ASSESSING VARIATION IN STRESS AMONG AND WITHIN AMERICAN PIKA (*OCHOTONA PRINCEPS*) TERRITORIES

by

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B.S., Colorado State University, 2011

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

Master of Arts

Department of Ecology and Evolutionary Biology

2019

This thesis entitled:

Assessing variation in stress among and within American pika (*Ochotona princeps*) territories

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Assessing variation in stress among and within American pika (*Ochotona princeps*) territories

Thesis directed by Professor Katharine Suding and Research Associate Chris Ray

ABSTRACT

As climate change alters ecosystems, how wild animals respond to changes in their habitats will determine their ability to persist into the future. By monitoring stress levels of individuals, we can concentrate conservation efforts on populations at most risk of decline before population loss is apparent. I used the American pika (*Ochotona princeps*), a highly territorial small mammal, to investigate the stress response to habitat quality. First, I investigated individual level differences in stress among territories of pika through time. Second, I investigated population level differences in mean stress of pika occupying two types of habitats. Observed patterns in stress across landscapes in our study suggest that stress could be used to evaluate seasonal variation in habitat quality. Our approach could be used in other wildlife studies to refine our understanding of habitat quality and its effect on individual stress levels as a driver of population decline.

DEDICATION

This thesis is dedicated to my family. Thank you for always believing in me.

AKNOWLEDGEMENTS

It really takes a village to successfully complete a graduate program. I would like to first thank my advisory committee; Drs Chris Ray, Katie Suding, Rebecca Safran, and Carol Wessman. Thank you for your insight and for guiding me through the thesis process. A special thank you to Dr. Chris Ray. Thank you, Chris, for your unwavering patience and thoughtful guidance. You challenged me to be a better scientist, writer, and student and I have learned so much from you. Thank you to all the people who helped me in the field and lab; Max Wasser, Nadiv Edelstein, Ryan Agabani, Micaela Seaver, Claire Atkins, Airy González, Brad Schrom, and Tyler Lampard. All of you had great attitudes and your hard work made my research possible. A special thank you to the Suding lab as well. Your advice and insights were invaluable in helping me think more deeply about my research. Each of you are amazing scientists, I learned so much from our discussions and in learning how you develop your own research. Most importantly, I would like to thank my family for their support and for always believing in me. I would not have been able to do any of this without the support of my husband, Elliot Martin. Elliot was my field assistant, cheerleader, and confidant through the whole process. He made so many sacrifices to help me realize my dreams. It was a difficult and tiring road, but we did it!

Many labs provided me with space and expertise to conduct fecal stress hormone analysis. First, thank you Dr. Johanna Varner for teaching me laboratory techniques and for always being available for questions. Second, thank you to the Martin, Schmidt, and Lowry labs for allowing me to use their lab space and lab equipment. Lastly, thank you to the Alisa Hove lab at Warren Wilson College, and the Jessica Castillo Vardaro lab at the University of California, Berkeley for providing genetic analysis of fecal samples.

Funding for this research was provided by the CU Boulder Department of Ecology and Evolutionary Biology Graduate Student Research grant, Indian Peaks Wilderness Alliance Paddon/Gellhorn Wilderness Research grant, and the Boulder County Nature Association Research grant. In-kind funding was provided by the National Parks Service (Task Agreement P18AC00585) and the Niwot Ridge Long-Term Ecological Research site.

CONTENT

TABLES

Chapter 1:

Chapter 2:

FIGURES

Chapter 1:

Chapter 2:

Chapter 1

TEMPORAL ANALYSIS OF FECAL GLUCOCORTICOID METABOLITES TO EXPLORE VARIATION WITHIN AND AMONG TERRITORIES OF A CLIMATE-SENSITIVE SMALL MAMMAL

ABSTRACT

How a wild animal responds to stressors in its environment can tell us a lot about individual health. However, few studies characterize how stress varies in wild animals over time and among habitat types. Here, we use the American pika (Ochotona princeps), a highly territorial small mammal, to investigate inter- and intra-individual differences in stress. For individually territorial animals such as pika, differences in habitat quality among territories should lead to differences in the levels of chronic stress exhibited by territory owners. In this study, we collected feces non-invasively from pika territories every two weeks from June to September of 2018. We indexed stress by measuring stress hormone metabolites in feces. We looked for two trends: 1) differences in mean stress level, suggestive of differences in territory quality; and 2) coherent variation in stress among territory owners over time, suggesting seasonal effects on physiology independent of territory quality. We used linear mixed-effects models to model temporal variation in stress as a fixed effect of day-of-year, and to model spatial variation in stress using fixed effects of broad habitat characteristics (elevation, aspect and site) as well as local habitat characteristics (depth of talus, size of rocks, and available forage types). We found that stress varied more through time than among territories in our population of pika, indicating similar habitats among territories and shared seasonal stressors among individuals. Our approach

could be used in other wildlife studies to refine our understanding of habitat quality and its effect on individual stress levels as a driver of population decline.

INTRODUCTION

Reversing the current, unprecedented rate of human-caused biodiversity loss (IUCN 2019) will depend in part on identifying stressed populations before they are lost, as well as determining how the environment can mediate stress. A growing body of work categorized as conservation physiology provides tools for discovering the mechanisms that support biological diversity and govern individual and population response to environmental change and stressors (Cooke et al. 2013). Almost half of the work published in the journal 'Conservation Physiology' over the last five years has focused on stress physiology. In a summary of 287 papers published on stress physiology in wildlife from 1990 to 2007 (Baker et al. 2013) the majority focused on the stress response to capture and handling. Only a small number of studies focused on stress response to changes in land use or environmental influences (Baker et al. 2013), such as habitat quality. If low quality habitats do not provide the energy needed to sustain allostasis (physiological stability in the presence of actual or perceived stressors), then additional energy demands could push an individual into allostatic overload (a cumulative effect of chronic stress) and reduce individual fitness (McEwen & Wingfield 2003). For individually territorial animals, differences in habitat quality among territories should lead to differences in the levels of chronic stress exhibited by territory owners. Therefore, any consistent difference in metrics of chronic stress among territory owners would suggest that physiological stress can be used as a metric of habitat quality.

There are many factors that can affect stress in wild organisms, such as extreme weather events or exposure to predators, and it has been shown that stress varies seasonally in many free-ranging vertebrates (Baker et al. 2013). If the stress level of each individual in a population

varies predictably in response to an environmental stressor, then that response can be used as a metric of population state (Madliger & Love 2014). However, few studies have quantified seasonal patterns in stress within and among individuals. A study of the capercaillie, a non-migratory grouse, found that sex and individual identity accounted for as much as 45% of the variance in stress among free-living individuals, while environmental variables accounted for only 5% of the variance (Coppes et al. 2018). By identifying individuals, Coppes et al. (2018) were able to detect strong seasonal variation within individuals that differed between sexes, highlighting the need to track stress in known individuals, especially when individuals are non-territorial and might share common habitats. Here, we argue that differences in the habitat or environment surrounding each individual should affect differences in the mean stress level among individuals over time, while individuals occupying similar habitats should exhibit more similar mean stress levels over time. Individuals in similar habitats might also exhibit temporally coherent variations in stress, while individuals in dissimilar habitats might experience 'incoherent' variations in stress that differ in phase and frequency (Fig. 1). If stress varies coherently among individuals over time, then climate or seasonal processes are important covariates of stress (Fig. 1a). If stress varies among individuals, then each individual is experiencing different stressors. Differences in mean stress level among individuals might signal long-term differences in exposure to stressors, such as differences in habitat quality. Of course, these processes are not mutually exclusive (Fig. 1b).

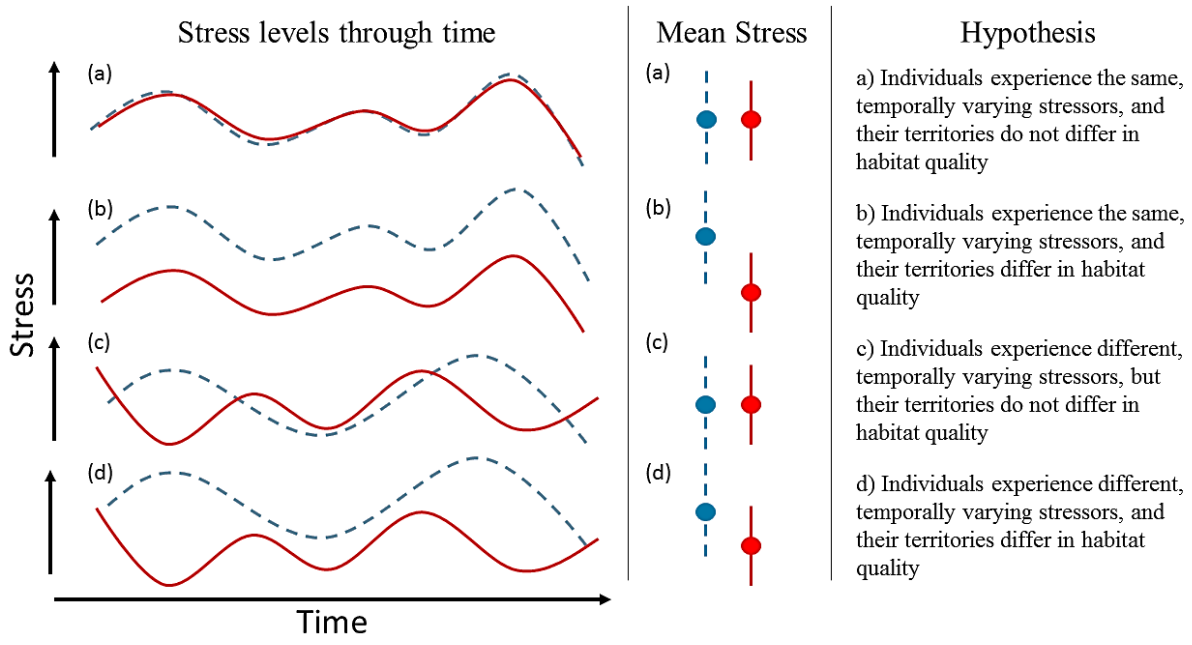

Figure 1: Examples of hypothesized variation in stress for two hypothetical individuals (blue symbols = individual A, red symbols = individual B). Temporal variation (left-hand panel) that is coherent (a and b) or incoherent (c and d) is superimposed on potentially habitat-mediated differences in mean stress level (central panel). Mean stress levels in territorial animals should be affected by habitat quality. In this example, individual A shows higher stress in (b) and (d), suggesting that it might occupy a territory of lower quality.

Our ability to measure effects of habitat quality on stress might vary with the scale of analysis and with our understanding of what constitutes high-quality habitat. For capercaillie, effects of habitat quality (proportion of open forest) on stress were apparent at very local scales (within a 20-m radius), but not at home-range scales (400-m radius) (Coppes et al. 2018). Canopy cover was patchy within each home range, so the strength of its effect was difficult to detect when canopy cover was averaged at that scale (Coppes et al. 2018). In a study of spotted salamanders, Homan et al. (2003) failed to find effects of habitat quality on individual stress levels, but also questioned whether the metrics of habitat quality used in their study (levels of canopy cover and soil moisture) were adequate indicators of habitat quality for spotted salamanders. Considerations of study scale and habitat metrics should be more straightforward

for territorial species with relatively small and permanent territories, as well as for species with well-characterized habitat needs and physiological limits.

We used the American pika (*Ochotona princeps*) as a model to explore variation in stress within and among individuals. The American pika (hereafter, pika) is a small (~150-g) mammal that lives in rocky habitats in western North America, including talus slopes, boulder-fields and lava beds. Pika are individually territorial and do not migrate after establishing a territory. Pika territories are relatively small (14-34 m in diameter; Smith and Weston 1990) and are generally centered on a winter food cache or 'haypile' of graminoids (such as grasses) and forbs (flowering plants) that are harvested during the growing season and consumed over winter. Pika do not migrate or hibernate, so each is exposed year-round to the habitat contained within its territory. Pika occurrence and persistence appears to be influenced by snowpack, association with sub-surface ice features, and climate variables (Hafner 1994, Millar and Westfall 2010, Wilkening et al. 2015, Yandow et al. 2015, Johnston et al. 2019). Experiments have shown that pika cannot tolerate temperatures above 24°C without immediate access to cooler temperatures, which likely explains their strong affinity for rocky habitats that harbor relatively cool and mesic sub-surface microclimates (MacArthur & Wang 1974, Smith 1974, Hafner 1993, 1994; Henry et al. 2012; Smith et al. 2016).

Pika are currently experiencing range retractions (Beever et al. 2016, Jeffress et al. 2017) and local extirpations (Nichols et al. 2016, Jeffress et al. 2017, Stewart et al. 2017). Climate has been implicated as a driver of pika losses (Hafner 1994; Grayson 2005; Beever et al. 2003, 2010, 2011; Wilkening et al. 2011, 2015; Stewart et al. 2017; Rodhouse et al. 2018), and several range projections suggest dramatic losses in response to climate change during this century (Galbreath et al. 2009, Calkins et al. 2012, Stewart et al. 2015). However, climate vulnerability predictions

for pika in eight national parks (Schwalm et al. 2016) suggest a more complex future in which some populations might persist into the next century, while others are extirpated. This potential complexity was corroborated by a range-wide analysis showing that the determinants of pika occurrence appear to vary by ecoregion (Smith et al. 2019). However, we note that all studies of pika range dynamics have relied on occurrence data to determine past and present pika habitat. None have used metrics of individual stress to refine our understanding of pika habitat quality. All predict pika vulnerability in terms of local extirpation, rather than the potential for population declines that might be reversed (before extirpation) through management interventions informed by an understanding of how habitat quality mediates stress in individuals. This deficit in our understanding of habitat-mediated stress, combined with the complexity of projected pika futures, highlights the potential for studies of physiological stress to advance pika conservation. The potential to characterize the relative phase and frequency of changes in stress, as well as differences in mean stress level (Fig. 1) should make stress analyses valuable for differentiating purely temporal drivers of stress from those with a spatial component suggestive of habitat effects.

