University of Nevada, Reno

An integrated investigation of the population genetics, physiological stress, and movement patterns in the American pika (*Ochotona princeps*)

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Abstract

The objective of this dissertation was to examine how ecological and micro-evolutionary factors may influence the response of alpine mammals to reductions in habitat quality projected under climate change using the American pika (*Ochotona princeps*) as a model system. I begin with an overview of anthropogenic impacts on global ecology and ecosystem functioning with an emphasis on the vulnerability of mountain systems to environmental change. I then introduce the American pika and discuss contemporary population loss across the species' range particularly at the southern end of its distribution in the Great Basin and northeastern California where the regional effects of climate change are expected to increase the co-occurrence of warm and dry conditions over the next few decades. In Chapter 1, I explore the effects of site, season, territory quality (microclimate temperature data) on patterns of physiological stress (via corticosterone stress hormones) in two high-elevation sites in the eastern Sierra Nevada. In Chapter 2, I examine the effects of habitat fragmentation on patterns of long-term pika occupancy as measured by 40 years of census data, and its association with habitat quality (patch perimeter and size), physiological stress, and climate variables calculated from local weather station data for a pika population located at the southern end of the species' range. In Chapter 3, I track changes in the structure and distribution of genetic variation, effective population size, and movement patterns over the last 65 years within this same vulnerable pika population through the use of historical and contemporary genetic datasets.

In Chapter 1, I found that patterns of stress differed significantly among pikas at two sites despite close geographic proximity and that the effects of site, year, territory, and individual diversity on patterns of pika stress were substantial compared to the relatively minor effects of microclimate predictors. In Chapter 2, I discovered significant associations among pika occupancy, temperature patterns, and stress hormone concentrations that suggest a role of individual physiologic response in demographic stability. Finally, in Chapter 3, I provide evidence of genetic erosion and its putative relationship with local climate for a genetically unique pika population at risk of extirpation.

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Introduction

Informing predictions of the future persistence of an alpine mammal through investigation of genetic variation, physiological health, and habitat quality

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Human-induced modification of global ecology

For the last 300 years, the interacting effects of linear population growth (i.e. 7 billion humans) as well as advances in technology have allowed humans to manipulate their surrounding resources thereby effectively marking the beginning of the Anthropocene epoch or the "age of humans" (Crutzen 2002; Steffen et al. 2007; Rochstrom et al. 2009; Smith and Zeder 2013; Dirzo et al. 2014; Corlett, 2015; Goudie 2015). The extent to which human activities are a primary driver in global-scale processes is evident by the continued modification of terrestrial land cover, burning of fossil fuels, overharvesting, and dumping of synthetic fertilizers resulting in consequences for ecosystem function and biodiversity (Braje and Erlandson 2013; McGill et al. 2015). While climate warming has been the major example of anthropogenic stress on the environment, the degree to which humans have transformed ecological processes encompasses a much greater pool of under-recognized ecological harm ranging from deforestation (Feeley and Rehm 2012; Larsen 2012), introduction of invasive species (Wallach et al. 2010; Cucherousset and Olden 2011), defaunation (particularly animals with larger body sizes) (Seddon 2014), desertification (Okin et al. 2009; Mainguet 2012), and noise pollution (Francis et al. 2009; Ortega 2012). Comparable to the five previous mass extinctions in Earth's history, these human activities are responsible for what is known as the "sixth extinction wave" with certain regions and taxonomic groups considered to be particularly sensitive and vertebrate species loss over the last century estimated at 100 times higher than the background rate (Dirzo et al. 2014; Ceballos et al. 2015; Urban 2015).

According to biogeographic theory, the majority of species will be required to respond to climate change by moving to more suitable conditions, acclimating through behavioral flexibility, adapting in *situ* over short time periods (i.e. local adaptation), or succumbing entirely (Hill et al. 2011; Van Buskirk 2012; Staudinger et al. 2013; Merilä and Hendry 2014; Duputié et al. 2015; Beever et al. 2017). In fact, the impact of changing environmental conditions is already apparent in natural populations through range and elevational shifts (Thomas et al. 2006; Kelly and Goulden 2008; Moritz et al. 2008; Loarie et al. 2009; Chen et al. 2011), behavioral shifts (Sih et al. 2010; Snell-Rood 2013), advanced phenology (Menzel et al. 2006; Cleland et al. 2007; Brown et al. 2016), and genetic change (Bálint et al. 2011; Habel et al. 2010; Alsos et al. 2011; Rubidge et al. 2012; Jay et al. 2012).

Alpine ecosystems and the American pika (Ochotona princeps): monitoring the impacts of environmental change on habitat quality

Projections of future climate are dependent upon both historical, present, and future anthropogenic emissions as well as natural climate variability; however, for all scenarios except the most optimistic, global surface temperature change for the end of the 21st century (2081-2100) is likely to exceed 2°C relative to 1985-2005 (Smith et al. 2007; Loarie et al. 2009; IPCC 2014). In contrast to global trends, recent warming is not consistent across time or space especially when considering the complexity of highly variable topography and corresponding microclimates therefore the consequences of a warming climate will vary regionally (LaDochy et al. 2007). It is likely that these non-uniform patterns in climate change within and across regions will contribute to heterogeneity in ecological dynamics, trophic interactions, and biogeographic responses across systems (Brooks et al. 2006; Rapacciuolo et al. 2014). As a result, threats to biodiversity are also distributed unevenly and many sub-regions will experience varying effects of climate change depending on the extent of land use change, population density, elevation, and proximity to ocean conditions (LaDochy et al. 2007).

Some of the most pronounced effects of climate change may occur in mountain systems in the form of altered alpine species distributions, retreat of glaciers, or disruption of hydrological cycles (Thuiller et al. 2005; Bradley et al. 2006; Nogués-Bravo et al. 2007). As some of the most fragile systems on the globe, mountains often harbor evolutionarily distinct assemblages while serving as a source of water and ecosystem services for adjacent lowlands (Diaz et al. 2003; Korner 2004; Nogués-Bravo et al. 2007; La Sorte and Jetz 2010; McCain and Colwell 2011; Graham et al. 2014). High elevation species are particularly threatened largely due to thermal sensitivity and dispersal limitations that hamper the opportunity for local altitudinal shifts (Inouye 2008; Balint et al. 2011; Jay et al. 2012). In fact, geographic isolation, local adaptation, and limited habitat area at the highest elevations may also hinder dispersal even for more vagile species due to reductions in the quantity and quality of suitable habitat which in turn, is expected to constrain population sizes (La Sorte and Jetz 2010).

The American pika is an alpine species considered useful as a model system for investigating the effects of climate change because they are locally abundant, highly detectable, and investigations are not confounded by altered habitat structure (Aho et al. 1998; Beever et al. 2016). Pikas are particularly challenged by environmental stochasticity due to several life history characteristics such as intolerance to high ambient temperatures, habitat specialization, limited vagility, and asocial behavior (Beever et al. 2003; Erb et al. 2011; and Nichols et al. 2016). Broadly distributed across the western United States and southwestern Canada, pikas inhabit boulder-fields, lava beds, and other habitats where cooler air is trapped in pockets between the rocks (Smith and Weston 1990). The thermal constancy of the subsurface talus environment enables pikas to behaviorally thermoregulate and survive both summer and winter temperature extremes (Smith and Weston 1990; Ray et al. 2012).

Given the American pikas' thermal sensitivity, numerous studies have predicted extreme range reduction as a result of climate change (Beever et al. 2010; Calkins et al. 2012; Johnston et al. 2012; Stewart et al. 2015); however, most of the observed extirpations have occurred in the warmer regions of the species' range (Beever et al. 2003; Beever et al. 2011; Stewart et al. 2015; Nichols et al. 2016). The relationship between pika occupancy and climate is complex (Erb et al. 2011; Jeffress et al. 2013; Varner and Dearing 2014) due to environmental heterogeneity at fine scales that enables pikas to persist outside their bioclimatic envelope (Simpson 2009; Rodhouse et al. 2010; Millar et al. 2013; Shinderman 2015; Schwalm et al. 2016; Varner et al. 2016). In the Great Basin and Sierra Nevada, distributional shifts and local extirpation of pika populations have been linked to both anthropogenic climate change and glacial-interglacial dynamics during the Pleistocene (Beever et al. 2003; 2010; 2011; 2016; Grayson 2005; Erb et al. 2011; Wilkening et al. 2011; Stewart et al. 2015; Nichols et al. 2016). Evidence of distributional loss (local extirpations, upslope retractions and encounter of old sign only) is prevalent in both the hydrographic Great Basin and Sierra Nevada regions but variables related to temperature and water balance explain extirpations within the Great Basin only (Beever et al. 2016). Recent work has suggested that the relative influence of climatic factors may be greater in more insular areas (Great Basin) compared to mainland areas (Sierra Nevada) where habitat extent appears to be a stronger determinant of population persistence (Stewart et al. 2015; Beever et al. 2016). Using historical records (pre-1991) and resurveys (1994-2013 and 2014-2015), Beever et al. (2016) recently determined that the rate of pika extirpation has increased through time in the hydrographic Great Basin beginning with 24% of all sites surveyed extirpated by 1999 to 44.1% of all sites (15 of 34) extirpated by 2008. Furthermore, the amount of habitat was a poor predictor of presentence in the Great Basin which suggests that despite the presence of suitable habitat, climate is constraining pika distribution and preventing recolonization (Beever et al. 2016). Northeastern California has suffered extirpation in 37.9% of all sites resurveyed (11/29) with very recent reports of complete extirpation from both Zion National Park and Cedar Breaks National Monument in southern Utah (another insular area) despite occupancy as recent as 2011-2012.

Unfortunately, according to climate modeling predictions for the state of California, the likelihood of warm and dry conditions co-occurring will continue to increase over the next few decades as a result of anthropogenic climate change enhancing the probability of high-atmospheric pressure in California (Diffenbaugh et al. 2015). Despite the official end of the drought emergency in California in 2017, the years of 2012-2014 are considered more severe than any conditions experienced during the last century (Griffin and Anchukaitis 2014). Thus, due to the severity off the ongoing and forecasted climate change in this region, the Sierra Nevada represents an important focus of investigation for American pika populations already considered vulnerable to lineage extinction (Galbreath et al. 2009; 2010) as a result of increasing rates of recent extirpation (Beever et al. 2003; 2011; Stewart et al. 2015; Nichols et al. 2016). Therefore, within the context of the current warm-dry period in California and the subsequent risks to the natural alpine systems of the Sierra Nevada, this dissertation set out to examine the interacting effects of habitat quality, physiological health, movement patterns, and population genetics on pika persistence across varying spatial and temporal scales in the American west.

How might site- or seasonally-specific effects influence the physiological stress response in pikas?

The majority of research investigating the effects of anthropogenic disturbance (habitat fragmentation, climate change, invasive species) on imperiled species like the pika focus on how the distribution of a species might be influenced by changing habitat factors (temperature, precipitation, elevation) (Beever et al. 2003; 2010; Grayson 2005; Calkins et al. 2012; Wilkening et al. 2013). While temperature and precipitation appear to be relatively important in determining the pika's current range across the western U.S., there is a need to more fully understand how habitat features (surface and sub-surface microclimates, forb-dominated vegetation communities, spatial configuration of talus) influence individual pika fitness and the resulting population trajectory (Wilkening et al. 2013). Considering the reliance of this non-hibernating alpine mammal on the quality of interstitial habitat for thermoregulation, range-wide predictions of future persistence that fail to incorporate behavioral flexibility may be limited. An alternative method for characterizing the habitat or climatic variables important in range dynamics is to track physiological stress among individuals and between populations (Wilkening et al. 2013).

Since fine-scale, site-specific factors influence pika persistence (Erb et al. 2011; Jeffress et al. 2013; Schwalm et al. 2016), it is likely that populations of the American pika will vary in their ability to respond to a changing environment depending on factors such as spatial configuration of talus habitat, genetic connectivity with other populations, and elevation. The hormonal stress response is a widely-used approach for incorporating a physiological biomarker into monitoring projects through non-invasive measurement of glucocorticoids (i.e. stress hormones) because they are released as a strategy for coping with negative or threatening conditions ((McEwen and Wingfield 2003; Romero 2004; Korte et al. 2005; Albano et al. 2012). Indeed, recent work has determined that patterns of physiological stress in pikas differ significantly among individuals and between genders with habitat features such as sub-surface ice associated with reduced individual stress (Wilkening et al. 2013; 2015). Furthermore, elevated physiological stress in individuals has been reliably linked with lower annual survival (Wilkening et al. 2016); this relationship with a fitness consequence lends support to the idea that physiological monitoring of individuals over time might help to identify populations at risk of extirpation. Despite these advances in characterizing stress physiology in pikas, almost all pika stress studies have occurred within the Northern Rocky mountains which is a region considered more robust to changing conditions compared to the warmer, southern portions of the range. Greater stress hormone sampling is needed across all of the five major mountain systems within the range in order to define regionally-specific ranges of baseline and elevated hormone concentrations.

In the Sierra Nevada, recent stress hormone work has highlighted the importance of fine-scale features in determining patterns of stress hormone concentrations for one population sampled repeatedly at two sites (Chapter 1). Although located at the same elevation and separated by only two km of continuous talus habitat, pikas maintained significantly elevated stress levels at the site associated with less stable and more shallow rock, a lack of sub surface ice, and no central alpine meadow. The finding that patterns of physiological stress can vary significantly within the dispersal distance of an individual pika is meaningful especially since high genetic connectivity between the two sites suggests that these differences may be related to phenotypic plasticity in the pika stress response rather than physiological differences related to genetic predisposition (Chapter 1). In addition, spatial variation in habitat quality across individual territories (proximity to foraging opportunities, stability of the interstitial microclimate, and defense from predators) was not found to be associated with significant differences in average stress hormone levels. Therefore, instead of finding that territory quality varies across a talus slope, it appears that generations of pika habitat selection has resulted in a number of territories that provide adequate physiological conditions for occupation by pikas. Furthermore, although this study found a relationship between physiological stress in pikas and fine-scale metrics of heat and cold, the relative influence of these climate predictors remained minor compared to the larger effects of site, year (season), territory quality and individual diversity of patterns of stress (Chapter 1).

Can habitat quality be characterized by a physiologic metric and how do patterns of physiological stress relate to demographic and fine-scale climate data?

