climate change effects on this charismatic mammal, and provides park managers with baseline information that can be incorporated into long term monitoring studies.

INTRODUCTION

American pikas (*Ochotona princeps*) are charismatic inhabitants of mountainous and rocky areas in many national parks throughout the western United States (Figure 1). These cold adapted members of the rabbit family, commonly known as rock rabbits, are sensitive to warmer temperatures and rely on access to cooler microclimates located underneath rocky (e.g. talus)

Figure 1. An American pika (*Ochotona princeps*) in Rocky Mountain National Park. Photo by Dick Orleans.

habitats (Hafner 1993). As a result of recent climate-mediated population declines and extirpations, pikas have become widely considered a sentinel species for detecting ecological effects of climate change (Beever *et al*. 2011). Projections suggest that populations will continue to decline (Ray *et al*. 2012), and the species has been considered for listing as threatened or endangered at the state and federal level. There has been a growing interest in documenting changes in pika distribution; in response, the National Park Service (NPS) recently funded a research program (Pikas in Peril) designed to assess vulnerability of pikas in parks to future climate scenarios.

The Pikas in Peril (PIP) program is a collaborative research effort among multiple university researchers and park staff from eight western national parks. The main goals of the program are to document pika occurrence patterns and distribution, measure gene flow and connectivity of populations, and predict impacts of future change on pika populations (Garett *et al.* 2011). Occupancy surveys were conducted in each of eight western parks in both 2010 and 2011. Surveyor crews recorded evidence of pika presence or absence at dozens of plots within each park each year, and also evaluated pika habitat and collected fresh fecal pellets for DNA analysis. Pikas are ideal candidates for occupancy surveys, because their presence is easy to detect. Data generated from these studies are being used to relate pika occupancy to habitat characteristics (Jeffress *et al*. 2013), which will allow park managers to identify prime habitat and predict future distribution under climate change scenarios (Garett *et al*. 2011). However, the PIP study focuses on patterns of pika presence and absence that may be confounded by transient processes and source-sink dynamics. For example, pikas may experience temporary extirpation in suitable habitats due to demographic or environmental stochasticity (Morrison & Hik 2007). Stochastic events can have strong effects on small populations such as are typical for a territorial

animal like the pika which inhabits relatively small patches of habitat. Pikas may also occur temporarily following colonization of an unsuitable habitat patch. Source-sink dynamics may create a more pervasive bias in occupancy data, obscuring effects of habitat quality on species presence-absence if the species occurs in poor habitats through overflow from good ones. To supplement presence-absence data, and to predict trends in occupancy that may not yet be apparent, it is useful to have data on the physiological response of pikas to their local habitats.

One method for identifying potential stressors directly affecting individuals is to measure physiological stress. Analyses of stress hormone metabolites found in fecal samples are increasingly used to evaluate stress in sensitive populations of mammal and birds. In response to a stressor, animals release glucocorticoid hormones (such as cortisol or corticosterone, respectively) into the bloodstream. These hormones circulating in the body are metabolized by the liver, and then excreted as metabolites into the gut. Glucocorticoid metabolites (GCMs) can be detected in the excrement of birds and mammals (Mostle & Palme 2002). Fecal samples can be collected relatively easily and non-invasively, important considerations for a species in decline such as pikas. There are caveats associated with non-invasive sample collection, though, such as the potential influence of environmental factors on GCM concentration. Environmental conditions such as temperature or humidity can alter microbial activity, which can result in increased decomposition of steroid metabolites and biased measurements of metabolite concentration (Millspaugh & Washburn 2004). Therefore it can be difficult to accurately compare GCM concentration measured in samples collected from across the western United States, since environmental conditions can vary considerably in different regions. Similarly, sample storage techniques have been found to alter GCM concentration in fecal samples (Khan *et al.* 2002), and must be tested in order to allow for reliable interpretation of results.

Here we present a practical application of recently developed techniques for non-invasively measuring stress in pikas, using fresh fecal samples that were collected as part of the PIP program. In this study, our first objective was to examine the effects of storage methods on stress hormone metabolite concentration (glucocorticoid metabolite; GCM) in pika fecal pellets. By comparing GCM concentration measured in control samples to those that were placed in storage envelopes for varying amounts of time, we tested whether storage methods directly alter GCM concentrations measured in samples. Our second objective was to non-invasively measure physiological stress (GCM concentration) in pikas living in national parks throughout the western United States. We present baseline values of GCM concentration for pikas within eight national parks, which can be used to identify changes in physiological stress levels for this climate sensitive species. Finally, we provide additional information regarding how our established techniques can contribute to long term monitoring and management programs related to pikas and other NPS key vital signs.

METHODS

Testing effects of sample storage (objective 1): To obtain fecal samples, pikas at the Niwot Ridge Long Term Ecological Research Site (LTER) in Colorado were live trapped during summer 2012. Fresh fecal pellets were collected only from adult female pikas (to control for observed differences in GCM concentration resulting from gender and age), and individuals were later released back into their home territories. Trapping and sampling procedures were reviewed and authorized by Colorado Parks and Wildlife (license no. TR2014) and procedures followed those approved by the University of Colorado-Boulder Institutional Animal Care and Use Committee (protocol 1104.06). Fecal samples were kept on ice in the field and transferred within 12 h to a −20°C freezer. Pellets collected from 6 different individuals were then pooled and

divided at random into 14 samples: 2 controls and 12 samples slated for experiment. Each sample consisted of approximately 9 pellets placed inside a small paper envelope (coin envelope). Envelopes were kept at room temperature in a dry location, and storage methods mimicked those already in use by National Park Service staff and trained project volunteers (Jeffress & Garrett 2011). Typical storage length varied from 1 -12 months; thus each envelope was stored for a different number of months (up to 1 year), and envelopes were numbered according to the length of storage time (for example: envelope 1 = 1 month, 2 = 2 months, etc.). After each specified time period, samples were removed from envelopes and placed in storage vials in a -20°C degree freezer. Control samples were maintained in a -20°C freezer during the entire period and GCM analysis was performed on all samples at the same time.

GCM extraction proceeded according to protocols we previously validated for pikas (Wilkening *et al.* 2013). Comparative analysis of GCM concentration in samples was conducted using a commercially available Corticosterone Enzyme Immunoassay Kit (Arbor Assay Design, Inc., Ann Arbor, MI; catalogue no. K014-H1). Final concentrations of fecal GCM were expressed as ng GCM/g dry feces. One-way ANOVA was used to test for differences in GCM concentration among control samples and experimental samples (those stored in envelopes). Prior to analysis, data were checked for outliers, normal probability plots were examined, and a Shapiro-Wilk statistic was calculated to test for normality. All statistical analyses were conducted using R 3.0.1 (R Core Team 2013) and significance was assessed at the α=0.05 level.

Comparing pika stress hormone levels among parks (objective 2): Fresh scat samples were collected from pika-occupied habitats in each of eight National Park Service units included in the PIP research program: Crater Lake National Park (CRLA) in Oregon, Craters of the Moon National Monument and Preserve (CRMO) in Idaho, Great Sand Dunes National Park and

Preserve (GRSA) and Rocky Mountain National Park (ROMO) in Colorado, Grand Teton National Park (GRTE) in Wyoming, Lava Beds National Monument (LABE) and Lassen Volcanic National Park (LAVO) in California, and Yellowstone National Park (YELL) located in portions of Montana, Idaho, and Wyoming (Figure 2). Habitats within these parks represented a broad range of those preferred by American pikas, including high elevation talus slopes and boulder fields and lower elevation lava flows underlain by ice features.

Samples were collected either within or enroute to sites randomly selected for pika occupancy surveys during 2010 and 2011. Collection protocols followed those established by Jeffress and Garrett (2011), and fresh fecal pellets were collected only from currently occupied pika territories. Pikas maintain established latrines within territories, thus fecal pellets can be identified as fresh by color, consistency and relative positioning (Figure 3). Surveyors within all parks attended group trainings that described collection and storage protocols, and were also given detailed field manuals for reference. Fecal pellets were transferred into coin envelopes using twigs or similar devices to avoid human contamination. Envelopes were labeled with the collection date, time, and location (GPS coordinates) and sealed with tape or stickers. To ensure that each sample represented a unique individual, we did not analyze samples that were collected within 50 meters of each other. Care was taken to prevent pellets from being crushed and collection envelopes were kept in a cardboard box or similar receptacle. Samples (N = 88) were kept at room temperature in a dry environment and later mailed to the University of Colorado in Boulder, where extraction and analysis procedures followed those detailed above.

RESULTS

In our test of sample storage methods (Objective 1), GCM concentration did not differ significantly either between controls (stored at -20C) and treatments (stored at room temperature

Figure 2. National parks in the western United States with pika populations, and the eight national parks being studied as part of the Pikas in Peril (PIP) program. Map created by Meghan Lonneker and Gordon Discus with the Upper Columbia Basin Network.

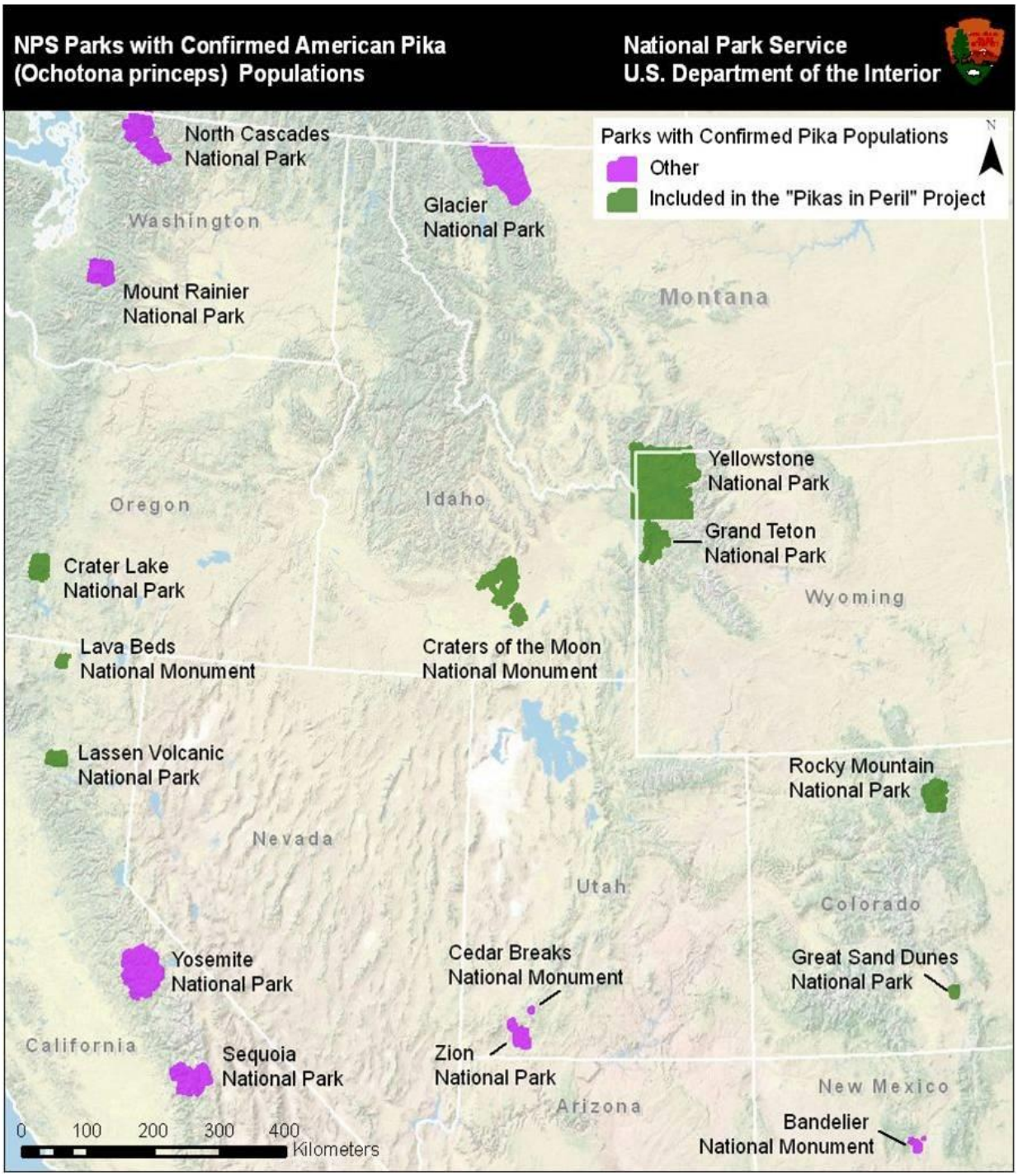


Figure 3. Pika scat stuck to a rock with urine that can be identified as fresh (deposited within the last 8 days) based upon its position. Photo by Johanna Varner.

for 1-12 months), or between samples placed in envelopes for different amounts of time $F_{(12,15)}$ = 1.84, p-value = 0.13 (Figure 4). These results indicate that sample storage methods did not bias GCM concentration, thus validating storage protocols used by NPS surveyors. In our comparison of samples among parks (objective 2), we found considerable variation in GCM concentration (Figure 5). Mean GCM concentration was lowest at ROMO (7.13 ng/g) and highest at LABE (19.3 ng/g). There was also relatively less variation in GCM measurements at LABE, with most values being in the higher range (17.4-21.2 ng/g, Table 1). In contrast, the range of GCM values recorded at several other parks was large, such as CRMO (7.7-28.8 ng/g) and ROMO (2.8-21.2 ng/g, Table 1).

DISCUSSION AND MANAGEMENT IMPLICATIONS

Having established that GCM concentrations were not influenced by sample storage techniques, we propose several hypotheses that could explain variation in GCM concentration observed both within and between different parks. First, the range of GCM values measured within each park includes samples collected from adult male and female pikas. GCM concentration has been shown to vary considerably between the sexes, with measurements in samples collected from males being twice as high (Wilkening *et al.* 2013). Second, temperature and precipitation regimes are not the same across different parks, and these factors can influence GCM concentration in samples collected non-invasively. For example, Table 1 displays the large range of precipitation and elevation values measured in each park. Elevation is often considered a proxy variable for temperature, with lower elevation areas correlating with higher temperatures. Average GCM concentration is highest in parks with lower elevation habitat (CRMO and LABE; Table 1), indicating that high temperatures may be increasing GCM concentration in exposed samples, or pikas subject to higher temperatures may be experiencing higher stress. Finally, diet

Figure 4. Mean GCM concentration measured in control samples (blue) and samples placed in storage envelopes for differing time periods (grey).GCM concentration is expressed in nanograms/gram. Sample letter C corresponds to control samples; sample number corresponds to the number of months samples were placed in storage envelopes.

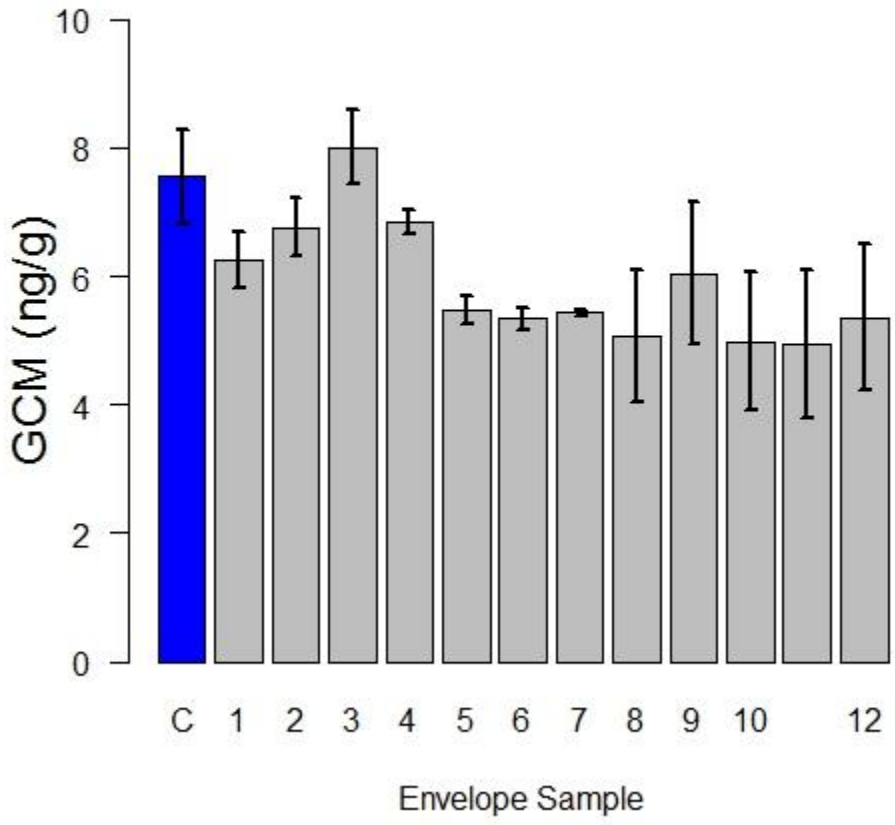


Figure 5. GCM concentration measured in samples collected from national park service units (see Table 1 for park abbreviations) from 2010-2011. Sample size per park ranges from 6-12. Boxes depict medians and 25% and 75% quartiles. Whiskers extend through the 95% interquartile range. GCM concentration is expressed in nanograms/gram.