We focused on a common metric of physiological stress that can be measured non-invasively from fecal samples deposited by free-living animals, to address questions about the spatio-temporal pattern of stress in pika occupying high-elevation habitats in Boulder County, Colorado. Glucocorticoids are an important class of stress hormones in many animals (Madliger et al. 2018). Chronically high glucocorticoid levels can lead to decreased body mass, reduced reproduction, and increased mortality (Dantzer et al. 2014). To explore hypotheses presented in Figure 1, we used data on stress measured over time across a number of pika territories to model temporal variation in stress as a function of day-of-year, and spatial variation in stress as a

function of broad habitat characteristics (elevation, aspect and site) as well as local habitat characteristics (depth of talus, size of rocks, and available forage types) to explore differences in mean stress level driven by territory quality. We predicted that samples collected from different territories would differ in mean stress level, allowing us to model stress as a function of local habitat characteristics. Our approach could be used in other studies to refine our understanding of habitat quality and its effect on individual stress levels as a driver of population decline.

MATERIALS AND METHODS

Study System

Fecal pellets were collected from pika living above and below treeline within the Niwot Ridge Long-Term Ecological Research site (40° 3'N 105° 36'W). On Niwot Ridge, pika occupy talus (broken rock fields) with varying rock size (up to 2 m long) and talus depth (0.5 m to 1.5 m deep). Fecal pellet sampling stations (hereafter, stations) were established at haypiles separated by at least 50 m, which we assumed to belong to different individuals occupying distinct territories. Stations (one per territory) were located in 20 territories at varying elevations (3279 - 3616 m) and aspects (six north facing, six south facing, and eight east facing) in three study sites: Cable Gate (CG), West Knoll (WK), and Long Lake (LL) (Fig. 2). WK was above treeline on a knoll with taluses facing east, north and south. CG and LL were below treeline with taluses facing south (CG) and north (LL). CG contained four stations, WK thirteen, and LL three (Fig. 2).

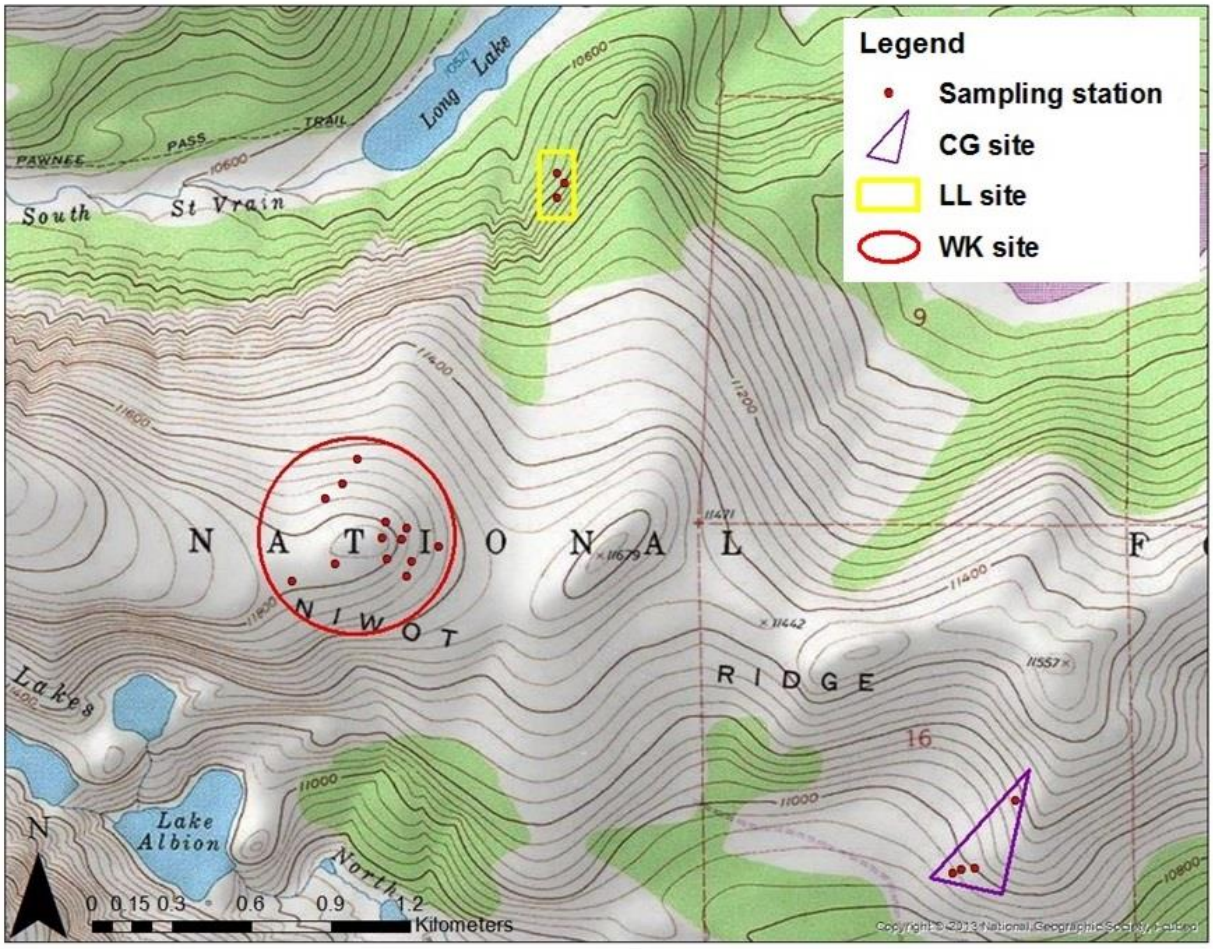

Figure 2: Study site locations on Niwot Ridge, Boulder County, Colorado, USA. Each red dot within a site is a fecal pellet sampling station (n=20) located within a unique territory.

Collection Method

Pika produce two types of feces, the first being a soft cecal feces that is re-ingested and later deposited as firm pellets (Smith and Weston 1990; Nichols 2010). Pellets change color and texture as they age (Nichols 2010), so we collected only "fresh" pellets that were greenish in color and still fibrous inside. In addition, we collected only adult pellets (>2.75 mm in diameter) because stress levels can vary between age classes (Wilkening et al. 2013). Each station was visited once every two weeks from June 8 through September 4, 2018. During each visit, fresh pellets were collected if available, and other pellets were cleared to facilitate identification of

fresh pellets on subsequent visits. Samples were stored in paper envelopes labeled with sampling date and coordinates. To avoid contamination between samples, we scooped up pellets using the envelope flap or pushed pellets into the envelope using a stick or rock. We attempted to collect 10-15 pellets per sample (0.1 grams total) to facilitate GCM analyses (Wilkening et al. 2013). Samples were stored at -20°C.

Sex determination

Because stress levels can vary between sexes (Wilkening et al. 2013), we split each sample (when sample size allowed) to allow for pika sex determination through genetic analysis. Subsamples were sent to Warren Wilson College (Alisa Hove Lab) for DNA extraction to determine the sex of the pika depositing fecal pellets at each sampling station. Briefly, the Hove Lab used a Qiagen DNeasy Fast Stool kit to extract DNA from fecal samples. DNA was amplified through multiplex PCR, targeting both *SRY* and *Ocp10* genes using primer sequences found in Table 1 (Lamb et al. 2014). PCR was performed three times for each sample, generating three replicates of the PCR product. The recipe for each PCR reaction contained: 11 µL GoTaq Green Master Mix (2x), 1 µL of each 10 µM *SRY* primer (forward and reverse), 1 µL of each 10 µM *Ocp10* primer, and 2 µL of extracted sample DNA. Two negative control reactions, lacking extracted sample DNA, were included for each grouping of PCRs to ensure that contamination leading to sex misidentification was not occurring. PCRs were performed using the following cycling parameters: 94˚C for 2 min, 40 cycles of 94˚C for 30 s, 51˚C for 30 s, and 72˚C for 30 s, and 72˚C for 5 min. After completion of all cycles, samples were held at 4˚C. Gel electrophoresis was performed on a 2% agarose gel. Samples were identified to sex using to the following criteria: male = *Ocp10* fragments were present in at least one of the three replicates, and *SRY* fragments were present in at least two of the replicates, or *Ocp10* and *SRY* fragments

were both present in the same replicate; female = *Ocp10* fragments were present in at least one of the three replicates, and *SRY* fragments were never present; unknown = only *SRY* fragments were present across all of the replicates, or target genes never amplified**.**

Table 1: Primers used in this study targeting *O. princeps*–specific Y-linked and autosomal regions (adapted from Lamb et al. 2014).

Region	Primer sequence	Size (bp)	References
Sex-determining region Y-chromosome (SRY)	F: AATGCATTCATACTATGGTC	117	Lamb et al 2014
	R: CTCTGTAAGCTTTTTCCACTG		Lamb et al 2014
Autosomal microsatellite (Ocp10)	F: TCCCAGTCACGACGTCCAATTTGGCTGTTA	179-203	Peacock and Kirchoff, unpublished (GQ461705)
	R: GTTTCTTCCAGTGTCTGGCATACGGTAAGC		Peacock and Kirchoff, unpublished (GQ461705)

Hormone analysis

Glucocorticoid metabolites (GCMs) are metabolized stress hormones present in fecal material that can be used as a measure of glucocorticoids in the body (Keay et al. 2006; Homyack 2010; Dantzer et al. 2014). GCMs in feces represent a time-averaged metric of glucocorticoids in the body (Romero and Reed 2005; Sheriff et al. 2011; Dantzer et al. 2014). GCMs are often used as a metric of relatively "chronic" stress (Keay et al. 2006; Dantzer et al. 2014), as opposed to metrics of "acute" stress such as the concentration of corticosterone in a blood sample (Romero and Reed 2005; Sheriff et al. 2011; Ellis et al. 2012). GCM concentrations were measured in fecal pellet samples (hereafter, samples) using a commercially available Corticosterone Enzyme Immunoassay Kit (cat. no. K014-H5; Arbor Assay Design, Inc., Ann Arbor, MI) previously validated for *O. princeps* (Wilkening et al. 2013). Each step in the laboratory process was completed according to the standard kit protocol. Briefly, samples were first dried in a DNA SpeedVac on medium heat for 30 minutes. Dried samples were weighted to ± .02g and combined with 200 proof ethanol in proportion to sample weight (e.g.,

1200µl of ethanol per 0.12g fecal sample). Samples were mashed in ethanol using a spatula and then shaken on a vortex shaker for 30 minutes. After shaking, samples were centrifuged and the supernatant was drawn off and transferred to a new vial. Supernatant was concentrated using a DNA SpeedVac on medium heat for 1 hour. Concentrated supernatant was stored in a -20°C freezer until all samples were prepared for the GCM assay. Frozen supernatant was thawed and reconstituted to its original volume using a mix of assay buffer and ethanol (<5% of volume) before loading onto enzyme immunoassay plates. Optical densities were read with a Thermo Scientific Multiskan EX Microplate Reader at 450nm using Ascent Software version 2.6. Samples, standards, and controls were assayed in triplicate and the average of each triplicate was used to calculate GCM concentrations. Samples were compared with seven standards of known corticosterone concentrations (5000, 2500, 1250, 625, 312.50, 156.25, and 78.125 pg/ml) and with non-specific binding (NSB) and maximum binding (B_o) controls. If a sample reading was outside of the range of the NSB or B_o averages, then the reading was dropped from analysis. Sample concentrations were calculated using a standard curve delineated by the standard concentrations. Final fecal GCM concentrations were reported as picogram/gram of dried feces. Inter-assay coefficients of variation were 2.6 and 8.5% for the low and high binding controls and 14.7 and 6.3% for low (78.125) and high (5000) standards, respectively. Intra-assay coefficients of variation were between 4.2 and 8.95% for five plates.