Environmental heterogeneity mediates the interactions between organisms and changing ecological conditions and can result in varying trajectories for populations that occur across variable habitat quality within a species' range (Walther et al. 2002; Doddington et al. 2013; Otto et al. 2014; Messer et al. 2016). Habitat quality has most commonly been estimated using population-level fitness proxies (i.e. population growth rate) however demographic parameters can sometimes be difficult to interpret if a heavily used site is associated with low abundance but high reproductive success (Knutson et al. 2006; Albano et al. 2012). Instead, by measuring the physiological response of individuals to changing environmental conditions, it is possible to identify relatively high or low quality habitat especially if studied within the context of potentially important habitat or climatic features.

Recent work characterizing habitat quality for a spatially fragmented pika population investigated whether a physiologic metric (i.e. stress hormones) was associated with a long-term demographic parameter (i.e. patch occupancy) as well as regional climate metrics. As the first study of stress hormone patterns for pika populations within the vulnerable Great Basin lineage (*O. princeps schisticeps*), this work also compared average physiological stress with other sampling sites that differed in habitat features such as rock type, perimeter size, and relative isolation. Located at the southern end of the species' range, this metapopulation is distributed across ore dump habitat in sagebrush steppe and has suffered declines in patch occupancy over the last 40 years which were significantly associated with changes to local climate. Pika occupancy has declined as average summer maximum temperature increased, and minimum winter temperature decreased (Chapter 2) which suggests that warming temperatures as well as acute cold negatively affect the number of patches occupied during each summer season. Furthermore, stress hormone levels were positively and significantly related to the extinction-recolonization ratio, patch size (i.e. talus perimeter), and increasing average monthly maximum temperatures as well as minimum monthly temperature during summer months. Together, these results suggest that warmer conditions as well as cold summer lows are physiologically stressful for pikas, and that individual physiologic responses to changes in temperature eventually translate to demographic consequences in terms of the level and distribution of patch occupancy that occurs during each summer season (Chapter 2). Comparisons of stress patterns between this spatially fragmented population and other sites within the region revealed that ore dump habitat is not inherently low quality and may provide a similar quality of habitat compared to natural talus outcrops. Our assumptions regarding the types of pika habitat that constitute low (ore dump, lava tubes, sea-level) or high quality (alpine talus) should continue to be challenged especially since the lack of physical disturbance to pika habitat does not prevent its functional degradation by climate change. Considering pikas' vulnerability to extreme temperatures and the thermoregulatory function that habitat heterogeneity and microclimate variability provide for this species, greater investigation of habitat quality across the species range is needed.

How might changes in habitat quality lead to genetic erosion and loss of evolutionary potential?

The effects of climate change on biotic patterns have often been considered at the ecosystem and species scales primarily through distributional shifts; however much less is known about how environmental change will impact intraspecific genetic diversity (Bálint et al. 2011). Cryptic evolutionary lineages represent a fundamental level of biodiversity and recent work suggests that investigating the genetic effects of climate change merely at the level of morphospecies likely underestimates the magnitude of diversity loss that will occur within these lineages depending on the extent of range shifts (Bálint et al. 2011). In fact, the severity of reductions in intraspecific diversity may differ among leading-edge, central, and rear-edge populations with some evidence suggesting that rear-edge populations may be especially important for species persistence (Hampe 2005; Bálint et al. 2011; Hill et al. 2011). Rear edge populations, or those populations located at the margins of a species' range, may harbor the majority of species-level genetic diversity as a consequence of climate-driven range shifts and their location within less suitable habitats (Hampe 2005). As small, often isolated populations, within-population genetic diversity is typically low; however, because rear edge populations were not source populations for post-glacial expansion, they may harbor relatively distinct genetic diversity under selection for local adaptation (Petit et al. 2003; Hampe 2005).

Therefore, an understanding of how intraspecific genetic variation changes over multiple generations in response to declining habitat quality can be an important tool for assessing population-level extirpation risk. Recent work investigating loss of intraspecific genetic diversity in nine aquatic insect species of European mountain regions (Bálint et al. 2011) revealed insular distribution patterns that has led to strong genetic differentiation and found support for the prediction that species with strong population structure (such as pikas) are more like to suffer substantial losses of cryptic diversity. For a pika population inhabiting highly fragmented, low-elevation habitat at the southwestern end of the species' range, collapse of suitable habitat and the associated reduction in patch occupancy over the last 40 years has likely contributed to the patterns of genetic erosion observed (Chapter 3). In fact, these losses of genetic diversity, as measured by declining allelic diversity, increased population substructure, and a reduced Ne, were linked to 120-year trends of warming maximum and minimum temperatures as well as reductions in mean annual precipitation (Chapter 3). Since regional scale climate changes can be mediated by local topographic heterogeneity (Hampe 2005; Lundquist and Cayan 2007; Daly et al. 2009), this evidence for a strong association between population genetic patterns and regional climate patterns is intriguing and suggests that investigation of local microclimate patterns that better reflect on-site temperature conditions might strengthen this putative relationship. The extent to which ongoing range shifts and regional population extirpations will impact a species' genome-wide genetic variability and adaptive capacity is unclear and will likely require a combination of both large-scale and local-scale exploratory investigations (Vucetich and Waite 2003; Hampe 2005; Eckert et al. 2008). Genetic comparisons with other pika populations within the Bodie Hills and eastern Sierra Nevada regions indicate that loss of this peripheral pika population may result in the disappearance of unique genetic diversity (Chapter 3); therefore, maintaining the greatest number of local populations within each of the five major genetic lineages of the American pika should be promoted in future conservation work. Furthermore, genetic monitoring programs aimed at understanding adaptive trait variation across all five subspecies would help to parse out the effects of gene flow constraints on population viability at finer scales along elevational or environmental gradients across the entirety of the species' range.

References

Aho, K., Huntly, N., Moen, J., & Oksanen, T. (1998). Pikas (*Ochotona princeps*: Lagomorpha) as allogenic engineers in an alpine ecosystem. *Oecologia*, *114*(3), 405–409. doi:10.1007/s004420050463

Albano, N. (2012). Conservation physiology tools: their role in assessing habitat quality in birds.

Ardeola, *59*(2). doi: 10.13157/arla.59.2.2012.197

Alsos, I. G., Ehrich, D., Thuiller, W., Eidesen, P. B., Tribsch, A., Schönswetter, P., … Brochmann, C. (2012). Genetic consequences of climate change for northern plants. *Proceedings. Biological Sciences*, *279*(1735), 2042–51. doi:10.1098/rspb.2011.2363

Bálint, M., Domisch, S., Engelhardt, C. H. M., Haase, P., Lehrian, S., Sauer, J., … Nowak, C. (2011). Cryptic biodiversity loss linked to global climate change. *Nature Climate Change* *1*(6), 313–318. doi: 10.1038/nclimate1191

Beever, E. A., Brussard, P. F., & Berger, J. (2003). Patterns of apparent extirpation among isolatied populations of pikas (*Ochotona princeps*) in the Great Basin. *Journal of Mammalogy*, *84*(1), 37–54. doi:10.1644/1545-1542(2003)084<0037:POAEAI>2.0.CO;2

Beever, E. A., Ray, C., Mote, P. W., & Wilkening, J. L. (2010). Testing alternative models of climate-mediated extirpations. *Ecological Applications*, *20*(1), 164–178. doi: 10.1890/08-1011.1

Beever, E., Ray, C., & Wilkening, J. (2011). Contemporary climate change alters the pace and drivers of extinction. *Global Change Biology*, *17*, 2054–2070. doi:10.1111/j.1365-2486.2010.02389.x

Beever, E. A., Perrine, J. D., Rickman, T., Flores, M., Clark, J. P., Waters, C., … Collins, G. H. (2016). Pika (*Ochotona princeps*) losses from two isolated regions reflect temperature and water balance, but reflect habitat area in a mainland region. *Journal of Mammalogy*, *97*(6), 1495–1511. doi:10.1093/jmammal/gyw128

Beever, E. A., Hall, L. E., Varner, J., Loosen, A. E., Dunham, J. B., Gahl, M. K., … Lawler, J. J. (2017). Behavioral flexibility as a mechanism for coping with climate change. *Frontiers in Ecology and the Environment*, *15*(6), 299–308. doi:10.1002/fee.1502

Belmecheri, S., Babst, F., Wahl, E. R., Stahle, D. W., & Trouet, V. (2015). Multi-century evaluation of Sierra Nevada snowpack. *Nature Climate Change*, *6*(1), 2–3. doi; 10.1038/nclimate2809

Bradley, R. S., Vuille, M., Diaz, H. F., & Vergara, W. (2006). Climate change threats to water supplies in the tropical Andes. *Science (New York, N.Y.)*, *312*(5781), 1755–6. doi: 10.1126/science.1128087

Brooks, T. M. (2006). Global biodiversity conservation priorities. *Science*, *313*(5783), 58–61. doi:10.1126/science.1127609

Brown, C. J., O’Connor, M. I., Poloczanska, E. S., Schoeman, D. S., Buckley, L. B., Burrows, M. T., … Richardson, A. J. (2016). Ecological and methodological drivers of species’ distribution and phenology responses to climate change. *Global Change Biology*, *22*(4), 1548–1560. doi: 10.1111/gcb.13184

Calkins, M. T., Beever, E. A., Boykin, K. G., Frey, J. K., & Andersen, M. C. (2012). Not-so-splendid isolation: modeling climate-mediated range collapse of a montane mammal *Ochotona princeps* across numerous ecoregions. *Ecography, 35*(9), 780–791. doi: 10.1111/j.1600-0587.2011.07227.x

Ceballos, G., Ehrlich, P. R., Barnosky, A. D., Garcia, A., Pringle, R. M., & Palmer, T. M. (2015). Accelerated modern human-induced species losses: entering the sixth mass extinction. *Science Advances*, *1*(5), e1400253–e1400253. doi:10.1126/sciadv.1400253

Chen, I.-C., Hill, J. K., Ohlemüller, R., Roy, D. B., & Thomas, C. D. (2011). Rapid range shifts of species associated with high levels of climate warming. *Science (New York, N.Y.), 333*(6045), 1024–6. doi:10.1126/science.1206432

Cleland, E. E., Chuine, I., Menzel, A., Mooney, H. A., & Schwartz, M. D. (2007). Shifting plant phenology in response to global change. *Trends in Ecology & Evolution*, *22*(7), 357–365. doi:10.1016/J.TREE.2007.04.003

Corlett, R. T. (2015). The Anthropocene concept in ecology and conservation. *Trends in Ecology & Evolution*, *30*(1), 36–41. doi: 10.1016/j.tree.2014.10.007

Creel, S., Creel, N. M., & Monfort, S. L. (1997). Radiocollaring and stress hormones in African wild dogs. *Conservation Biology 11*: 544–548. doi: 10.2307/2387628

Crutzen, P. J. (2002). The "anthropocene." *Journal de Physique IV (Proceedings)*, *12*(10), 1–5. doi: 10.1051/jp4:20020447

Cucherousset, J., & Olden, J. D. (2011). Ecological impacts of nonnative freshwater fishes. *Fisheries*, *36*(5), 215–230. doi:10.1080/03632415.2011.574578

Daly, C., Conklin, D. R., & Unsworth, M. H. (2009). Local atmospheric decoupling in complex topography alters climate change impacts. *International Journal of Climatology*, *30*(12), 1857-1864. doi:10.1002/joc.2007

Diaz, H. F., Grosjean, M., & Graumlich, L. (2003). Climate variability and change in high elevation regions: past, present and future. *Climatic Change*, *59*(1/2), 1–4. doi: 10.1023/A:1024416227887

Diffenbaugh, N. S., Swain, D. L., & Touma, D. (2015). Anthropogenic warming has increased drought risk in California. *Proceedings of the National Academy of Sciences of the United States of America*, *112*(13), 3931–6. doi: 10.1073/pnas.1422385112

Dirzo, R., Young, H. S., Galetti, M., Ceballos, G., Isaac, N. J. B., & Collen, B. (2014). Defaunation in the Anthropocene. *Science (New York, N.Y.), 345*(6195), 401–6. doi: 10.1126/science.1251817

Doddington, B. J., Bosch, J., Oliver, J. A., Grassly, N. C., Garcia, G., Schmidt, B. R., … Fisher,

M. C. (2013). Context-dependent amphibian host population response to an invading pathogen. *Ecology*, *94*(8), 1795–1804. doi: 10.1890/12-1270.1

Duputié, A., Rutschmann, A., Ronce, O., & Chuine, I. (2015). Phenological plasticity will not help all species adapt to climate change. *Global Change Biology*, *21*(8), 3062–3073. doi:10.1111/gcb.12914

Eckert, C. G., Samis, K. E., & Lougheed, S. C. (2008). Genetic variation across species' geographical ranges: the central–marginal hypothesis and beyond. *Molecular Ecology*, *17*(5), 1170–1188. doi:10.1111/j.1365-294X.2007.03659.x

Erb, L. P., Ray, C., & Guralnick, R. (2011). On the generality of a climate-mediated shift in the distribution of the American pika (*Ochotona princeps*). *Ecology*, *92*(9), 1730–1735. doi:10.1890/11-0175.1

Feeley, K. J., & Rehm, E. M. (2012). Amazon's vulnerability to climate change heightened by deforestation and man-made dispersal barriers. *Global Change Biology*, *18*(12), 3606–3614. doi:10.1111/gcb.12012

Francis, C., Ortega, C., Cruz, A. (009). Noise pollution changes avian communities and species interactions. *Current Biology 19*(16), 1415-1419 doi:10.1016/j.cub.2009.06.052

Galbreath, K. E., Hafner, D. J., & Zamudio, K. R. (2009). When cold is better: climate-driven elevation shifts yield complex patterns of diversification and demography in an alpine specialist (American pika, *Ochotona princeps*). *Evolution*, *63*(11), 2848–2863. doi:10.1111/j.1558-5646.2009.00803.x

Galbreath, K. E., Hafner, D. J., Zamudio, K. R., & Agnew, K. (2010). Isolation and introgression in the Intermountain West: contrasting gene genealogies reveal the complex biogeographic history of the American pika (*Ochotona princeps*). *Journal of Biogeography*, *37*(2), 344–362. doi:10.1111/j.1365-2699.2009.02201.x

Goudie, A. (2015). *The human impact on the natural environment : past, present and future*. West Sussex, UK: Wiley-Blackwell.