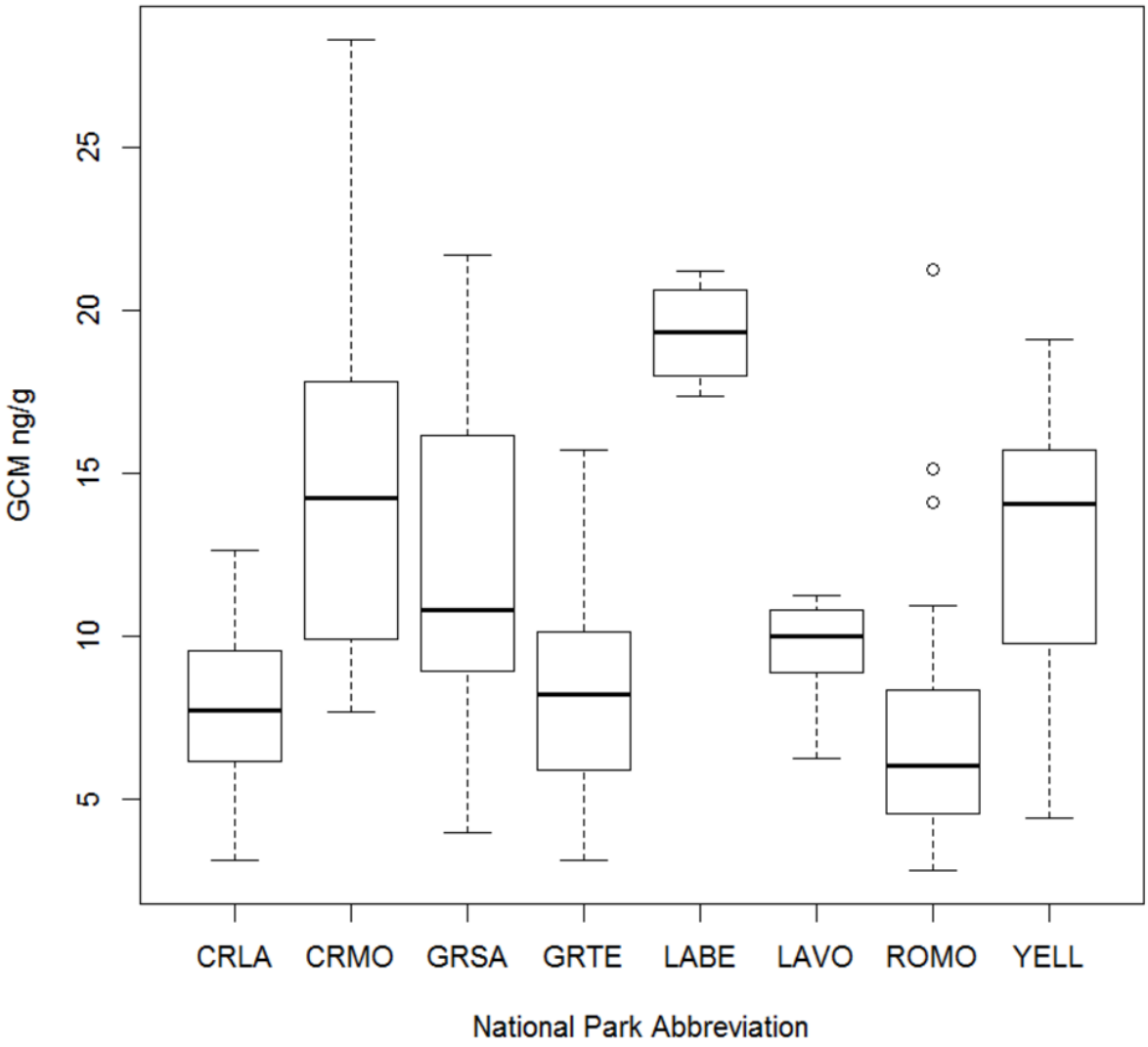


Table 1. Table 1. Comparison of stress hormone concentration and environmental variables at national parks. Means are given, followed by range of values measured from each park. Annual precipitation data were obtained from the PRISM website (www.prism.oregonstate.edu), which provides grid based estimates at 800 meter resolution for years 1971-2000 using parameter-elevation regressions on independent slope models.

Park	Abbreviation	GCM (ng/g)	Elevation (m)*	Precipitation (cm)*
Crater Lake National Park	CRLA	7.9 (3.2-12.6)	2115 (1701-2530)	156.7 (104.5-183.1)
Craters of the Moon National Monument and Preserve	CRMO	15.0 (7.7-28.28)	1700 (1511-1833)	32.7 (23.1-37.4)
Great Sand Dunes National Park and Preserve	GRSA	12.1 (4.0-21.7)	3427 (2647-3987)	86.2 (38.9-104.1)
Grand Teton National Park	GRTE	8.4 (3.1-15.7)	2815 (2090-3635)	171.9 (95.9-235.5)
Lava Beds National Monument	LABE	19.3 (17.4-21.2)	1463 (1249-1717)	43.3 (31.2-58.0)
Lassen Volcanic National Park	LAVO	9.5 (6.3-11.3)	2282 (1731-3089)	218.0 (85.0-308.4)
Rocky Mountain National Park	ROMO	7.13 (2.8-21.2)	3462 (2572-3795)	98.4 (45.2-130.8)
Yellowstone National Park	YELL	12.7 (4.4-19.1)	2424 (1651-3088)	94.5 (33.2-140.4)

*Data from Jeffress *et al*. 2013

can alter GCM concentration, since hormones pass through the digestive system (Touma & Palme 2005). Pikas are generalist herbivores and feed primarily on locally abundant vegetation, which varies by region and between parks. Additional analyses investigating the influence of habitat characteristics specific to each park may help to explain the variation we observed.

This is the first study to present multi-regional values of a physiological stress metric measured in pikas, an important indicator species occurring within a large number of western parks. Additionally, the spatial extent of our research is unique, since there are no other studies measuring physiological stress within a species across such a large and varied geographic area. Samples collected non-invasively should represent baseline values of GCM concentration in each park. Previous research has shown that exposure to environmental conditions does not influence GCM concentration, if samples are collected within a single eco-region during the same season (Wilkening *et al*.; *in review*). Pika fecal samples are typically collected during the summer months (due to weather constraints), and eco-regions do not vary within parks. Thus, park managers can use baseline values given here to document any changes in this stress metric over time measured within a particular park. Results from our study add a vital physiological component to methods of estimating population vulnerability, helping to improve our understanding of the potential for effects of climate change on this species.

Chapter 4

WHEN CAN WE MEASURE STRESS NON-INVASIVELY? POST-DEPOSITION EFFECTS ON A FECAL STRESS METRIC CONFOUND A MULTI-REGIONAL ASSESSMENT OF PHYSIOLOGICAL STRESS

ABSTRACT

The measurement of glucocorticoid stress hormone metabolites in fecal samples has become a common method to assess physiological stress in wildlife populations. Glucocorticoid metabolite (GCM) measurements can be collected relatively easily without disturbing the animal, and studies relating this stress metric to anthropogenic disturbance are increasing. However, post-deposition environmental conditions can alter GCM concentration measured in fecal samples. This confounding effect can make it difficult to separate environmental factors causing physiological stress in an individual pre-deposition, from those acting on a fecal sample post-deposition. We used fecal samples from American pikas (*Ochotona princeps*) to examine the influence of environmental conditions on GCM concentration by 1) Comparing GCM concentration measured in freshly collected control samples to those placed in natural habitats for timed exposure, and 2) Relating local environmental characteristics (climate data) to measured GCM concentration in samples collected non-invasively throughout the Western United States. We also explored whether local habitat and/or climate could explain GCM concentration in pikas after accounting for post-deposition effects. Our *in situ* exposure trials revealed that post-deposition exposure to environmental factors in different eco-regions influences GCM concentration in pika feces. We also found significant effects of maximum temperature, minimum temperature, and precipitation during the month of sample collection on GCM concentrations in pika feces collected in currently occupied habitats across the species'

range. Metrics of physiological stress in pikas measured during the pre-deposition period failed to explain residual variance in fecal GCM concentration after accounting for post-deposition effects on our samples. Together, these results indicate that we were unable to detect a signal of climate induced physiological stress in pikas, and that non-invasive measurement of physiological stress in pikas across the Western US may be confounded by climatic conditions in the post-deposition environment. Our results emphasize the importance of considering environmental influences on this stress metric in multi-regional comparisons; however, comparisons of measured GCM concentration within an eco-region or climatic envelope may prove useful for population monitoring.

INTRODUCTION

As human populations continue to increase, wildlife species around the world will be faced with additional declines in habitat quality and resource availability. Much of the current wildlife conservation research focuses on identifying impacts of anthropogenic habitat loss, fragmentation and degradation on species health and persistence. Habitat can be defined as the set of environmental conditions (e.g. temperature, precipitation) and resources (e.g. shelter, food) that determine the presence, survival and reproduction of a population (Block & Brennan 1993; Hall *et al.* 1997). Environmental conditions within natural systems can fluctuate widely, and all organisms have evolved a series of strategies to survive changes resulting from seasonal variation or stochastic events (Levins 1968). Physiological stress can occur as a result of environmental change (Munck *et al.*1984; Moberg 2000; Hik *et al.* 2001) among other factors, and an evolved stress response is often what enables animals to successfully respond to these expected or infrequent events (Romero 2002). However, the effects of increased physiological

stress resulting from prolonged environmental change associated with anthropogenic disturbance are still unknown for many species.

One of the most widely used methods is the measurement of glucocorticoid (GC) stress hormones and their metabolites in physiological samples such as blood, feces, or urine. In response to a physical or psychological stressor, vertebrates release glucocorticoid (cortisol or corticosterone) hormones into the blood stream. This release of glucocorticoids (GCs) in response to short term stress permits rapid energy mobilization and behavioral change, which can result in improved survival and fitness. However, continued release of GCs resulting from persistent chronic stress can lead to neuronal cell death, hyperglycemia, muscle and bone atrophy, hypertension, immunosuppression and reduced reproduction, which ultimately decrease survival and fitness (Riley 1981; Munck *et al.* 1984; McEwen & Sapolsky 1995; von Holst 1998; Wingfield & Sapolsky 2003; Engelmann *et al.* 2004; Romero 2004; Boonstra 2005; Korte *et al.* 2005). Additionally, behavioral responses to stressors (such as hyper-vigilance or avoidance behaviors) are energy intensive and distract an individual from normal behaviors (e.g., feeding or reproduction) necessary to sustain health and fitness.

Interest in the effects of anthropogenic disturbance on the endocrine stress response has grown, and recent studies have focused on how habitat fragmentation (Rangel-Negrin *et al.* 2009), degradation (Rizo-Aguilar *et al*. 2014; Balestri *et al*. 2014) and proximity to urban environments (Fokidis *et al*. 2011; French *et al.* 2008; Zhang *et al*. 2011) influence physiological stress levels in wildlife species. Similarly, researchers are establishing relationships between GCs and habitat characteristics, and the quantification of physiological stress in some species has been used as a bio-indicator of habitat quality. For example, pollution has been linked to elevated GCs in southern toads (Hopkins *et al.* 1997) and Galapagos marine iguanas (Wikelski *et*

al. 2001), and differences in forest habitat structure resulting from past logging activity and proximity to roads has been correlated with higher GC concentration in Northern Spotted Owls (Wasser *et al*. 1997). This use of a physiological stress measure as a bio-monitor has the potential to improve our ability to predict which properties of habitat quality will have the greatest impact on populations.

GCs circulating in the body are typically metabolized by the liver, and then excreted into the gut as metabolites (Taylor 1971; Palme *et al*. 1996; Mostl & Palme 2002). Although some portion of the hormone can be broken down by microbes or reabsorbed into the blood stream while in the gut, the skeletal structure of the hormone is not degraded (Taylor 1971; MacDonald *et al*. 1983). Consequently, stress hormone metabolites, such as glucocorticoid metabolites (GCMs), can be detected in the feces of birds and mammals. This non-invasive method of assessing stress in wildlife populations is advantageous in several ways. First, fecal samples can be collected relatively easily without disturbing or endangering the animal, which facilitates repeated sampling over time (Millspaugh & Washburn 2004; Mostl & Palme 2002). Second, fecal samples collected non-invasively are not biased by capture-induced stress, and may provide a more precise assessment of the endocrine condition of an animal (Harper & Austad 2000; Millspaugh *et al*. 2001; Touma & Palme 2005). Finally, GCM measurements typically reflect an average level of circulating glucocorticoids over time, and these measurements may be less affected by the pulsatile nature of hormone secretion (Palme *et al.* 1996, 2005; Harper & Austad 2000). Therefore, GCM concentrations may represent the average stress level of an animal more accurately than a plasma or similar sample taken at a single point in time (Touma & Palme 2005).

One major caveat associated with fecal GCM measurement is that environmental characteristics can alter GCM concentration in exposed feces. For example, fluctuating temperature and humidity levels can influence bacterial enzymes within the feces that decompose steroid metabolites, thus increasing or decreasing the relative concentration of GCM measured in samples collected from different environments (Kahn *et al*. 2002; Terio 2002; Millspaugh & Washburn 2004; Palme 2005). Thus, effects of the environment on exposed samples can be difficult to separate from effects of the environment on the animal—effects that increase physiological stress. Diet can also bias GCM measurements, since excretion rates of stress hormone metabolites can be affected by diet composition (Wasser *et al*. 1993; von der Ohe & Serveen 2002). This is particularly true for herbivores and omnivores, where variability in the consumption of plant fiber may alter GCM concentration.

Here we present results from the first multi-regional assessment of physiological stress in an indicator species, the American pika (*Ochotona princeps*). Pikas are a mammalian habitat specialist related to rabbits, which occur only in mountainous or rocky areas throughout the western United States. Due to their narrow thermal tolerance and recent population declines, they have been identified as an indicator species for identifying effects of anthropogenic climate change (McDonald & Brown 1992; Hafner 1993, 1994; Lawlor 1998; Beever *et al.* 2003; Smith *et al.* 2004; Grayson 2005).

Our first objective was to investigate the influence of environmental conditions on GCM concentration in fecal samples. Fecal samples collected fresh from several pikas were pooled and split at random into treatment and control samples. Treatment samples were placed outside for timed exposure, at multiple sites across the Western United States. By comparing GCM concentration measured in exposed fecal samples to control samples (which were kept in cold

storage), we determined whether exposure to varying environmental conditions altered GCM concentration in pika feces.

Our second objective was to compare GCM concentration measured in samples collected non-invasively from pikas across the Western US to evaluate factors that might predict GCM concentration. Habitat and climatic characteristics varied considerably between collection areas, and the total study area spanned 1300 km in both latitude and longitude, representing much of the species range. Using samples collected from a broad array of currently occupied sites, we analyzed relationships between GCM concentration and local climatic and vegetation characteristics. Our goal was to determine whether local habitat and/or climate correlated with physiological stress levels (GCM) in pikas after accounting for any post-deposition effects. We accounted for post-deposition effects by regressing GCM concentration on climate during the month of sample collection and then explored whether residual variation was related to pre-deposition metrics of climate and habitat characteristics (e.g., vegetation) experienced by each sampled individual. Our results suggest strong post-deposition effects of the environment that outweigh any pre-deposition effects on physiological stress in this species, with important implications for the use and interpretation of non-invasive stress measurement in wildlife populations.

METHODS

Pikas produce two types of feces: cecal feces, which are commonly re-ingested, and fecal pellets, which are not re-ingested (Conner 1983). Fecal pellets are commonly observed within pika territories, and pellet age can be determined by observation (Nichols 2010). In the current study, we focused on fecal pellets as a convenient means for non-invasive sampling.

Influence of environment on GCM concentration

Fecal samples were collected from pikas at two study sites in the Rocky Mountains: the Niwot Ridge Long Term Ecological Research Site and the Brainard Lake Recreation Area, Boulder County, Colorado, USA. Pikas were live trapped, sampled under light anesthesia (inhalant anesthetic, Isoflurane) during a 20-minute handling process, and released at point of capture. Trapping and sampling procedures were reviewed and authorized by Colorado Parks and Wildlife (license no. TR2014) and procedures followed those approved by the University of Colorado-Boulder Institutional Animal Care and Use Committee (protocol 1104.06).

A fecal sample consisting of approximately 20-40 pellets deposited naturally during the handling procedure was collected from each of 11 pikas. Only adult females were sampled, to control for known effects of age and sex on GCM concentration in pikas (Wilkening *et al.* 2013). Pellets were kept on ice in the field, and transferred within 12 h to a -20°C freezer. All pellets were pooled and divided at random into 3 controls plus 24 samples slated for experimental exposure. Each "exposure sample" consisted of approximately10 pellets inside a lidded, plastic, food-storage container (9 cm × 9 cm × 12 cm) modified for aeration by mesh panel inserts (6 cm x 6 cm) on all six sides.

Timed exposures were conducted in triplicate at each of 8 sites in 2 eco-regions. Exposure sites in the Rocky Mountains were Emerald Lake (EL) in Montana and 3 sites in Colorado: Niwot Ridge Long Term Ecological Research Site (NWT), Brainard Lake Recreation Area (BRLA), and Rocky Mountain National Park (RMNP). Sites in the Oregon Cascades were Laurence Lake (LL) on Mount Hood and 3 sites in the Columbia River Gorge: Wyeth (WY), Herman Creek (HC), and Mosier Pass (MP; Table 1). All sites contained numerous patches of typical pika habitat, characterized by large regions of broken rock (talus) interspersed with areas of vegetation. Sites represented a broad range of the habitats associated with pikas, from basalt

Table 1. Sites where pika fecal samples were exposed to test effects of environment on GCM concentration. Mean latitude and longitude refer to the average location in decimal degrees within each site where exposure boxes were placed. Elevation range gives the range of elevations of locations within each site where samples were exposed.

Eco-region	Site Name	Site Abbrev.	Mean Latitude	Mean Longitude	Elevation range (m)
Rocky Mountains	Niwot Ridge LTER	NWT	40.06	105.60	3587-3625
	Brainard Lake Recreation Area	BLRA	40.07	105.59	3300-3326
	Rocky Mountain National Park	RMNP	40.40	105.67	3246-3313
	Emerald Lake	EL	45.41	110.93	2748-2846
Oregon Cascades	Laurance Lake	LL	45.43	121.67	928-933
	Wyeth	WY	45.69	121.80	160-172
	Herman Creek	HC	45.67	121.84	250-266
	Mosier Pass	MP	45.68	121.41	60-62

lava flows located in river valleys to granite talus slopes above tree line (Appendix III). Ranging 60-3625 meters above sea level, sites also included a variety of microclimatic conditions and vegetative communities. Rocky Mountain sites in Colorado and Montana were dominated by alpine tundra flanked by forests of Lodgepole pine, sub-alpine fir and spruce trees. Oregon sites in the Columbia River Gorge were characterized by high relative humidity and annual rainfall, high moss cover, and a dense Douglas fir canopy. Those near Mount Hood were surrounded by subalpine forest dominated by western hemlock, western red cedar and Douglas Fir.

All sites except MP were occupied by pikas during this study. MP was approximately 25 km east of the distributional edge of pikas in the Columbia River Gorge (Simpson 2009). MP was similar to occupied regions of the Gorge, including WY and HC, in terms of average annual temperatures (ca. 10°C), but received considerably less annual precipitation (approximately 111 cm/year, vs 190 cm/year) over the past year. We included this unoccupied site specifically to investigate the effects of relative humidity and precipitation within this eco-region.

At occupied sites, exposure samples were positioned next to fresh pika feces. At the unoccupied site (MP), samples were positioned in the talus under boulders as in a naturally occurring pika latrine. Pellets were kept on ice until placed into exposure containers on-site. Containers were placed under rocks to shield pellets from direct sunlight and rain, mimicking the typical environment of a natural latrine. All exposures were conducted during August 2012. After two weeks *in situ*, each exposure sample was stored on ice and transferred to the lab for analysis. Control samples were stored in a -20°C freezer during the entire exposure period, and GCM analysis was performed on all samples at the same time.

Extraction and analysis of GCMs was conducted using protocols previously developed and validated for pikas (Wilkening *et al.* 2013). Samples were first lyophilized and ground into

powder using a mortar and pestle. The extraction process followed the steroid solid extraction protocol provided by Arbor Assay Design, Inc. Comparative analysis of GCM levels in samples was conducted using a commercially available Corticosterone Enzyme Immunoassay Kit (Arbor Assay Design, Inc., Ann Arbor, MI; cat. no. K014-H1). Extracted samples were run in triplicate alongside a standard curve of seven known concentrations of corticosterone (5000 - 78.125 pg/ml) during each assay. Values for each extracted sample were generated using a micro plate reader (BioTek Microplate Reader Synergy HT; 2005 Biotek Industries, Inc.) and Gen 5 1.11 Data Analysis software. Intra-assay coefficients of variation were less than 10% and inter-assay coefficients of variation were less than 15%. Final concentrations of fecal GCM were expressed as ng GCM/g dry feces.