Linear mixed-effects models

Fecal GCM concentration was log-transformed and used as the response variable in linear mixed-effects models to characterize variation in stress among stations (territories) and within stations over time. We fit a set of models based on up to six fixed effects (Table 2) plus random effects of station (to account for repeated measures at each station) and date (to account for

spatially coherent effects of weather). Predictor variables were selected to explore fine scale habitat characteristics after accounting for effects of lab procedures (lab), inter-individual differences (sex), temporal trends, and broad habitat characteristics (Table 2). Fixed effects of day of year (DOY, from 0 to 364) and DOY^2 were used to model any seasonal trend, peak or trough in stress. Fixed effects of site, aspect, and elevation were included to model broad scale spatial effects on stress that might affect groups of adjacent territories or territories in sufficient proximity. Interaction terms of day of year squared and elevation, and day of year and site were also explored as we hypothesize that elevation and site location will influence the timing of seasonal stressors such as snow melt or plant senescence. A subset of models included fixed effects representing fine scale habitat differences between territories, each measured within a 12-m radius of the haypile. These fixed effects included talus depth (depth in meters of deepest crevice, estimated visually from bottom of surface rock to ground under talus), size of largest rocks (L_{R1} and L_{R10}, where L = length of longest axis in meters, $R1$ = largest rock, and $R10$ = tenth-largest rock), variation in size of largest rocks ($[L_{R1} - L_{R10}] / [0.5(L_{R1} + L_{R10})]$), and extent of six land-cover classes (visual estimate of percent cover of rock, bare ground, graminoids, forbs, shrubs, and trees) (Table 2). Multiple effects of rock characteristics were explored because we lacked prior knowledge of how rock structure may affect stress in pika. Fixed effects of pika sex were also expected (Wilkening et al. 2013). Sex was categorized as male, female, or unknown. Unknown sex could stem from failure to determine sex through genetic analysis or inability to perform genetic analysis due to small sample size (too few pellets to conduct both genetic and stress analyses). Finally, a fixed effect of enzyme immunoassay plate was also considered to account for any potentially spurious effects of our laboratory procedures that might affect each subset of samples that was analyzed on the same plate (and day). Predictor variable

covariance was calculated using Pearson's rho (continuous variables), Spearman's rank correlation coefficient or Kendall's tau, and variables with high correlation (>0.7) were not included in the same model (Appendix I).

Table 2: Candidate predictor variables used in a set of 20 linear mixed-effects models to predict glucocorticoid metabolite (GCM) concentrations, plus the hypothesized effects of each predictor variable.

Predictor	Hypothesized effect
Plate	Lab
Sex	Inter-individual
DOY^2	Temporal
DOY	Temporal
Elevation (m)	Broad scale habitat
Aspect (^{0})	Broad scale habitat
Site	Broad scale habitat
Variation of 10 biggest rocks	Fine scale habitat
10th biggest rock	Fine scale habitat
Mean rock size of 10 largest rocks	Fine scale habitat
Biggest rock	Fine scale habitat
Graminoid : Forb	Fine scale habitat
Deepest crevice	Fine scale habitat
Percent forb cover	Fine scale habitat
Combined percentage of graminoid, forb, and shrub cover	Fine scale habitat

To address our original hypotheses (Fig. 1), if day of year covariates alone garnered highest support in our models, then temporally coherent variation in stress is an important process in our system (Fig. 1a). If day of year covariates are not supported, then the variation in stress is temporally incoherent or negligible (Fig. 1c). If both day of year and broad or local habitat covariates are supported, then stress varies coherently over time but differs by territory quality (Fig. 1b). If only broad or local habitat covariates are supported, then differences in territory quality are a dominant process in our system (Fig. 1d).

Models were fit using the lme4 package (Bates et al. 2015) in R 3.5.1 (R Core Team 2018). We ranked models using Akaike Information Criterion adjusted for a small sample size (AICc) (Burnham and Anderson 2002). Relative support for each model was calculated using ΔAICc = focal model AICc – minimum AICc of all models considered here. We assume that models with ΔAICc > 2 have lower support than the "top" model (ΔAICc = 0).

RESULTS

Of the 109 samples collected, 20 were collected from Cable Gate (CG), 9 from Long Lake (LL), and 80 from West Knoll (WK). The elevation of stations ranged from 3337 m to 3407 m at CG, 3277 m to 3307 m at LL, and 3559 m to 3617 m at WK. Three stations at CG had southwest facing aspects and one station south facing; all stations at LL had northwest facing aspects; WK had two stations with north facing aspects, two northeast facing, four east facing, two southeast facing, one south facing, one west facing, and one northwest facing.

Sufficient fecal sample size allowed for genetic analysis of samples collected from 17 of the 20 sampling stations to determine the sex of each territory owner. Genetic analysis of these 17 samples revealed that 11 were deposited by males, and the other six were deemed unknown due to DNA amplification failure (Fig. 3). No females were identified from the available fecal samples. The three sampling stations with insufficient sample size for genetic analysis were also categorized as unknown sex so they could be incorporated in model analysis.

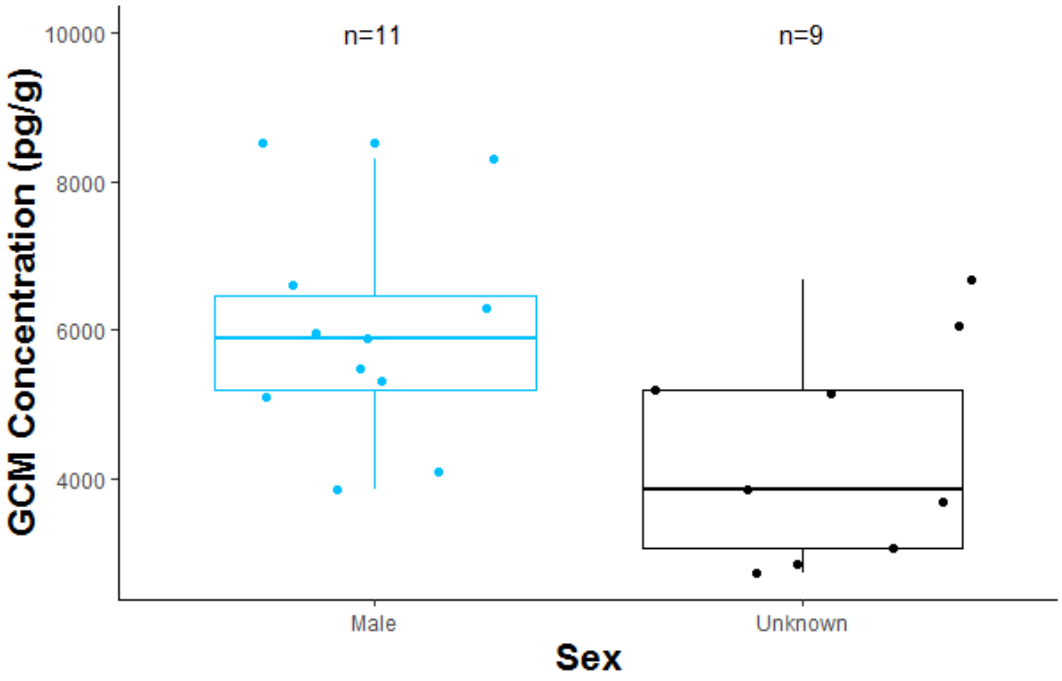

Figure 3: Glucocorticoid metabolite (GCM) concentration by sex. Eleven out of 20 territory owners were male, while the other remaining nine territory owners were of undetermined sex due to DNA amplification failure or insufficient sample size.

Tracking stress through time using a repeated measures study, we found that stress varied more within stations than among stations (Fig. 4). The null model, with only random effects of date and station, revealed that more of the random variance was explained by date (50.16%) than by station (17.82%). A scatterplot also suggests that GCM varied mainly within stations over time rather than among stations (Fig. 4).

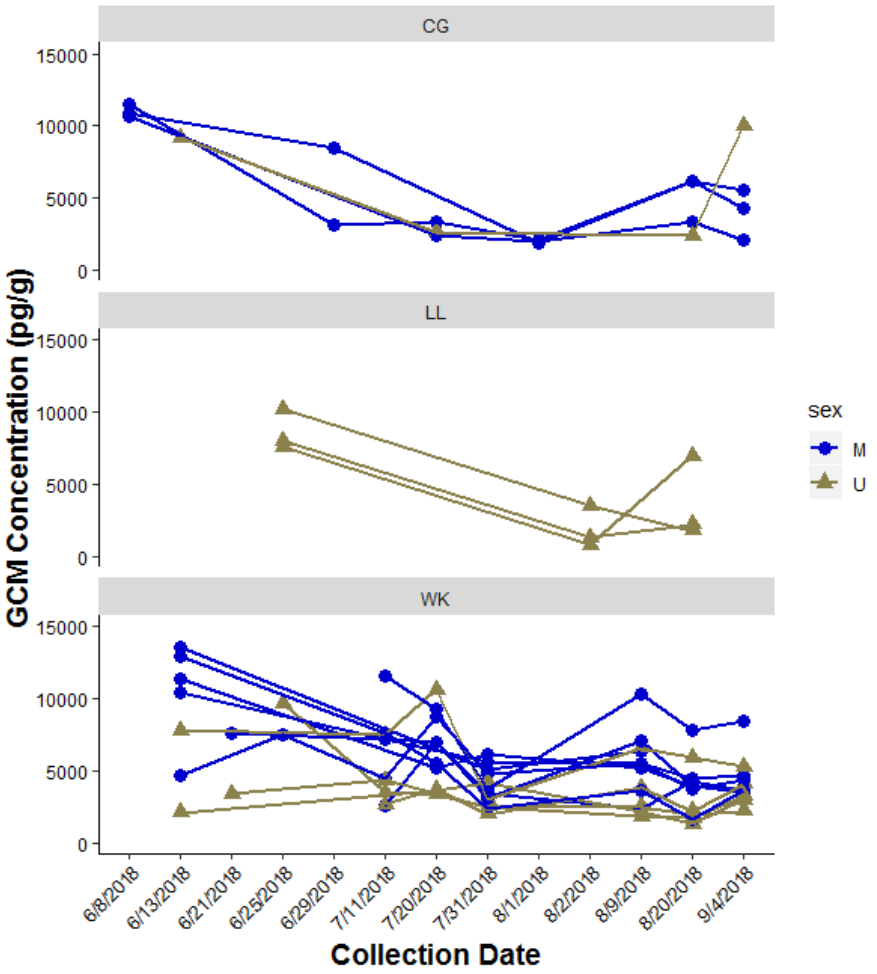

Figure 4: Glucocorticoid metabolite (GCM) concentration in picograms per gram (pg/g) by collection date. Each panel displays results from a different site with line color denoting sex. From top to bottom, GCM concentration from collection stations at Cable Gate (CG), Long Lake (LL), and West Knoll (WK).

When we compared models using ΔAICc (Table 3), the model of GCM with best support included a positive effect of male sex, a negative effect of DOY, and a positive effect of DOY^2. Other well-supported models included similar terms to the most-supported model. Models based on predictors related to broad habitat features (elevation, site, aspect) garnered much lower support (equivalent to the null model), and models based on territory-specific habitat features (rock size, crevice depth, vegetation characteristics) garnered very weak support.

Table 3: Relative support for models of glucocorticoid metabolite (GCM) concentration (log-transformed) collected from sampling stations at the Niwot Ridge LTER. Models are ranked in order of increasing

AICc values (Akaike Information Criterion adjusted for a small sample size, Burnham and Anderson 2002). ΔAICc is the difference between the focal model AICc value and the model with the lowest AICc value. The respective effect sign of each covariate is reported as positive (+), negative (-), or varied (var). Sample size (n) was 109 for each model.

Model	k	AICc	ΔAICc	effect sign	n
DOY, DOY^2, sex	7	164.9347	0	(-) , (+), (+)	109
Sex	5	166.7092	1.7745	(+)	109
DOY, DOY^2	6	166.7871	1.8524	(-), (+)	109
DOY^2 * elevation	7	169.1070	4.1723	(-)	109
Null	4	169.1527	4.2180	(+)	109
DOY^2 * site	9	169.7961	4.8614	var	109
Elevation, DOY, DOY^2, sex	8	170.5654	5.6307	(+). (-) , (+), (+)	109
Aspect, DOY, DOY^2, sex	8	170.7624	5.8277	(-), (-) , (+), (+)	109
Variation of 10 biggest rocks	5	171.4653	6.5306	(+)	109
10th biggest rock	5	172.8084	7.8737	(-)	109
Mean rock size of 10 largest rocks	5	173.0485	8.1138	(-)	109

Sampling date and EIA plate were somewhat confounded during analysis. Thus, we added plate as a fixed effect to the null model. The plate model ranked thirteenth amongst models (ΔAICc = 8.265), providing little support for a confounding effect of plate. Plate 3 contained samples from mid-season and exhibited most of the lowest glucocorticoid metabolite concentrations; however, the ranks of the top three models did not change when all samples from plate 3 were removed from the analysis.

DISCUSSION

While the time series of stress metrics we measured include pairs of individuals that match each of our four hypotheses (compare Figs. 1 and 4), our results mainly support hypothesis (a), that individuals experience the same, temporally varying stressors, and their territories do not differ in habitat quality. Our study revealed that glucocorticoid metabolites (GCMs) vary more over time within territories than between territories. Mean stress levels varied coherently through time and did not differ between territories, suggesting pika are experiencing shared physiological or

climate drivers rather than independent temporal processes within each territory. Low variation in stress among territories suggests that different territory owners might be exposed to similar stressors. Samples used in this study were collected in relatively close proximity (all within 3 km of each other) and did not vary greatly in elevation. Additionally, presence of water or ice under talus has a strong correlation with pika presence and persistence (Millar and Westfall 2010; Erb et al. 2011), but on Niwot Ridge permafrost is unlikely below 3700m (Janke 2005). Our highest sample location was at 3600m, suggesting that all of our samples were collected from locations likely without permafrost. Pika within our study sites might be experiencing similar habitats, and thus similar stressors, leading to lower variation among territories than within territories.