Graham, C. H., Carnaval, A. C., Cadena, C. D., Zamudio, K. R., Roberts, T. E., Parra, J. L., … Sanders, N. J. (2014). The origin and maintenance of montane diversity: integrating evolutionary and ecological processes. *Ecography*, *37*(8), 711–719. doi:10.1111/ecog.00578

Grayson, D. K. (2005). A brief history of Great Basin pikas. *Journal of Biogeography*, *32*(12), 2103–2111. doi:10.1111/j.1365-2699.2005.01341.x

Griffin, D., & Anchukaitis, K. J. (2014). How unusual is the 2012-2014 California drought? *Geophysical Research Letters*, *41*(24), 9017–9023. doi:10.1002/2014GL062433

Habel, J. C., Rödder, D., Schmitt, T., & Néve, G. (2011). Global warming will affect the genetic diversity and uniqueness of *Lycaena helle* populations. *Global Change Biology*, *17*(1), 194–205. doi: 10.1111/j.1365-2486.2010.02233.x

Hampe, A., & Petit, R. J. (2005). Conserving biodiversity under climate change: the rear edge matters. *Ecology Letters*, *8*(5), 461–467. doi: 10.1111/j.1461-0248.2005.00739.x

Hill, J. K., Griffiths, H. M., & Thomas, C. D. (2011). Climate change and evolutionary adaptations at species' range margins. *Annual Review of Entomology*, *56*(1), 143–159. doi:10.1146/annurev-ento-120709-144746

Homyack, J. A. (2010). Evaluating habitat quality of vertebrates using conservation physiology tools. *Wildlife Research*, *37*(4), 332–342. doi:10.1071/WR08093

Inouye, D. W. (2008). Effects of climate change on phenology, frost damage, and floral abundance of montane wildflowers. *Ecology*, *89*(2), 353–362. doi:10.1890/06-2128.1

IPCC, 2014: Climate Change 2014: Synthesis report. Contribution of working groups I, II and III to the fifth assessment report of the intergovernmental panel on climate change [Core Writing Team, R.K. Pachauri and L.A. Meyer (eds.)]. IPCC, Geneva, Switzerland, 151 pp.

Jay, F., Manel, S., Alvarez, N., Durand, E. Y., Thuiller, W., Holdregger, R., … Francois, O. (2012). Forecasting changes in population genetic structure of alpine plants in response to global warming. *Molecular Ecology*, *21*(10), 2354–2368. doi:10.1111/j.1365-294X.2012.05541.x

Jeffress, M. R., Rodhouse, T. J., Ray, C., Wolff, S., & Epps, C. W. (2013). The idiosyncrasies of place: geographic variation in the climate-distribution relationships of the American pika. *Ecological Applications*, *23*(4), 864–878. doi:10.1890/12-0979.1

Johnston, K. M., Freund, K. A., & Schmitz, O. J. (2012). Projected range shifting by montane mammals under climate change: implications for Cascadia's National Parks. *Ecosphere*, *3*(11), 1-51. doi:10.1890/ES12-00077.1

Kelly, A. E., & Goulden, M. L. (2008). Rapid shifts in plant distribution with recent climate change. *Proceedings of the National Academy of Sciences of the United States of America*, *105*(33), 11823–6. doi:10.1073/pnas.0802891105

Knutson, M. G., Hines, R. K., Powell, L. A., Friberg, M. A., & Niemi, G. J. (2006). An assessment of bird quality using population growth rates. *The Condor 108*(2), 301-314. doi: 10.1650/0010-5422(2006)108[301: AAOBHQ]2.0.CO;2

Körner, C. (2004). Mountain biodiversity, its causes and function. *Ambio 13*, 11-17.

Korte, S. M., Koolhaas, J. M., Wingfield, J. C., & McEwen, B. S. (2005). The Darwinian concept of stress: benefits of allostasis and costs of allostatic load and the trade-offs in health and disease. *Neuroscience & Biobehavioral Reviews*, *29*(1), 3–38. doi:

10.1016/J.NEUBIOREV.2004.08.009

La Sorte, F. A., & Jetz, W. (2010). Projected range contractions of montane biodiversity under global warming. *Proceedings of the Royal Society B: Biological Sciences*, *277*(1699), 3401–3410. doi:10.1098/rspb.2010.0612

LaDochy, S., Medina, R., & Patzert, W. (2007). Recent California climate variability: spatial and temporal patterns in temperature trends. *Climate Research 33*(2), 159-169. doi:10.2307/24869330

Larsen, T. H. (2012). Upslope range shifts of Andean dung beetles in response to deforestation: compounding and confounding effects of microclimatic change. *Biotropica*, *44*(1), 82–89. doi: 10.1111/j.1744-7429.2011.00768.x

Loarie, S. R., Duffy, P. B., Hamilton, H., Asner, G. P., Field, C. B., & Ackerly, D. D. (2009). The velocity of climate change. *Nature*, *462*(7276), 1052–1055. doi:10.1038/nature08649

Looking forward, looking back: Humans, anthropogenic change, and the Anthropocene. (2013). *Anthropocene*, *4*, 116–121. doi:10.1016/J.ANCENE.2014.05.002

Lundquist, J. D., & Cayan, D. R. (2007). Surface temperature patterns in complex terrain: Daily variations and long-term change in the central Sierra Nevada, California. *Journal of Geophysical Research*, *112*(D11), D11124. doi:10.1029/2006JD007561

Mainguet, M. (2012). *Desertification: natural background and human mismanagement*. Berlin, Germany: Springer-Verlag.

McCain, C. M., & Colwell, R. K. (2011). Assessing the threat to montane biodiversity from discordant shifts in temperature and precipitation in a changing climate. *Ecology Letters*, *14*(12), 1236–1245. doi:10.1111/j.1461-0248.2011.01695.x

McEwen, B. S., & Wingfield, J. C. (2003). The concept of allostasis in biology and biomedicine. *Hormones and Behavior*, *43*(1), 2–15. doi:10.1016/S0018-506X(02)00024-7

McGill, B. J., Dornelas, M., Gotelli, N. J., & Magurran, A. E. (2015). Fifteen forms of biodiversity trend in the Anthropocene. *Trends in Ecology & Evolution*, *30*(2), 104–113. doi:10.1016/J.TREE.2014.11.006

Menzel, A., Sparks, T. H., Estrella, N., Koch, E., Aasa, A., Ahas, R., … Zust, A. (2006). European phenological response to climate change matches the warming pattern. *Global Change Biology*, *12*(10), 1969–1976. doi:10.1111/j.1365-2486.2006.01193.x

Merilä, J., & Hendry, A. P. (2014). Climate change, adaptation, and phenotypic plasticity: the problem and the evidence. *Evolutionary Applications*, *7*(1), 1–14. doi:10.1111/eva.12137

Messer, P. W., Ellner, S. P., & Hairston, N. G. (2016). Can population genetics adapt to rapid evolution? *Trends in Genetics*, *32*(7), 408–418. doi:10.1016/J.TIG.2016.04.005

Millar, C. I., Westfall, R. D., & Delany, D. L. (2013). New records of marginal locations for American pika (*Ochotona princeps*) in the western Great Basin. *Western North American Naturalist*, *73*(4), 457–476. doi:10.3398/064.073.0412

Moritz, C., Patton, J. L., Conroy, C. J., Parra, J. L., White, G. C., & Beissinger, S. R. (2008). Impact of a century of climate change on small-mammal communities in Yosemite National Park, USA. *Science (New York, N.Y.)*, *322*(5899), 261–4. doi:10.1126/science.1163428

Nichols, L. B., Klingler, K. B., & Peacock, M. M. (2016). American Pikas (*Ochotona princeps*) extirpated from the historic masonic mining district of eastern California. *Western North American Naturalist*, *76*(2), 163–171. doi:10.3398/064.076.0203

Nogués-Bravo, D., Araújo, M. B., Errea, M. P., & Martínez-Rica, J. P. (2007). Exposure of global mountain systems to climate warming during the 21st Century. *Global Environmental Change*, *17*(3–4), 420–428. doi:10.1016/J.GLOENVCHA.2006.11.007

Okin, G. S., Parsons, A. J., Wainwright, J., Herrick, J. E., Bestelmeyer, B. T., Peters, D. C., & Fredrickson, E. L. (2009). Do changes in connectivity explain desertification? *BioScience*, *59*(3), 237–244. doi:10.1525/bio.2009.59.3.8

Ortega, C. P. (n.d.). Effects of noise pollution on birds: a brief review of our knowledge. doi:10.1525/om.2012.74.1.6

Otto, S. A., Diekmann, R., Flinkman, J., Kornilovs, G., & Möllmann, C. (2014). Habitat heterogeneity determines climate impact on zooplankton community structure and dynamics. *PLoS ONE*, *9*(3), e90875. doi:10.1371/journal.pone.0090875

Petit, R. J., Aguinagalde, I., de Beaulieu, J.-L., Bittkau, C., Brewer, S., Cheddadi, R., … Vendramin, G. G. (2003). Glacial refugia: hotspots but not melting pots of genetic diversity. *Science (New York, N.Y.)*, *300*(5625), 1563–5. doi: 10.1126/science.1083264

Rapacciuolo, G., Maher, S. P., Schneider, A. C., Hammond, T. T., Jabis, M. D., Walsh, R. E., … Beissinger, S. R. (2014). Beyond a warming fingerprint: individualistic biogeographic responses to heterogeneous climate change in California. *Global Change Biology*, *20*(9), 2841–2855. doi:10.1111/gcb.12638

Ray, C., Beever, E., & Loarie, S. (2012). Retreat of the American pika: up the mountain or into the void. *In Wildlife Conservation in a Changing Climate* (1st ed., pp. 245-270). Chicago: The University of Chicago Press.

Rockström, J., Steffen, W., Noone, K., Persson, Å., Chapin, F. S., Lambin, E. F., … Foley, J. A. (2009). A safe operating space for humanity. *Nature*, *461*(7263), 472–475. doi:10.1038/461472a

Rodhouse, T. J., Beever, E. A., Garrett, L. K., Irvine, K. M., Jeffress, M. R., Munts, M., & Ray, C. (2010). Distribution of American pikas in a low-elevation lava landscape: conservation implications from the range periphery. *Journal of Mammalogy*, *91*(5), 1287–1299. doi:10.1644/09-MAMM-A-334.1

Romero, L. M. (2004). Physiological stress in ecology: lessons from biomedical research. *Trends in Ecology & Evolution*, *19*(5), 249–255. doi:10.1016/j.tree.2004.03.008

Rubidge, E. M., Patton, J. L., Lim, M., Burton, A. C., Brashares, J. S., & Moritz, C. (2012). Climate-induced range contraction drives genetic erosion in an alpine mammal. *Nature Climate Change*, *2*(4), 285–288. doi:10.1038/nclimate1415

Schwalm, D., Epps, C. W., Rodhouse, T. J., Monahan, W. B., Castillo, J. A., Ray, C., & Jeffress, M. R. (2016). Habitat availability and gene flow influence diverging local population trajectories under scenarios of climate change: a place-based approach. *Global Change Biology*, *22*(4), 1572–1584. doi:10.1111/gcb.13189

Shinderman, M. (2015). American pika in a low-elevation lava landscape: expanding the known distribution of a temperature-sensitive species. *Ecology and Evolution*, *5*(17), 3666–3676. doi:10.1002/ece3.1626

Sih, A., Stamps, J., Yang, L. H., McElreath, R., & Ramenofsky, M. (2010). Behavior as a key component of integrative biology in a human-altered world. *Integrative and Comparative Biology*, *50*(6), 934–944. doi:10.1093/icb/icq148

Simpson, W. G. (2009). American pikas inhabit low-elevation sites outside the species' previously described bioclimatic envelope. *Western North American Naturalist*, *69*(2), 243–250. doi:10.3398/064.069.0213

Smith, A. T., & Weston, M. L. (1990). *Ochotona princeps*. *Mammalian Species*, (352), 1. doi:10.2307/3504319

Smith, B., Zeder, M.A. (2013). The onset of the Anthropocene. *Anthropocene 4*, 8-13. doi.org/10.1016/j.ancene.2013.05.001

Smith, D. M., Cusack, S., Colman, A. W., Folland, C. K., Harris, G. R., & Murphy, J. M. (2007). Improved surface temperature prediction for the coming decade from a global climate model. *Science (New York, N.Y.)*, *317*(5839), 796–9. doi:10.1126/science.1139540

Snell-Rood, E. C. (2013). An overview of the evolutionary causes and consequences of behavioural plasticity. *Animal Behaviour*, *85*(5), 1004–1011. doi:10.1016/J.ANBEHAV.2012.12.031

Staudinger, M. D., Carter, S. L., Cross, M. S., Dubois, N. S., Duffy, J. E., Enquist, C., … Turner, W. (2013). Biodiversity in a changing climate: a synthesis of current and projected trends in the US. *Frontiers in Ecology and the Environment*, *11*(9), 465–473. doi:10.1890/120272

Steffen, W., Crutzen, P. J., & McNeill, J. R. (2007). The Anthropocene: are humans now overwhelming the great forces of nature. *AMBIO: A Journal of the Human Environment 36*(8), 614-621. doi:10.1579/0044-7447(2007)36[614:TAAHNO]2.0.CO;2

Stewart, J. A. E., Perrine, J. D., Nichols, L. B., Thorne, J. H., Millar, C. I., Goehring, K. E., … Wright, D. H. (2015). Revisiting the past to foretell the future: summer temperature and habitat area predict pika extirpations in California. *Journal of Biogeography*, *42*(5), 880–890. doi:10.1111/jbi.12466

Thomas, C. D., Franco, A. M. A., & Hill, J. K. (2006). Range retractions and extinction in the face of climate warming. *Trends in Ecology & Evolution*, *21*(8), 415–416. doi:10.1016/J.TREE.2006.05.012

Thuiller, W., Lavorel, S., Araújo, M. B., Sykes, M. T., & Prentice, I. C. (2005). Climate change threats to plant diversity in Europe. *Proceedings of the National Academy of Sciences of the United States of America*, *102*(23), 8245–50. doi:10.1073/pnas.0409902102

Urban, M. C. (2015). Accelerating extinction risk from climate change. *Science 348* (6234), 571–3. doi: 10.1126/science.aaa4984

Van Buskirk, J. (2012). Behavioural plasticity and environmental change. *In Behavioural responses to a changing world: mechanisms and conseqeunces* (1st ed, pp.145-158). Oxford, Oxford University Press.