Climate data with 800-meter resolution were obtained for each site from the PRISM climate group (www.prism.oregonstate.edu). From these data, maximum temperature, minimum temperature and precipitation were calculated for the exposure month (August 2012) at each site.

Prior to analysis, data were checked for outliers, normal probability plots were examined, and a Shapiro-Wilks statistic was calculated to test for normality. One-way ANOVA was used to test for differences in GCM concentration among control samples and those exposed at different sites. Mean GCM concentration by site was regressed on maximum temperature, minimum temperature and precipitation by site for the exposure month (August 2012). All statistical analyses were conducted using R 3.0.1 (R Core Team 2013) and significance was assessed at the α=0.05 level.

Multi-regional assessment of GCM concentration

Fresh fecal pellets (Nichols 2010; Jeffress *et al*. 2013) were collected from 114 sites during summer 2010-2012. Our collection area spanned almost the entire range of pika distribution in

the western United States, and included samples from Oregon, California, Idaho, Montana, Wyoming, Colorado and Utah (Appendix IV). Habitat and climatic characteristics differed considerably between collection areas, with sites ranging from high elevation talus slopes and boulder fields to lower elevation lava beds.

The majority of samples were collected from western national parks by crews trained in a single collection and storage protocol designed to minimize contamination and maximize collection of fresh samples from unique individuals (Jeffress & Garrett 2011). Previous experiments demonstrated no effect of storage on GCM concentration measured in samples collected using this protocol (Wilkening & Ray; *in review*). Extraction and analysis procedures followed those detailed above.

Given each sample's date and GPS location, PRISM climate data were summarized for 3 distinct periods to represent known pika stressors, including higher average summer temperatures, higher maximum summer temperatures, lower winter minimum temperatures, and lower amounts of winter precipitation (Beever *et al*. 2010, 2011; Erb *et al.* 2011; Wilkening *et al.* 2011; Jeffress *et al.* 2013). Fecal samples defined as "fresh" by the collection protocol were most likely deposited within the month prior to collection, so we first characterized the "post-deposition" period by calculating PRISM estimates of maximum temperature, minimum temperature, and precipitation during the month and year in which the sample was collected. We also calculated a second "summer stress" period as June to August of the year that the sample was collected. In this period, we calculated average temperature and average maximum temperature. Finally, we calculated a third "winter stress" period from October to May just prior to the summer in which the sample was collected. For this period, we calculated average minimum temperature and precipitation.

Vegetation data were collected at a sub-sample of randomly selected sites (n= 31) during peak flowering period following protocols in Jeffress and Garrett (2011). Each of these 31 sites included a 12 meter radius plot, which encompassed a fecal sample collection location. Cover within the plot was assigned into one of the six following categories: rock, bare ground, forb (flowering herbaceous plants), shrub (woody pants), grass (graminoids), and trees. Percent cover was classified using a modified Daubenmire scale (Daubenmire 1959), with values ranging from 0 (0%) - 7 (100%) (Jeffress *et al.* 2013). Cover classes were later transformed into mid-point percent values, for use in linear regressions. Only vegetation characteristics previously associated with pika dynamics were used as predictors, and these included forb cover (Wilkening *et al*. 2011), grass cover (Rodhouse *et al.* 2010), and the ratio of grass to forb cover (Erb *et al.* 2014). Because pikas are generalist herbivores, shrub cover was also included in order to test hypotheses suggesting that plant nutritional contents (e.g., fiber) may influence GCM concentration in fecal samples (Wasser *et al.* 1993).

We used linear regressions to assess environmental effects on GCM concentration, and also to explain variation in GCM concentration measured in samples from across the western US. For the "post-deposition" period, we regressed GCM concentration on maximum temperature, minimum temperature and precipitation during the month a sample was collected, in order to account for effects of environment on measured GCM after a sample was deposited. Model residuals from the best of these regressions were then regressed on additional predictors calculated using climate data from the "summer stress" and "winter stress" periods, to account for factors potentially affecting pika physiological stress before the sample was deposited. Models were compared using an information-theoretic framework (AIC). Predictor variables were examined for collinearity, and highly correlated (Spearman's $r > 0.5$) predictors were not

used in the same model. Interaction terms among predictors were omitted if not significant ($p > 0.10$). Residual plots were visually inspected for deviations from homoscedasticity and normality, and GCM concentration was log-transformed to correct for observed heteroscedasticity. All statistical analyses were conducted using R 3.0.1 (R Core Team 2013) and significance was assessed at the $\alpha=0.05$ level.

RESULTS

Influence of environment on GCM concentration

In our exposure experiment, GCM concentration varied significantly among control groups and samples exposed at different sites ($F(8,18) = 4.39$, $p < 0.001$; Figure 1). A post-hoc Tukey test showed significant differences in mean GCM concentration between the following samples: Mosier Pass-Control ($p = 0.03$), Mosier Pass-Niwot ($p = 0.00$), Mosier Pass-Rocky Mountain National Park ($p = 0.04$), and Wyeth-Niwot ($p = 0.03$). Mean GCM concentration in samples exposed at all other locations did not differ significantly, either between different sites or controls. Linear regression revealed significant relationships between GCM concentration and climate during the exposure month, including positive effects of maximum temperature ($R^2 = 0.54$, $F(1, 22) = 25.44$, $p < 0.001$) and minimum temperature ($R^2 = 0.40$, $F(1, 22) = 14.96$, $p <0.001$), and a negative effect of precipitation ($R^2 = 0.45$, $F(1, 22) = 17.84$, $p = 0.004$; Figure 2).

Multi-regional assessment of GCM concentration

Our multi-regional comparison revealed considerable variation in GCM concentration measured in samples from across the western US (Figure 3). Among climate predictors used to assess environmental effects on GCM concentration in the “post-deposition” period, monthly estimates

Figure 1. Mean GCM concentration and standard error measured in control samples and samples exposed at 8 sites across two ecoregions. Site abbreviation is defined in Table 1. Asterisks identify sites where GCM concentration differed significantly as indicated by a post-hoc Tukey test.

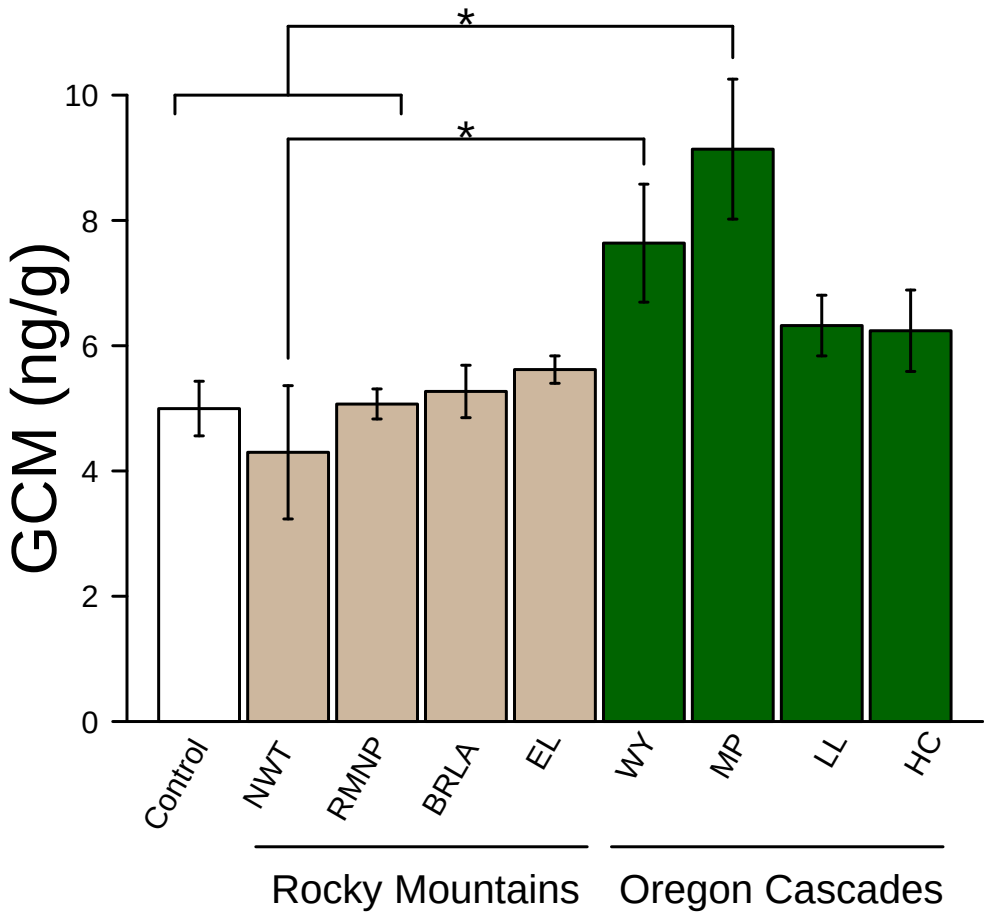


Figure 2. Mean and standard error of GCM concentration measured in samples exposed at different sites and (A) maximum and (B) minimum temperatures, and (C) precipitation during the month of exposure. Point color reflects ecoregion.

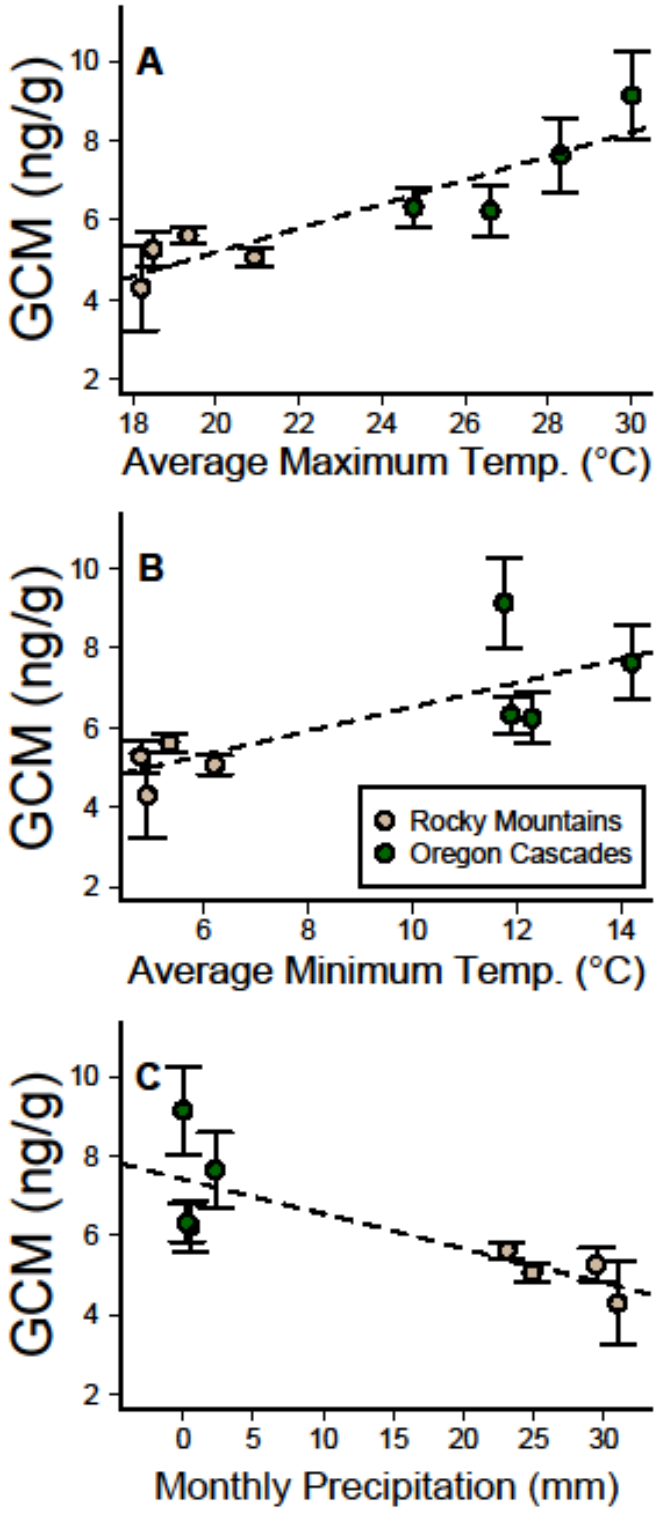
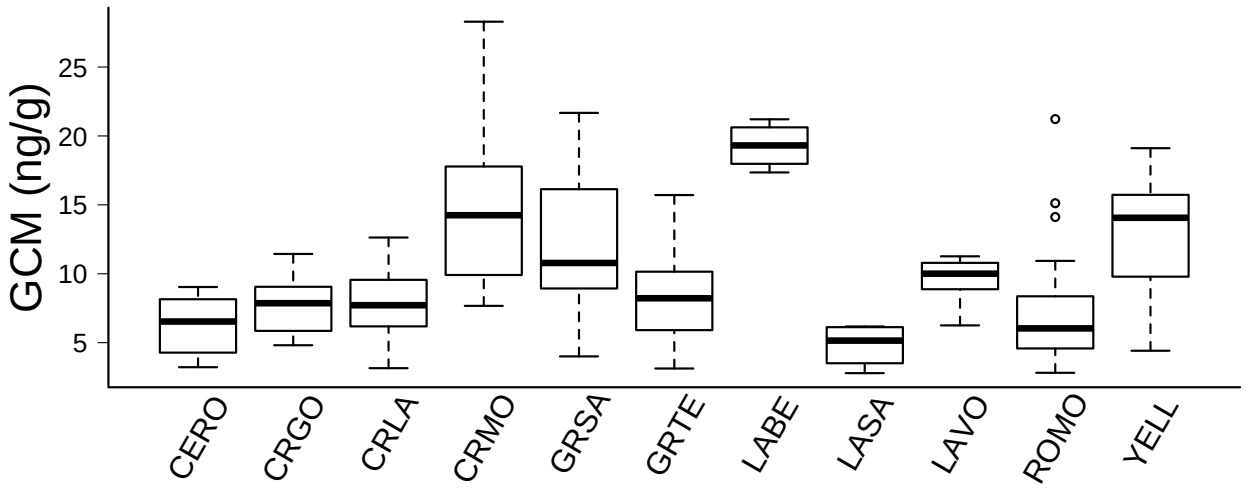


Figure 3. GCM concentration measured in samples collected from sites across the western US during 2010-2012. Boxes depict medians and 25% and 75% quartiles. Whiskers extend through the 95% interquartile range. Sites are grouped according to either national park or designated eco-region. Abbreviations (in alphabetical order) are as follows: CERO = Central Rockies, CRGO= Columbia River Gorge, CRLA = Crater Lake National Park, CRMO = Craters of the Moon National Monument and Preserve, GRSA = Great Sand Dunes National Park and Preserve, GRTE = Grand Teton National Park, LABE = Lava Beds National Monument, LASA = La Sal Mountain Range, LAVO = Lassen Volcanic National Park, ROMO = Rocky Mountain National Park, YELL = Yellowstone National Park.



of maximum and minimum temperature were highly correlated and did not appear in the same model. Interaction terms were not significant, and were removed from all models. Linear regressions revealed significant effects of maximum temperature ($R^2 = 0.14$, $F(1, 112) = 17.75$, $p < 0.001$), minimum temperature ($R^2 = 0.05$, $F(1, 112) = 6.11$, $p = 0.015$) and precipitation ($R^2 = 0.05$, $F(1, 112) = 5.88$, $p < 0.017$) during the month of sample collection on measured GCM concentration (Figure 4).

Maximum temperature and minimum temperature were positively related to GCM concentration, while precipitation was negatively related. Maximum temperature also appeared as a predictor in the two models with highest support (Table 2). Overall, maximum temperature alone was the best model that predicted post-deposition variation in GCM concentration, as determined by the lowest AIC score (Table 2).

To detect pre-deposition effects of climate on physiological stress in pikas, after accounting for post-deposition effects on GCM, we used the residuals from the best post-deposition model (MaxTemp in Table 2) as the response variable in further regressions. Among climate metrics representing stressors in the pre-depositional "summer stress" and "winter stress" periods, average summer temperature was correlated with maximum summer temperature and minimum winter temperature, so these predictors appeared only in separate models (Table 3). Overall, the null model was a better predictor of our pre-depositional physiological stress response variable than any of the climate predictors used in this model set. All hypothetical models had very low R^2 values and high AIC scores relative to the null model (Table 3). The null model was rivaled by a model with a significant positive effect of maximum summer temperature and a significant negative effect of minimum winter temperature, as predicted, but this model explained only 1% of the variance in the response variable (Table 3).

Figure 4. GCM concentration (log transformed) versus post-deposition climate metrics: (A) maximum and (B) minimum temperatures, and (C) precipitation during the month when samples were collected. Temperatures and precipitation were determined from monthly PRISM climate data.

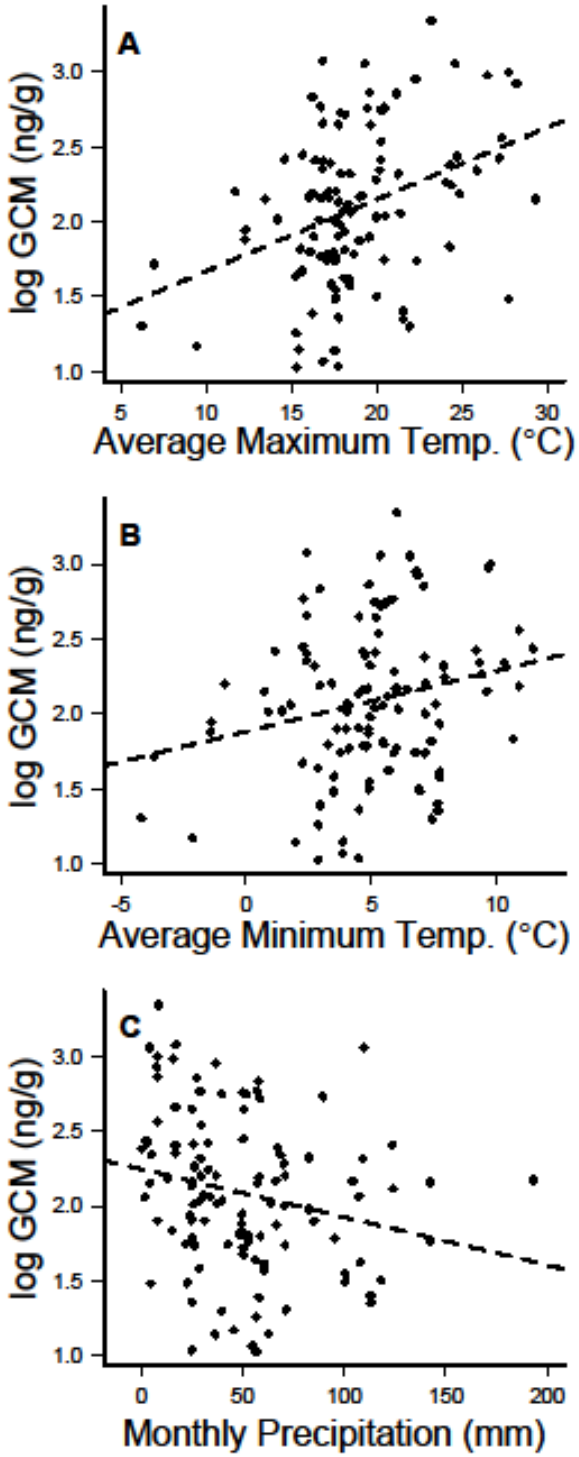


Table 2. Relative support for models of post-deposition environmental effects on fecal GCM concentration measured in samples collected non-invasively across multiple eco-regions. Model predictors include Precipitation (mm), MaximumTemperature (°C) and MinimumTemperature (°C) during the month in which each samples was collected. Models are ranked in order of increasing AIC values. Delta AIC is the difference between the indicated model and the best model (the model with lowest AIC). B, t, and p-values are given for model coefficient(s).