Temporal variation in our metric of stress was high within stations, suggesting that pika stressors might vary seasonally. We hypothesize that abiotic factors such as sub-surface talus temperature, and biotic factors such as animal interactions could be driving variation. Additionally, our most supported linear mixed-effects model supported the fixed effect of day of year (quadratic and linear), suggesting season to be an important driver of stress in this system (Table 3). Specifically, the quadratic day of year term fit the U-shaped curve seen in the data (Fig. 5). We hypothesize that GCM concentrations are highest in the beginning of June because winter haypiles are likely depleted and territories are being actively defended from new dispersers (young-of-the-year). Starting in mid-June adult pika initiate haypile storage activities (Krear 1965) and through September 25-55% of their surface activity is spent constructing haypiles (Dearing 1997). At this time, pika are settling into claimed territories, likely reducing GCM concentrations. Haypiles are developing by mid-August in our system (Dearing 1997), coinciding with when GCM concentrations are lowest (Fig. 5, week 4). GCM concentrations

may have increased again after sampling period 4 because pika were defending their newly developed haypiles.

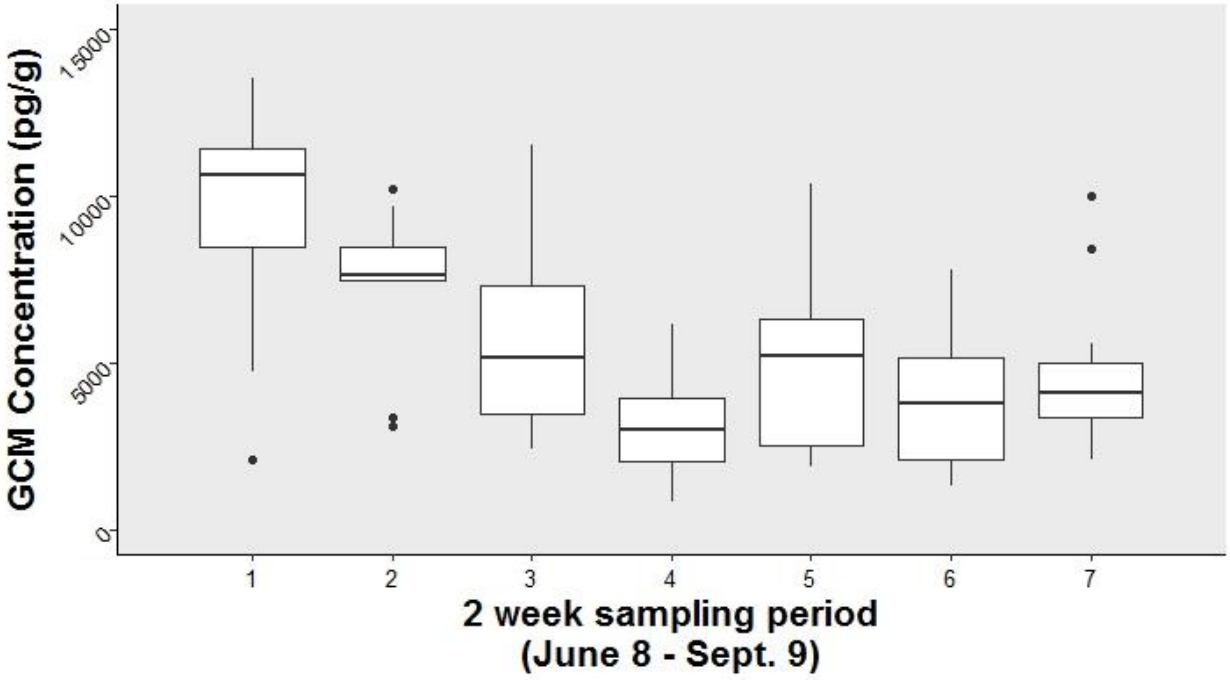

Figure 5: Glucocorticoid metabolite (GCM) concentration in picograms/gram for all 20 sampling stations grouped by two-week sampling periods. When available, fecal samples were collected from each station every two weeks from June 8 to September 4, 2018.

Stress levels in our study are within range of stress levels found in other studies of pika (2177 - 15800 pg/g; Wilkening et al. 2013, 2015; Wilkening & Ray 2016). Additionally, our results echo findings in other small mammals. Our average stress concentration was 5176 pg/g, similar to the baseline stress concentrations found in North American red squirrels (*Tamiasciurus hudsonicus*) (6040 pg/g, Dantzer et al. 2010). Our findings that stress was highest in the spring and decreased as the summer progressed have also been found in arctic ground squirrels (*Urocitellus parryii*), yellow-bellied marmots (*Marmota flaviventris*), and chipmunks (*Tamias* spp.) (Sherriff et al. 2012, Smith. et al. 2012, Hammond et al. 2018, respectively).

Sex determination, in every case, was based on samples from one time point during the collection period. Therefore, it is possible that the sex of the territory owner changed throughout the season and could have affected the variation in stress. Turnover rates in site occupancy are highly dependent on local influences but can be as high as 50% in some populations (Rodhouse et al. 2018). Genetic analysis revealed that the majority of territory owners within our study were male. However, six analyzed samples were designated "unknown" due to the lack of amplification of the target sex genes (Fig. 3). We do not attribute the lack of amplification due to errors in the laboratory procedures; rather, samples may not have contained enough genetic material. It is possible that females could be present in the six unknowns. Also, our sex ratios may be skewed towards males because males may be more territorial (Krear 1965) and could be more likely to mark their territories with conspicuous piles of fecal pellets that we would notice and collect. It's also possible that because males and females usually occupy adjacent territories (Krear 1965, Sharp 1973, Tapper 1973, Smith and Ivins 1984, Brandt 1989) that the spacing of our sampling stations may have skipped over the females.

We recognize that other factors such as reproductive status and social status that were not addressed in this study can affect stress (Baker et al. 2013; Dantzer et al. 2014). High levels of stress in the spring, as found in this study, could be attributed to the timing of reproduction. In pika, the first litter is usually conceived one month before snowmelt (Millar 1972, Smith 1978), coinciding with our first sampling period. Additionally, a social hierarchy may exist among territory owners, where some individuals are more aggressive and dominant over others (Kawamichi, 1976; Sharp 1973; Tapper 1973). In the spring when territories are empty from winter mortality, aggressive behavior may be highest as resident pika defend territories from unfamiliar immigrants (Smith and Ivins 1987). Using non-invasive techniques to measure stress

makes it difficult to determine the identity and sex of individuals, reducing our ability to analyze effects of individual level characteristics. But we believe that the methods for collecting stress data at the population level outlined here is accessible to managers and could be easily adapted to already established pika monitoring efforts, allowing us to capture much needed baseline stress data for the species.

Our results suggest that a pika population can coherently respond to seasonal stressors and recommend that conservationists assess potential climate stressors when managing populations. Though we saw more variation temporally than spatially, we suggest that other studies be done within more heterogeneous landscapes and at larger scales to determine if this pattern holds true. Further studies will also help determine whether non-invasive analysis of fecal pellets could provide insights on the relative quality of pika territories. Using stress as a metric of habitat quality could help manage and preserve the species before population declines become evident, and the effects of habitat quality on stress is an understudied topic in conservation physiology (Homyack 2010; Baker et al. 2013) that holds promise for a more mechanistic understanding of population health.

Chapter 2

ASSESSING HABITAT QUALITY USING STRESS MEASUREMENTS IN AMERICAN PIKA POPULATIONS

ABSTRACT

As climate change progresses, a range of biotic and abiotic influences will inevitably change ecosystems. How animals cope will depend on whether these changes disrupt ecosystem processes. If climate change degrades habitat quality, and habitat quality becomes a chronic stressor, population health can decline. We investigated habitat quality by looking for differences in fecal stress hormone levels between two habitat types. We use the American pika (Ochotona princeps) to explore effects of habitat quality because pika persistence has been linked to climate-related variables like vegetation quality and the presence of sub-surface ice. Previous research has suggested stress level differences between populations living with or without the presence of sub-surface ice. We collected feces non-invasively from pika territories in the spring and fall of 2018 to characterize variation in stress within and among 8 populations occupying paired habitat types—active and fossil rock glaciers. In active rock glaciers, a carapace of fractured-rock "talus" helps maintain sub-surface ice, while fossil rock glaciers no longer maintain ice. We found support for either elevation (a proxy of habitat quality) or rock glacier type (correlated with elevation) as important covariates of stress in linear mixed-effects models. Stress was higher at lower elevations and in fossil rocks glaciers, but this effect was evident only for samples collected in the spring. Sub-surface temperature sensors were placed in talus habitats to characterize temperature-driven differences among habitats. We explored temperature metrics previously shown to influence pika occupancy such as acute and chronic cold stress, as well as

metrics to explore pika response to pre- and post-winter sub-surface temperatures. We found no support for temperature differences between habitat types, or evidence of temperature-driven variation in stress. Our results provide evidence that stress metrics can be used to assess seasonal variation in habitat quality.

INTRODUCTION

Climate change is predicted to influence range dynamics for many species over the coming decades, with general trends showing upslope and poleward shifts in species distributions (Chen et al. 2011). Developing methods for evaluating those predictions in the short term, rather than waiting for range adjustments, would speed the refinement of conservation efforts. Wildlife management has long relied on monitoring techniques that measure population size and distribution to understand population health. However, these techniques can only detect population decline after the fact (Ellis et al. 2012). In a summary of 950 recent papers on relationships of animals (invertebrates and vertebrates) to a measure of habitat (Ellis et al. 2012), the majority focused on distribution-related responses such as presence/absence or abundance. Only 36 studies measured a process-driven response, like stress, to habitat factors (Ellis et al. 2012). Wildlife populations live in a patchwork of habitats across landscapes, and constantly experience changes in localized environments. How animals cope with these changing conditions can affect their fitness (Sheriff et al. 2011). Animals in low quality habitats may not have access to the energy required for allostasis (physiological stability in the presence of actual or perceived stressors), resulting in allostatic overload (a cumulative effect of chronic stress) that reduces individual fitness (McEwen & Wingfield 2003). Stress in individuals can therefore signal effects of habitat quality, and the stress response of a population can be used as a metric of population health (Madliger & Love 2014).

Stress physiology metrics have the potential to help conservationists understand habitat-mediated effects on populations (Homyack 2010). For example, in seabirds called red-legged kittiwakes (*Rissa brevirostris*), young who were fed poor-quality diets exhibited higher levels of stress (Kitaysky et al. 2006). In capercaillie (*Tetrao urogallus)*, a non-migratory grouse, stress was significantly lower in small-scale open canopy habitats (a metric of good quality habitat for the species) (Coppes et al. 2018). In many species, chronically high glucocorticoid levels can lead to a decrease in body mass, reduced reproduction, and increased mortality (Dantzer et al. 2014).

Understanding which habitats produce higher average stress at the population level could help managers prioritize conservation efforts. Here, we ask whether glucocorticoid metrics can be used to understand habitat quality. Specifically, we ask whether the amount of glucocorticoid metabolites measured non-invasively in the feces of free-roaming individuals of a species will vary with metrics of habitat quality that are understood to limit the species' range. We focus on a species long-studied as a model for biogeographic response to climate, with strong habitat associations and a recent history of population losses that have been attributed to impacts of climate on habitat quality.

The American pika (*Ochotona princeps*) has long been understood to require the relatively cool and mesic habitats offered by fractured-rock habitats known as taluses, boulder fields and lava beds (Hafner 1994; Henry et al. 2012; Smith et al. 2016). Most of these habitats are produced at higher elevations by freeze-thaw processes, and pika are generally associated with permafrost and peri-glacial features (Hafner 1994; Millar & Westfall 2010). Range retractions (Beever et al. 2016; Jeffress et al. 2017) and local extirpations (Nichols et al. 2016; Jeffress et al. 2017; Stewart et al. 2017) have been documented for pika, with climate being

implicated as a driver of these losses (Hafner 1994; Beever et al. 2003, 2010, 2011; Grayson 2005; Wilkening et al. 2011, 2015; Stewart et al. 2015, 2017; Rodhouse et al. 2018). Several range projections suggest dramatic losses in response to climate change during this century (Galbreath et al. 2009; Calkins et al. 2012; Stewart et al. 2015). However, climate vulnerability predictions for pika in eight national parks suggest a more complex future in which some populations might persist into the next century, while others are extirpated. It is important to note that each of these predictions were based on surface climate projections and did not consider sub-surface microclimates that can extend the range and persistence of this species (Varner & Dearing 2014).

We use presence of sub-surface ice as a metric of good-quality habitat for pika. We make this assertion based on evidence that pika cannot tolerate temperatures above 24°C without immediate access to cooler temperatures (MacArthur & Wang 1974; Smith 1974; Hafner 1993, 1994; Henry et al. 2012; Smith et al. 2016), as well as the growing body of work that argues for a close relationship between rock-ice features (RIFs) and pika distribution. A survey of pika sign (current or old) at 420 sites in the southwestern United States found that the majority of occurrences were on RIFs (Millar & Westfall 2010). In the southern Rocky Mountains, rocky slopes are hypothesized to have been created and maintained by alpine permafrost and, as recently as 1994, it was estimated that current populations of pika were within 5 km of current permafrost (Hafner 1994). RIFs provide sub-surface microclimates that are more moderate than ambient temperatures, being cooler in the summer, warmer in the winter, and less variable year-round (Millar & Westfall 2010; Millar et al. 2013; Wilkening et al. 2015). Furthermore, pika inhabiting an area associated with RIFs exhibited lower stress than those in an area without RIFs (Wilkening et al. 2015).