Varner, J., & Dearing, M. D. (2014). Dietary plasticity in pikas as a strategy for atypical resource landscapes. *Journal of Mammalogy*, *95*(1), 72–81. doi:10.1644/13-MAMM-A-099.1

Varner, J., Horns, J. J., Lambert, M. S., Westberg, E., Ruff, J. S., Wolfenberger, K., … Dearing, M. D. (2016). Plastic pikas: behavioural flexibility in low-elevation pikas (*Ochotona princeps*). *Behavioural Processes*, *125*, 63–71. doi:10.1016/J.BEPROC.2016.01.009

Vucetich, J. A., & Waite, T. A. (2003). Spatial patterns of demography and genetic processes across the species' range: null hypotheses for landscape conservation genetics. *Conservation Genetics*, *4*(5), 639–645. doi:10.1023/A:1025671831349

Wallach, A. D., Johnson, C. N., Ritchie, E. G., & O'Neill, A. J. (2010). Predator control promotes invasive dominated ecological states. *Ecology Letters*, *13*(8),1008-1018. doi:10.1111/j.1461-0248.2010.01492.x

Walther, G., Post, E., Convey, P., Menzel, A., & Parmesan, C. (2002). Ecological responses to recent climate change. *Nature 416*, 389-395.doi: 10.1038/416389a

Wilkening, J. L., Ray, C., Beever, E. A., & Brussard, P. F. (2011). Modeling contemporary range retraction in Great Basin pikas (*Ochotona princeps*) using data on microclimate and microhabitat. *Quaternary International*, *235*(1–2), 77–88. doi:10.1016/j.quaint.2010.05.004

Wilkening, J. L., Ray, C., & Sweazea, K. L. (2013). Stress hormone concentration in

RockyMountain populations of the American pika (*Ochotona princeps*). *Conservation Physiology*, *1*(1), 1–13. doi:10.1093/conphys/cot027

Wilkening, J. L., Ray, C., & Varner, J. (2015). Relating sub-surface ice features to physiological stress in a climate sensitive mammal, the American pika (*Ochotona princeps*). *PLoS ONE*, *10*(3), 1–17. doi:10.1371/journal.pone.0119327

Wilkening, J. L., Ray, C., & Varner, J. (2016). When can we measure stress noninvasively? Postdeposition effects on a fecal stress metric confound a multiregional assessment. *Ecology and Evolution*, *6*(2), 502–513. doi:10.1002/ece3.1857

CHAPTER 1 The effects of site, season, and territory variability on fecal GCM concentrations for the American pika (*Ochotona princeps*) within high-elevation talus habitat in Mono County, California, USA

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Abstract

Major environmental perturbations that disrupt the predictability and phenology of ecological cues can have significant impacts on a species' persistence. Conservation physiology aims to identify the effects of disturbance on wildlife through their physiological response and downstream effects on behavior, survival, and reproduction. In response to a stressor, vertebrates release glucocorticoid hormones (such as cortisol or corticosterone) through activation of the hypothalamic-pituitary-adrenal axis. The amount of glucocorticoid hormones and their metabolites in plasma, saliva, hair, or feces provides a temporal record of an animal's endocrine condition and is considered a useful metric for monitoring population level health. Despite widespread measurement of physiological stress, interpretation is heavily dependent upon a demonstrated understanding of individual fitness effects across a range of glucocorticoid concentrations which may vary throughout a species' range and even between seasons. This study monitored endocrine condition in the American pika (*Ochotona princeps*) through collection of fecal glucocorticoid metabolites in two high-elevation sites within the eastern Sierra Nevada of California. Pikas are a small lagomorph species considered vulnerable to climate change largely because of an inability to easily dissipate heat without substrate-enabled behavioral thermoregulation. By pairing endocrine information with genetic and microclimate temperature data, we examined the effects of site and season-specific stressors on pika endocrine condition through repeated measurements of stress at occupied territories. In addition to revealing an association between endocrine condition and physiologically relevant temperature metrics, our results suggest an important role for seasonal temperature variation, site, and territory in patterns of individual and population-level pika stress.

Keywords (in alpha order): American pika, glucocorticoid metabolites, physiological stress, mixed effects modeling

Introduction

Concern regarding the detrimental effects of anthropogenic disturbance on wildlife populations has resulted in efforts to quantify levels of physiological stress associated with perturbations such as habitat fragmentation (Martínez-Mota et al. 2007; Janin et al. 2011), anthropogenic noise (Anderson et al. 2011; Blickley et al. 2012a, b; Rolland et al. 2012), habitat quality (Wasser et al. 1997; Newcomb Homan et al. 2003; Cash and Holberton 2005; Hopkins and DuRant 2011), and climate change (Cockrem 2013). The vertebrate stress response has evolved to provide animals with the ability to respond appropriately to predictable daily or seasonal environmental variability through the release of glucocorticoid hormones (GCs; Romero 2002). In response to a stressor, such as a predation attempt, resource limitations or challenging climatic conditions, the hypothalamic-pituitary-adrenal (HPA) axis is activated with secretions of GCs occurring for several minutes to multiple hours depending on the event duration (Saplosky 1992; Sapolsky et al. 2000; Wingfield and Romero 2001). The release of GCs, such as cortisol or corticosterone, though energetically expensive, enables behavioral and physiological responses to the stressor and may ultimately result in increased survival in the short term (Romero 2002; 2004). However, the sustained release of GCs as a result of chronic stress can be maladaptive and lead to immunosuppression, decreased reproduction, and the occurrence of pathologies (Boonstra et al. 1998; Blas et al. 2007; Sheriff et al. 2009). Thus, an understanding of the physiological response to stress and the subsequent effects on behavior, survival, and reproduction can be used to improve predictions regarding impacts of ongoing and future disturbances on population viability (Danzer 2014).

The concentration of GCs has traditionally been measured using blood plasma (Wingfield et al. 1992; Sapolsky et al. 2000; Romero 2002; Wilkening et al. 2013). However, if the blood sample is collected more than three minutes after capture, circulating hormone levels can increase rapidly as a direct response to capture stress, thus biasing the GC measurement and the subsequent interpretation (Wilkening et al. 2013). The associated stress of capturing and handling with most vertebrate species highlights the need for development of non-invasive approaches to GC measurement especially for elusive or endangered species that may be highly vulnerable to extinction. Fortunately, steroid hormones, such as corticosterone, are metabolized by the liver and eventually excreted as metabolites through the gut and therefore can be detected in animal waste products. Since GCs or their metabolites can be measured in hair or feces (Sheriff et al. 2011), wildlife studies are increasingly using such samples to measure physiological stress in individuals and populations in order to understand the effects of both natural (e.g. storm events, predators, disease, and conspecific competition) and anthropogenic (e.g. noise pollution and invasive species) stressors on survival and reproductive success (Hadinger et al. 2015; Tennessen et al. 2015; Wilkening and Ray 2016).

The collection of fecal glucocorticoid metabolites (GCMs) provides two advantages over plasma samples in that they: (i) can be collected noninvasively and repeatedly over time with less direct impact to the individual, and (ii) integrate over time and thus minimize the effects of small changes in daily hormone secretion which enables more accurate detection of broad-scale patterns (Wilkening et al. 2013; Palme 2005). Because stress hormones are important components in how vertebrates interact with their environment, long-term monitoring programs that incorporate tracking of an individual’s stress response (through either GCs or GCMs) can help provide an ‘early warning system’ for at-risk populations if baseline thresholds are surpassed (Lindemayer et al. 2013; Danzter 2014). Therefore, the measurement of stress hormone concentrations within individuals is often done to quantify and evaluate differences in stress within and among populations (Wingfield et al. 1994; Creel et al. 1997; Wasser et al. 2000; Romero 2004; Wilkening and Ray 2016).

Species-specific patterns of GC secretion and interpretation of the putative effects on population viability is only possible if baseline concentrations have been established (Millspaugh and Washburn 2004; Wilkening and Ray 2016). Wilkening et al. (2013) recently validated the use of a corticosterone enzyme immunoassay for measuring GCMs in American pika (*Ochotona princeps*) feces and established the directionality of the relationship such that GCMs increase in response to the stress of capture. The American pika (*Ochotona princeps*; hereafter, pikas) is a small (~150 g), cold-adapted lagomorph associated with high-elevation rocky substrates distributed across the western United States and Canada. As a thick-furred and non-hibernating mammal, pikas are considered sensitive to warming temperatures associated with climate change due to their reliance on microclimate refugia of interstitial talus space in order to thermoregulate (Smith and Weston 1990; Beever et al. 2013). The American pika has suffered significant population losses during the 20th and 21st centuries, especially across the southern portion of their range (e.g. Great Basin and Sierra Nevada; Beever et al. 2003, 2011; Wilkening et al. 2011; Stewart et al. 2015; Nichols et al. 2016). In these regions, pikas are demonstrably retreating upslope with the lowest elevation of pika occurrence haven risen approximately 145 m per decade since the beginning of the 21st century (Beever et al. 2011). As a result, pikas have been petitioned for listing as threatened or endangered at both federal and state levels (United States Fish and Wildlife Service 2010; Osborn and Applebee 2011; California Department of Fish and Wildlife 2013). Although summer mean temperature has been associated with patterns of pika persistence (Stewart et al. 2015; Beever et al. 2003, 2010, 2011, 2013; Wilkening et al. 2011; Calkins et al. 2012), it is also clear that habitat features such as talus area, habitat configuration, and functional connectivity significantly mediate the impacts of climate for this species and likely enable persistence of populations that occur outside the species' bioclimatic envelope (Jeffress et al. 2013; Shinderman et al. 2015; Schwalm et al. 2016).

Wilkening et al. (2013) was the first study to measure stress hormone metabolites in any species of pika and provided baseline (pre-peak), peak (highest), and post-peak GCM concentrations for pikas in Rocky Mountain populations. Environmental factors, such as temperature or precipitation, can modify concentrations of GCMs in exposed fecal samples collected at some point post-defecation (Khan et al. 2002; Millspaugh and Washburn 2004; Palme 2005; Shutt et al. 2012; Wilkening et al. 2016). An inability to parse the confounding effects of environmental biases from the true endocrine signal is a serious concern for interpretation of stress data; therefore, multiregional comparisons may be challenging because of differing local climatic conditions. In fact, the majority of research focusing on endocrine patterns in pikas has occurred in the Rocky Mountains, a region where pika habitats are considered to be more buffered against changing climatic conditions than in other parts of the range (Erb et al. 2011; Jeffress et al. 2013; Schwalm et al. 2016). As a result, we do not have a standard range of baseline GCM concentrations established across the species' distribution which includes five major mountain systems (Galbreath et al. 2009; Calkins et al. 2012). Fortunately, GCM concentrations are considered reliable indicators of population health if used as a monitoring tool within an eco-region and collected within the same season (small spatial and short temporal scales) (Wilkening et al. 2016).

Recent work has also established that physiological stress in pikas differs significantly among individuals and appears to be influenced by habitat characteristics (Wilkening et al. 2015), such as sub-surface ice features, that significantly decrease individual stress perhaps due to lower mean summer temperatures provided by mediating microclimates (Wilkening et al. 2015). Importantly, Wilkening and Ray (2016) also established a fitness link by determining that fecal GCM concentrations were a better predictor of annual survival in pikas than plasma GC and that those individuals with higher baseline GCM levels were less likely to survive through the next year. Inter-individual variation in baseline corticosterone levels has been linked to differences related to life history stage (Williams et al. 2008; Love et al. 2014), food availability (Kitaysky et al. 1999; Lanctot et al. 2003), individual experience (Beletsky et al. 1989; de Kloet et al. 2005; Angelier et al. 2007), and predator pressure (Scheuerlein et al. 2001; Clinchy et al. 2004). In fact, in some species, there has been evidence suggesting a heritable component underlying variation in corticosterone levels (Satterlee and Johnson 1988; Federenko et al. 2004; Evans et al. 2006; Jenkins et al. 2014) and plasticity of stress-coping phenotypes (Koolhaas et al. 1999; Overli et al. 2007; Ouyang et al. 2011; Love et al. 2014). As a result GC or GCM concentrations may be indicative of individual quality with lower quality individuals maintaining higher average baseline levels in response to perceived challenges (Husak and Moore 2008; Wada et al. 2008; Bonier et al. 2009a,b). Evidence of intra-individual variation in corticosterone concentrations suggests that repeated sampling of an individual's endocrine condition may be necessary to understand differences in hormonal phenotypes (Romero and Reed 2008; Vitousek et al. 2008; Angelier et al. 2010; Rensel and Schoech 2011; Ouyang et al. 2011). Genetic markers, can be used with fecal DNA collected simultaneously with fecal GCMs, to identify repeatedly sampled individuals in order to establish fine-scale GCM patterns among individuals through time. Additionally, we used these genetic data to obtain a measure of individual variability in genetic background and to evaluate genetic connectivity among sampling sites for an understanding of plasticity of the stress response within the dispersal distance of an individual pika.

Here, we use stress hormone concentrations as a measurement of endocrine condition and integrate these physiological data with paired genetic and microclimate information to assess individual and population level physiological stress in pikas. Using a time-series of noninvasively collected fecal GCMs, this study aimed to characterize and compare natural fluctuations in GCM concentrations at both the individual and population-level scales over time (i.e. within and across seasons) at two sites located in the Sierra Nevada during July through September of 2013-2015. This is a region in which pika GCM patterns have not previously been described, but it is considered to support a relatively vulnerable pika lineage and is located where the magnitude of climate change effects to pikas are expected to be significant (Galbreath et al. 2009; 2010). Because local site conditions influence pika persistence (Jeffress et al. 2013; Schwalm et al. 2016), we hypothesized that populations likely differ in their capacity to respond to environmental change. Specifically, we predicted that: (i) **site-level patterns** in average GCM concentrations would differ due to fine-scale habitat features and within and between-season variation in resource availability; (ii) **individual variation** in the stress response (Bonier et al. 2009a,b) occurs and may be associated with differences in territory turnover rates and/or individual recapture rates ; (iii) **sexes** (as determined by microsatellite sexing) would significantly differ in relative GCM levels with males experiencing higher GCM levels than females due to an increase in the release of glucocorticoids as a result of testosterone as has been previously reported (Wilkening et al. 2013); and finally that (iv) **territory-specific microclimates** (surface and interstitial) across one talus slope will vary in terms of heat and cold-related temperature stress and therefore be associated with significantly different average GCM concentrations as well as average turnover rates.

Methods

Study sites

During the summers (July-September) of 2013-2015, fresh fecal samples were collected repeatedly from occupied pika territories in multiple populations located in the Sierra Nevada to compare variation in fecal GCM concentrations: (i) among individuals; (ii) between sexes; (iii) among territories; and (iv) among seasons and years. We simultaneously collected fresh fecal material for population genetic analyses. Two high-elevation sites separated by 2 km of semi-continuous talus, Cabin Slope (3031 m in elevation, 3.9 ha in area) and Pipet Tarn (3170 m in elevation, 14.8 ha in area) were our main sampling locations. These sites are located in the Harvey Monroe Hall Natural Area (Inyo County, California, USA), a 1400-ha United States Forest Service natural preserve bordered by Yosemite National Park to the south, and which have large continuous talus patches typical of pika habitat (Fig. 1). Each territory in Pipet Tarn was also outfitted with two temperature data loggers in order to calculate the respective microclimates within interstitial space and under ambient surface conditions. Multiple intervening locations were also sampled for genetic analysis.