Model	**AIC**	**Δ AIC**	**Multiple R^2**	**Adjusted R^2**	**B**	**t value**	**p value**
MaxTemp	159.88	-----	0.14	0.13	0.05	4.21	<0.00
Precipitation, MaxTemp	160.32	0.44	0.15	0.13	-0.00, 0.04	-1.23, 3.59	0.22, 0.00
Precipitation, MinTemp	167.06	7.18	0.10	0.08	-0.00, 0.04	-2.35, 2.40	0.02, 0.02
MinTemp	170.60	10.72	0.05	0.04	0.04	2.47	0.02
Precipitation	170.81	10.93	0.05	0.04	-0.00	-2.43	0.02
Null	174.65	14.77	------	------	------	------	------

Table 3. Relative support for models of pre-deposition climate effects on physiological stress in pikas. The response variable consists of residual fecal GCM concentrations derived from the top model in Table 2 (i.e., accounting for post-deposition effects). Candidate models represent factors predictive of pika thermal stress in previous studies including mean summer temperature (MeanSummerTemp, °C), mean maximum summer temperature (MaxSummerTemp, °C), mean minimum winter temperature (MinWinterTemp, °C) and total winter precipitation (WinPrecip, mm) all measured during the year preceding deposition of the sample. Models are ranked in order of increasing AIC. Delta AIC is the difference between the indicated model and the best model (the model with lowest AIC). B, t, and p-values are given for model coefficient(s).

Model	**AIC**	**Δ AIC**	**Multiple R^2**	**Adjusted R^2**	**B**	**t value**	**p value**
Null	156.32	------	------	------	------	------	------
MaxSummerTemp, MinWinterTemp	157.94	1.62	0.04	0.01	0.07, -0.20	2.01, -1.99	0.04, 0.04
MaxSummerTemp	158.00	1.68	0.00	-0.01	0.01	0.56	0.58
MeanSummerTemp	158.06	1.74	0.00	-0.01	0.01	0.51	0.61
MinWinterTemp	158.31	1.99	0.00	-0.01	0.00	0.11	0.91
WinPrecip	158.32	2.00	<0.00	-0.01	<0.00	0.03	0.98
MinWinterTemp, WinPrecip	161.08	4.76	0.01	-0.02	0.03, -0.00	1.04, -0.87	0.30, 0.38
MeanSummerTemp, WinPrecip	161.13	4.81	0.01	-0.02	0.03, 0.00	1.07, 0.94	0.29, 0.35
MaxSummerTemp, WinPrecip	161.43	5.11	0.01	-0.02	0.02, 0.00	0.93, 0.74	0.35, 0.46
MaxSummerTemp, MinWinterTemp, WinPrecip	164.73	8.41	0.05	-0.02	<0.00, <0.00, <0.00	1.33, -1.07, 0.56	0.19, 0.29, 0.58

Similarly, we found no evidence that vegetation cover predicted pre-depositional physiological stress in pikas. Due to collinearity, the grass:forb ratio was not included in models with forb cover or grass cover. None of the vegetation related variables were significant predictors of our pre-depositional physiological stress response variable, and vegetation models did not explain any residual variance in GCM concentrations (Table 4). The null model had the lowest AIC score, and R^2 values for all models were low.

DISCUSSION

Our results clearly indicate that post-deposition exposure to environmental factors influences GCM concentration in pika feces, and that non-invasive measurement of physiological stress in pikas across the Western US may be confounded by the influence of localized environmental conditions. To our knowledge this is the first study to test for post-deposition effects on fecal GCM *in situ*, outside of a laboratory. Results from laboratory experiments have varied for other species, but we found that GCM concentration measured in pika fecal samples is sensitive to natural and ecologically relevant variation in temperature and precipitation (Figure 2). This result was corroborated by linear regression of measured GCM on climate during the month of sample collection in currently occupied pika habitats throughout the western United States, which also revealed significant post-deposition effects of temperature and precipitation (Table 1). Finally, metrics of physiological stress suggested by previous studies and measured prior to sample deposition failed to explain residual variance in fecal GCM concentration after accounting for post-deposition effects (Table 2). Together, these results indicate that we were not able to detect a signal of climate-induced physiological stress in pikas across the western US, given the confounding effect of localized climate on fecal samples after deposition.

Table 4. Relative support for models of pre-deposition effects of available forage on physiological stress in pikas. The response variable consists of residual fecal GCM concentrations derived from the top model in Table 2 (i.e., accounting for post-deposition effects). Candidate models represent factors predictive of pika dynamics in previous studies such as percent cover of forbs (ForbCover), graminoids (GrassCover), and shrubs (ShrubCover), as well as the ratio of graminoid to forb cover (GrassForbRatio). Models are ranked in order of increasing AIC values. Delta AIC is the difference between the indicated model and the best model (the model with lowest AIC). B, t, and p-values are given for model coefficient(s).

Model	**AIC**	**Δ AIC**	**Multiple R^2**	**Adjusted R^2**	**B**	**t value**	**p value**
Null	35.12	------	------	------	------	------	------
ForbCover	36.65	1.53	0.02	-0.02	-0.00	-0.67	0.51
GrassCover	36.74	1.62	0.01	-0.02	0.00	0.60	0.55
ShrubCover	37.11	1.99	0.00	-0.03	0.00	0.10	0.92
GrassForbRatio	37.12	2.00	<0.00	-0.03	<0.00	0.00	0.99
ForbCover, GrassCover	39.73	4.61	0.04	-0.06	<0.00, <0.00	-0.91, 0.32	0.37, 0.75
ForbCover, ShrubCover	39.95	4.83	0.04	-0.07	-0.01, -0.00	-0.97, -0.33	0.34, 0.75
ShrubCover, GrassForbRatio	41.11	5.99	0.00	-0.11	<0.00, <0.00	0.08, 0.01	0.94, 0.99
ForbCover, GrassCover, ShrubCover	46.91	11.79	0.07	-0.22	<0.00, <0.00, <0.00	-1.01, 0.18, -0.23	0.32, 0.86, 0.82

Our results suggest that environmental characteristics specific to pika habitat influence measured GCM concentration after deposition. Specifically, warmer temperatures appear to increase measured GCM during controlled exposures in pika habitat (Figure 2). This was consistent with the results of our multi-regional study, in which we detected a pattern of increased GCM concentration with increasing minimum and maximum temperature during the month of sample collection. This pattern has also been observed in manipulative laboratory experiments using fecal samples from other mammals. Experimental heating of fecal samples from white tailed deer (*Odocoileus virginianus*, Millspaugh *et al.* 2003) and cheetahs (*Acinonyx jubatus*, Terio *et al.* 2002) resulted in higher GCM concentration when compared to control samples. This pattern can most likely be explained by increased microbial activity in response to warmer temperatures, which decompose more of the steroid metabolite in the fecal sample (Terio *et al.* 2002; Millspaugh *et al.* 2003).

We also found that precipitation negatively influenced GCM concentration in our multiregional analysis, with lower GCMs measured in samples exposed to higher precipitation during the month of sample collection (Table 1). This result is also consistent with our exposure trials, in which the warmest and driest site (MP) had the highest measured GCM concentration (Figure 1).Other research related to the impacts of precipitation (or increased moisture) on GCM concentration has reported mixed effects. A study on white tailed deer (*Odocoileus virginianus*) feces found an increase in GCM concentration when samples were exposed to a simulated rainfall event. These increases were apparent even in samples exposed for short time periods, and the additional moisture probably stimulated growth of the microbes that break down steroids into metabolites (Washburn & Millspaugh 2002). However, another study related to environmental effects on GCMs in grizzly bear (*Ursus arctos horribilis*) and American black bear (*Ursus*

americanus) scat saw decreased GCM in response to precipitation (Stetz *et al.* 2013). This effect was attributed to steroid metabolite degradation in response to increased water on fecal samples, which could also account for our observed pattern.

While our results suggest consistent post-deposition environmental effects on GCMs across our broad study region, effects of local environmental conditions within the same eco-region were not as pronounced. Half of our exposure sites were in the Rocky Mountains (NWT, BLRA, RMNO, EL), and the remaining sites were located in the Oregon Cascades (MP, LL, WY, HC). When comparing exposure samples, GCM concentration differed significantly only between those samples exposed in different eco-regions, and many of these differences involved the one unoccupied site (MP; Figure 1). We included this site because it is similar in macroclimate to other low-elevation lava bed habitats that are occupied by pikas. However, microclimatic conditions in occupied, low-elevation talus (e.g., WY and HC) may experience significant discrepancies from the prevailing ambient conditions (Varner & Dearing 2014a). Thus, the microclimatic conditions that our exposure samples experienced at MP may not actually represent ecologically relevant exposure conditions for pika scat. Finally, among samples exposed to environmental conditions in the Rocky Mountains, there were no significant differences between groups, nor were there differences between any group and the controls (fresh samples collected directly from Rocky Mountain pikas and stored in the freezer). These results suggest that it may be possible to make valid comparisons among fresh samples collected within the same eco-region during the same season. Numerous recent studies have reliably correlated GCM concentration with habitat characteristics for a single population or several populations within the same region, where environmental variables are not likely to differ

considerably (Bauer *et al.* 2013; Balestri *et al.* 2014; Castilla-Navarro *et al.* 2014; Davies *et al.* 2014; Rizo-Aguilar *et al.* 2014).

Although there were considerable differences in regional vegetation communities across our sample collection area, vegetation factors were not predictive when explaining residual GCM concentration, after accounting for post-deposition effects (Table 3). Furthermore, the grass:forb cover ratio varied substantially across our sampling locations (from 0-30), yet this ratio did not predict residual GCM concentration in pikas. This result is surprising, given previous results suggesting that pikas thrive preferentially in locations with higher forb cover (Wilkening *et al.* 2011; Jeffress *et al.* 2013; Erb *et al.* 2014). Although pikas are generalist herbivores, they tend to consume plant species according to relative abundance and nutritional content (Huntly *et al.*1986; Dearing 1996, 1997; Smith & Erb 2014), and are increasingly selective when foraging in more extreme climates (Smith & Erb 2014). The use of broad cover classes might weaken statistical relationships, but strong effects of forb and grass cover on pika occupancy have been detected using similar data (Jeffress *et al*. 2013).

This fact that vegetation did not explain residual GCM concentration (after accounting for post-deposition effects) may be due to the fact that dietary fiber content does not differ considerably between the vegetation cover classes (i.e., food items) measured in this study (Varner & Dearing 2014b). Dietary fiber may affect fecal mass and gut passage time, or it may produce influential changes in gut microbial activities post-deposition that alter steroid metabolite structure (Goldin *et al*. 1982; MacDonald *et al.* 1983; von Der Ohe & Serveen 2002). Specifically, in herbivores, the level of fiber in the diet directly affects fecal bulk, and can therefore dilute GCM concentration in fecal samples (Goymann 2005). For example, in baboons (*Papio cynocephalus)*, an increase in dietary fiber led to a decrease in excreted progesterone

metabolites (Wasser *et al.* 1993), and adding fiber to food decreased testosterone and corticosterone (GCM) in feces from European stonechats (Goymann 2005). However, testosterone and corticosterone (GCM) concentration in fecal samples increased when the diet of red squirrels (*Tamiasciurus hudsonicus*) was supplemented with additional fiber (Dantzer *et al*. 2011). The effect of dietary fiber on GCM concentration requires further investigation.

Interestingly, pika diet in the Columbia River Gorge (i.e., WY and HC) is comprised largely of mosses (Varner & Dearing 2014b), which are extremely high in fiber. We did not measure moss cover in this study because most plots where scat samples were collected had no measurable moss cover. Surprisingly, GCM concentrations measured in samples non-invasively collected at WY and HC were relatively high, contrary to the prediction above that increased dietary fiber should decrease GCM concentration. This result may instead reflect microclimatic conditions, which are known to differ substantially from macroclimate at these two sites (Varner & Dearing 2014a). Another contributing factor may be our relatively small sample size, since vegetation surveys were conducted at only 31 fecal sampling locations; additional research is needed to understand the influence of diet on GCM concentration in pikas.

Although proximate mechanisms are not well-understood, variation in GCM concentration has been explained by diet type in some species. The capercaillie (*Tetrao urogallus*), a European bird species, excreted higher concentration of GCM when living in pine forests than when living in spruce forests and this may be due to diet differences between the two habitat types (Thiel *et al.* 2011). Similarly, for Alaskan brown bears (*Ursus arctos horribilis*), which can feed on a range of diets including pure grass, berries, or meat, a study examining multiple factors found the best predictor of fecal GCM concentration was diet type (von der Ohe *et al*. 2004). Additionally, a more recent study on bears found that higher quality diets (berries

and meat) were associated with lower GCMs, identifying a nutritional influence on GCM concentration measured in fecal samples (Stetz *et al.* 2013). However, as we have shown, multi-regional comparisons of GCM concentration between populations with access to different types or quality of food and different climatic conditions can be difficult to interpret, since changes in both dietary composition and climate may influence hormone metabolite concentration.

In conclusion, field studies related to non-invasive measurement of physiological stress to anthropogenic disturbance are increasing, and our results have several important implications. First, post-deposition environmental effects on exposed samples must be taken into consideration, especially for studies that have large geographical or seasonal scope. Second, comparisons within the same eco-region may be reliable, particularly if the sample size is large and there is knowledge of baseline values for regional populations.

Finally, our multi-regional comparison is an important step towards understanding how landscape scale patterns in climate and habitat might affect a species in decline across the western US. Previous pika studies have relied on the establishment of relationships between habitat characteristics and persistence or occupancy. However, the effects on populations start with individuals, and our use of a stress metric can be applied in future research to indicate habitat quality from the perspective of an individual. With further calibration to control for post-deposition effects, the baseline values that we measured for multiple eco-regions should prove useful for population monitoring and for identifying environmental stressors before populations begin to decline. The prevalence of habitat loss and degradation underscores the importance of integrating such physiological measures into wildlife population assessments.

Chapter 5

STRESS HORMONE CONCENTRATION PREDICTS SURVIVAL IN THE AMERICAN PIKA

ABSTRACT

The measurement of stress hormone levels is increasingly used to assess the health of wildlife populations. But for many species we do not have a good understanding regarding what range of increases in GC levels, and over what time period, is detrimental to an animal. An infrequent increase in stress hormone levels can allow animals to redirect behavior or activities, which can promote individual survival. However, chronic elevation of GC levels results in deleterious health effects that most likely reduce survival. Interpretations of varying GC levels can lead to opposite conclusions, and therefore it is necessary to have some understanding about how individual survival relates to GC concentration for a particular species. We related survival in American pikas (*Ochotona princeps*) to two different stress metrics, glucocorticoid metabolite (GCM) concentration in fecal samples and GC concentration in plasma samples. Annual survival was analyzed in relation to stress metrics, physiological metrics, and habitat characteristics at several sites in the Rocky Mountains. Our results indicated that GCM concentration was the strongest predictor of annual survival in pikas, suggesting that individuals with higher baseline GCM level may be less likely to survive. Given the limited time and resources that characterize many wildlife conservation projects, it is important to identify which endocrine metrics are the most informative for a species. These results contribute to a better understanding of key factors affecting survival in American pikas.

INTRODUCTION

The endocrine condition of an animal correlates to individual health, and the measurement of stress hormone (glucocorticoid) levels is often used to assess the health of a population (Wingfield *et al*. 1994; Creel *et al.* 1996; Wasser *et al.* 2000; Romero 2004). However, for most species, we do not know how high and how long glucocorticoid (GC) levels must rise to do harm. An increase in GC secretion typically enables an animal to redirect behavior, an adaptive response which can be interpreted as favorable for many individuals (Sapolsky *et al.* 2000). Thus, elevated GC levels do not always indicate distress (Moberg 2000; Romero 2004), and an evolved stress response is often what allows an individual to cope with environmental change or stochastic events. Some studies have suggested that elevation of GC levels provides adaptive advantages to the individual such as enhanced survival or reproductive opportunities (Boonstra *et al.* 2001; Cote *et al.* 2006). Others show that chronic elevation of GC levels results in deleterious effects that ultimately reduce survival, such as diminished resistance to disease, or impaired growth and development (Sapolsky *et al.* 2000; Romero & Wikelski 2001; Rogovin *et al.* 2003). Additionally, it is generally accepted that an increase in GC levels leads to a reduction in reproductive activities in favor of self-preservation behaviors, therefore promoting individual survival over current reproductive success (DeNardo & Licht 1993; Breuner & Hahn 2003; Lanctot *et al.* 2003; Lynn *et al.* 2003; Breuner *et al.* 2008). To better interpret the consequences and ultimate effects of varying GC levels, it is necessary to understand how individual demographic rates relate to GC concentration for a particular species.

Few studies have assessed whether GC levels can predict individual survival in mammals (Ebensperger *et al.* 2013). According to the Cort-Fitness Hypothesis, high baseline GC level should be negatively associated with metrics of relative fitness such as survival, due to the numerous adverse consequences previously mentioned. For example, a recent review (Bonier *et*

al. 2009) found that baseline GC concentration was negatively correlated with survival in approximately half of the studies involving diverse bird and animal species, such as marine iguanas, cliff swallows, and ring-tailed lemurs. However, approximately a quarter of the studies reported a positive correlation between baseline GC concentration and survival, as exhibited in side-blotched lizards, European starlings, and European wild rabbits; furthermore, in the remaining quarter of the studies, there appeared to be no relationship between survival and baseline GC level for several other species including European white storks and Yellow-legged gulls (Bonier *et al.* 2009). These and similar findings suggest that survival can be predicted by baseline GC level, but the relationship is not consistent across all species. Increased GC levels may be an indicator of a population in distress, but we must first understand the relationship between the magnitude of GC secretion and metrics of individual performance for a species.

Here we related survival in American pikas (*Ochotona princeps*) to two different stress metrics, glucocorticoid metabolite (GCM) concentration in fecal samples and GC concentration in plasma samples. Pikas, cold-adapted members of the rabbit family, occur only in rocky areas across the western United States. They rely on access to cool sub-surface microclimates to escape warmer temperatures, and are widely considered a sentinel species for detecting ecological effects of climate change (McDonald & Brown 1992; Hafner 1993, 1994; Lawlor 1998; Beever *et al.* 2003; Smith *et al.* 2004; Grayson 2005).