Previous studies have confirmed that fecal hormone analysis can be used to assess physiological stress in pika (Wilkening et al. 2013, 2015; Wilkening & Ray 2016). Fecal stress hormones increase in response to an induced stressor (such as capture and handling) and the measurement of fecal hormones is sensitive enough to detect inter-individual stress level differences in pika (Wilkening et al. 2013). Further, pika that exhibited higher fecal hormone levels were found to be less likely to survive one year after capture (Wilkening & Ray 2016), pointing to the utility of fecal hormones as an indicator of individual fitness After appropriate validation, Wilkening et al. (2015) found that stress varied between two sites, one with and one without sub-surface ice. The use of stress as a potential indicator of habitat quality could provide information about environmental change that may be missed by using abiotic metrics alone (Wilkening et al. 2015).

Here, we investigated whether stress could be an indicator of habitat quality for pika and whether the results of Wilkening et al. (2015) are generalizable across landscapes. We focused on fecal hormone levels to address questions about habitat quality and its effect on stress in pika occupying RIF habitats in Boulder County, Colorado. Expanding on Wilkening et al. (2015), we sampled pika inhabiting several "active" rock glaciers considered to harbor sub-surface ice and several "fossil" rock glaciers considered devoid of sub-surface ice. We predicted that fecal samples collected from active and fossil rock glaciers would differ in mean stress levels, allowing us to model stress as a function of habitat-driven differences such as temperature (acute or chronic cold and heat stress, average winter temperature, and variation in winter temperatures) after accounting for effects of sex and seasonality. Temperature-sensing data loggers were placed under the surface of the talus to record the sub-surface temperatures experienced by pika in spring and fall at each study site. Results from this study could add evidence that RIFs are

important microhabitats for the species. Further, knowledge of habitat quality and its effect on population stress could help refine conservation efforts and inform predictions of future pika distributions.

MATERIALS AND METHODS

Study system

The American pika (hereafter, pika) is a small (~150-g) mammal that lives in rocky habitats (talus slopes, boulder fields, and lava beds) in western North America. Pika defend relatively small territories (14-34 m in diameter; Smith & Weston 1990) and do not migrate after establishing a territory. Furthermore, pika do not hibernate, so each individual is exposed year-round to the habitat contained within its territory. Each territory owner usually builds a winter food cache or 'haypile' of graminoids (such as grasses) and forbs (flowers) that is created during the growing season and consumed over winter (Dearing 1997). Haypiles are typically located near the centroid of a pika's territory (Huntly et al. 1986; Roach et al. 2001). We determined territory locations by the presence of fresh haypiles or fresh defecations at old haypile sites

Fecal pellets were collected from pika territories located in active and fossil rock glaciers within two sites along the Front Range of Colorado: Rocky Mountain National Park (40° 19'N 105° 39'W) and adjacent to Niwot Ridge Long-Term Ecological Research site (40° 3'N 105° 36'W). Rock glacier type (active, inactive or fossil) was determined using geographic data layers created by Janke (2005), who used a combination of photographs, field investigations, and previous studies of terminus (leading-edge) motion to classify rock glaciers along the Front Range. Active rock glaciers were classified as moving, exhibiting steep frontal (leading-edge) slopes and pronounced ridges and furrows due to the presence of ice (Janke 2005). Both inactive

and fossil rock glaciers were classified by the absence of movement, indicated by gentler frontal slopes. Fossil rock glaciers were further indicated by increasing cover of vegetation within the boundaries of a formerly active rock glacier (Janke 2005). Inactive rock glaciers may still contain ice, while fossil rock glaciers are thought to contain no ice (Whalley & Martin 1992; Barsch 1996). We chose to study only active and fossil (rather than inactive) rock glaciers because these represented the most extreme differences in probable ice content. Active and fossil rock glaciers were paired in the same drainage when possible to help control for differences in environments and conditions. Four study sites (two active and two fossil rock glaciers) were chosen in Rocky Mountain National Park, and four study sites (two active and two fossil) were chosen adjacent to Niwot Ridge Long-Term Ecological Research site (Fig. 1). Study sites occurred at a range of elevations and aspects (Table 1).

At each of the eight sites, we aimed to collect fecal pellets from at least 10 unique territories, sampling each territory in both spring and fall. Fecal pellet sampling stations (hereafter, stations) were separated by at least 50 m, which exceeds the distance between territory centers (Smith & Weston 1990) and should ensure that we sampled unique individuals occupying distinct territories.

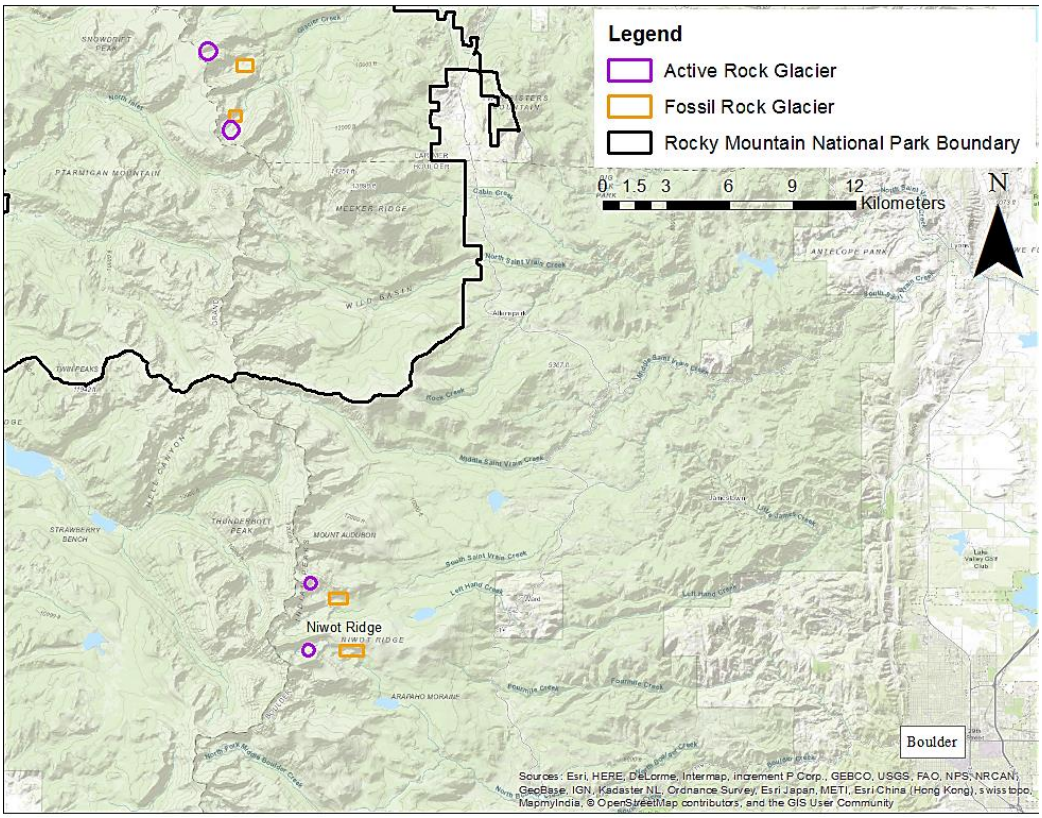

Figure 1: Study site locations within Rocky Mountain National Park (Larimer County) and adjacent to Niwot Ridge (Boulder County), Colorado, USA.

Table 1: Paired active and fossil rock glaciers studied in Rocky Mountain National Park (ROMO) and Niwot Ridge Long-Term Ecological Research site (NWT), Boulder County, Colorado, USA.

Site Name	Abbreviation	Location	Rock Glacier Type	Elevation(m)	Predominate Aspect
Tyndall Gorge	A1	ROMO	Active	3534	East
Lake Haiyaha	F1	ROMO	Fossil	3122	East
Sky Pond	A2	ROMO	Active	3342	East
Sky Pond	F2	ROMO	Fossil	3321	South
Lake Isabelle	A3	NWT	Active	3560	Southeast
Lake Isabelle	F3	NWT	Fossil	3356	South
Green Lake 5	A4	NWT	Active	3620	North
Green Lake 1	F4	NWT	Fossil	3438	South

Sample collection

We indexed stress in individuals by measuring the metabolites of an important class of stress hormones called glucocorticoids. Glucocorticoid metabolites (GCMs) can be assayed from

fecal pellets collected non-invasively from natural habitats. Pika, like all lagomorphs, create two types of feces. Cecal feces are the soft portion of waste that has passed through the gut only once. After re-ingestion and further digestion, cecal feces are re-excreted as firm fecal pellets (Smith & Weston 1990; Nichols 2010). Fecal pellets change color and texture as they age, and fresh pellets are greenish in color and fibrous inside (Nichols 2010). We collected only greenish, fibrous (fresh) fecal pellets, because fecal glucocorticoid metabolite levels decline with time after pellet deposition (Wilkening et al. 2016). We also collected only adult pellets (>2.75mm in diameter) because stress levels can vary between age classes (Wilkening et al. 2013).

At each site (n=8), multiple collection stations were established in the spring and were revisited in the fall. After the collection of fresh pellets in the spring, any remaining pellets were cleared from each station to facilitate identification of fresh pellets in the fall. Samples were stored in paper envelopes labeled with sampling date and coordinates. To avoid contamination between samples, we scooped up pellets using the envelope flap or pushed pellets into the envelope using a stick or rock. We attempted to collect 10-15 pellets (0.1 grams total) per sample to facilitate GCM analyses (Wilkening et al. 2013). Samples were stored at -20°C.

Sex determination

Because stress levels can vary between sexes (Wilkening et al. 2013), we used portion a of each spring sample for pika sex determination through genetic analysis. When sample size allowed, a subsample of each spring sample was sent to Warren Wilson College (Alisa Hove lab) or to the University of California, Berkeley (Jessica Castillo Vardaro lab) for DNA extraction to determine sex at each sampling station.

Briefly, the Hove lab used a Qiagen DNeasy Fast Stool kit to extract DNA from fecal samples. DNA was amplified through multiplex PCR, targeting both *SRY* and *Ocp10* genes using primer sequences found in Table 2 (Lamb et al. 2014). PCR was performed three times for each sample, generating three replicates of the PCR product. The recipe for each PCR reaction contained: 11 µL GoTaq Green Master Mix (2x), 1 µL of each 10 µM *SRY* primer (forward and reverse), 1 µL of each 10 µM *Ocp10* primer, and 2 µL of extracted sample DNA. Two negative control reactions, lacking extracted sample DNA, were included for each grouping of PCRs to ensure that contamination leading to sex misidentification was not occurring. PCRs were performed using cycling parameters of: 94˚C for 2 min, 40 cycles of 94˚C for 30 s, 51˚C for 30 s, and 72˚C for 30 s, and 72˚C for 5 min. After completion of all cycles, samples were held at 4˚C. Gel electrophoresis was performed on a 2% agarose gel.

Following a similar protocol, the Jessica Castillo Vardaro lab used a Qiagen DNeasy Blood and Tissue kit to extract DNA from fecal samples. DNA was amplified through multiplex PCR, targeting both *SRY* and *Ocp12* genes using primer sequences found in Table 2 (Castillo et al. 2014). Each PCR reaction contained: 15 mM Tris-HCL, 50 mM KCl, 1.5 mM MgCl2, 0.2 µM each *SRY* primer (forward and reverse), 0.8 µM each *Ocp12* primer, 0.2 mM of each dNTP, 8 µg bovine serum albumen (BSA), 4% DMSO, and 1 µL of template DNA in a 10 µL reaction. One negative control and one positive control reaction, lacking DNA and containing DNA from animals of known sex, respectively, were included for each PCR. PCRs were performed using cycling parameters of: 95˚C for 10 min, 40 cycles of 95˚C for 30 s, 53˚C for 30 s, and 72˚C for 30 s, and 72˚C for 7 min. After completion of all cycles, samples were held at 8˚C. Gel electrophoresis was performed on a 1.5% agarose gel. At a minimum, PCR was performed three times for each sample.

In both labs, sex was determined based on consensus across all PCR replicates to account for allelic dropout and PCR errors resulting from low quality and low quantity DNA template. Samples were identified as male or female according to the following criteria: male = *Ocp10/Ocp12* fragments were present in at least one of the three replicates and *SRY* fragments in at least two of the replicates or *Ocp10/Ocp12* and *SRY* fragments were both present in the same replicate; female = *Ocp10/Ocp12* fragments were present in at least one of the three replicates and *SRY* fragments were never present; unknown = only *SRY* fragments were present across all of the replicates or target genes never amplified.

Table 2: Primers used in this study targeting *O.princeps*–specific Y-linked and autosomal regions (adapted from Lamb et al 2014).

Region	Primer sequence	Size (bp)	References
Sex-determining region Y-chromosome (SRY)	F: AATGCATTCATACTATGGTC	117	Lamb et al 2014
	R: CTCTGTAAGCTTTTTCCACTG		Lamb et al 2014
Autosomal microsatellite (Ocp10)	F: TCCCAGTCACGACGTCCAATTTGGCTGTTA	179-203	Peacock and Kirchoff, unpublished (GQ461705)
	R: GTTTCTTCCAGTGTCTGGCATACGGTAAGC		Peacock and Kirchoff, unpublished (GQ461705)
Autosomal microsatellite (Ocp12)	F-GCAGGTCTTTGGGGAATAAAA	184-248	Castillo et al 2014 (GQ461707)
	R-CCTGCTCTACAACCATCTGGA		Castillo et al 2014 (GQ461707)

Temperature data collection

At each site, one or two HOBO temperature-sensing data loggers (hereafter, sensors) were placed under the surface of the talus to record sub-surface temperatures. All sensors (model H08 pro, H08, or H02; Onset Computer Corporation, onsetcomp.com) recorded temperature (±0.2° Celsius) every one or two hours for one year starting in September 2018. Sensors were placed at similar depths (~0.75 m) and at similar aspects within each of 11 territories (1-2

territories per study site). Each site (Table 1) received at least one sensor, positioned near the center of the rock glacier near pika sign. Several sites (n=3) received an additional sensor, positioned near the edge of the rock glacier near pika sign. Sensor location and elevation were recorded with a handheld GPS unit and photos were taken to aid in retrieval of the sensor. A generalized additive model (GAM) was used to estimate mean, maximum, and minimum daily temperatures from the interval data recorded by each sensor. GAMs were fit using the *mgcv* package (Wood 2011) in R 3.5.1 (R Core Team 2018).