Paired fecal GCM and fecal DNA sample collection

For fecal GCM samples, a minimum of ten fresh fecal pellets (indicated by golden-green color and wet from urination) were collected during each visit and stored in coin envelopes and on ice until return to the lab where they were stored in a -20°C freezer at the University of Nevada, Reno prior to processing. For genetic samples, additional fresh fecal pellets were identified using the protocol of Nichols et al. (2011) and collected using sterilized tweezers (dipped in 70% ethanol between samples). Two to five pellets were transferred to a 2.0 ml vial of 500 µl of ATL tissue lysis buffer (DNeasy96 blood and tissue extraction kits, QIAGEN) in the field, and 400 µl of supernatant from each sample was transferred to ice-cold proteinase K for storage until DNA extraction was performed in the laboratory. We genotyped fecal DNA collected from individual pikas at ten nuclear and neutral microsatellite markers that were optimized for multiplexing with low quality fecal DNA (Peacock and Kirchoff 2002). Non-invasive collection of fecal DNA allows for collection of a larger sample size and tracking of a subset of individual pikas over time at each territory through genetic “ID tags”.

Extraction of hormone metabolites and enzyme immunoassays

Each GCM fecal sample was lyophilized and approximately six pellets per sample were randomly chosen and ground into powder using a mortar and pestle. Steroid metabolites were extracted using a steroid solid extraction protocol developed by Arbor Assay Design, Inc. (Ann Arbor, MI, USA). Approximately 0.05 g of dried fecal material was immersed in 1.5 ml of 90% aqueous ethanol and placed on a rocker for 1 hour. Samples were centrifuged for 15 minutes at 5000 rpm and 1.0 ml of the supernatant was transferred to a clean screw-on tube. The supernatant solution was then evaporated to dryness under nitrogen and extracts were stored in a -20°C freezer. Before enzyme immunoassay analysis, extracts were reconstituted with 50 µl of 90% aqueous ethanol and 950 µl of assay buffer and mixed thoroughly using a vortex shaker.

Corticosterone, rather than cortisol, is the primary GC in lagomorphs (Teskey-Gerstl et al. 2000; Monclus et al. 2006; Rehnus et al. 2009; Sheriff et al. 2009; Scarlata et al. 2011), therefore corticosterone metabolites were measured in this study following the Wilkening et al.

(2013) protocol. GCM levels were measured using a commercially available corticosterone enzyme immunoassay kit (Arbor Assay Design, Inc.; catalogue no. K014-H1 or K014-H5). This assay, which utilized a sheep poly-clonal antibody specific for corticosterone and was developed to estimate total corticosterone in many sample types including fecal extracts, has been validated for the American pika (Wilkening et al. 2013). The extracted samples were run in duplicate during each assay with seven known concentrations of corticosterone (5000, 2500, 1250, 625, 312.50, 156.25, and 78.125 pg/ml), and the value for each extracted sample was read using a microplate reader at 420 nm. A standard curve was then generated using the averaged results from the known corticosterone concentrations, and GCM concentrations were then calculated from the standard curve.

Fecal DNA isolation, extraction, and microsatellite genotyping

Total genomic DNA was isolated using DNeasy96 Blood and Tissue Kits (QIAGEN, Inc.) according to the manufacturer's protocol with minor alterations specific to each sample type. Fourteen neutral di, tri, and tetranucleotide repeat, polymorphic microsatellite loci have been developed for the American pika (Peacock et al. 2002; Peacock and Kirchoff 2009; unpublished locus sequences – Genbank accession numbers GQ461714.1-461723.1). After PCR optimization, samples were amplified with ten polymorphic microsatellite markers that consistently amplified using low quality fecal DNA (Table S1). All microsatellite PCR reactions were completed on a MBS Satellite 0.2G thermal cycler (Thermo Electron Corporation) and included a 15-minute hot start at 95°C, followed by 41 cycles of 95°C for 30 seconds, a touch down annealing temperature of 65°C to 55°C for 90 seconds and an elongation step at 72°C for 30 seconds. The touch down annealing temperature starts with 7 cycles of 65°C, 7 cycles at 61°C, 7 cycles at 58°C, and 20 cycles at 55°C. The first 21 cycles amplified the locus specific primer and the final 20 cycles added the fluorescently labeled M13 tail to the PCR product.

All PCR products were diluted to an appropriate concentration, typically 1:5 PCR product to deionized water. One microliter of the diluted PCR product was then transferred to 19 μl of HiDye Formamide/LIZ500 size standard (Applied Biosystems). Fragment size analysis was carried out on an Applied Biosystems 3730 DNA Genetic Analyzer (Nevada Genomics Center, http://www.ag.unr.edu/genomics/), and alleles were scored, binned, and genotyped using ABI Prism GeneScan (version 3.5.1) and GeneMapper (version 3.7) software. To avoid genotyping and sample ID error using low-quality fecal samples, individual identity was only assigned if a consensus genotype was achieved after three independent PCRs for each multi-locus panel (per-locus genotype was consistent across at least two separate runs) (Paetkau 2003; Pederson 2012).

Fecal GCM comparisons among individuals, between sites, and across seasons

Endocrine data were analyzed separately for individuals, territories, and sites across time in order to explore fecal GCM patterns at both individual and population levels (Wilkening et al. 2013). Prior to analysis, GCM data were checked for outliers, and the relationship between variables was visualized using *ggplot2* (Wickham 2009; R core team 2016). Normality was tested using a Shapiro-Wilks statistic and F-tests were used to evaluate equality of variance between samples. A Welch’s t-test was applied to assess overall differences in GCM concentrations among samples within and between the Pipet Tarn and Cabin Slope sites (Wilkening et al. 2015). Individual-specific variation in stress hormone concentrations was assessed across territories and seasons through opportunistic genetic mark-recapture efforts using microsatellite genotypes as individual identifiers. To investigate stress levels that could be associated with territory quality, we compared GCM levels for individuals who successively occupied the same territory for multiple seasons during the study.

We identified sex per individual using a PCR approach to amplify Y chromosome-specific fragments or homologous fragments derived from both sex chromosomes (Fontanesi et al. 2008). In pikas, DNA obtained from hair samples has been used successfully to sex individuals at the ZFX and ZFY loci (Henry et al. 2011). Recently, we developed a method for sexing pikas using the amplification of fragments of the ZFX/ZFY locus by fluorescently labeling the commercially available SRY microsatellite (sex determining region of Y chromosome). The SRY primer was optimized such that it could be run simultaneously with the OCP 10 microsatellite locus, which served as a PCR amplification control. Male-specific SRY locus fragments amplify a homozygous peak at 117 bp, whereas females fail to amplify any fragments. In order to determine a sex for an individual, each sample was run at least twice and had to consistently amplify a homozygous peak at 117 bp to be considered a male. Females were identified if samples failed to amplify during both runs at SRY, but consistently amplified OCP 10 during the same PCR reaction. If after two runs, the data were inconsistent the sample was considered an unknown sex and not included in any sex-specific analyses.

For each territory, the average rate of turnover was calculated as the total number of new captures divided by the total number of sampling visits associated with an individual genetic ID. Recaptures occurred when two genetic samples collected at the same territory matched across a minimum threshold of at least 18/22 total microsatellite alleles (including the corresponding sexing locus). This allowed for recaptures to be considered despite failed amplification or allelic mismatch at 1-2 total loci per fecal DNA sample. Allelic mismatch often occurred due to failure to obtain a consensus genotype at a locus despite repeated amplification (at least 2 to 3 independent PCRs per locus). Turnover rate (%) was defined as the number of new individuals divided by the total number of sampling visits that resulted in the successful genotyping of an individual genetic ID. Individual recapture rates and the average territory turnover rates were then associated with measures of GCM concentrations at both the territory and individual scales.

Genetic population structure

Genetic data were analyzed for each of three sites across the 2 km of continuous, alpine talus habitat to test for connectivity among sampling sites. Linkage disequilibrium was tested using the heterozygosity deficit test implemented in Genepop 4.0 (Rousset 2008). FSTAT (version 2.9.3.2; Goudet 2001) was used to quantify the number of alleles (N_A), heterozygosity (He, Ho) and to test for deviations from Hardy-Weinberg equilibrium (F_{IS}) per locus per population. If a locus exhibited systematic patterns of deviation from HWE or there was evidence of null alleles or allelic dropout, it was removed from the analysis (Micro-Checker 2.2.3; Van Oosterhout et al. 2004). To assess levels of genetic differentiation across this continuous habitat, pairwise F_{ST} values were calculated using FSTAT. To examine population structure without a priori population designations, a principal coordinates analysis was performed to visualize the spatial patterns of genetic differentiation among the two sampling sites using the program GenAlEx 6.5 (Peakall and Smouse 2006; 2012).

Microclimate characterization using temperature loggers

Temperature data were collected to assess variation in territory microclimate across the Pipet Tarn site and to calculate temperature metrics considered relevant to pika physiology for inclusion in mixed-effects modeling of pika fecal GCMs (Wilkening et al. 2011; Wilkening et al. 2015; Schwalm et al. 2016). At the Pipet Tarn site only, 38 LogTag TRIX-16 Temperature

Recorders (ThermoWorks, Utah, U.S.; 32K bytes of memory; -40°C to 85°C temperature measurement range with 0.5°C accuracy) were programmed to record a temperature reading in degrees Celsius every 35 minutes and placed at 19 occupied territories within the 14.8 ha talus area. One data logger was placed adjacent to each fresh haypile at the surface (i.e. ambient microclimate) and a second logger was placed at ~3 meters below the surface of the talus (i.e. interstitial microclimate). Each pair of temperature loggers located at an individual territory were at a minimum of 30 m from the next nearest territory, with the greatest distance between occupied territories approximately 421 m (Fig. 1). All temperature loggers were secured with wire cable at an angle that prevented exposure to direct sunlight, and approximate locations recorded with hand held GPS loggers.

QA/ QC of temperature data

To minimize bias and ensure quality control of these sensor data, all temperature data were examined manually for inconsistencies (e.g. extreme outliers, missing data) by plotting each year separately. There were no missing data detected, although complete logger failure occurred during the study period for one surface logger and one depth logger, respectively. The data from the failed loggers were removed from further analysis. The binary treatment (surface and depth loggers) enabled a more accurate understanding of the average daily and monthly temperature patterns for identification of outlier values that should be considered extreme either for ambient or interstitial conditions, respectively. The analysis period was restricted to 8 Oct 2012 to 25 Aug 2015 since the specific deployment period varied by a day or two per logger per year.

To initially process the raw data from each logger and ensure the same number of sampling readings per day, we deleted the first reading each logger recorded after midnight (anywhere between 12:00-12:35 am), which resulted in a total of 40 readings per day per logger. Despite the issue of defining each day due to a recording frequency of 35 minutes rather than every 30 minutes, all loggers were started simultaneously and therefore captured similar daily variability. Monthly averages (±1 SD) were first determined for each logger to visually check for any discrepancies prior to calculation of specific, physiologically relevant temperature metrics. We combined data from all years of the study (ca. 2012-2015) for each logger and then removed any temperature data associated with days prior to 8 Oct 2012, and 25 Aug 2015. For each logger, we checked: 1) that Tmax was always greater than Tmin, (Reek et al. 1992; Blandford et al. 2008), 2) for the presence of missing data, and 3) the frequency of acute heat stress (days above 26°C or 28°C) experienced per logger. The microclimate temperature metrics calculated for this study were selected based on previous pika microclimate studies (Beever et al. 2010; Wilkening et al. 2011; 2015; Schwalm et al. 2016) and are referred to in terms of their physiological relevance to pikas: acute or chronic high temperature stress and acute or chronic low temperature stress. These temperature metrics included: temperature seasonality (difference between annual maximum and minimum temperature), average summer temperature (average of all temperatures during Jun-Sept) (heat stress), number of days below -5°C (cold stress), number of days below -10°C (cold stress), number of days above 26°C (heat stress), number of days above 28°C (heat stress), mean temperature of coldest quarter (cold stress), mean temperature of warmest quarter (heat stress), maximum temperature in July (heat stress), and minimum temperature in January (cold stress). All metrics were calculated at each territory for surface and interstitial microclimate locations. Comparisons were made using one-way ANOVAs to determine whether significant temperature differences exist: (i) between surface and interstitial microclimates, (ii) among individual territories, and (iii) within territories over time.

Most of the temperature metrics calculated for this study were monthly or annual averages for comparison between the ambient and subsurface microclimates and for association with physiological stress data. For the four daily metrics used in this study, we calculated the acute cold (db5; db10), and acute heat (da26, and da28) metrics for each logger by assigning a "1" if an acute reading within a 24-hour period occurred and then summing these values per day (0 or 1) across all days per year for an annual estimate per metric.

For fecal samples collected opportunistically or at territories without temperature loggers, a distance matrix was used to associate GCM data with distance-weighted average temperature metrics following Wilkening et al. (2015). The average minimum distance between sample locations without data loggers and the nearest data logger was 253.39 meters (SD=108.2 m). Since microclimate data were not collected at the Cabin Slope site, a distance matrix was not applied because we considered a distance of 2 km from the nearest temperature logger too far to accurately extrapolate microclimate conditions for these territories.

Characterization of population-level fecal GCM patterns using linear mixed effects modeling

To determine whether there was a relationship between physiological stress in pikas and the variables measured in this study (i.e. gender, sex, individual heterozygosity, and territory quality), we used a mixed effects modeling approach to account for repeated sampling within territories, sites, and years, which violates the assumption of independence (Buckley et al. 2003; Zuur et al. 2009). Mixed effects modeling was performed for only a subset of the stress data from Pipet Tarn that included microclimate temperature data, individual heterozygosity, gender, year, and elevation as the explanatory variables. GCM concentrations in Cabin Slope were used for stress hormone comparisons with Pipet Tarn within and across seasons and genetic samples were used to determine whether these two sites constituted one population. The relative support for each predictor variable was evaluated using an information-theoretic approach and ten total candidate models based on biologically relevant hypotheses (Zuur et al. 2009) (Table S2).

We used a stepwise variance inflation factor (VIF) approach to deal with collinearity among explanatory variables. All predictor variables were assessed for correlation using a stepwise variance inflation factor (VIF) function, and variables with a variance inflation factor > 6 were removed from further analysis (vif_fun.r, *MASS, ClusterGeneration packages*, program R*)*. Models that were assigned an AIC_c score 0-2 units higher than the lowest score were considered to have similar support, whereas scores greater than 2 were ranked lower (Burnham and Anderson 2002; Wilkening et al. 2015). Predictor variables were ranked and akaike weights calculated using the *MuMIn* package (Bartoń 2013), and models were fit using *lme4* in R (Bates et al. 2014; R core team 2016).