Survival can be influenced by a species response to acute or chronic stress, so the inclusion of both acute and chronic stress metrics should improve overall understanding of the stress response in pikas (Breuner *et al.* 2008; Bonier *et al.* 2009). In our study, blood samples were collected during a period of acute stress response after capture. Fecal samples were collected non-invasively to measure baseline GCM level indicative of a chronic stress response.

We also analyzed individual survival in relation to several other indicators of physiological condition (weight, ecto-parasite load) and habitat characteristics (elevation, slope aspect) previously found to affect pika population dynamics. We hypothesized that the probability of survival would be higher for individuals with lower GCM, under the assumption that these pikas were experiencing lower levels of chronic physiological stress. We also hypothesized that survival would be higher for pikas with higher GC levels, since this measurement represents the ability of an individual to react to a stressor. A failure to release GCs in response to a stressful event could indicate poor health or other factors leading to low survival (Busch & Hayward 2009). Finally, we hypothesized that survival would be higher for individuals with greater body mass and fewer ecto-parasites, and those living in habitats characterized by favorable microhabitat conditions.

METHODS

Study area and sample collection

Pikas (N = 156) were sampled at 4 sites in the Rocky Mountains: Emerald Lake (EL) and Swan Creek (SC) in Gallatin County, Montana, and Long Lake (LL), Mitchell Lake (ML) and the Niwot Ridge Long Term Ecological Research Site (NWT) in Boulder County, Colorado. Sampling occurred during summer 2009-2013 at each site except SC, which was only sampled in 2013, and EL which was not sampled in 2013 (due to forest fire). Pikas were live trapped and sampled under light anesthesia (inhalant anesthetic, Isoflurane) during a 20-minute handling process. Individuals were marked with unique, color coded ear tags and released at point of capture. Gender and age class (adult or juvenile) were determined, and physiological data for each individual were collected including weight, rectal body temperature, cover of mites on the outer ear (none, low, medium, high), and number of fleas observed. Additionally, the

microhabitat associated with each marked pika was characterized in terms of microclimatic variables (elevation, slope aspect, GPS location). The territory of each tagged pika and all adjacent territories were revisited annually through 2014, to determine survival through multiple re-sight attempts by multiple observers.

Blood and fecal samples were obtained at a range of times after initial capture as a result of our field protocol (traps were checked at intervals and trapped pikas were handled in the order they were discovered). The range of sampling times (20-180 minutes after initial capture) ensured that GC samples represented an acute stress response (sampling for baseline GC must occur within 3 minutes of capture; Touma & Palme 2005) and GCM samples represented baseline level (gut passage time is >3 hours in lagomorphs; Stott 2008). Blood was collected when possible via retro-orbital bleeding, and samples were separated into plasma on-site using a portable centrifuge. Fecal samples were collected when produced during handling or capture, resulting in the collection of both plasma and fecal samples from 62 individuals. Pikas produce two types of feces: ceacal feces which are commonly re-ingested, and fecal pellets, which are not re-ingested. Previous analyses identified significant differences in GCM concentration between the two types of feces (Wilkening, *unpublished data*). Only fecal pellets were included here because the majority of samples were of this type. Physiological samples were held on ice in the field and stored at minus 20º in the laboratory.

Sample analysis

Comparative analysis of GC and GCM concentration in samples was done using a Correlate-EIA Corticosterone Enzyme Immunoassay Kit (Arbor Assay Design, Inc., Ann Arbor, MI; cat. no. K014-H1). Extraction and analysis protocols followed those established by Arbor Assays, Inc., which were validated specifically for pikas by Wilkening *et al.* 2013. Prior to the start of the

plasma samples were first diluted with an equal volume of the supplied dissociation reagent, then diluted further with the supplied assay buffer. Fecal samples were lyophilized and ground into powder using a mortar and pestle. A portion of the total dried fecal material sample (0.100 g) was blended with 1 ml of 90% aqueous ethanol using a vortex shaker (Vortex Genie 2; Scientific Industries, Inc.). Samples were then centrifuged at 5000 rev/min for 15 minutes, and the resulting supernatant was drawn off and transferred to a clean tube for evaporation. The supernatant solution was evaporated to dryness in a vacuum centrifuge and extracts were stored at -20°C. Immediately prior to running the assay, extracts were reconstituted with a mixture of ethanol and assay buffer and were mixed completely using a vortex shaker.

During each assay, samples were run in triplicate alongside a standard curve of seven known concentrations of corticosterone (5000, 2500, 1250, 625, 312.50, 156.25, 78.125 pg/ml.) Values for each extracted sample were generated using a micro plate reader (BioTek Microplate Reader Synergy HT; 2005 Biotek Industries, Inc.) and Gen 5 1.11 Data Analysis software. Intra-assay coefficients of variation were less than 10% and inter-assay coefficients of variation were less than 15%. Final concentrations of plasma GC were expressed as pg/ml, and fecal GCM were expressed as pg/g.

Data Analysis

Juveniles were omitted from analyses, due to significant differences between juveniles and adults in both GC and GCM concentration (Wilkening *et al.* 2013). Survival rate (number of individuals that survived/total number of individuals marked) was calculated per year for each site, and only adult pikas were included in survival analyses. We used F-tests to assess the equality of variance between samples, and Welch's t-test to identify differences in either GC or GCM concentration between individuals that survived and those that did not. Prior to analysis,

data were checked for outliers, normal probability plots were examined, and a Shapiro-Wilks statistic was calculated to test for normality. Several predictors (GC, GCM, number of fleas observed) were log transformed to correct for observed heteroscedasticity. All statistical analyses were conducted using R 3.0.1 (R Core Team 2013) and significance was assessed at the $\alpha=0.05$ level.

Survival Models

For models of annual survival, candidate predictor variables represented physiological metrics shown to influence survival in other species (Arnold & Anja 1993; Brown *et al.* 1995; Vander Haegen 2013), and habitat characteristics identified in previous studies of the American pika (Beever *et al.* 2003; Erb *et al.* 2011; Jeffress *et al.* 2013). Physiological metrics included GC and GCM concentration, body weight, and ectoparasite abundance (number of fleas and cover of ear mites). Habitat characteristics included elevation classed as low, medium and high with respect to latitude- and longitude-adjusted values for pikas (Hafner 1993) and slope aspect (north or south facing) of an individual's territory. Trapping locations were pre-selected to represent these classes of elevation and aspect. Site (Emerald Lake, Niwot, Long Lake, Mitchell Lake, Swan Creek), year (2009-2013), sex (male or female), and state (sub-species)were also included as predictors, to account for any differences due to these factors. Predictors were abbreviated as:

GC = GC concentration

GCM = GCM concentration

Wt = body weight

Mite = ear mite coverage

Flea = number of fleas observed

Elev = elevation

Asp = slope aspect

Annual survival was used as the response variable in logistic regression models . Models were developed and compared using an information-theoretic framework. Relative support for each predictor variable was evaluated using 31 candidate models (Table 2) with various combinations of predictors. Highly correlated predictors (Spearman's r> 0.5) did not appear in the same model. Our data set included GC or GCM measurements for all individuals (N = 109), and both GC and GCM for a subset of individuals (N = 62). To retain the largest sample size and maximize the number of models considered, two different model sets were used to examine survival: "GC models" included all individuals sampled successfully for GC (N = 89), and "GCM models" included all individuals sampled successfully for GCM (N = 82).

The relative support for each model was calculated separately using an information criterion (AIC; Burnham & Anderson 2002). A lower AIC score indicated that the model was better supported by the data, and the best model had the lowest AIC score within the model set (GC or GCM). We considered models with an AIC score 0-2 units higher than the lowest score to have support similar to the best model, while models with a score 4 or more units higher had little support. Models were first analyzed with all interaction terms, and interaction terms were omitted if not significant (p-value > 0.10).

RESULTS

Survival Rate

Re-sight data from repeated observations indicated a high probability (>0.9) of detecting surviving pikas, similar to the high probability of detecting habitat patch occupancy (also >0.9) in other pika studies (Rodhouse *et al*. 2010; Jeffress *et al*. 2013). Annual survival rate among adult pikas sampled for GC or GCM was highest every year in Montana, and consistently lower

at sites in Colorado (NWT, LL, ML; Table 1). However, the difference between annual Montana and mean annual Colorado survival rates 2010-2013 was only marginally significant in a paired *t*-test ($t = 2.4801$, df = 3, p-value = 0.089). The lowest annual survival rate in 2010 and 2013 was at LL, and the lowest survival rate in 2011 and 2012 was at ML. In Montana, survival rate was highest in 2012 (EL) and lowest in 2009 (EL). Among Colorado sites, annual survival was lowest during 2010 at NWT and LL, and at ML it was lowest during 2012. Among Colorado sites, the highest rate of annual survival was recorded during 2011 at NWT and LL, and during 2013 at ML (Table 1).

Comparison between GC and GCM

Among individuals that survived and those that did not, an F test revealed a significant difference in the variances of GC ($F(33,54) = 0.30$, p-value < 0.001) and GCM ($F(32,48) = 11.32$, p-value < 0.001) concentration. GC concentration was significantly higher in males than in females (Welch's t-test; p-value <0.001, df 38), and additional tests examining survival and GC concentration were split by gender. Among males, mean GC concentration was higher in pikas that survived (735.62 pg/ml) than those that did not (551.24 pg/ml), although this difference was not significant (Welch's t-test; p-value = 0.311, df 33). Mean GC concentration was roughly the same among females that survived (373.57 pg/ml) and those that did not (379.58 pg/ml; Welch's t-test; p-value = 0.841, df 50). There was no difference in GCM concentration between males and females. Mean GCM concentration was significantly lower in individuals that survived (2167.57 pg/g) than in those that did not (5324.34 pg/g; Welch's t-test; p-value < 0.001, df 36).

Survival Models

Among predictor variables used in GC models, elevation was positively correlated with weight and number of fleas observed, and negatively correlated with ear mite cover. Ear mite coverage

Table 1. Annual survival rate, and sample size given in parantheses, estimated for adult pikas included in this study at four sites in the Rocky Mountains.

	Montana		Colorado		
Year	Emerald Lake (EL)	Swan Creek (SC)	Niwot (NWT)	Long Lake (LL)	Mitchell Lake (ML)
2009	0.67 (3)	NA	NA	NA	NA
2010	0.78 (9)	NA	0.40 (5)	0.00 (4)	0.40 (5)
2011	0.73 (11)	NA	0.63 (19)	0.86 (7)	0.50 (4)
2012	0.90 (3)	NA	0.50 (8)	0.67 (6)	0.14 (7)
2013	NA	0.71 (7)	0.60 (10)	0.50 (4)	0.70 (4)

Table 2. Candidate models. Predictors in each candidate model include GC concentration, GCM concentration, body weight (Wt), percentage of earmite coverage (Mite), number of fleas observed (Flea), elevation (Elev), slope aspect (Asp), site, year, sex, and state. No interaction terms were found to be significant, and are not included in models.

Model	Predictors
1	GC or GCM
2	Elev
3	Asp
4	Wt
5	Mite
6	Flea
7	Null
8	GC or GCM + Elev
9	GC or GCM + Asp
10	GC or GCM + Wt
11	GC or GCM + Mite
12	GC or GCM + Flea
13	GC or GCM + Sex
14	GC or GCM + Site
15	GC or GCM + Year
16	GC or GCM + Elev + Sex
17	GC or GCM + Asp + Sex
18	GC or GCM + Asp + Site
19	GC or GCM + Wt + Sex
20	GC or GCM + Wt + Sex
21	GC or GCM + Wt + Site
22	Wt + Site
23	GC or GCM + Mite + Sex
24	GC or GCM + Mite + Site
25	Mite + Site
26	GC or GCM + Flea + Sex
27	Flea + Sex
28	Flea + Site
29	Site
30	State
31	GC or GCM + State

was also negatively correlated with weight and number of fleas observed, while weight was positively correlated with number of fleas observed. The best model included weight and site as predictors, followed by two other models that included site alone and flea and site (Table 3). GC concentration appeared as a predictor in two equally supported models (AIC differed by <2 from the top model), along with aspect and site, and weight and site (Table 3).

Among predictor variables used in GCM models, elevation was positively correlated with number of fleas observed, and negatively correlated with ear mite coverage. Ear mite coverage was also negatively correlated with number of fleas observed. In this analysis, the best model included GCM and number of fleas observed as predictors, which were both negatively correlated with survival (Table 4; Figure 1). Sex was another predictor which appeared in the two models with similarly high support (AIC differed by <2 from the top model). GCM concentration appeared in all supported models in this set (AIC differed by < 4 from the top model; Table 4). State (sub-species) received less overall support as a predictor of survival.

DISCUSSION

In order to better understand wildlife population dynamics, we need information related to key factors affecting patterns and rates of individual survival. This is particularly imperative for a species in decline, such as the American pika, which has been considered for listing as threatened or endangered at the state and federal level. However, vulnerable wildlife species are often those most difficult to locate or capture, and collecting demographic data can be challenging. The numerous non-invasive or minimally invasive sampling techniques which have been recently developed to assess the endocrine condition of an animal are being used increasingly in wildlife conservation research (Cockrem 2005; Heath & Frederick 2005;

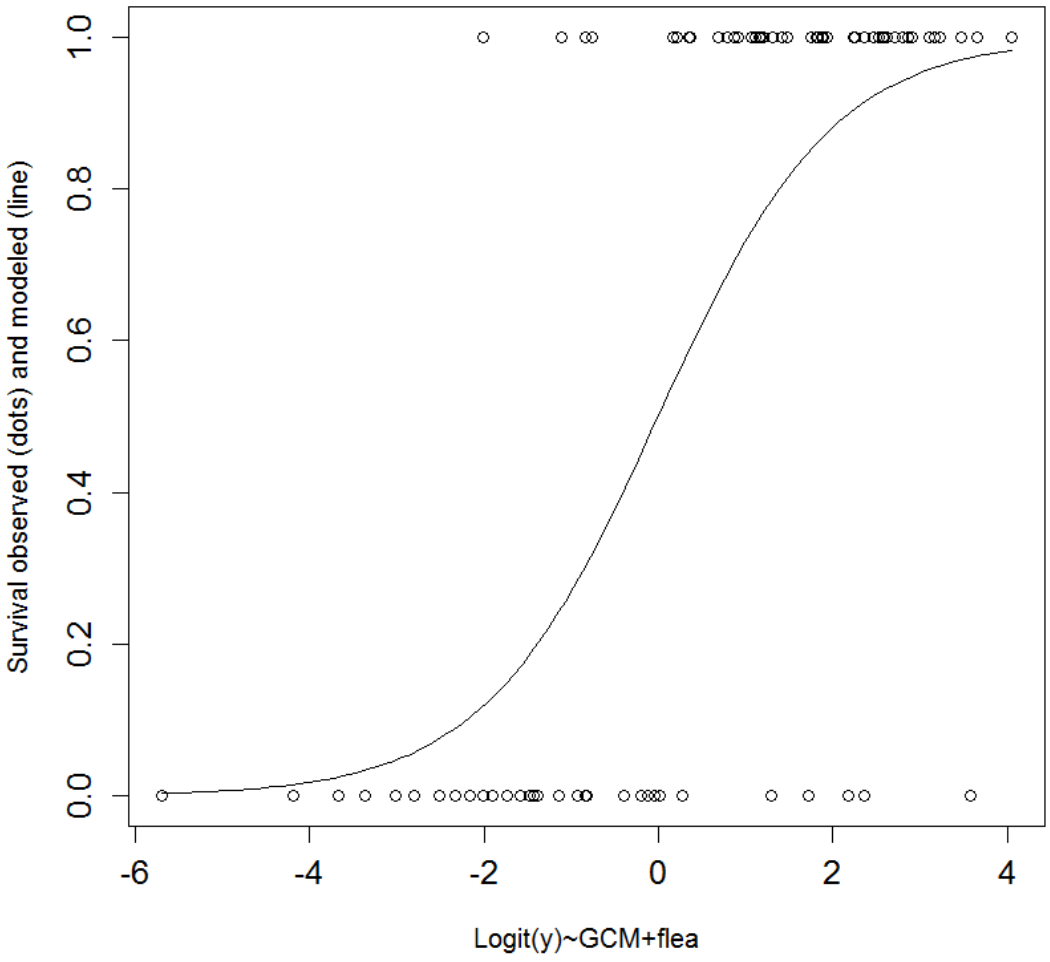
Table 3. Relative support for survival models and GC concentration measured in pikas at Rocky Mountain sites. Models are ranked in order of increasing AIC values (Akaike's information criterion). L denotes likelihood. Delta AIC is the difference between the indicated model and the best model (the model with lowest AIC). Models with Delta AIC > 4 are not shown.

Model	AIC	Delta AIC
Wt + Site	112.95	-----
Site	113.27	0.32
Flea + Site	114.43	1.48
GC + Asp + Site	114.47	1.52
GC + Wt + Site	114.52	1.57
GC + Site	114.82	1.87
Mite + Site	115.2	2.25
State	116.4	3.45
GC + Mite + Site	116.75	3.80
Null	120.38	7.43

Table 4. Relative support for survival models and GCM concentration measured in pikas at Rocky Mountain sites. Models are ranked in order of increasing AIC values (Akaike's information criterion). L denotes likelihood. Delta AIC is the difference between the indicated model and the best model (the model with lowest AIC). Models with Delta AIC > 4 are not shown.

Model	AIC	Delta AIC
GCM + Flea	72.92	-----
GCM + Flea + Sex	73.36	0.44
GCM	74.32	1.40
GCM + Sex	75.01	2.09
GCM + Year	75.32	2.40
GCM + State	75.32	2.40
GCM + Wt	75.43	2.51
GCM + Elev	76.26	3.34
GCM + Mite	76.28	3.36
GCM + Asp	76.30	3.38
Null	112.53	39.61

Figure 1. Observed survival and modeled probability of survival of pikas in the Rocky Mountains. The best model of survival (ΔAIC = 0; Survival ~ GCM + Flea) is shown here.



Wikelski & Cooke 2006). Information gleaned from endocrinology tools provide invaluable insight regarding the current physiological condition of an animal, but interpretation of results can be complicated. Given that increases in GC levels sometimes predict mortality and decreased reproduction, one would expect that high GC levels indicate stress. But research has shown that GC levels do not always change in a predictable pattern in response to unfavorable conditions (Breuner *et al.* 2008), and that the relationship between GC levels and survival or reproductive success is often not linear (Busch & Hayward 2009). It is likely that part of the problem lies with methodologies, but there might also be a physiological basis for these inconsistencies. Therefore, it is important to identify which endocrine metrics are the most informative for a species, given the limited time and resources that characterize many wildlife conservation research projects.