Hormone analysis

Glucocorticoid metabolite (GCM) concentrations were measured in fecal pellet samples (hereafter, samples) using a commercially available Corticosterone Enzyme Immunoassay Kit (cat. No. K014-H5; Arbor Assay Design, Inc., Ann Arbor, MI) previously validated for *O. princeps* (Wilkening et al. 2013). Each step in the laboratory process was completed according to the standard kit protocol. Briefly, samples were first dried in a DNA SpeedVac on medium heat for 30 minutes. Dried samples were weighted to ± .02g and combined with 200 proof ethanol in proportion to sample weight (e.g., 1200µl of ethanol per 0.12g fecal sample). Samples were mashed in ethanol using a spatula and then shaken on a vortex shaker for 30 minutes. After shaking, samples were centrifuged and the supernatant was drawn off and transferred to a new vial. Supernatant was concentrated using a DNA SpeedVac on medium heat for 1 hour. Concentrated supernatant was stored in a -20°C freezer until all samples were prepared for the GCM assay. Frozen supernatant was thawed and reconstituted to its original volume using a mix of assay buffer and ethanol (<5% of volume) before loading onto enzyme immunoassay plates. Optical densities were read with a Thermo Scientific Multiskan EX Microplate Reader at 450nm using Ascent Software version 2.6. Samples, standards, and controls were assayed in triplicate

and the average of each triplicate was used to calculate GCM concentrations. Samples were compared with seven standards of known corticosterone concentrations (5000, 2500, 1250, 625, 312.50, 156.25, and 78.125 pg/ml) and with non-specific binding (NSB) and maximum binding (B_o) controls. If a sample reading was outside of the range of the NSB or B_o averages, then the reading was dropped from analysis. Sample concentrations were calculated using a standard curve delineated by the standard concentrations. Final fecal GCM concentrations were reported as picogram/gram of dried feces. Inter-assay coefficients of variation were 3.6 and 13.9% for the low and high binding controls, respectively, and 8.9% for pooled fecal extracts. Intra-assay coefficients of variation were between 5.0 and 11.6% for nine plates.

Linear mixed-effects models

Fecal GCM concentration was log-transformed and used as the response variable in linear mixed-effects models to characterize variation in stress among and within rock glacier habitats. We fit two sets of candidate models, each based on up to two fixed effects (Table 3) plus random effects of rock glacier (to account for location differences) and territory (to account for repeated measures in spring and fall).

In one set of candidate models, we explored qualitative effects of habitat and season. Habitat quality was modeled as a fixed effect of rock glacier type (active or fossil) or elevation in meters. We expected stress levels to be higher in pika living in fossil rock glacier habitats. Alternatively, we used elevation as a proxy for the many factors that vary with elevation, including rock glacier type. We also included fixed effects of location and season, because our study glaciers were clustered in two latitudinal groups (ROMO or NWT; Fig. 1), and because stress is known to vary seasonally in many animals (Baker et al. 2013). Fixed effects of pika sex

were also expected (Wilkening et al. 2013). Sex was categorized as male, female, or unknown. Unknown sex stemmed from failure to determine sex through genetic analysis.

In our second set of candidate models, we considered fixed effects of specific sub-surface temperature metrics that we hypothesized would induce physiological stress in pika, based on previous results (Wilkening et al. 2015; Wilkening & Ray 2016). These metrics were intended to model acute cold stress (number of days below -5°C or -10°C), acute heat stress (number of days above 10°C or 15°C), chronic temperature stress (CV of temperatures recorded during the month after fecal samples were collected in the fall), and chronic cold stress (mean and variation of temperatures during two months at the beginning and end of winter, 10/23/2018-12/23/2018 and 4/01/2019-6/01/2019, respectively). Our metric of chronic cold stress represents a period of time when snowpack is variable in our study area, which could reduce the buffering capacity of talus to outside cold temperatures. Once a thick blanket of snow covers the talus, sub-surface temperatures barely deviate from freezing, providing a stable refuge for pika during extremely cold ambient temperatures. Variation (CV) was calculated as the mean of temperatures recorded during the specified period divided by the standard deviation of those same temperatures. Each sub-surface temperature model included either: i) a single fixed effect of one of our temperature metrics; or ii) fixed and interacting effects of one temperature metric and rock glacier type, to account for potential differences in sub-surface temperatures between active and fossil rock glaciers.

Predictor variable covariance was calculated using Pearson's rho (continuous variables), Spearman's rank correlation coefficient or Kendall's tau, and variables with high correlation (>0.7) were not included in the same model (Appendix II). Models were fit using the *lme4* package (Bates et al. 2015) in R 3.5.1 (R Core Team 2018). We ranked models using Akaike's

Information Criterion adjusted for a small sample size (AICc) and compared Akaike weights to assess the relative support for each predictor variable (Burnham & Anderson 2002). Relative support for each model was calculated using ΔAICc = focal model AICc – minimum AICc of all models considered here. We assume that models with ΔAICc > 2 have lower support than the "top" model (ΔAICc = 0). Akaike weight, or the relative likelihood of a model *i*, was calculated using $w_i = \exp(-0.5 * \Delta AICc_i)/\Sigma_i(\exp(-0.5 * \Delta AICc_i)$.

Table 3: List of covariates and associated hypotheses used to construct linear mixed-effects models of glucocorticoid metabolite concentrations measured from fecal sampling of individual territories of the American pika.

Predictor	Hypothesis
Sex	Inter-individual
Glacier type	Habitat
Elevation (m)	Habitat
Season	Temporal
Location	Geographic clustering
# of days below -5°C	Acute cold stress
# of days below -10°C	Acute cold stress
# of days above 10°C	Acute heat stress
# of days above 15°C	Acute heat stress
Average temperature beginning of winter (Oct-Dec)	Chronic cold stress
Average temperature end of winter (April-June)	Chronic cold stress
Beginning of winter temperature variation (Oct-Dec)	Chronic cold stress
End of winter temperature variation (April-June)	Chronic cold stress
Variation of temperatures one month after fecal collection	Chronic stress

RESULTS

During the summer of 2018, a total of 190 fecal samples were collected from 100 sampling stations in eight rock glaciers along the Front Range of Colorado. Of these samples, 79 were collected from active rock glaciers (41 in the spring, 38 in the fall) and 111 were collected from fossil rock glaciers (58 in the spring, 53 in the fall). The elevation of sampled territories ranged from 3122 m to 3703 m (Fig. 2).

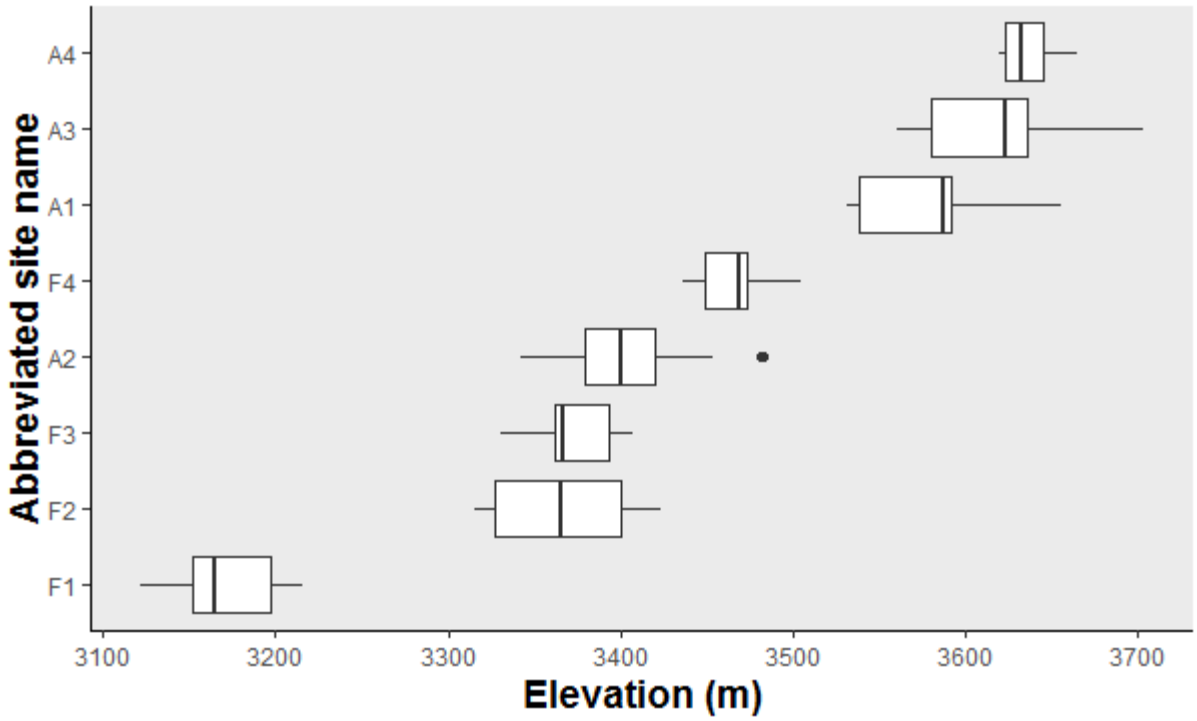

Figure 2: Elevation range of sampled territories grouped by site (n = 8). Abbreviated site names that start with an "F" are fossil rock glacier sites; site names that start with an "A" are active rock glacier sites.

Sample size for our fitted models was reduced by equipment failure and difficulties with DNA amplification. Of the 11 temperature sensors deployed in rock glaciers in the fall of 2018, the single sensor deployed in A3 failed to record temperature, resulting in a reduction in sample size (n=174) for temperature models. Sufficient fecal sample size allowed for genetic analysis of samples from 88 of the 100 sampling stations to determine the sex of the putative territory owner. These analyses revealed that 35 of the samples were deposited by males and 19 by females. However, sex was classified as unknown for samples from the remaining 34 territories, due to DNA amplification failure (Fig. 3). For these reasons, sample size was reduced to n=151 for models accounting for temperature and sex.

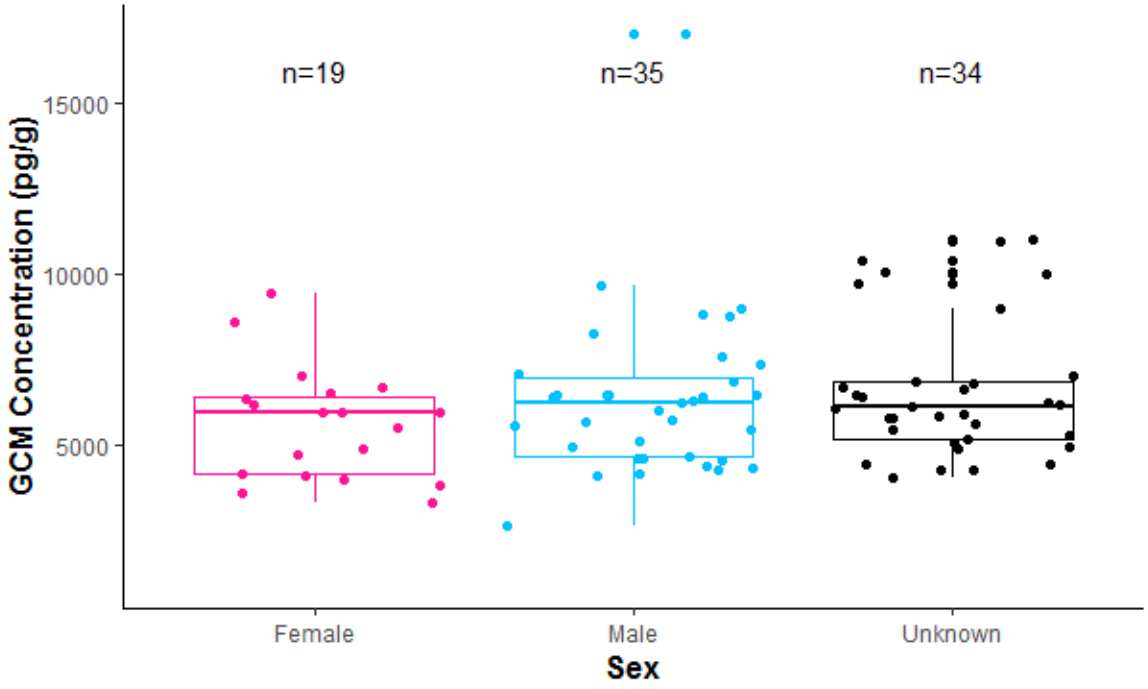

Figure 3: Glucocorticoid metabolite (GCM) concentration by sex. Genetic analysis of feces collected from 88 territories determined that 19 territory owners were female, and 35 territory owners were male. Due to DNA amplification failure, sex was undetermined for 34 territory owners.