We started with a global additive model that included all possible predictor variables without any interaction terms. Random intercept and slope effects were held constant for each model, but fixed effects differed by model (representing a priori competing hypotheses, Table S2). All models incorporated a random structure through the use of a random intercept to take into account by-site, by-territory, or by-year variability in fecal GCM concentrations. This approach assumes that the effects of physiological stress will be the same for all pikas in the study. However, considering that the effects of physiological stress on measures of pika fitness are not yet well documented, this assumption may not be valid. Instead, it may be that the effect of physiological stress is different for each individual such that some animals may naturally maintain higher baseline stress levels than others. There are likely intrinsic factors (e.g. genetics and sex) as well as extrinsic factors (e.g. territory location, foraging access, and predator abundance) that contribute to an individual's endocrine condition. As a result, in addition to the random intercept effects held constant for all models (e.g. (1| Year), a random slope effect was also included, where observed heterozygosity was used as a surrogate measure of individual diversity, to test our hypothesis that the physiological stress response in general may be different across individuals depending on an animal's genetic predisposition (Baayen et al. 2008; Schielzeth and Forstmeier 2008; Barr et al. 2013; Winter 2013).

Although we did track individuals across time using genetic IDs for comparison of territory-specific turnover and individual recapture rates, we did not include this genetic metric in the fecal GCM linear mixed effects modeling due to small sample size (very few recaptures). Instead, we used observed heterozygosity as a surrogate measure of individual diversity, although we do not propose that variation at microsatellite loci reflect adaptive trait variation in the pika stress hormone response. Observed heterozygosity was calculated for each fecal DNA sample regardless of whether that individual was later recaptured. By incorporating a random slope using individual observed heterozygosity and territory (1+ Hindiv | Territory), differing baseline-levels of physiological stress (the intercept, represented here by 1) as well as differing responses based on individual variability (here represented as Hindiv) and territory can be modeled. This random structure was kept constant in all tested models and since only fixed effects differed among models, maximum likelihood was used for estimating coefficients rather than restricted maximum likelihood estimation.

Pseudo R^2 values were also estimated for each model as a measure of goodness-of-fit (Nakagawa and Schielzeth 2013) and parsed into a marginal R^2_M (i.e. variance explained entirely by the fixed effects) and a conditional R^2_C (i.e. variance explained by both the fixed and random effects). However, to confirm that the most parsimonious model was selected, models were ranked by BIC as well as ΔAIC value (Wilkening et al. 2015). Finally, Moran's I was calculated in *ape* package (Paradis et al. 2004; R core team 2016) to test for spatial autocorrelation in model residuals, which were also visually inspected for any deviations from homoscedasticity or normality.

Results

A total of 182 fecal GCM samples (147 for Pipet Tarn, 35 for Cabin Slope) and 102 paired fecal DNA samples were processed from repeatedly sampling of 25 territories (n=19 at Pipet Tarn, n=6 at Cabin Slope). Despite collecting fecal DNA samples at all territories, amplification issues with degraded DNA and the need for rigorous individual differentiation resulted in only 13 of the 25 territories having reliably paired genetic data that could be examined with fecal GCM patterns at both the individual level and across multiple seasons.

Characterization of genetic population structure and connectivity

Observed heterozygosities were not statistically different among sites (Pipet Tarn, Ho= 0.612, the intervening talus sites, Ho=0.6805, and Cabin Slope, Ho=0.621; $F_{2,27}$=0.275, P = 0.762); however, average allelic richness was significantly lower in Cabin Slope compared to Pipet Tarn and the intervening 2 km of talus ($F_{2,27}$=3.747, P=0.037; Table S3). F statistics suggest that there is very little genetic differentiation among these sites (F_{ST}=0.025, P=0.01), and AMOVA results reveal that most of the genetic variation is partitioned within (70%) rather than among individuals (28%, Fig. 4). Principal coordinates analysis revealed a lack of genetic clustering by geographic site suggesting a single panmictic population across the sampling sites (Fig. 4).

Characterization of territory quality through comparisons of microclimate variation

Local temperature patterns for the two types of microclimate studied, interstitial and surface, were distinct at Pipet Tarn for many of the metrics measured. For heat metrics, interstitial conditions were significantly cooler than surface microclimates both in terms of average summer temperature ($F_{1, 52}$=57.22, *P*=0.000) and mean temperature of the warmest quarter (June-August) ($F_{1, 52}$=32.85, *P*=0.000). For cold metrics, interstitial microclimates remained significantly warmer than surface conditions in terms of fewer number of days below -5°C ($F_{1,52}$=8.095, *P* =0.006), warmer average values for mean temperature of the coldest quarter ($F_{1,52}$=90.12, *P*=0.000), and warmer average values for minimum temperature in January ($F_{1,52}$=92.29, *P*=0.000). Interestingly, temperature seasonality was significantly greater in interstitial microclimates compared to surface conditions ($F_{1, 52}$=52.35, *P* =0.000)

Minimum temperature in January differed significantly among years (2013-2015) both at the surface ($F_{2, 51}$=25.15, p=0.000) and within the interstitial microclimates ($F_{2, 51}$=35.9, *P* =0.000). In 2015, the last year of the study, some of the warmest temperatures within interstitial microclimates were recorded. Average summer temperature ($F_{2, 51}$=3.51 *P* =0.037) and the number of days below 5°C ($F_{2, 51}$=5.79, *P* =0.005) increased significantly within the interstitial microclimate during the duration of the study. Other metrics of cold stress also varied significantly across years for both surface and interstitial microclimates in terms of mean temperature of the coldest quarter (surface $F_{2,51}$=16.91, *P* =0.000; interstitial $F_{2, 51}$=31.39, p<0.000) and minimum temperature in January ($F_{2, 51}$=35.9, *P* =0.000; $F_{2,51}$=25.15, *P* =0.000).

Temperature patterns were also compared among territories (2013-2015) within the Pipet Tarn site. Territories differed most frequently in terms of acute and chronic heat stress within the interstitial microclimate: maximum temperature in July (interstitial) ($F_{17,36}$=3.84, P =0.0003), mean temperature of the warmest quarter (interstitial) ($F_{17, 36}$=5.77, P =0.000), number of days above 28°C (interstitial) ($F_{17,36}$=2.07, p=0.032), number of days above 26°C (interstitial) ($F_{17,36}$=3.73, P=0.0004), and average summer temperature (interstitial) ($F_{17,36}$=5.66, P=0.000). Significant differences among territories also occurred in terms of chronic heat stress within surface microclimates: average summer temperature (June through September) (surface) ($F_{17, 36}$=8.54, P =0.000) and mean temperature of the warmest quarter (surface) (July-Sep) ($F_{17, 36}$=5.06, P =0.000). The only temperature metric related to cold stress that differed significantly among territories was mean temperature of the coldest quarter (Nov-Jan) (surface) ($F_{17, 36}$=2.02, P =0.0378). No days below -10°C were recorded within this talus slope during 2012-2015 and only a subset of territories experienced any acute heat stress within interstitial microclimates in terms of number of days above 26°C (n=3) and number of days above 28°C (n=3).

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Population-level fecal GCM comparisons and patterns of individual variation

On average, pikas inhabiting the Cabin Slope site had significantly higher GCM concentrations throughout the summer season compared to Pipet Tarn ($F_{1, 226}$=73.15, P =0.000). No significant differences in average GCMs were detected among years (2013-2015). There were monthly differences in GCM concentrations with sites exhibiting different patterns in concentrations over the summer months (July-September). Pikas at Cabin Slope appear to experience significantly lower average physiological stress during July than in August or September ($F_{2, 67}$=17.11, P =0.000), whereas September is significantly less stressful for pikas at Pipet Tarn ($F_{2, 130}$=3.36, P =0.032; Fig. 2). No statistically significant difference in fecal GCMs was detected between males and females at Cabin Slope, however, the sexes were statistically different at Pipet Tarn ($F_{1, 42}$ =7.046, P =0.0112). Female pikas at Pipet Tarn had higher concentrations of GCMs than males, but both Cabin Slope and Pipet Tarn males exhibited a greater range of GCM values. The repeated genotyping necessary for accurate molecular sexing resulted in failure to produce consensus genotypes for some individuals. As a result we has a smaller sample size for females (n=15) compared to males (n=47) due to the need for paired samples for both GCM and genetic analysis.

For the subset of individuals repeatedly tracked over the summer season (Pipet Tarn, n=9; Cabin Slope, n=4), there were significant differences in turnover rates among territories within sites (Pipet, $F_{8, 24}$=6.05, P =0.000; Cabin Slope, $F_{3, 8}$=345.016, P =0.000; Fig. 3); however, these differences in territory turnover rates were not associated with significant differences in average fecal GCM concentrations among individuals within sites. Similarly, although fecal GCM concentrations differed significantly between sites as described above, average turnover rates did not ($F_{1,43}$= 1.74, P =0.194). Individual recapture rates differed significantly among territories within both sites (Pipet Tarn, $F_{7, 8}$=1.41, p<0.000; Cabin Slope, $F_{3, 6}$=5.36, p<0.000; Fig. 3) and were significantly higher at Cabin Slope compared to Pipet Tarn ($F_{1, 24}$ =15.04, P =0.000) despite significantly higher average fecal GCM concentrations.

Effects of site, year, and microclimate on pika stress response

For surface and interstitial microclimate temperature data, eight variables were below the VIF threshold: temperature seasonality (surface), average summer temperature (surface and interstitial), number of days below -5°C (surface), number of days above 26°C (depth), number of days above 28°C (surface), mean temperature of the coldest quarter (Nov-Jan) (surface), and maximum temperature in July (interstitial). Therefore, these eight non-collinear temperature variables were included as fixed effects for linear regression and model fitting. Each model included sex as an additive fixed effect and intercepts for territory and year, as well as a by-heterozygosity random slope for the effect of individual diversity. Delta AIC (ΔAIC < 2) identified similar and strong support for the top two candidate models, which each incorporated a metric of acute heat stress. AICc was lowest for model 7 which include the number of days above 26°C (interstitial) ($AIC_{C\,=}$ -219.3) and model 8 which included maximum temperature in July (interstitial) ($AIC_{C\,=}$ -219.2; Table 1). Mean temperature of the coldest quarter (surface) and temperature seasonality (surface) were moderately supported (ΔAIC <4). The highest R^2_M and R^2_C were found for models containing a measure of heat stress (number of days above 26°C and maximum temperature in July) or cold stress (mean temperature of the coldest quarter (Nov-Jan) (Table 1). Akaike weights were highest for the number of days above 26°C (0.37) and maximum temperature in July (0.36), which were both positively correlated with fecal GCM concentrations, whereas mean temperature of the coldest quarter (surface) was negatively correlated with fecal GCM concentrations (Table 2; Fig. 5). The Q-Q plots and histograms of the model residuals were relatively evenly distributed without any clear violations of normality (Winter 2013). Moran I's statistic also did not exhibit any evidence of spatial autocorrelation in residuals (P =0.2611).

Discussion

The effects of climate change have most often been quantified by changes in species distributions, disruptions in phenology, and altered species interactions (Parmesan et al. 1999; Walther et al. 2002; Parmesan and Yohe 2003; Visser and Both 2005; Inouye 2008). However, stress hormone monitoring offers a more direct method for examining how individual physiological response may translate into population level demographic consequences. Because pikas generally inhabit isolated alpine areas that have not experienced intense anthropogenic landscape modification, but appear to be affected by changing temperature and precipitation regimes, they represent a valuable model system and potential bio-indicator for assessing the impacts of climate change in alpine ecosystems (Beniston 2003; Parmesan and Yohe 2003; Cannone et al. 2007). The mounting evidence of contemporary extirpation at the southern end of the range (Beever et al. 2003; Stewart et al. 2015; Nichols et al. 2016) suggests that the functional quality of pika habitat (e.g. thermal buffering capacity, protection from predators) is being impacted by a changing climate. Pikas are considered a keystone species (Aho et al. 1998; Wilkening et al. 2015), therefore monitoring stress levels in pika populations through non-invasive collection of fecal samples that integrate both physiological and genetic metrics as well as associated microclimate-scale climate data may represent one strategy for tracking the health of alpine ecosystems.

Site-specific patterns of fecal GCM concentrations despite high genetic connectivity

The fine-scale spatial design of this study revealed the importance of local, site-specific effects on patterns of stress hormone concentrations for two pika populations separated by only two km of continuous alpine habitat. The population genetic data support regular movement between these two sites with very little genetic differentiation having occurred across this habitat over the last 25 years (Peacock 1997). Despite this high level of connectivity and close geographic proximity, there was evidence for both annual and within-season temporal variation in stress hormone concentrations, and our site-specific results suggest that the temporal patterns of physiological stress may vary significantly within eco-regions and within the dispersal distance of an individual pika (Peacock 1997). On average, pikas at Cabin Slope had significantly higher concentrations of stress hormones regardless of season or year. Monthly patterns were also site-specific, such that fecal GCM concentrations increased across each summer season in Cabin Slope, whereas endocrine patterns in Pipet Tarn plateaued until September when a decline in fecal GCM concentrations suddenly occurred. Cabin Slope is a steep, south-facing talus slope characterized by small, unstable rock habitat. This site lacks a central alpine meadow near its base and pika territories are located primarily on the periphery of the talus with access only to edge vegetation. In contrast, Pipet Tarn contains sub-surface ice features as well as an active waterfall that feeds a subterranean stream and central tarn adjacent to the meadow. It is possible that these alpine water resources underlying the occupied territories at Pipet Tarn provide a cooling interstitial mechanism, and also indirectly enable a greater diversity and abundance of alpine forbs compared to Cabin Slope. This evidence of within-population variability in the stress hormone response may be suggestive of plasticity in the individual coping style and hormonal phenotype across genetically related individuals.

Individual variability in fecal GCM patterns and recapture rates

Although non-invasive methods for investigating stress hormone patterns in feces enable collection of physiological data without direct capture of animals, there are caveats associated with this type of sampling due to inherent differences among sexes, diet, and individuals in metabolic rate and hormone metabolite regulation (Goymann 2012). In uncontrolled environments, free-living animals may vary in terms of hormone metabolite concentrations simply as a result varying food availability across seasons, changing metabolic rates with ambient temperature conditions, and even individual bacterial composition which is responsible for hormone metabolism in the gut (Goymann 2005; Touma and Palme 2005; Palme et al. 2005;

Dantzer et al. 2011; Goymann 2012). Repeated and multiple-season measures of corticosterone at both individual and population scales may help to parse out the varying effects of these factors on non-invasive measures of corticosterone (Wasser et al. 1997; Foley et al. 2001; Millspaugh et al. 2001; Macbeth et al. 2010; Sheriff et al. 2011; Rolland et al. 2012) by providing an understanding of the inter-annual consistency of the hormonal response among individuals (Ouyang et al. 2011).