Here our results indicated that GCM concentration was the strongest predictor of annual survival in pikas, suggesting that individuals with higher baseline GCM level may be less likely to survive. Among commonly used endocrine metrics, those measuring baseline GCM level are the most readily related to survival, since this metric reflects the allostatic load of the individual and may be indicative of chronic stress (McEwan & Wingfield 2003; Korte *et al.* 2005). There is support for the expected negative relationship between high baseline GCM levels and survival in the literature (Busch and Hayward 2009), although studies pertaining to lagomorph species are rare. Research related to pygmy rabbits (*Brachylagus idahoensis*) has shown that higher GCM concentration measured in adult females was related to reduced reproduction and survival of subsequent litters (Scarlata *et al*. 2012). However, another study on captive European rabbits (*Oryctolagus cuniculus*) found that higher GCM concentration (fecal CORT) was positively associated with survival and negatively associated with body condition (Tablado *et al.* 2012). In this case, rabbits were kept in captivity for 2-4 weeks and then re-introduced into a new area.

Measurements collected during the captivity period most likely reflect the stress response of individuals, rather than an accurate measurement of chronic stress. This is corroborated by the fact that higher GC levels (plasma CORT) were also positively related to survival, and these measurements were not considered baseline (Tablado *et al.* 2012). Examples such as this illustrate the importance of standardized definitions of baseline measurements and chronic stress. In our study, GCM samples were collected from free-ranging individuals, and capture associated stress was not reflected in fecal samples since they were collected within 3 hours after an animal was captured, too soon to allow for excretion of metabolites resulting from elevated GC (Wilkening *et al.* 2013).

Contrary to our prediction, we did not find a significant positive relationship between GC levels (plasma CORT) and survival. This metric was considered an acute stress response in our study, rather than a baseline measurement, given that we were measuring how an individual responded to capture stress more than 3 minutes after capture. It is widely assumed that an adaptive acute stress response is beneficial for individuals, but this is not strongly supported by the literature. Many studies have documented increased GC levels in vertebrates in response to a stressor, but very few have related this increase to positive or negative effects on fitness. Most studies have focused on the deleterious impacts of chronic stress, or they fail to take into consideration how the natural variability in GC levels relates to fitness. A recent review examining studies related to the acute stress response and two metrics of fitness (survival and reproduction) found that survival could be predicted (nonlinearly) by high or low GC levels, and that the direction of the relationship was often confounded by the reproductive state of the individual (Breuner *et al.* 2008). Busch and Hayward (2009) offer a modified log-quadratic relationship between survival, reproduction, and measures of stress to explain inconsistencies

across studies. They suggest that baseline GCs (fecal or plasma) need to be high enough to support the extra physiological demands of reproduction, but not so high as to disrupt required behavioral processes. Similarly, an acute stress response should protect the individual from dying in the pursuit of reproduction, but should not always favor individual survival over survival of young (Busch and Hayward 2009). When placed in the context of survival and reproduction, high GCs are not always detrimental. Recognizing that elevated GCs can sometimes promote survival is necessary for proper interpretation of GC measurements. GCs typically need to be elevated for long periods of time before they cause harmful effects in wildlife populations (Sapolsky *et al.* 2000). Additional information related to reproductive status could help to clarify the relationship between GC levels and survival in pikas.

We also found that pika survival was negatively related to the number of fleas measured on an individual. A high numbers of fleas may indicate individuals in poor health that are less likely to survive (Devevey & Christie 2009), or could identify individuals more susceptible to mortality as a result of diseases transmitted through fleas (Collinge & Ray 2006). Little is known about disease and pikas, although past studies have revealed that pikas harbor 66 species of ecto-parasites (Severaid 1955). Long term demographic studies in Montana have documented significant population declines in some years, most likely resulting from epidemics (C.Ray *unpublished data*). Fleas are known to carry plague, and the spread of plague could play a role in pika survival (Biggins and Kosoy 2001). Pika susceptibility to plague is unknown, but survival for other mammals, such as Prairie dogs, has significantly increased when colonies were sprayed for fleas (Biggins *et al.* 2010).

Predator abundance has been shown to positively correlate with both baseline and stress induced GC measurements, and the GC response to predators appears to be fairly similar across

species. Individuals can experience chronic stress in response to sustained high levels of predation (Boonstra *et al.* 1998; Scheuerlein *et al.* 2001; Clinchy *et al.* 2004), and also rely on an elevated acute stress response as a means of escape (Mateo 2007). High poaching pressure is positively related to GCM concentration measured in African elephants (*Loxodonta africana*; Gobush *et al.* 2008), and direct chase by humans evokes a stress response in red deer (*Cervus elaphus*) and the California sheephead (*Semicossyphus pulcher*), a Pacific ocean fish (Bateson and Bradshaw 1997; Galima *et al.* 2005). Among lagomorphs, a study related to survival patterns in European rabbits (*Oryctolagus cuniculus*) found that predation was greater for newborns and juveniles, and that predation rates depended somewhat on population density and occurrence of disease outbreaks (Tablado *et al.* 2012). Pikas are preyed upon by weasels (Smith & Weston 1990), and it is likely that predation pressure influences both survival and GC response in pikas. Future studies could incorporate measurements estimating local predator density or abundance.

Additionally, microclimatic factors measured here were not predictive of annual survival. This could be a result of microclimatic variables (elevation and aspect) being placed into categories, rather than direct measurements. Future studies could incorporate more specific data on microclimate, rather than estimated proxy variables such as elevation and aspect. Another factor that could be affecting GCM measurements and pika survival is diet, since this has been shown to influence GCM concentration in fecal samples from other species (von der Ohe *et al*. 2004; Thiel *et al.* 2011; Stetz *et al.* 2013), and pika occupancy and persistence has been related to diet (Wilkening *et al.* 2011; Jeffress *et al.* 2013; Erb *et al.* 2014). Regional variation among sites in vegetation type and abundance could partly explain patterns of survival and GCM excretion observed here, and additional analyses could include metrics of vegetation cover. Our study included two sub-species of pika located in separate states (Colorado and Montana), and

state was used as a predictor to encompass variation in GC metrics that could result from genetic influences (Sapolsky *et al.* 2000). Although it was not predictive here, the relationship between GC level and sub-species could be investigated in more detail in studies related to survival and stress in pikas. Ultimately, identifying factors that cause stress in pikas, as indicated by elevated GCM concentration, may allow us to alleviate negative stressors contributing to population decline and increase the chance of survival.

BIBLIOGRAPHY

Arnold, W. & V.L. Anja. 1993. Ectoparasite loads decrease the fitness of alpine marmots (*Marmota marmota*) but are not a cost of sociality. Behavioral Ecology 4:36-39.

Ashley, N.T., P.S. Barboza, B.J. Macbeth, D.M. Janz, M.R.L. Cattet, R.K. Booth & S.K. Wasser. 2011. Glucocorticosteroid concentrations in feces and hair of captive caribou and reindeer following adrenocorticotropic hormone challenge. General Comparative Endocrinology 172:382-391.

Ayres, K.L., R.K. Booth, J.A. Hempelmann, K.L. Koski, C.K. Emmons, R.W. Baird, K. Balcomb-Bartok, M.B. Hanson, M.J. & S.K. Wasser. 2012. Distinguishing the Impacts of Inadequate Prey and Vessel Traffic on an Endangered Killer Whale (*Orcinus orca*) Population. PloS ONE 7, e36842. doi:10.1371/journal.pone.0036842

Bates, D., M. Maechler M & B. Bolker .2013. Ime4: Linear mixed effects models using S4 classes. R package version 0.999999-2. http://CRAN.R-project.org/package=lme4

Beever, E.A., J. Berger & P.F. Brussard. 2003. Patterns of apparent extirpation among isolated populations of pikas (*Ochotona princeps)* in the Great Basin. Journal of Mammalogy 84:37-54.

Beever , E.A., C. Ray, P.W. Mote & J.L. Wilkening. 2010. Testing alternative models of climate-mediated extirpations. Ecological Applications 20:164-178.

Beever, E.A., C. Ray C, J.L. Wilkening, P.F. Brussard & P.W. Mote . 2011. Contemporary climate change alters the pace and drivers of extinction. Global Change Biology 17:2054-2070.

Beniston, M. 2003. Climate change in mountain regions: a review of possible impacts. Climate Change 59: 5–31

Berkeley, E.V., J.F. Kirkpatrick, N.E. Schaffer, W.M. Bryant & W.R. Threlfall. 1997. Serum and fecal steroid analysis of ovulation, pregnancy, and parturition in the black rhinoceros (*Diceros bicornis*). Zoo Biology16:121-132.

Biggins, D.E., J.L. Godbey, K.L. Gage, L.G. Carter and J.A. Montenieri. 2010. Vector control improves survival of three species of prairie dogs (*Cynomys)* in areas considered epizootic for plague. Vector-Borne and Zoonotic Diseases 10:17-26.

Biggins, D., & M. Kosoy. 2001. Influences of introduced plague on North American mammals; implications from ecology of plague in Asia. Journal of Mammalogy 82:906–16.

Block, W.M. & L. A. Brennan. 1993. The habitat concept in ornithology. Current Ornithology 11: 35–91.

Bonier, F., P.R. Martin, I.T. Moore & J.C. Wingfield. 2009. Do baseline glucocorticoids predict fitness? Trends in Ecology and Evolution 24:634-642.

Boonstra, R., D. Hik, G.R. Singleton & A. Tinnikov. 1998. The impact of predator induced stress on the snowshoe hare cycle. Ecological Monographs 68: 371–394.

Boonstra, R., C.J. McColl & T.J. Karels. 2001. Reproduction at all costs: the adaptive stress response of male arctic ground squirrels. Ecology 82:1930-1946.

Boonstra, R. 2005. Equipped for life: the adaptive role of the stress axis in male mammals. Journal of Mammalogy 86:236-247.

Bosson, C.O., R. Palme & R. Boonstra. 2009. Assessment of the Stress Response in Columbian Ground Squirrels: Laboratory and Field Validation of an Enzyme Immunoassay for Fecal Cortisol Metabolites. Physiological and Biochemical Zoology 82:291-301.

Braganza, K., D. J. Karoly & J. M. Arblaster. 2004. Diurnal temperature range as an index of global climate change during the twentieth century. Geophysical Research Letters 31:1-4.

Breuner, C.W. & T.P Hahn. 2003. Integrating stress physiology, environmental change, and behavior in free-living sparrows. Hormones and Behavior 43:115-123.

Breuner, C.W., S.H. Patterson & T.P. Hahn. 2008. In search of relationships between the acute adrenocortical response and fitness. General and Comparative Endocrinology 157:288-295.

Briones, M. J. I., P. Ineson & T. G. Piearce. 1997. Effects of climate change on soil fauna: responses of enchytraeids, *diptera* larvae and tradigrades on a transparent experiment. Applied Soil Ecology 6:117-134.

Brown, C.R., M. B. Brown & B. Rannala. 1995. Ectoparasites reduce long-term survival of their avian host. Proceedings of the Royal Society Biological Sciences 262:313-319.

Brown, C.R., M. B. Brown, S. A. Raouf, L. C. Smith & J. C. Wingfield. 2005. Effects of endogenous steroid hormone levels on annual survival in Cliff Swallows. Ecology 86: 1034-1046.

Buckley, Y. M., D. T. Briese & M. Rees. 2003. Demography and management of the invasive plant species *Hypericum perforatum. I.* Using multi-level mixed-effects models for characterizing growth, survival and fecundity in a long-term data set. Journal of Applied Ecology 40:481-493.

Burger, K.C., J. J. Degenhardt & J. R. Giardino. 1999. Engineering geomorphology of rock glaciers. Geomorphology 31:93-132.

Burnham, K. P. & D. R. Anderson. 2002. Model Selection and Multimodel Inference: A Practical Information-Theoretic Approach. Springer. 488 p.

Busch, D.S. & L.S. Hayward. 2009. Stress in a conservation context: A discussion of glucocorticoid actions and how levels change with conservation-relevant variables. Biological Conservation 142:2844-2853.

Chelini, M.O. M, E. Otta, C. Yamakita & R. Palme. 2010. Sex differences in the excretion of fecal glucocorticoid metabolites in the Syrian hamster. Journal of Comparative Physiology 180:919-925.

Chinnadurai, S.K., J.J. Millspaugh, W.S. Matthews, K.Canter, R. Slotow, B.E. Washburn & R.J. Woods. 2009. Validation of Fecal Glucocorticoid Metabolite Assays for South African Herbivores. Journal of Wildlife Management 73:1014-1021.

Christian, J.J. 1980. Endocrine functions in population regulation. In *Biosocial mechanisms of population regulation:* 55-115. Cohen, M.N., Malpass, R.S. & Klein, H.G. (Eds). New Haven, CT: Yale University Press.

Clark, D.H., M.M. Clark & A.R. Gillespie. 1994. Debris-covered glaciers in the Sierra Nevada, California and their implications for snowline reconstruction. Quaternary Research 41:139-153.

Clinchy, M., L. Zanette, R. Boonstra, J.C. Wingfield & J.M. Smith. 2004. Balancing food and predator pressure induces chronic stress in songbirds. Proceedings of the Royal Society of London B 271:2473–2479.

Cockrem, J.F. 2005. Conservation and behavioral neuroendocrinology. Hormones and Behavior 48:492–501.

Collinge, S. K. & C. Ray. 2006. Disease ecology: community structure and pathogen dynamics. Oxford University Press. 227 pp.

Convey, C., W. Block & H. J. Peat. 2003. Soil arthropods as indicators of water stress in Antarctic terrestrial habitats? Global Change Biology 9:1718-1730.

Cook, C.J., D.J. Mellor, P.J. Harris, J.R. Ingram & L.R. Matthews. 2000. Hands-on and hands-off measurement of stress. In: Moberg GP, Mench JA eds. The Biology of Animal Stress. CABI Publishing, New York, NY.

Cote, J., J. Clobert, S. Meylan & P.S. Fitz. 2006. Experimental enhancement of corticosterone levels positively affects subsequent male survival. Hormones and Behavior 49:320-327.

Creel, S., N.M. Creel & S.L. Monfort. 1996. Social stress and dominance. Nature 379:212.

Creel, S., J.E. Fox, A. Hardy, J. Sands, B. Garrott & R.O. Peterson. 2002. Snowmobile activity and glucocorticoid stress responses in wolves and elk. Conservation Biology 16:809-814.

Cyr, N.E. & L.M. Romero. 2008. Fecal glucocorticoid metabolites of experimentally stressed captive and free-living starlings: Implications for conservation research. General and Comparative Endocrinology 158:20-28.

Dantzer, B., A.G. McAdam, R. Palme, Q.E. Fletcher, S. Boutin , M.M. Humphries & R. Boonstra. 2010. Fecal cortisol metabolite levels in free-ranging North American red squirrels: Assay validation and the effects of reproductive condition. General and Comparative Endocrinology 167:279-286.

Dantzer, B., A. G. McAdam, R. Palme, S. Boutin & R. Boonstra. 2011 How does diet affect fecal steroid hormone metabolite concentrations? An experimental examination in red squirrels. General and Comparative Endocrinology 174:124–131.

Daubenmire, R.F. 1959. Canopy coverage method of vegetation analysis. Northwest Science 33: 43-64.

Davies, N. A., G. Gramotnev, C. McAlpine, L. Seabrook , G. Baxter, D. Lunney, J. R. Rhodes & A. Bradley. 2013. Physiological stress in Koala populations near the arid edge of their distribution. PloS ONE 8(11):1-12.

Davinroy, T.C. 2000. Hydrological and biogeochemical characteristics of alpine talus, Colorado Front Range. Dissertation, University of Colorado, Boulder.

Dearing, D.E. 1995. Factors governing diet selection in a herbivorous mammal, the north American pika (*Ochotona princeps*). Dissertation, University of Colorado, Boulder. 166 pages.

Dearing, M.D. 1996. Disparate determinants of summer and winter diet selection in a generalist herbivore, *Ochotona princeps*. Oecologia 108:467-478.

Dearing, M.D.1997. The manipulation of plant toxins by a food-hoarding herbivore, *Ochotona princeps.* Ecology 78:774-781.

Dehnhard, M., M. Clauss, M. Lechner-Doll, H.H. D. Meyer & R. Palme R. 2001. Noninvasive monitoring of adrenocortical activity in roe deer (*Capreolus capreolus*) by measurement of fecal cortisol metabolites. General and Comparative Endocrinology 123:111-120.

DeNardo, D.F. & P. Licht. 1993. Effects of corticosterone on social behavior of male lizards. Hormones and Behavior 27:184-199.

Devevey, G. & P. Christie. 2009. Flea infestation reduces the life span of the common vole. Parasitology 136 :1351-1355

Diaz, H. F. & R. S. Bradley. 1997. Temperature variations during the last century at high elevation sites. Climate Change 36: 253–279.

Ebensperger, L. A., D. Tapia, J. Ramirez-Estrada, C. Leon, M. Soto-Gamboa & L. D. Hayes.

2013. Fecal cortisol levels predict breeding but not survival of females in the short lived rodent, *Octodon degus*. General and Comparative Endocrinology 186:164-171.

Engelmann, M., R. Landgraf & C.J. Wotjak. 2004. The hypothalamic-neurohypophysial system regulates the hypothalamic-pituitary-adrenal axis under stress: an old concept revisited. Frontiers in Neuroendocrinology 25:132-149.

Erb, L.P., C. Ray & R. Guralnick. 2011. On the generality of a climate-mediated shift in the distribution of the American Pika (*Ochotona princeps*). Ecology 92:1730-1735.

Erb, L.P., Ray, C. & Guralnick, R. 2014. Determinants of pika population density vs. occupancy in the Southern Rocky Mountains. Ecological Applications 24:429-435.

Fletcher, Q.E. & R. Boonstra. 2006. The impact of live-trapping on the stress response of the meadow vole (*Microtus pennsylvanicus*). Journal of Zoology 270:473–478.

Fokidis, H.B., L. Hurley, C. Rogowski, K. L. Sweazea & P. Deviche. 2011. Effects of captivity and body condition on plasma corticosterone, locomotor behavior, and plasma metabolites in curve-billed thrashers. Physiological and Biochemical Zoology 84:595–606.

Foley, C. A., H., S. Papageorge & S. K. Wasser. 2001. Noninvasive stress and reproductive measures of social and ecological pressures in free-ranging African elephants. Conservation Biology 15:1134-1142.

French, S.S., H. B. Fokidis & 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 B 178:997–1005

Galima, M.M., C.G. Lowe, & K.M. Kelley. 2005. Catch-and-release stress: impacts on the endocrine physiology of the California sheephead, *Semicossyphus pulcher*. Society for

Integrative and Comparative Biology, San Diego.

Ganswindt, A., R. Palme, M. Heistermann, S. Borragan & J.K. Hodges. 2003. Non-invasive assessment of adrenocortical function in the male African elephant (*Loxodonta africana).* General and Comparative Endocrinology 134:156-166.

Garrett, L., M. Jeffress, M. Britten, C. Epps, C. Ray & S. Wolff. 2011. Pikas in peril: Multiregional vulnerability assessment of a climate-sensitive sentinel species. Park Science 28: 9-13.

Gobush, K.S., B.M. Mutayoba, & S.K. Wasser. 2008. Long-term impacts of poaching on relatedness, stress physiology, and reproductive output of adult female African elephants. Conservation Biology 22:1590–1599.