Using samples with complete information (n=151), we fitted all models based on 1-2 covariates in Table 3 and found weak support for sex as a driver of the variation in stress (Table 4). Using samples with complete temperature information (n=174), we found no support for temperature as a driver of the variation in stress (Table 5). Because we saw no support for either sex or temperature as predictor variables, we fitted a final set of models using all available samples (n=190) to explore qualitative effects of habitat and season on stress (Table 6).

Table 4: Relative support for models of (log-transformed) glucocorticoid metabolite concentration from all sampling stations in the Front Range of Colorado with complete information on focal covariates, including microclimatic variables and sex of the putative territory owner. Models were fitted with and without the addition of sex and microclimatic variables as fixed effects, and are ranked by AICc (Akaike's Information Criterion adjusted for a small sample size, Burnham and Anderson 2002). ΔAICc is the difference between the focal model AICc value and the lowest AICc value for models within the candidate set. Akaike weight is the relative support for each model. The effect sign associated with each respective covariate in the model is reported as positive (+), negative (-), or varied (var) across models. Sample size (n) and number of fitted parameters (k) are also shown for each model.

model	k	AICc	ΔAICc	Weight	Effect sign	n
Elevation * Season	7	115.15	0.00	0.46	(+)	151
Glacier Type * Season	7	116.27	1.12	0.26	(-)	151
Season	5	116.60	1.45	0.22	(-)	151
Location, Glacier Type * Season	8	121.14	5.99	0.02	var, (-)	151
Elevation, Glacier Type * Season	8	121.98	6.83	0.02	(+), (-)	151
Elevation * Season, Sex	9	124.41	9.26	0.00	(+), var	151
Glacier Type * Season, Sex	9	125.15	10.00	0.00	(-), var	151

Table 5: Relative support for models of (log-transformed) glucocorticoid metabolite concentration from all sampling stations in the Front Range of Colorado with complete information on all focal covariates except sex of the putative territory owner. Models were fitted with and without the addition of microclimatic variables as fixed effects, and are ranked by AICc (Akaike's Information Criterion adjusted for a small sample size, Burnham and Anderson 2002). ΔAICc is the difference between the focal model AICc value and the lowest AICc value for models within the candidate set. Akaike weight is the relative support for each model. The effect sign associated with each respective covariate in the model is reported as positive (+), negative (-), or varied (var) across models. Sample size (n) and number of fitted parameters (k) are also shown for each model.

model	k	AICc	ΔAICc	Weight	Effect sign	n
Season	5	125.55	0	0.44	(-)	174
Elevation * Season	7	126.11	0.562	0.33	(+)	174
Glacier Type * Season	7	127.12	1.567	0.20	(-)	174
Location, Glacier Type * Season	8	132.19	6.64	0.02	var, (-)	174
Elevation, Glacier Type * Season	8	133.01	7.458	0.01	(+), (-)	174
Average temperature end of winter (April-June)	5	158.40	32.85	0.00	(+)	174

When we compared models based on qualitative effects of habitat and season (Table 6), two models were well supported: one based on a positive interaction of elevation and season (Fig. 4), followed by a similar model based on a negative interaction of glacier type and season (Fig. 5). The latter model garnered about half the Akaike weight of the former, suggesting the effect of elevation was the more explanatory predictor of pika stress. A model containing a positive effect of season alone had only slight support. Location (ROMO or NWT) garnered very weak support.

Table 6: Relative support for models of (log-transformed) glucocorticoid metabolite concentration by habitat and season, using data from all sampling stations in the Front Range of Colorado. Models were fitted without sex and microclimatic variables as fixed effects, and are ranked by AICc (Akaike's Information Criterion adjusted for a small sample size, Burnham and Anderson 2002). ΔAICc is the difference between the focal model AICc value and the lowest AICc value for models within the

candidate set. Akaike weight is the relative support for each model. The effect sign associated with each respective covariate in the model is reported as positive (+), negative (-), or varied (var) across models. Sample size (n) and number of fitted parameters (k) are also shown for each model.

model	k	AICc	ΔAICc	Weight	Effect sign	n
Elevation * Season	7	139.26	0.00	0.59	(+)	190
Glacier Type * Season	7	140.72	1.46	0.28	(-)	190
Season	5	142.80	3.54	0.10	(-)	190
Location, Glacier Type * Season	8	146.26	7.00	0.02	(+), (-)	190
Elevation, Glacier Type * Season	8	146.82	7.56	0.01	(+), (-)	190

DISCUSSION

In our study, variation in stress was best explained by the interaction of elevation and season. These results show that pika living at higher elevations are less stressed and that stress is higher in the spring than in the fall (Fig. 4). Pika occupancy surveys have documented population loss at lower elevations (Beever et al. 2016; Jeffress et al. 2017; Stewart et al. 2017) and our study suggests that these range retractions might be due to processes causing physiological stress (Fig. 4). Furthermore, our results suggest that metrics of physiological stress might be useful for predicting populations at risk, given that we detected higher stress levels in populations at lower elevations typical of recent extirpations. We hypothesize that abiotic and biotic factors could be driving seasonal trends across elevations such as the timing of snowmelt or plant green-up. A thick blanket of snow can buffer sub-surface talus temperatures from extreme cold ambient temperatures. In the spring when cold snaps are common in the mountains, lower elevations may not have snow cover needed to buffer pika from acute cold stress. Also, the timing of plant green-up occurs sooner at lower elevations, which could influence the timing of plant senescence later in the summer, when pika are harvesting food for the winter. The timing of the period during which pika can acquire highly nutritious food might be important in determining haypile quality and pika stress levels in the following spring.

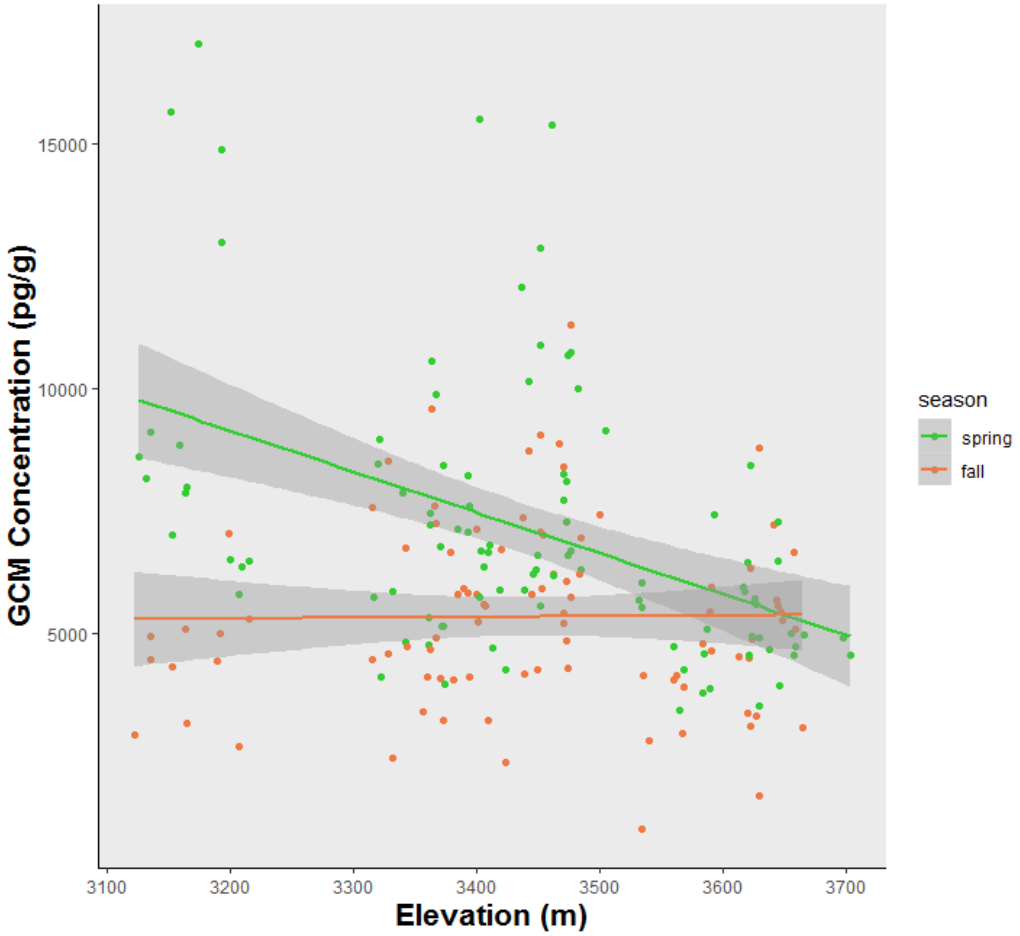

Figure 4: Glucocorticoid metabolite (GCM) concentration in picograms/gram by elevation and season. Each point is a different fecal sample and is colored by the season at which the sample was collected. Lines show mean GCM concentration by season with 95% confidence intervals.

The interaction of glacier type and season was also highly supported as a predictor of pika stress. This model supports our hypothesis that stress is higher in fossil rock glaciers than in active rock glaciers, and refines our understanding by showing that this effect on stress is evident mainly in the spring (Fig. 5). The coherent difference in stress between rock glacier types indicates that habitat quality correlates with stress in our system. Results from this study expand on previous research comparing pika GCM levels on and off rock ice features (RIFs) at NWT (Wilkening et al. 2015). Our study echoed previous results showing that pika exhibit lower stress on RIFs (Wilkening et al. 2015), and results hold true when replicated across space, indicating

that RIFs may constitute higher quality habitats that result in lower stress and higher fitness for pika.

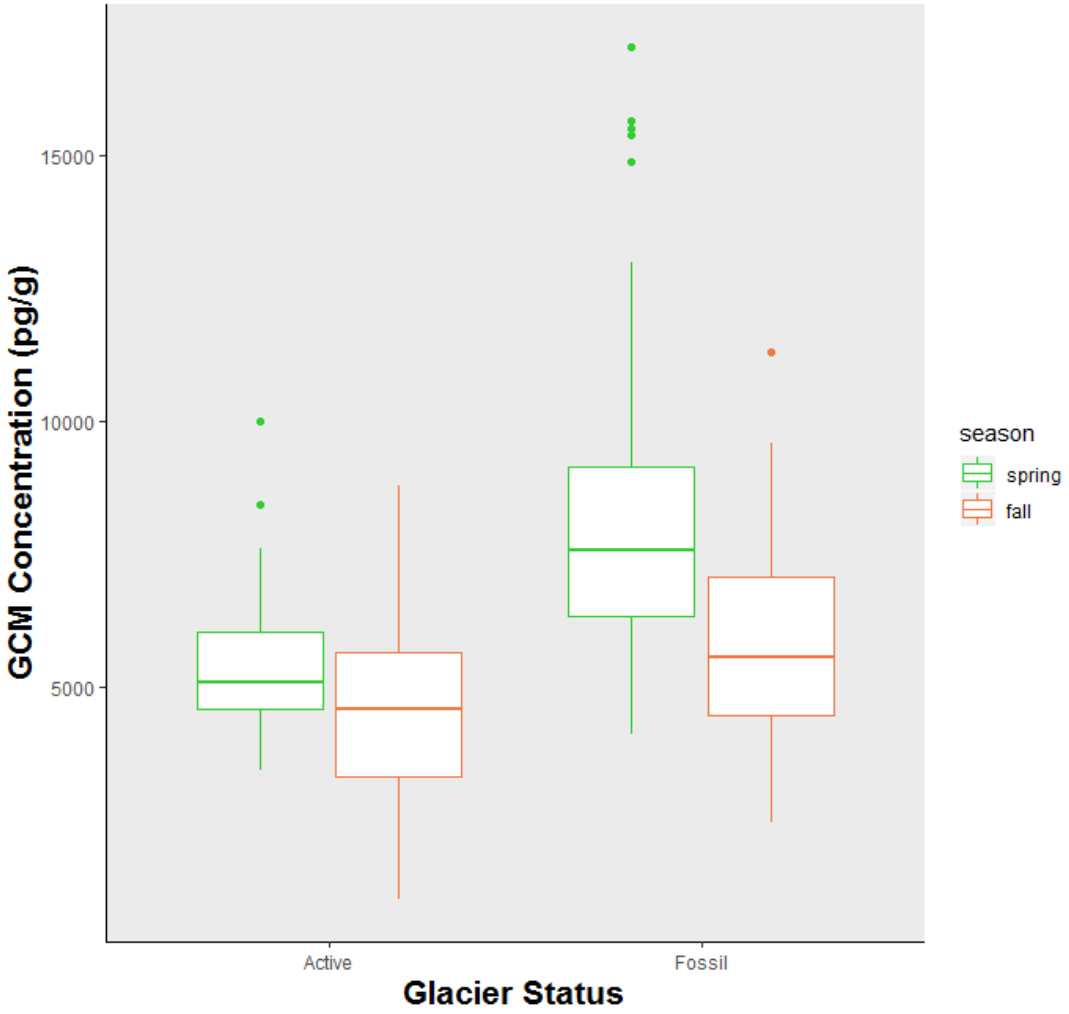

Figure 5: Glucocorticoid metabolite (GCM) concentration in picograms/gram for all sampled territories grouped by glacier type and season. When available, fecal samples were collected from each territory in the spring and in the fall of 2018 from locations along the Front Range of Colorado.