We were able to characterize patterns of fecal GCMs and turnover rate at nine occupied territories at Pipet Tarn and four occupied territories at Cabin Slope over the course of the study. Within each site, territories differed significantly in average turnover rate suggesting that there may be inherent differences in territory quality within a talus slope. However, fecal GCM concentrations were not predictive of low or high quality territories. Turnover rate represents just one component of territory quality and may be the result of stochastic mortality events and therefore finer-scale measures of territory quality (e.g. predator pressure, microclimate variability, proximity to forage) may be more closely linked to the individual stress hormone response especially because of the integrated nature specific to GCMs. In addition, despite measuring significantly higher average fecal GCM concentrations for pikas in Cabin Slope, these concentrations do not yet translate into significantly higher per-territory turnover rates. In fact, individual recapture rates were actually higher at Cabin Slope compared to Pipet Tarn despite the higher average fecal GCM concentrations. Furthermore, across territories within both sites, individuals that maintained similar average fecal GCM concentrations (not significantly different) often experienced significantly different recapture rates. Together, these results suggest that fecal GCM concentrations are not yet high enough at Cabin Slope to affect fitness consequences (i.e. habitat still maintain high quality functionality for pikas to persist there), and/or limited sampling sizes within Cabin Slope may not reflect the true variability in turnover rates that occur there.

The degraded nature of fecal DNA makes reliable identification of individuals challenging therefore this non-invasive study was limited by only being able to follow a few individuals across multiple years and only during the summer season. In addition, we used turnover and recapture rates as proxies for apparent survival across seasons, but there may be other variables related to high average fecal GCM concentrations, such as reproductive success, predation escape, or immunological defenses that affect fitness outcomes that we were not able to measure during this study. Continued monitoring of per-territory fecal GCMs and territory turnover rates for a larger number of territories across a broader elevational gradient within the Sierra Nevada region, may help to elucidate what relative concentrations of fecal GCMs constitute a “high risk” for population persistence.

Influence of microclimate variability on fecal GCM concentrations and territory turnover rates

Spatial variation in habitat quality exists across a species’ distribution due to multiple factors including slope, aspect and geographic location, as well as climatic and ecological variability (Vucetich and Waite 2003: Bean et al. 2014; Valladares et al. 2014; Wood et al. 2014). For territorial species, it is equally probable that habitat quality varies across territories at relatively small spatial scales with differences related to proximity to foraging opportunities, safety from predation, or even the relative stability of the interstitial microclimate (Manica et al. 2014). This study examined only one component of pika territory quality (surface and interstitial microclimate variability), while recognizing that other territory-related factors exist such as distance to the talus-vegetation interface, proximity to potential mates, and access to cover from both avian and mustelid predators that will influence individual pika fitness.

Not surprisingly, interstitial microclimates of pika territories effectively buffered ambient conditions by providing cooler temperatures during summer months and warmer temperatures during the winter months. In addition, occupied territories differed significantly and most frequently in metrics of heat stress rather than cold stress within both associated interstitial and surface microclimates. Extreme temperatures were rarely recorded within occupied territories with only three territories ever experiencing a day with temperatures greater than 26 or 28°C during 2012-2015. Despite differences among territories in terms of microclimate temperatures, the finding that territories did not differ significantly in average fecal GCM concentrations suggests that all occupied territories effectively buffered ambient temperatures well enough to provide similar physiological conditions. However, considering the significant differences in turnover rate between territories within sites, perhaps due to non-physiological factors, territory availability and selection during a juvenile's dispersal stage is likely to play an important role in future survivorship. The characterization of variability in territory microclimates emphasizes the finite aspect of territory availability after generations of pikas have selected and thus defined territories that qualified as suitable habitat within this talus slope. A next step in characterizing thermal variability across a talus slope should be to characterize unselected "non-territories" to determine whether interstitial microclimates associated with pika territories buffer ambient conditions more effectively than random locations within a talus slope.

Relating individual variation and territory quality to site-level patterns of fecal GCMs

Ultimately, this study set out to determine whether patterns of fecal GCM concentrations were associated with territory-specific surface and/or interstitial microclimate conditions. In addition, we were interested in exploring the utility of this research design (simultaneous collection of fecal DNA and fecal GCMs) for understanding patterns of individual level variability (e.g., sex and genetic variation). Perhaps most important was the finding that although fine-scale metrics of heat (e.g. number of days above 26°C and maximum temperature in July) as well as cold (mean temperature of the coldest quarter, Nov-Jan) may contribute to physiological stress in pikas, the influence of climate predictors remained minor compared to the larger effects of site, year, territory quality, and individual diversity on patterns of fecal GCMs concentrations (Table 1, R^2_M vs. R^2_C). In fact, higher fecal GCMs were significantly associated with territories that experienced greater heat within interstitial microclimates in terms of both maximum temperature in July as well as number of days above 26°C. Model support was strongly improved by accounting for annual climatic variability as well as a surrogate measure of individual genetic diversity.

Overall, these results suggest that specific habitat conditions play a significant role in determining the magnitude of physiological stress experienced by an individual. Despite strong genetic connectivity among sampling sites located at similar elevations across continuous talus habitat, individuals from different sites differed significantly in levels of fecal GCM concentrations, which may be a result of variation in territory quality. Although we did follow a subset of individuals over the duration of the study, a larger sample size and longer time frame would improve the estimates of turnover and recapture rates per site. By linking demographic parameters to individuals and territories across longer time frames, non-invasive genetic-mark-recapture studies would enable the establishment of the scale at which fecal GCM are most appropriate for evaluating extirpation risk without costly and time-intensive trapping for GC samples.

Conclusions

This study represents the first attempt to characterize patterns of physiological stress in pika populations within the Sierra Nevada, as well as the first effort towards long-term monitoring of physiological stress at both individual and territory scales. We compared two populations within close geographic proximity across the same seasons to control for any differences in elevation or annual resource availability and used genetic ID tags and molecular sexing techniques to explore and control for individual or sex-based differences in fecal GCMs. Despite accounting for the potentially serious issues associated with noninvasive monitoring of steroid hormone metabolites found in fecal material, there are still likely to be metabolite differences among individuals due to gut bacteria, flea and tick load, genetic predisposition, and life history stage that we could not control for without transferring animals into a laboratory setting, which is not ethically feasible for this species of special concern in the state of California. Future studies should focus on establishing the effects of environmental exposure within and across populations in different eco-regions and in habitats that vary in structure and spatial configuration. In considering the influence of territory quality on pika stress more generally, additional factors that may contribute to territory quality should be considered (e.g., distance to foraging or food availability). In addition, fecal GCM concentrations may also be influenced by relative predator diversity and abundance, changing disease dynamics, and pika population density (Wilkening et al. 2015). Since cold stress has previously been identified as an important predictor of physiological stress in pikas (Wilkening et al. 2015), there is also a need to address the inherent sampling bias of the summer field season. Winter physiology in pikas is virtually unknown, and it is likely that we are failing to capture some of the most physiologically stressful months especially for pregnant females (i.e. early spring).

Although the genetic implications of these results are limited by the use of a small number of neutral genetic markers, this study design highlights the potential of using this low-cost and integrated fecal sampling protocol to assess plasticity of the stress response as well as genetic variation and gene flow among multiple study sites within eco-regions for greater comparative power. Ideally, future research will incorporate the use of non-neutral markers through development of species-specific SNPs in order to identify adaptive trait loci associated with physiological trait variation. In concert with scientific researchers, pika citizen science programs are well suited to implement fecal GCM and DNA sampling protocols in long-term monitoring efforts.

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References

Aho, K., Huntly, N., Moen, J., & Oksanen, T. (1998). Pikas (*Ochotona princeps*: Lagomorpha) as allogenic engineers in an alpine ecosystem. *Oecologia*, 114(3), 405–409. doi:10.1007/s004420050463

Anderson, P. A., Berzins, I. K., Fogarty, F., Hamlin, H. J., & Guillette, L. J. (2011). Sound, stress, and seahorses: The consequences of a noisy environment to animal health. *Aquaculture*, *311*(1–4), 129–138. doi: 10.1016/j.aquaculture.2010.11.013

Angelier, F., Weimerskirch, H., Dano, S., & Chastel, O. (2007). Age, experience and reproductive performance in a long-lived bird: a hormonal perspective. *Behavioral Ecology and Sociobiology*, *61*(4), 611–621. doi:10.1007/s00265-006-0290-1

Angelier, F., Wingfield, J.C., Weimerskirch, H., and Chastel O. 2010. (2010). Hormonal correlates of individual quality in a long-lived bird: a test of the "corticosterone–fitness hypothesis." *Biology Letters* 6 (2010), 846-849. doi: 10.1098/rsbl.2010.0376

Ardia, D., Parmentier, H., & Vogel, L. (2011). The role of constraints and limitation in driving individual variation in immune response. *Functional Ecology*, *25*(1), pp.61-73. doi:10.1111/j.1365-2435.2010.01759.x

Bartoń, K. (2013). MuMIn: Multi-model inference. R package version 1.9. 13. *The Comprehensive R Archive Network (CRAN).*

Bates, D., Maechler, M., Bolker, B., Walker, S., Christensen, R.H.B., Singmann, H., Dai, B., Grothendieck, G., E.Eigen, C. & Rcpp, L. (2014). Package 'lme4'. *R Foundation for Statistical Computing, Vienna, 12.*

Baayen, R.H., Davidson, D.J., & Bates, D.M. (2008). Mixed-effects modeling with crossed random effects for subjects and items. *Journal of Memory and Language*, 59(4), 390–412. doi: 10.1016/J.JML.2007.12.00

Barr, D.J., Levy, R., Scheepers, C. & Tily, H.J. (2013). Random effects structure for confirmatory hypothesis testing: keep it maximal. *Journal of Memory and Language*, 68(3), 255–278. doi: 10.1016/J.JML.2012.11.001

Bean, W. T., Prugh, L. R., Stafford, R., Butterfield, H. S., Westphal, M., & Brashares, J. S. (2014). Species distribution models of an endangered rodent offer conflicting measures of habitat quality at multiple scales. *Journal of Applied Ecology, 51*(4), 1116–1125.doi: 10.1111/1365-2664.12281

Beever, E. A., Brussard, P. F., & Berger, J. (2003). Patterns of apparent extirpation among isolated populations of pikas *(Ochotona princeps*) in the Great Basin. *Journal of Mammalogy, 84*(1), 37–54. doi: 10.1644/1545-1542(2003)084<0037: POAEAI>2.0.CO; 2

Beever, E. A., Ray, C., Mote, P. W., & Wilkening, J. L. (2010). Testing alternative models of climate-mediated extirpations. *Ecological Applications*, *20*(1), 164–178. doi: 10.1890/08-1011.1

Beever, E., Ray, C., & Wilkening, J. (2011). Contemporary climate change alters the pace and drivers of extinction. *Global Change Biology*, *17*(6), 2054-2070. doi:10.1111/j.1365-2486.2010.02389.x

Beever, E. A., Dobrowski, S. Z., Long, J., Mynsberge, A. R., & Piekielek, N. B. (2013). Understanding relationships among abundance, extirpation, and climate at ecoregional scales. *Ecology*, *94*(7), 1563–1571. doi: 10.1890/12-2174.1

Beniston, M. (2003). Climatic change in mountain regions: a review of possible impacts. *Climatic change*, *59*(1), 5-31.

Beletsky, L., Orians, G., & Wingfield, J. (1989). Relationships of steroid hormones and polygyny to territorial status, breeding experience, and reproductive success in male red-winged blackbirds. *The Auk, 106*(1), 107-117. doi:10.2307/4087763

Blandford, T. R., Humes, K. S., Harshburger, B. J., Moore, B. C., Walden, V. P., Ye, H., & Ye, H. (2008). Seasonal and synoptic variations in near-surface air temperature lapse rates in a mountainous basin. *Journal of Applied Meteorology and Climatology*, 47(1), 249–261. doi: 10.1175/2007JAMC1565.1

Blas, J., Bortolotti, G. R., Tella, J. L., Baos, R., & Marchant, T. A. (2007). Stress response during development predicts fitness in a wild, long lived vertebrate. *Proceedings of the National Academy of Sciences*, *104*(21), 8880–8884. doi: 10.1073/pnas.0700232104

Blickley, J. L., Blackwood, D., & Patricelli, G. L. (2012a). Experimental evidence for the effects of chronic anthropogenic noise on abundance of Greater sage-grouse at leks. *Conservation Biology*, *26*(3), 461–471. doi:10.1111/j.1523-1739.2012.01840.x

Blickley, J. L., Word, K. R., Krakauer, A. H., Phillips, J. L., Sells, S. N., Taff, C. C, Wingfield, J.C. & Patricelli, G. L. (2012b). Experimental chronic noise is related to elevated fecal corticosteroid metabolites in lekking male Greater sage-grouse (*Centrocercus urophasianus*). *PLoS ONE*, *7*(11), e50462. doi: 10.1371/journal.pone.0050462

Bonier, F., Martin, P. R., Moore, I. T., & Wingfield, J. C. (2009a). Do baseline glucocorticoids predict fitness? *Trends in Ecology and Evolution*, *24*(11), 634–642. doi: 10.1016/j.tree.2009.04.013

Bonier, F., Moore, I. T., Martin, P. R., & Robertson, R. J. (2009b). The relationship between fitness and baseline glucocorticoids in a passerine bird. *General and Comparative Endocrinology*, *163*(1–2), 208–213. doi: 10.1016/j.ygcen.2008.12.013

Boonstra, R., Hik, D., & Singleton, G. (1998). The impact of predator-induced stress on the snowshoe hare cycle. *Ecological Monographs, 68*: 371–394. doi:10.1890/0012 9615(1998)068[0371:TIOPIS]2.0.CO; 2

Buckley, Y. M., Briese, D. T., & Rees, M. (2003). Demography and management of the invasive plant species *Hypericum perforatum. I.* Using multi-level mixed-effects models for characterizing growth, survival and fecundity in a long-term data set. *Journal of Applied Ecology*, *40*(3), 481–493. doi: 10.1046/j.1365-2664.2003.00821.x

Burnham, K., & Anderson, D. (2003). *Model selection and multimodel inference: a practical information-theoretic approach*. Springer Science and Business Media.