Goldin, B.R., H. Adlercreutz, S. L. Gorbach, J. H. Warram, J. T. Dwyer, L. Swenson & M. N. Woods.1982. Estrogen excretion patterns and plasma-levels in vegetarian and omnivorous women. New England Journal of Medicine 307:1542–1547.

Goldman, B.D. 1999. The circadian timing system and reproduction in mammals. Steroids 64:679–685.

Goymann, W., E. Mostl, T. Van't Hoff T, M.L. East & H. Hofer. 1999. Non-invasive fecal monitoring of glucocorticoids in spotted hyenas (*Crocuta crocuta*). General and Comparative Endocrinology 114:340-348.

Goymann,W. 2005. Non-invasive monitoring of hormones in bird droppings: biological validations, sampling, extraction, sex differences, and the influence of diet on hormone metabolite levels. Annals of the New York Academy of Sciences 1046:35–53.

Grayson, D.K. 2005. A brief history of Great Basin pikas. Journal of Biogeography 32:2103-

2111.

Hafner, D.J. 1993. North American pika (*Ochotona princeps)* as a late Quaternary biogeographic indicator species. Quaternary Research 39:373-380.

Hafner, D.J. 1994. Pikas and permafrost: post-Wisconsin historical zoogeography of *Ochotona* in the southern Rocky Mountains, U. S. A. Arctic and Alpine Research 26:375-382.

Hall, L.S., P. R. Krausman & M. L. Morrison.1997. The habitat concept and a plea for standard terminology. Wildlife Society Bulletin 25:173–182.

Harper, J.M. & S.N. Austad. 2000. Fecal Glucocorticoids: A Noninvasive Method of Measuring Adrenal Activity in Wild and Captive Rodents. Physiological and Biochemical Zoology 73:12-22.

Harper, J.M. & S.N. Austad. 2001. Effect of Capture and Season on Fecal Glucocorticoid Levels in Deer Mice (*Peromyscus maniculatis*) and Red-Backed Voles (*Clethrionomys gapperi*). General and Comparative Endocrinology 123:337-344.

Heath, J.A. & P.C. Frederick. 2005. Relationships among mercury concentrations, hormones, and nesting effort of white ibises (*Eudocimus albus*) in the Florida everglades. The Auk 122:255–267.

Hik, D.S., C. J. McColl & R. Boonstra. 2001. Why are Arctic ground squirrels more stressed in the boreal forest than in alpine meadows? Ecoscience 8:275-288.

Hogan, L.A., A.T. Lisle, S.D. Johnston & H. Robertson. 2012. Non-invasive assessment of stress in captive numbats, *Myrmecobius fasciatus* (Mammalia: Marsupialia), using faecal cortisol measurement. General and Comparative Endocrinology 179:376-383.

Homan, R.N., J.V. Regosin, D. M. Rodrigues, J. M. Reed, B. S. Windmiller & L. M. Romero. 2003. Impacts of varying habitat quality on the physiological stress of spotted salamanders (*Ambystoma maculatum).* Animal Conservation 6:11-18.

Hopkins, W.A., M. T. Mendonca & J. D. Congdon. 1997. Increased circulating levels of testosterone and corticosterone in southern toads, *Bufo terrestris*, exposed to coal combustion waste. General and Comparative Endocrinology 108: 237-246.

Howell-Stephens, J.A., J.S. Brown, D. Bernier, D. Mulkerin & R.M. Santymire. 2012. Characterizing adrenocortical activity in zoo-housed southern three-banded armadillos (*Tolypeutes matacus*). General and Comparative Endocrinology 178:64-74.

Hughes, L. 2000. Biological consequences of global warming: is the signal already apparent? Trends in Ecology and Evolution 15:56-62.

Hunt, K.E., A.W. Trites & S.K. Wasser. 2004. Validation of a fecal glucocorticoid assay for Stellar sea lions (*Eumetopias jubatus*). Physiology and Behavior 80:595-601.

Huntley, N.J., A. T. Smith & B. L. Ivins. 1986. Foraging behavior of the pika (*Ochotona princeps*), with comparisons of grazing versus haying. Journal of Mammalogy 67:139-148.

IPCC, 2014: Summary for Policymakers, In: Climate Change 2014, Mitigation of Climate Change. Contribution of Working Group III to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change [Edenhofer, O., R. Pichs-Madruga, Y. Sokona, E. Farahani, S. Kadner, K. Seyboth, A. Adler, I. Baum, S. Brunner, P. Eickemeier, B. Kriemann, J. Savolainen, S. Schlömer, C. von Stechow, T. Zwickel and J.C. Minx (eds.)]. Cambridge University Press, Cambridge, United Kingdom and New York, NY, USA.

Ives, J. & B. Fahey. 1971. Permafrost occurrence in the Front Range, Colorado Rocky Mountains, U.S.A. Journal of Glaciology 10:105-111.

Ives, J. 1973. Permafrost and its relationship to other environmental parameters in a mid-latitude, high altitude setting, Front Range, Colorado Rocky Mountains. Permafrost: the North American Contributions to the 2nd International Permafrost Conference. National Academy of Science 121-125.

Jeffress, M. & L. Garrett. 2011. Pika monitoring under way in four western parks: The development of a collaborative multipark protocol. Park Science 28(2):18-20.

Jeffress, M. R., T. J. Rodhouse, C. Ray, S. Wolff & C. W. Epps. 2013. The idiosyncrasies of place: geographic variation in the climate-distribution relationships of the American pika. Ecological Applications 23: 864-878.

Jurke, M. H., N.M. Czekala, D.G. Lindburg & S.E. Millard. 1997. Fecal corticoid metabolite measurement in the cheetah (*Acinonyx jabatus*). Zoo Biology 16:133-147.

Karl, T. R., G. Kukla, V. N. Razuvayev, M. J. Changery, R. G. Quayle, R. R. Heim, D. R. Easterling & C. B. Fu. 1991. Global warming: Evidence for asymmetric diurnal temperature range. Geophysical Research Letters 18: 2253-2256.

Kenagy, G.J. & N.J. Place. 2000. Seasonal changes in plasma glucocorticosteroids of free-living female yellow-pine chipmunks: effects of reproduction and capture and handling. General and Comparative Endocrinology 117:189–199.

Khan, M.Z., J. Altmann, S. S. Isani & J. Yu. 2002. A matter of time: evaluating the storage of fecal samples for steroid analysis. General and Comparative Endocrinology 128:57-64.

Kirkpatrick, J.F., J.C. McCarthy, D.F. Gudermuth, S.E. Shideler & B.L. Lasley. 1996. An assessment of the reproductive biology of Yellowstone bison (*Bison bison*) subpopulations using non capture methods. Canadian Journal of Zoology 74:8-14.

Koolhaas, J.M., S.F. de Boer, B. Buwalda, B.J. van der Vegt, C. Carere & A.G. G. Groothius. 2001. How and why coping systems vary among individuals. In: D.M. Broom, eds. Coping with Challenge Welfare in Animals Including Humans. Dahlem Univ. Press, Dahlem, pp. 197–209.

Korte, S.M., J.M. Koolhaas, J.C. Wingfield & B.S. McEwen. 2005. The Darwinian concept of stress: benefits of allostasis and costs of allostatic load and the trade-off in health and disease. Neuroscience and Behavioral Reviews 29:3-38.

Krajick, K. 2004. All downhill from here? Science 303:1600-1602.

Krear, H.R. 1965. An ecological and ethological study of the pika (*Ochotona princeps saxatilis bangs*) in the front range of Colorado. Dissertation, University of Colorado, Boulder. 134 pages.

Lanctot, R.B., S.A. Hatch, V.A. Gill & M. Eens. 2003. Are corticosterone levels a good indicator of food availability and reproductive performance in a kittiwake colony? Hormones and Behavior 43:489-502.

Laternser, M. & M. Schneebeli. 2003. Long-term snow climate trends of the Swiss Alps (1931-99). International Journal of Climatology 23:733-750.

Laver, P.N., A. Ganswindt, S.B. Ganswindt & K.A. Alexander. 2012. Non-invasive monitoring of glucocorticoid metabolites in banded mongooses (*Mungos mungo*) in response to physiological and biological challenges. General and Comparative Endocrinology 179:178-183.

Lawlor, T.E. 1998. Biogeography of Great Basin mammals: paradigm lost? Journal of Mammalogy 79: 1111-1130.

Leopold, M., D. P. Dethier, J. Völkel, T. Corson Rickert, T. Raab & N. Caine. 2008. Using geophysical methods to study the shallow subsurface of a sensitive alpine environment, Niwot Ridge, Colorado Front Range, USA. Arctic, Antarctic, and Alpine Research 40:519-530.

Leopold, M., J. Völkel, D. P. Dethier, M. W. Williams & N. Caine. 2010. Mountain permafrost, a valid archive to study climate change? Examples from the Rocky Mountains, USA. Nova Act LC 112: 281-289.

Levins, R. 1968. Evolution in changing environments: Some theoretical explanations. Princeton University Press, 120 pages.

Lynn, S.E. 2003. The effects of short-term fasting on activity, corticosterone, and corticosterone binding globulin in a migratory songbird, Gambel's white-crowned sparrow (*Zonotrichia leucophrys gambelii*). Hormones and Behavior 43:150-157.

MacArthur, R.A. & L.C.H. Wang. 1973. Physiology of thermoregulation in the pika, *Ochotona princeps*. Canadian Journal of Zoology 51:11-16.

MacArthur, R.A. & L.C.H.Wang LCH. 1974. Behavorial thermoregulation in the pika, *Ochotona princeps:* a field study using radio-telemetry. Canadian Journal of Zoology 52:353-358.

MacDonald, I.A., V. D. Bokkenheuser, J. Winter, A. M. McLernon & E. H. Mosbach. 1983. Degradation of steroids in the human gut. Journal of Lipid Research 24:675-700.

Mai, D. H. 1995. Tertiare Vegetationsgeschichte Europas. G. Fischer, Stuttgart.

Mateo, J.M. 2007. Developmental and geographic variation in stress hormones in wild Belding's ground squirrels (*Spermophilus beldingi*). Hormones and Behavior 50:718–725.

Matthias, L., J. Völkel, D. P. Dethier & M. W. Williams. 2014. Changing mountain permafrost from the 1970s to today-comparing two examples from Niwot Ridge, Colorado Front Range, USA. Z Geomorphol Supplement 58: 137-157.

McDonald, K.A. & J.H. Brown. 1992. Using montane mammals to model extinctions due to global change. Conservation Biology 6:409-415.

McEwen, B.S. & R.M. Sapolsky. 1995. Stress and Cognitive Function. Current Opinion in Neurobiology 5: 205-216.

McEwan, B.S. & J. C. Wingfield. 2003. The concept of allostasis in biology and biomedicine. Hormones and Behavior 43:2-15.

Millar, C.I. &R. D. Westfall. 2008. Rock glaciers and periglacial rock-ice features in the Sierra Nevada; Classification, distribution, and climate relationships. Quaternary International 188:90-104.

Millar, C.I. & R.D. Westfall. 2010. Distribution and Climatic Relationships of the American Pika (*Ochotona princeps)* in the Sierra Nevada and Western Great Basin, U.S.A.: Periglacial Landforms as Refugia in Warming Climates. Arctic, Antarctic and Alpine Research 42:76-88.

Miller, M.W., N.T. Hobbs & M.C. Sousa. 1991. Detecting stress responses in Rocky Mountain bighorn sheep (*Ovis canadensis*): reliability of cortisol concentrations in urine and feces. Canadian Journal of Zoology 69:15-24.

Millspaugh, J.J., R. J. Woods, K.E. Hunt, K. J. Raedeke, G. C. Brundige, B. E. Washburn BE & S. K. Wasser. 2001. Using fecal glucocorticoid assays to study the physiological stress response of elk. Wildlife Society Bulletin 29:899-907.

Millspaugh, J.J., Washburn, B.E., Milanick, M.A., Beringer, J., Hansen, L.P., Meyer, T.M.

2002. Noninvasive techniques for stress assessment in white-tailed deer. Wildlife Society Bulletin 30:899–907.

Millspaugh, J.J., B. E. Washburn, M. A. Milanick, R. Slotow & G. van Dyck. 2003. Effects of heat and chemical treatments in fecal glucocorticoid measurements: implications for fecal sample transport. Wildlife Society Bulletin 31:399-406.

Millspaugh, J.J. & B.E. Washburn. 2004. Use of fecal glucocorticoid metabolite measures in conservation biology research: considerations for application and interpretation. General and Comparative Endocrinology 138:189-199.

Moberg, G.P. 2000. Biological response to stress: implications for animal welfare. In: Moberg GP, Mench JA, eds. The biology of animal stress. CABI Publishing, p. 43-76.

Molotch, N.P., T. Meixner & M.W. Williams. 2008. Estimating stream chemistry during the snowmelt pulse using a spatially distributed, coupled snowmelt and hydrochemical modeling approach. Water Resources Research 44:1-14.

Monclus, R., H.G. Rodel, R. Palme, D.V. Holst & J. de Miguel. 2006. Non-invasive measurement of the physiological stress response of wild rabbits to the odour of a predator. Chemoecology 16: 25-29.

Morrison, S.F. & D.S. Hik. 2007. Demographic analysis of a declining pika (*Ochotona collaris)* population: linking survival to broad scale patterns via spring snowmelt patterns. Journal of Animal Ecology 76:899-907.

Moritz, C., J.L. Patton, C.J. Conroy, J. L. Parra, G. C. White & S. R. Beissinger SR. 2008. Impact of a century of climate change on small-mammal communities in Yosemite National Park, USA. Science 322:261-264.

Morrow, C.J., E. S. Kolver, G. A. Verkerk & L. R. Matthews. 2002. Fecal glucocorticoid

metabolites as a measure of adrenal activity in dairy cattle. General and Comparative Endocrinology 126:229–241.

Mostl, E. & R. Palme. 2002. Hormones as indicators of stress. Domestic Animal Endocrinology 23:67-74.

Mostl, E., S. Rettenbacher & R. Palme. 2005.Measurement of Corticosterone Metabolites on Birds' Droppings: An Analytical Approach. Annals of the New York Academy of Science 1046:17-34.

Mote, P. W., A. F. Hamlet, M. P. Clark & D. P. Lettenmaier. 2005. Declining mountain snowpack in western North America. Bulletin of the American Metereological Society 86: 39-49.

Munck, A., P. M. Guyre & N. J. Holbrook. 1984. Physiological functions of glucocorticoids in stress and their relation to pharmacological actions. Endocrinology Review 5:25-44.

Naftz, D. L., D. D. Susong, P. F. Schuster, L. D. Cecil, M. D. Dettinger, R. L. Michel & C. Kendall. 2002. Ice core evidence of rapid air temperature increases since 1960 in alpine areas of the Wind River Range, Wyoming, United States. Journal of Geophysical Research 107: 10-29.

Nakagawa, S. & H. Schielzeth. 2013. A general and simple method for obtaining R2 from generalized linear mixed-effects models. Methods in Ecology and Evolution 4: 133-142.

Nichols, L. B. 2010. Fecal pellets of American pikas (*Ochotona princeps*) provide a crude chronometer for dating patch occupancy. Western North American Naturalist 70:500-507.

Osborn, S.D. & D. B. Applebee. 2011. Evaluation of the petition from the Center for Biological

Diversity to list the American pika (*Ochotona princeps*) as threatened. Department of Fish and Game. 32 pages.

Owen, M.A., R. R. Swaisgood, N. M. Czekala, K. Steinman & D. G. Lindburg. 2004. Monitoring stress in captive giant pandas (*Ailuropoda melanoleuca*): behavioral and hormonal responses to ambient noise. Zoo Biology 23:147–164.

Palme, R., P. Fischer, H. Schildorfer & M.N. Ismail. 1996. Excretion of infused ^{14}C-steroid hormones via faeces and urine in domestic livestock. Animal Reproduction Science 43:43-63.

Palme, R. 2005. Measuring fecal steroids: guidelines for practical application. Annals of the New York Academy of Science 1046:75-80.

Palme, R., S. Rettenbacher, C. Touma, S. M. El-Bahr & E. Mostl. 2005. Stress hormones in mammals and birds: comparative aspects regarding metabolism, excretion and noninvasive measurement in fecal samples. Annals of the New York Academy of Science 1040:162-171.

Paradis, E., J. Claude & K. Strimmer. 2004. APE: Analyses of phylogenetics and evolution in R language. Bioinformatics 20: 289-290.

Parmesan, C. & G. Yohe. 2003. A globally coherent fingerprint of climate change impacts across natural systems. Nature 421: 37-42.

Parmesan, C. 2006. Ecological and Evolutionary Responses to Recent Climate Change. Annual Review of Ecology, Evolution and Systematics 37:637-669.

Paul, F., A. Kaab, M. Maisch, T. Kellenberger & W. Haeberli. 2004. Rapid disintegration of alpine glaciers with observed satellite data. Geophysical Research Letters 31: L21402.

Piekarz, R. 1963. The influence of coprophagy on the time of passing of food down the alimentary tract in the domestic rabbit. Acta Physiol Pol *XIV:*337-348.

Pihl, L. & J. Hau. 2003. Faecal corticosterone and immunoglobulin A in young adult rats. Laboratory Animals 37:166–171.

Pinheiro, J. C. & D. M. Bates. 2000. Mixed-effects Models in S and S-Plus. Springer, New York, NY.

R Core Team. 2013. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL http://www.R-project.org/.

Rangel-Negrin, A., J. L. Alfaro, R. A. Valdez, M. C. Romano & J. C. Serio-Silva. 2009. Stress in Yucatab spider monkeys: effects of environmental condition on fecal cortisol levels in wild and captive populations. Animal Conservation 12:496-502.

Ray, C. & E.A. Beever EA. 2007. Distribution and abundance of the American Pika within Lava Beds National Monument. National Park Service Report, pp. 1-57.

Ray, C., E. A. Beever & S. R. Loarie. 2012. Retreat of the American pika: up the mountain or into the void? In: Brodie JF, Post E, Doak D, eds. Conserving wildlife populations in a changing climate. University of Chicago Press, Chicago, IL.

Reeder, D.M., N.S. Kosteczko, T. H. Kunz & E. P. Widmaier. 2004. Changes in baseline and stress-induced glucocorticoid levels during the active period in free ranging male and female little brown myotis, *Myotis lucifugus* (Chiroptera:Vespertilionidae). General and Comparative Endocrinology 136:260–269.

Rehnus, M., K. Hacklander & R. Palme. 2009. A non-invasive method for measuring glucocorticoid metabolites (GCM) in Mountain hares (*Lepus timidus*). European Journal of Wildlife Research 55:615-620.

Riley, V. 1981. Psychoneuroendocrine influences on immunocompetence and neoplasia. Science 212:1100-1109.

Rizo-Aguilar, A., J. A. Guerrero, A. M. P. Montoya-Lara & C. Valdespino. 2014. Physiological stress in volcano rabbit (*Romerolagus diazi*) populations inhabiting contrasting zones at the Corredor Biologico Chichinautzin, Mexico. Mammalian Biology 79:357-361.

Rodhouse, T. J., E. A. Beever, L. K. Garrett, K. M. Irvine, M. Munts , C. Ray, & M. R. Shardlow. 2010. Distribution of the Lava Beds pika (*Ochotona princeps Goldmani*): Conservation implications from the range periphery. Journal of Mammalogy 91:1287-1299.