Both of our top models included an interaction of season indicating that season is an important driver of stress in our study. Our results show that stress is highest in spring which suggests that winter could be a limiting factor for pika. Metrics of cold stress have been shown to affect pika stress, occupancy, and persistence in other studies (Beever et al. 2003, 2010, 2011; Wilkening et al. 2011, 2015; Jeffress et al. 2013; Rodhouse et al. 2017). We hypothesize that the

reduction in snowpack in the mountain west (Mote et al. 2005) along with the loss of sub-surface ice in our research area (Leopold et al. 2015) might be affecting the buffering effect of the pika's rocky habitats and causing a more stressful and unpredictable environment for pika. Our results agree with previous findings that snowpack and winter cold stress influence distribution and extinction risk for pika (Beever et al., 2010; Erb et al., 2011; Guralnick et al., 2012; Schwalm et al., 2016; Rodhouse et al., 2017), and add new evidence that individual stress levels are elevated as pika come out of the winter season. If replicated across years, this new evidence might suggest that localized pika losses are due to sustained stressors related to habitat quality, rather than stochastic events like epizootics or extreme weather. Alternatively, high levels of stress in the spring could be attributed to the timing of reproduction. In pika, the first litter is usually conceived one month before snowmelt (Millar 1972; Smith 1978), coinciding with our first sampling period.

Sex did not explain the variation in stress in our study. Samples used in genetic analysis were collected from territories in the spring, and we assumed that the sex of the territory owner stayed the same from spring into fall. Turnover rates in site occupancy are highly dependent on local influences but can be as high as 50% in some populations (Rodhouse et al 2018). However, previous studies suggest that territories are likely to retain an owner of the same sex through time (Krear 1965; Sharp 1973; Tapper 1973; Smith et al. 1983, 1984).

Sub-surface temperature sensors were placed in rock glaciers to capture fine scale habitat differences that could be driving habitat quality for pika. None of our temperature metrics were supported as predictors of the variation in stress in our system. Additionally, our estimated sub-surface temperature metrics did not differ between rock glacier types. Previous research has shown that rock glaciers with ice provide cooler sub-surface temperatures in the summer and

warmer sub-surface temperatures in the winter (Millar & Westfall 2010; Wilkening et al. 2015). We may have found no difference in sub-surface temperatures between rock glacier types because we collected data from only one or two sensors per glacier and for only nine months of the year (10/23/2018 – 7/19/2019). Further, we were unable to collect sub-surface temperature data during late summer, possibly the warmest time in the alpine and a possible driver of chronic heat stress in pika. Our temperature sensors were placed no more than 0.75 below the rock surface. It is possible that such shallow temperature readings did not capture the full range of temperatures a pika would experience. Previous geophysical studies on rock glacier A4 (Leopold et al. 2011) detected ice 4-5 m below the surface - depths that are likely accessible to pika, but often too difficult to outfit with sensors (Ray et al. 2016). We suggest that further data on sub-surface temperatures across rock glacier types through multiple years should be collected in order to fully characterize rock glacier microclimates. Rock glaciers can also be important for other animals and plants, warranting continued research on their potential to serve as a microhabitat in mountain ecosystems. During the summer, the ice within rock glaciers can continue to provide water to surrounding vegetation, creating dense plant communities (Millar & Westfall 2010; Millar et al. 2013). In the great basin, this dense vegetation has been shown to support a higher diversity of shrubs and herbaceous plants compared to surrounding alpine and subalpine habitats (Millar and Westfall 2010, Millar et al 2013). Additionally, the prolonged water resource can help surrounding plants survive during drought conditions and could provide biotic refugia for arthropods during warmer and drier climates (Millar & Westfall 2010; Millar et al. 2015).

Due to warmer temperatures in summer and fall, ice features such as rock glaciers and permafrost on and near the Niwot Ridge Long-Term Ecological Research site and our study sites

are declining (Leopold et al. 2015). It's estimated that if current average temperatures were to increase by 2°C, ~94% of permafrost would be lost along the Front Range of Colorado (Janke 2005). However, decline in ice will likely respond slowly to warming temperatures (Janke 2005), and possibly even slower in rock glacier habitats where ice is protected by a rocky carapace (Millar & Westfall 2008). For pika, which are predicted to be extirpated from some of our study sites by 2100 (Schwalm et al. 2016), active rock glaciers may serve as an overlooked microrefugia that could help the species persist.

Results from this study showed that stress in alpine populations of a small mammal was correlated not only with season but also with habitat metrics (elevation and rock glacier type). The pattern in stress that we observed across landscapes suggests that stress could be used to evaluate seasonal variation in habitat quality. The non-invasive sampling methods used in this study could be adapted easily for other observational studies to aid in further research on stress in many systems.

CONCLUSION

Earth is currently in a biodiversity crisis with extinction rates increasing sharply in the past century. In order to actively conserve species, we need conservation tools that can predict species decline before they become apparent. Before populations decline, we should be able to evaluate an increase in stress as individual health declines. By measuring stress and evaluating what causes an increase in stress, we may be able to link human-caused disturbance to population health. Animals who utilize poor habitats may be most susceptible to new threats and be the first to show population decline. Poor habitat quality could be a source of chronic stress. In this thesis we employed fecal stress hormone analysis to explore seasonal stressors and habitat quality effects on American pika (*Ochotona princeps*), hereafter pika.

Recent research on pika has shown evidence of population decline in parts of their range. However, the Southern Rockies in Colorado continue to be a stronghold for the species and have yet to see significant decline. The future of pika persistence appears to be more complex though. Recent climate vulnerability predictions estimate that pika could be extirpated from Rocky Mountain National Park by the end of the century. Considering these patterns, we have the opportunity to look for early evidence of population stress in this region.

In looking at stress levels through time among individuals, we found evidence of seasonal trends in stress. Our finding that stress varies coherently through time among pika was previously undocumented in the literature. In two studies (chapters 1 and 2), we found that stress was highest in the spring, in agreement with previous studies that have shown cold stress to be an important predictor of pika decline. In the spring, when snow levels are most variable, the lack of an insulating snow layer and high probability of cold snaps in temperature could be causing

stress in pika. In western North America, spring snowpack levels in the mountains have steadily decreased since the mid-twentieth century, and this trend is expected to continue as climate change progresses. In our study, the lower stress levels observed in summer, relative to spring, suggest that winter was more of a limiting factor than summer for pika. Although mean summer temperature has explained occupancy patterns in the Great Basin and Southern Rockies, there may be a summer temperature threshold that has not yet been reached in our system—at high elevations near Rocky Mountain National Park—to become a limiting factor for pika. An alternative hypothesis is that the annual timing of reproduction causes a pattern of higher stress in spring relative to summer. The lack of variation between spring and summer stress at higher elevations in one of our studies could be due to elevational differences in the timing of reproduction relative to our sampling.

Additionally, we found that individual stress levels decreased at higher elevations.This finding supports previous evidence of low-elevation range retractions from occupancy surveys in other studies. Our study suggests that these range retractions might be due to processes causing physiological stress. In our models, elevation serves as a proxy for many potential habitat differences such as snowpack, temperature, or forage availability. Timing of snowmelt and plant green-up are heavily tied to elevation and could be causing the variation we see in stress. Lower elevations may experience earlier snowmelt at a time when pika need the insulating blanket of snow to buffer extreme temperature swings typically experienced in the mountains in the spring. Also, the timing of plant green-up occurs sooner at lower elevations, which could influence the timing of plant senescence later in the summer, when pika are harvesting food for the winter. The timing of the period during which pika can acquire highly nutritious food might be important in determining haypile quality and pika stress levels in the following spring.

We also found that mean stress levels differ between habitat types. We compared mean stress levels in populations living on active rock glaciers (talus with sub-surface ice) and fossil rock glaciers (talus without sub-surface ice). We found that populations occupying active rock glaciers had lower levels of stress compared to populations occupying fossil rock glaciers. Previous studies have found that pika have a close association with permafrost and that talus with sub-surface ice provides a more moderate microclimate compared to ambient temperatures. In our study, we did not find sub-surface temperature to have any explanatory power in our models, showing no evidence of sub-surface temperature differences between habitat types. Evaluating other habitat differences between rock glacier types—such as talus depth, rock size, or vegetation availability—could help determine drivers of habitat quality for pika.

Results from our study showed that stress in pika was correlated not only with season but also with habitat metrics (elevation and/or presence of sub-surface ice). At the individual level (Chapter 1), individual pika within the same area were shown to coherently respond to seasonal stressors. In broadening our scope, we then looked at the population level (Chapter 2) and found that populations differed in mean stress levels between two habitat types. Together, both chapters provide evidence that we can evaluate seasonal variation in habitat quality using metrics of stress. Our study is among the few examples exploring the utility of stress metrics to evaluate habitat quality in wildlife We recommend that more studies be done to characterize habitat differences across elevations and between rock glacier types. The techniques we used here could be adopted within commonly used protocols for survey of pika habitats and analysis of pika response to habitat characteristics.

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APPENDIX I

Correlation Matrices. Matrices displaying correlations in Chapter 1 among all predictor variables for a) temporal and broad scale habitat models, and b) fine scale habitat models.

a)

	Aspect	Elevation	DOY2	DOY
Aspect	1			
Elevation	-0.4524	1		
DOY2	0.03471	-0.1365	1	
DOY	-0.087	0.070934	-0.3478	1

b)

	Variation of 10 biggest rocks	10th biggest rock	Mean rock size of 10 largest rocks	Biggest rock	Graminoid : forb	Deepest crevice	Percent forb cover	Combined percentage of graminoid, forb, and shrub cover
Variation of 10 biggest rocks	1							
10th biggest rock	-0.49823784	1						
Mean rock size of 10 largest rocks	-0.29323918	0.9737822	1					
Biggest rock	-0.12411922	0.9185488	0.984392007	1				
Graminoid : Forb	-0.20866437	0.1045122	0.075026515	0.0495224	1			
Deepest crevice	-0.0739662	0.3997253	0.434544034	0.4458849	0.134284061	1		
Percent forb cover	0.238145772	-0.578084	-0.571139103	-0.545268	-0.560439472	-0.112058153	1	
Combined percentage of graminoid, forb, and shrub cover	0.076058431	-0.577627	-0.601133229	-0.597743	0.112649905	-0.189486725	0.6768034	1

APPENDIX II

Correlation Matrices. Matrices displaying correlations in Chapter 2 among all predictor variables for a) qualitative effects of habitat and season, and b) sub-surface temperature metrics.

a)

	Sex	Glacier type	Elevation	Season	Location
Sex	1				
Glacier type	0.08546	1			
Elevation	0.02556	-0.6921745	1		
Season	-0.0007	0.0040929	0.00953	1	
Location	0.21972	-0.0132052	0.52943	0.01155	1

b)

	Elevation	# of days below -5°C	# of days below -10°C	# of days above 10°C	# of days above 15°C	CV temperature one month after fecal collection	Average temperature beginning of winter	Beginning of winter temperature variation	Average temperature end of winter	End of winter temperature variation
Elevation	1									
# of days below -5°C	-0.6999	1								
# of days below -10°C	-0.3199	0.39703	1							
# of days above 10°C	-0.3771	0.17788	0.46354	1						
# of days above 15°C	0.06816	0.0187	0.59124	0.82716	1					
CV temperature one month after fecal collection	-0.8809	0.92912	0.42844	0.24271	-0.0441	1				
Average temperature beginning of winter	-0.8024	0.7415	0.22077	-0.1147	-0.3938	0.888618102	1			
Beginning of winter temperature variation	-0.9232	0.87656	0.24034	0.21571	-0.1711	0.971143369	0.89049472	1		
Average temperature end of winter	-0.7397	0.46	0.31813	0.4505	0.00937	0.591058312	0.38457094	0.640589461	1	
End of winter temperature variation	-0.5424	0.10595	0.05733	0.39083	-0.0757	0.284890165	0.15032607	0.384705363	0.917313443	1

APPENDIX III

Observations from the field

In collecting fecal samples, I observed patterns in the timing of visible fecal deposits and the consistent use of fecal stations within pika territories. When I started collecting samples in the beginning of June for Chapter 1, it was very difficult to find any feces. From the beginning of June until mid-July, only a few dispersed pellets could be found in a territory and not all territories would have feces to collect. It was not until mid-July that I could reliably find feces in each territory. After mid-July it was like a switch had turned on and every territory had stacked pellets (pellets in one location instead of dispersed pellets within a territory). Each time I collected feces, I would clear the territory of any remaining feces to ensure that I could collect the freshest sample during the next sampling effort. For most of the territories, I could collect feces from the exact same location each visit, and a stack of pellets would be replaced each time. Interestingly, I observed a similar pattern in territories for Chapter 2. In Chapter 2, I collected fresh feces from the same territories in the spring and fall. In the spring, after collecting a fecal sample, I would brush away any remaining feces in the territory to ensure a fresh sample could be collected in the fall. Most territories I designated in the spring would have a pile of feces in the same location when I returned in the fall. I have two hypotheses for my above observations; 1) feces could reliably be found in the same location within a territory because pika use feces to communicate territory ownership, and 2) feces were hard to find prior to mid-July because pika were inactive to save energy when forage was scarce. Additionally, I observed that within some fecal piles, there were a mix of pellet sizes. It is possible that pika defecate in each other's

territory to communicate to neighbors. It's also possible that the territories with mixed pellet sizes were owned by females and their young would defecate in shared fecal stations. Future studies exploring the role of fecal stations in communication or as a sign of owner activity could help shed light into how individuals interact with each other within a population.