Rogovin, K., J. A. Randall, I. Kolosova & M. Moshkin. 2003. Social correlates of stress in adult males of the great gerbil, Rhombomys opimus, in years of high and low population densities. Hormones and Behavior 43:132-139.

Romero, L.M. & Wikelski M. 2001. Corticosterone levels predict survival probabilities of Galápagos marine iguanas during El Niño events. Proceedings of the National Academy of Science, USA 98:7366-7370.

Romero, L.M. 2002. Seasonal changes in plasma glucocorticoid concentrations in free-living vertebrates. General and Comparative Endocrinology 128:1-24.

Romero, L.M. 2004. Physiological stress in ecology: lessons from biomedical research. Trends in Ecology and Evolution 19:249-255.

Root, T.L., J. T. Price, K. R. Hall, S. H. Schneider, C. Rosenzweig & A. Pounds. 2003. Fingerprints of global warming on wild animals and plants. Nature 421: 57-60.

Rose, J. Q. & W. J. Jusko. 1979. Corticosteroid analysis in biological fluids by high-performance liquid chromatography. Journal of Chromatography B 162:273-280.

Rusterholtz, H.P. & A. Erhardt. 1998. Effects of elevated CO_2 on flowering phenology and nectar production of nectar plants important for butterflies of calcareous grasslands. Oecologia 113: 341-349.

Sapolsky, R.M, L. M. Romero & A. U. Munck. 2000. How do glucocorticoids influence stress responses? Integrating permissive, suppressive, stimulatory, and preparative actions. Endocrine Review 21:55-89.

Scarlata, C.D., B. A. Elias, J. R. Godwin, R. A. Powell, D. Shepherdson , L. A. Shipley & J. L. Brown. 2011. Characterizing gonadal and adrenal activity by fecal steroid analyses in pygmy rabbits (*Brachylagus idahoensis*). General and Comparative Endocrinology 171:373-380.

Scarlata, C.D., B.E. Elias, J.R. Godwin, R.A. Powell, D. Sheperdson, L.A. Shipley & J.L. Brown. 2012. Relationship between fecal hormone concentrations and reproductive success in captive pygmy rabbits (*Brachylagus idahoensis*). Journal of Mammalogy 93:759-770.

Scheuerlein, A., T.J. Van't Hof & E. Gwinner. 2001. Predators as stressors? Physiological and reproductive consequences of predation risk in tropical stonechats (*Saxicola torquata axillaris*). Proceedings of the Royal Society of London B 268:1575–1582.

Schrott, L. 1996. Some geomorphical-hydrological aspects of rock glaciers in the Andes (San Juan, Argentina). Zeitschrift fuer Geomophologie 104:161-173.

Service, R. F. 2004. As the west goes dry. Science 303:1124-1127.

Severaid, J.H. 1955. The natural history of the pikas (mammalian genus *Ochotona*). Dissertation. University of California, Berkeley, California, USA

Sheriff, M.J., C. O. Bosson, C. J. Krebs & R. Boonstra. 2009. A non-invasive technique for analyzing fecal cortisol metabolites in snowshoe hares (*Lepus americanus*). Journal of Comparative Physiology 179:305–313.

Shutt, K., J. M. Setchell & M. Heistermann. 2012. Non-invasive monitoring of physiological stress in the Western lowland gorilla: Validation of a fecal glucocorticoid assay and methods for practical application in the field. General and Comparative Endocrinology 179:167-177.

Simpson W.G. 2009. American pikas inhabit low elevation sites outside the species' previously described bioclimatic envelope. Western North American Naturalist 69:243-250.

Smith, A.T. 1974. The distribution and dispersal of pikas: influences of behavior and climate. Ecology 55:1368-1376.

Smith, A. T. & M.L. Weston. 1990. *Ochotona princeps*. Mammal Species 352:1-8.

Smith, R. I. L. 1994. Vascular plants as bioindicators of regional warming in Antarctica. Oecologia 99:322-328

Smith, A.T., W. Li & D. Hik. 2004. Pikas as harbingers of global warming. Species 41:4-5.

Smith, J.E., R. Monclús, D. Wantuck, G. L. Florant & D. T. Blumstein. 2012. Fecal glucocorticoid metabolites in wild yellow-bellied marmots: Experimental validation, individual differences and ecological correlates. General and Comparative Endocrinology 178:417-426.

Smith, J. A. & L. P. Erb. 2013. Patterns of selective caching behavior of a generalist herbivore, the American pika (*Ochotona princeps*). Arctic, Antarctic and Alpine Research 45:396-403.

Spercoski, K.M., R. N. Morais, R. G. Morato, R. C. de Paula, F. C. Azevedo, J. A. May-Júnior,

J. P. Santos, A. L. Reghelin, D. E. Wildt & N. Songsasen N. 2012. Adrenal activity in maned wolves is higher on farmlands and park boundaries than within protected areas. General and Comparative Endocrinology 179:232-240.

Stetz, J., K. Hunt, K. C. Kendall & S. K. Wasser. 2013. Effects of exposure, diet, and thermoregulation on fecal glucocorticoid measures in wild bears. PLoS ONE 8(2): e55967. doi:10.1371/journal.pone.0055967.

Stott. P. 2008. Comparisons of digestive function between the European hare (*Lepus europaeus*) and the European rabbit (*Oryctolagus cuniculus*): Mastication, gut passage, and digestibility. Mammalian Biology 73:276-286.

Tablado, Z., E. Revilla & F. Palomares. 2012. Dying like rabbits: general determinants of spatio-temporal variability in survival. Journal of Animal Ecology 81:150-161.

Taylor, W. 1971. The excretion of steroid hormone metabolites in bile and feces. Vitamins and Hormones 29:201-285.

Terio, K.A., S. B. Citin & J. L. Brown. 1999.Fecal cortisol metabolite analysis for noninvasive monitoring of adrenocortical function in the cheetah (*Acinonyx jubatus*). Journal of Zoo and Wildlife Medicine 30:484–491.

Terio, K. A., J. L.Brown, R. Moreland & L. Munson. 2002. Comparison of different drying and storage methods on quantifiable concentrations of fecal steroids in the cheetah. Zoo Biology 21:215-222.

Teskey-Gerstl, A., E. Bamberg, T. Steinbeck & R. Palme. 2000. Excretion of corticosteroids in urine and faeces of hares (*Lepus europaeus*). Journal of Comparative Physiology 170:163-168.

Thiel, D., S. Jenni-Eiermann, R. Palme. & L. Jenni. 2011. Winter tourism increases stress hormone levels in the Capercaillie (*Tetrao urogallus*). Ibis 153:122–133.

Thun, R., E. Eggenberger, K. Zerobin, T. Luscher & W. Vetter. 1981. Twenty-four-hour secretory pattern of cortisol in the bull: evidence of episodic secretion and circadian rhythm. Endocrinology 109:2208–2212.

Touma, C., N. Sachser, E. Mostl & R. Palme. 2003. Effects of sex and time of day on metabolism and excretion of corticosterone in urine and feces of mice. General and Comparative Endocrinology 130:267-278.

Touma, C., R. Palme & N. Sachser. 2004. Analyzing corticosterone metabolites in fecal samples of mice: a noninvasive technique to monitor stress. Hormones and Behavior 45:10-2.

Touma, C. & R. Palme. 2005. Measuring Fecal Glucocorticoid Metabolites in Mammals and Birds: The Importance of Validation. Annals of the New York Academy of Science 1046:54-74.

Turner Jr., J.W., R. Nemeth & C. Rogers. 2003. Measurements of fecal glucocorticoids in parrotfishes to assess stress. General and Comparative Endocrinology 133:341-352.

United States Fish and Wildlife Service (USFWS). 2010. Endangered and threatened wildlife and plants; 12-month finding on a petition to list the American pika as threatened or endangered. Federal Register 50 CFR 17:1–34.

Vander Haegen, W.M., G. R. Orth & M.J. Linders. 2013. Survival and causes of mortality in a northern population of western gray squirrels. Journal of Wildlife Management 77:1249 -1257.

Varner, J. & M. D. Dearing M.D. 2014a.The importance of biologically relevant microclimates in habitat suitability assessments. PLoS ONE, 9, e104648.

Varner, J. & M. D. Dearing 2014b. Dietary plasticity in pikas as a strategy for atypical resource landscapes. Journal of Mammalogy 95:72-81.

Viviroli, D., R. Weingartner & B. Messerli. 2003. Assessing the hydrological significance of the world's mountains. Mountain Research and Development 23:32-40.

von der Ohe, CG & C. Servheen. 2002. Measuring stress in mammals using fecal glucocorticoids: opportunities and challenges. Wildlife Society Bulletin 30:1215–1225.

von der Ohe, C.G., S. K. Wasser, K. Hunt & C. Servheen. 2004. Factors associated with fecal glucocorticoids in Alaskan brown bears (*Ursus arctos horribilis*). Physiological and Biochemical Zoology 77:313-320.

Von Holst, D. 1998. The concept of stress and its relevance for animal behavior. Advances and Studies in Behavior 27:1-131.

Walther, G.R. 2002. Weakening of climatic constraints with global warming and its consequences for evergreen broad-leaved species. Folia Geobotanica 37:129-139.

Walther, G.-R., E. Post, P. Convey, A. Menzel, C. Parmesan, T. J. C. Beebee, J.-M. Fromentin, O. Hoegh-Guldberg & F. Bairlein. 2002. Ecological responses to recent climate change. Nature 416:389-395.

Walther, G.-R., E. S. Gritti, S. Berger, T. Hickler, Z. Tang & M. T. Sykes. 2007. Palms tracking climate change. Global Ecology and Biogeography 16:801-809.

Wasser, S.K., R. Thomas, P. P. Nair, C. Guidry, J. Southers, J. Lucas, D. E. Wildt & S. L. Monfort. 1993. Effects of dietary fiber on faecal steroid measurements. Journal of Reproduction and Fertility 97:569–574.

Wasser, S. K., K. Bevis, G. King & E. Hanson. 1997. Non-invasive physiological measures of disturbance in the northern spotted owl. Conservation Biology 11:1019-1022.

Wasser, S.K., K.E. Hunt, J.L. Brown, K. Cooper, C.M. Crockett, U. Bechert, J.J. Millspaugh, S. Larson & S.L. Monfort. 2000. A generalized fecal glucocorticoid assay for use in a diverse array of nondomestic mammalian and avian species. General and Comparative Endocrinology 120:260-275.

Watt, A.D., J. B. Whitaker, M. Dorcherty, G. Brooks, E. Lindsay & D.T. Salt. 1995. The impact of elevated CO2 on insect herbivores. In: Harrington R, Stork NE, editors. Insects in a changing environment. San Diego: Academic Press. pp. 197-217.

Wikelski, M., L. M. Romero & H. L. Snell. 2001. Marine iguanas oiled in the Galapagos. Science 292:437-438.

Wikelski, M. & S.J. Cooke. 2006. Conservation physiology. Trends in Ecology and Evolution 21:38–46.

Williams, M.W., M. Knauf, N. Caine, F. Liu & P. L Verplanck. 2006. Geochemistry and Source Waters of Rock Glacier Outflow, Colorado Front Range. Permafrost and Periglacial Processes 17:13–33.

Wilkening, J. L., C. Ray, E. A. Beever & P. F. Brussard. 2011. Modeling contemporary range retraction in Great Basin pikas (*Ochotona princeps)* using data on microclimate and microhabitat. Quaternary International 27:1-12.

Wilkening, J.L., C. Ray & K. L. Sweazea. 2013. Stress hormone concentration in Rocky Mountain populations of the American pika (Ochotona princeps) Conservation Physiology 1:1-13.

Wingfield, J.C., C. M. Vleck & M. C. Moore. 1992. Seasonal changes of the adrenocortical response to stress in birds of the Sonoran desert. Journal of Experimental Zoology 264:419-428.

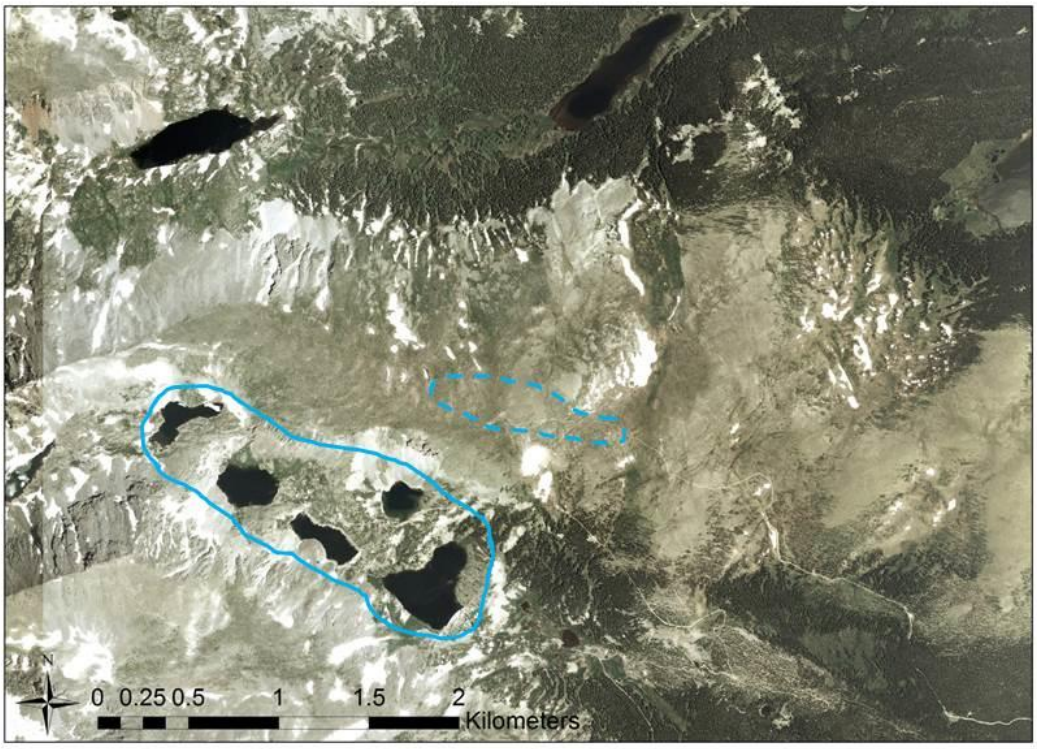
Wingfield, J.C., R. Suydam & K. Hunt. 1994. The adrenocortical responses in snow Buntings (*Plectrophenax nivalis*) and Lapland longspurs (*Calcarius lapponicus*) at Barrow, Alaska. Comparative Biochemical Physiology 108: 299-306.

Wingfield, J.C. & R. M. Sapolsky RM. 2003. Reproduction and resistance to stress: when and how. Journal of Neuroendocrinology 15:711-724.

Zhang, S., F. Lei, S. Liu, D. Li, C. Chen & P. Wang. 2011. Variation in baseline corticosterone levels of Tree Sparrow *(Passer montanus)* populations along an urban gradient in Beijing, China. Journal of Ornithology 152:801–806.

APPENDIX I

Map of the Rocky Mountains in the Colorado Front Range, depicting the location of sampling areas in Chapter 2; Green Lakes Valley Watershed (GLVW) with RIFs (solid line) and Niwot Ridge LTER (NWT) without RIFs (dashed line).



APPENDIX II

Correlation Matrices. Matrices displaying correlations in Chapter 2 among all predictor variables for Green Lakes Valley Watershed, Niwot Ridge LTER and Both Sites combined.

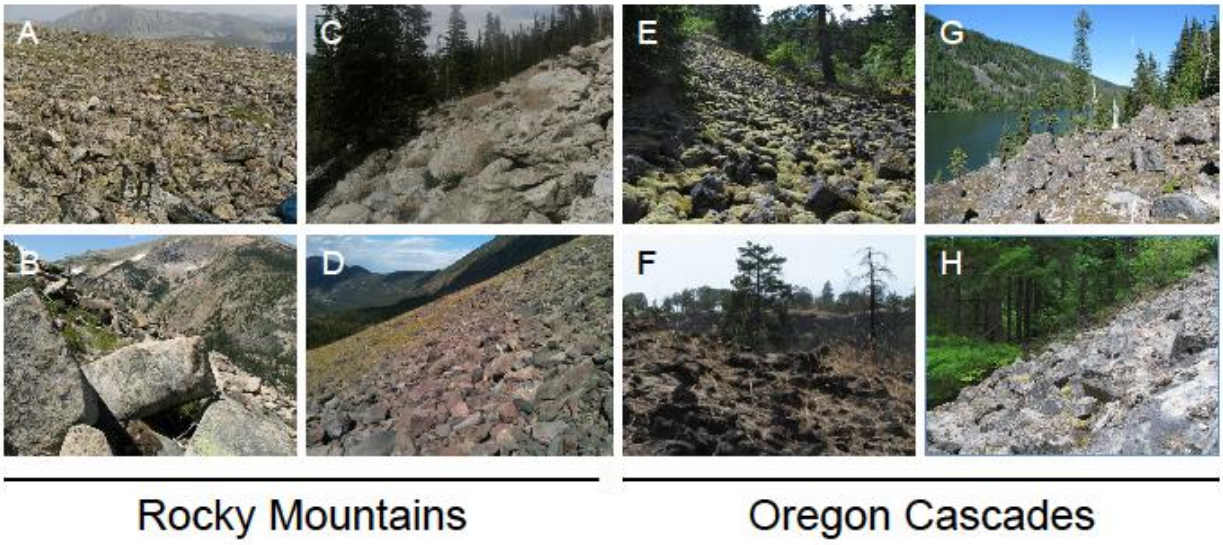
Analysis 1: GLVW	ELEV	AST	DB-10	PSG	SUMDTR
ELEV	1				
AST	-0.15923	1			
DB-10	0.271024	-0.00583	1		
PSG	0.722987	-0.13947	0.315352	1	
SUMDTR	0.254854	0.638894	0.098247	0.300302	1

Analysis 2: NWT	ELEV	AST	DB-10	PSG	SUMDTR
ELEV	1				
AST	-0.10914	1			
DB-10	-0.64878	0.365056	1		
PSG	0.525785	-0.37318	0.91084	1	
SUMDTR	-0.59191	0.552756	0.55643	0.58389	1

Analysis 3: Both Sites	ELEV	AST	DB-10	PSG	SUMDTR
ELEV	1				
AST	-0.06649	1			
DB-10	0.532501	0.506103	1		
PSG	0.61131	-0.52243	-0.34405	1	
SUMDTR	0.130451	0.650676	0.862297	-0.3143	1

APPENDIX III

Pika habitats where pika fecal samples were exposed as described in Chapter 4. Site abbreviations are listed in Table 1 (Chapter 4). In the Rocky Mountains: (A) NWT, (B) RMNP, (C) BRLA, (D) EL. In the Oregon Cascades: (E) WY, (F) MP, (G) LL, (H) HC.



APPENDIX IV

Map showing current distribution of the North American pika (*Ochotona* princeps) and pika habitat as described in Chapter 4.