Calkins, M. T., Beever, E. A., Boykin, K. G., Frey, J. K., & Andersen, M. C. (2012). Not-so-splendid isolation: modeling climate-mediated range collapse of a montane mammal *Ochotona princeps* across numerous ecoregions. *Ecography*, *35*(9), 780–791. doi:

10.1111/j.1600-0587.2011.07227.x

Cannone, N., & Sgorbati, S. (2007). Unexpected impacts of climate change on alpine vegetation. *Frontiers in Ecology and the Environment*, 5: 360–364. doi: 10.1890/1540-9295(2007)5[360: UIOCCO]2.0.CO;2

Cash, W. Ben, & Holberton, R. L. (2005). Endocrine and behavioral response to a decline in habitat quality: effects of pond drying on the slider turtle,*Trachemys scripta*. *Journal of Experimental Zoology Part A: Comparative Experimental Biology*, *303A*(10), 872–879. doi: 10.1002/jez.a.217

Clinchy, M., Zanette, L., Boonstra, R., Wingfield, J. C., & Smith, J. N. M. (2004). Balancing food and predator pressure induces chronic stress in songbirds. *Proceedings. Biological Sciences*, *271*(1556), 2473–9. doi: 10.1098/rspb.2004.2913

Cockrem, J. F. (2013). Corticosterone responses and personality in birds: individual variation and the ability to cope with environmental changes due to climate change. *General and Comparative Endocrinology*, *190*, 156–163. doi: 10.1016/J.YGCEN.2013.02.021

Cooke, S. J., Sack, L., Franklin, C. E., Farrell, A. P., Beardall, J., Wikelski, M., & Chown, S. L. (2013). What is conservation physiology? Perspectives on an increasingly integrated and essential science. *Conservation Physiology*, *1*(1), 1–23. doi: 10.1093/conphys/cot001

Creel, S., Creel, N. M., & Monfort, S. L. (1997). Radiocollaring and stress hormones in African wild dogs. *Conservation Biology*. doi:10.2307/2387628

Crespi, E. J., Williams, T. D., Jessop, T. S. & Delehanty, B. (2013). Life history and the ecology of stress: how do glucocorticoid hormones influence life-history variation in animals? *Funct Ecol*, 27: 93–106. doi:10.1111/1365-2435.12009

Dantzer, B., McAdam A.G., Palme, R., Boutin, S. & Boonstra, R. (2011). How does diet affect fecal steroid hormone metabolite concentrations? An experimental examination in red squirrels? *General and Comparative Endocrinology*, 174(2), 124-131. doi: 10.1016/j.ygcen.2011.08.010

Dantzer, B., Fletcher, Q. E., Boonstra, R., & Sheriff, M. J. (2014). Measures of physiological stress: A transparent or opaque window into the status, management and conservation of species? *Conservation Physiology*, *2*(1), 1–18. doi: 10.1093/conphys/cou023

de Kloet, E. R., Sibug, R. M., Helmerhorst, F. M., & Schmidt, M. (2005). Stress, genes and the mechanism of programming the brain for later life. *Neuroscience & Biobehavioral Reviews*, *29*(2), 271–281. doi: 10.1016/J.NEUBIOREV.2004.10.008

Diaz, H. F., & Bradley, R. S. (1997). Temperature variations during the last century at high elevation sites. In *Climatic Change at High Elevation Sites* (pp. 21–47). Dordrecht: Springer Netherlands. doi: 10.1007/978-94-015-8905-5_2

Erb, L. P., Ray, C., & Guralnick, R. (2011). On the generality of a climate-mediated shift in the distribution of the American pika (*Ochotona princeps*). *Ecology*, *92*(9), 1730–1735. doi: 10.1890/11-0175.1

Evanno, G., Regnaut, S., & Goudet, J. (2005). Detecting the number of clusters of individuals using the software STRUCTURE: a simulation study. *Molecular Ecology*, *14*(8), 2611–2620. doi: 10.1111/j.1365-294X.2005.02553.x

Evans, M. R., Roberts, M. L., Buchanan, K. L., & Goldsmith, A. R. (2006). Heritability of corticosterone response and changes in life history traits during selection in the zebra finch. *Journal of Evolutionary Biology*, *19*(2), 343–352. doi:10.1111/j.1420-9101.2005.01034.x

Federenko, I.S., Nagamine, M., Hellhammer, D.H., Wadhwa, P.D., & Wüst S. (2004). The heritability of hypothalamus pituitary adrenal axis responses to psychosocial stress is context dependent. *The Journal of Clinical Endocrinology & Metabolism*, 89 (12), 6244–6250, doi: 10.1210/jc.2004-0981

Foley, C. A. H., Papageorge, S., and Wasser, S. K. (2001). Noninvasive stress and reproductive measures of social and ecological pressures in free-ranging African elephants. *Conservation Biology*, 15(4), 1134–1142. doi:10.1046/j.1523-1739.2001.0150041134.x

Fontanesi, L., Tazzoli, M., Pecchioli, E., Hauffe, H.C., Robinson, T.J. & Russo, V. (2008). Sexing European rabbits (Oryctolagus *cuniculus*), European brown hares (Lepus *europaeus*) and mountain hares (Lepus *timidus*) with *ZFX* and *ZFY* loci. *Molecular Ecology Resources*, *8*(6), 1294–1296. doi: 10.1111/j.1755-0998.2008.02167.x

Galbreath, K. E., Hafner, D. J., & Zamudio, K. R. (2009). When cold is better: climate-driven elevation shifts yield complex patterns of diversification and demography in an alpine specialist (American pika, *Ochotona princeps*). *Evolution*, *63*(11), 2848–2863. doi: 10.1111/j.1558-5646.2009.00803.x

Galbreath, K. E., Hafner, D. J., Zamudio, K. R., & Agnew, K. (2010). Isolation and introgression in the Intermountain West: contrasting gene genealogies reveal the complex biogeographic history of the American pika (*Ochotona princeps*). *Journal of Biogeography*, *37*(2), 344–362. doi: 10.1111/j.1365-2699.2009.02201.x

Gilbert, K., Andrew, R., Bock, D., Franklin, M., Kane, N., Moore, J., & Vines, T. (2012). Recommendations for utilizing and reporting population genetic analyses: the reproducibility of genetic clustering using the program STRUCTURE. *Molecular Ecology*, *21*, 4925–4930.

Goudet, J. (2001). FSTAT; a program to estimate and test gene diversities and fixation indices version 2.9. 3. *Http://www. Unil. Ch/izea/softwares/fstat. Html*.

Goymann, W. (2005). Noninvasive monitoring of hormones in bird droppings: physiological validation, sampling, extraction, sex differences, and the influence of diet on hormone metabolite levels. *Annals of the New York Academy of Sciences*, 1046(1), 35–53. doi: 10.1196/annals.1343.005

Goymann, W. (2012). On the use of non-invasive hormone research in uncontrolled, natural environments: the problem with sex, diet, metabolic rate and the individual. *Methods in Ecology and Evolution*, 3(4), 757–765. doi: 10.1111/j.2041-210X.2012.00203.x

Grayson, D. K. (2005). A brief history of Great Basin pikas. *Journal of Biogeography*, *32*(12), 2103–2111. doi: 10.1111/j.1365-2699.2005.01341.x

Hadinger, U., Haymerle, A., Knauer, F., Schwarzenberger, F., & Walzer, C. (2015). Faecal cortisol metabolites to assess stress in wildlife: evaluation of a field method in free-ranging chamois. *Methods in Ecology and Evolution*, *6*(11), 1349–1357. doi: 10.1111/2041-210X.12422

Hafner, D., & Sullivan, R. (1995). Historical and ecological biogeography of Nearctic pikas (Lagomorpha: Ochotonidae). *Journal of Mammalogy*, *76*(2), 302-321. doi: 10.2307/1382343

Harper, J. M., & Austad, S. N. (2004). Fecal corticosteroid levels in free-living populations of deer mice (*Peromyscus maniculatus*) and southern red-backed voles (*Clethrionomys gapperi*). *The American Midland Naturalist*, *152*(2), 400–409. doi: 10.1674/0003-0031(2004)152[0400: FCLIFP]2.0.CO;2

Henry, P., & Russello, M. (2011). Obtaining high-quality DNA from elusive small mammals using low-tech hair snares. *European Journal of Wildlife Research*, *57*(3), 429-435. doi:10.1007/s10344-010-0449-y

Henry, P., Sim, Z., & Russello, M. A. (2012). Genetic evidence for restricted dispersal along continuous altitudinal gradients in a climate change-sensitive mammal: the American Pika. *PLoS ONE*, *7*(6), 1–10. https://doi.org/10.1371/journal.pone.0039077

Hopkins, W. A., & DuRant, S. E. (2011). Innate immunity and stress physiology of eastern hellbenders (*Cryptobranchus alleganiensis*) from two stream reaches with differing habitat quality. *General and Comparative Endocrinology*, *174*(2), 107–115. doi: 10.1016/J.YGCEN.2011.08.006

Houle, D. (1991). Genetic covariance of fitness correlates: what genetic correlations are made of and why it matters. *Evolution*, pp.630-648. doi:10.1111/j.1558-5646.1991.tb04334.x

Husak, J.F. &Moore, I.T. 2008. Stress hormones and mate choice. *Trends in Ecology & Evolution 23* (10), 532-534. doi:10.1016/j.tree.2008.06.007

Inouye, D. (2008). Effects of climate change on phenology, frost damage, and floral abundance of montane wildflowers. *Ecology*, *89*(2), pp.353-362. doi:10.1890/06-2128.1

Janin, A., Léna, J.-P., & Joly, P. (2011). Beyond occurrence: body condition and stress hormone as integrative indicators of habitat availability and fragmentation in the common toad. *Biological Conservation*, *144*(3), 1008–1016. doi: 10.1016/j.biocon.2010.12.009

Jeffress, M. R., Rodhouse, T. J., Ray, C., Wolff, S., & Epps, C. W. (2013). The idiosyncrasies of place: geographic variation in the climate-distribution relationships of the American pika. *Ecological Applications*, *23*(4), 864–878. doi: 10.1890/12-0979.1

Jenkins, B. R., Vitousek, M. N., Hubbard, J. K., & Safran, R. J. (2014). An experimental analysis of the heritability of variation in glucocorticoid concentrations in a wild avian population. *Proceedings. Biological Sciences*, *281*(1790), 20141302. doi: 10.1098/rspb.2014.1302

Khan, M. Z., Altmann, J., Isani, S. S., & Yu, J. (2002). A matter of time: evaluation the storage of fecal samples for steroid analysis. *General and Comparative Endocrinology*, *128*(1), 57–64. doi: 10.1016/S0016-6480(02)00063-1

Kitaysky, A. S., Wingfield, J. C., & Piatt, J. F. (1999). Dynamics of food availability, body condition and physiological stress response in breeding black-legged kittiwakes. *Functional Ecology*, *13*(5), 577–584. doi: 10.1046/j.1365-2435.1999.00352.x

Koolhaas, J.M., Korte, S.M., De Boer, S.F., Van Der Vegt, B.J., Van Reenen, C.G., Hopstser, H., De Jong, I.C., Ruis, M.A.W., & Blokhuis, H.J. (1999). Coping styles in animals: current status in behavior and stress-physiology. *Neuroscience & Biobehavioral Reviews 23* (7), 925-935. doi: 10.1016/S0149-7634(99)00026-3

Lancaster, L. T., Hazard, L. C., Clobert, J., & Sinervo, B. R. (2008). Corticosterone manipulation reveals differences in hierarchical organization of multidimensional reproductive trade-offs in r-strategist and K-strategist females. *Journal of Evolutionary Biology*, *21*(2), 556–565. doi: 10.1111/j.1420-9101.2007.01478.x

Lanctot, R.B., Hatch, S.A., Gill, V.A., & Eens, M. (2003). Are corticosterone levels a good indicator of food availability and reproductive performance in a kittiwake colony? *Hormones and Behavior* 43 (4), 489-502. Doi: 10.1016/S0018-506X (03)00030-8

Lanyon, S. M., & Thompson, C. F. (1986). Site fidelity and habitat quality as determinants of settlement pattern in male painted buntings. *The Condor*, *88*(2), 206–210. doi:10.2307/1368917

Love, O. P., Madliger, C. L., Bourgeon, S., Semeniuk, C. A. D., & Williams, T. D. (2014). Evidence for baseline glucocorticoids as mediators of reproductive investment in a wild bird. *General and Comparative Endocrinology*, *199*, 65–69. doi: 10.1016/J.YGCEN.2014.01.001

Macbeth, B. J., Cattet, M. R. L., Stenhouse, G. B., Gibeau, M. L., & Janz, D. M. (2010). Hair cortisol concentration as a noninvasive measure of long-term stress in free-ranging grizzly bears (*Ursus arctos*): considerations with implications for other wildlife. *Canadian Journal of Zoology*, *88*(10), 935–949. doi: 10.1139/Z10-057

Martínez-Mota, R., Valdespino, C., Sánchez-Ramos, M. A., & Serio-Silva, J. C. (2007). Effects of forest fragmentation on the physiological stress response of black howler monkeys. *Animal Conservation*, *10*(3), 374–379. doi: 10.1111/j.1469-1795.2007.00122.x

Mathewson, P. D., Moyer-Horner, L., Beever, E. A., Briscoe, N. J., Kearney, M., Yahn, J. M., & Porter, W. P. (2017). Mechanistic variables can enhance predictive models of endotherm distributions: the American pika under current, past, and future climates. *Global Change Biology*, *23*(3), 1048–1064. doi: 10.1111/gcb.13454

Romero, M.L. (2002). Seasonal changes in plasma glucocorticoid concentrations in free-living vertebrates. *General and Comparative Endocrinology*, *128*(1), 1–24. doi: 10.1016/S0016-6480(02)00064-3

Millspaugh, J., Woods, R., Hunt, K., & Raedeke, K. (2001). Fecal glucocorticoid assays and the physiological stress response in elk. *Wildlife Society Bulletin (1973-2006)*, *29*(3), 899-907.

Millspaugh, J. J., & Washburn, B. E. (2004). Use of fecal glucocorticoid metabolite measures in conservation biology research: considerations for application and interpretation. *General and Comparative Endocrinology*, *138*(3), 189–199. doi: 10.1016/j.ygcen.2004.07.002

Misenhelter, M. D., & Rotenberry, J. T. (2000). Choices and consequences of habitat occupancy and nest site selection in sage sparrows. *Ecology*, *81*(10), 2892. doi: 10.2307/177349

Monclús, R., Rödel, H. G., Palme, R., Von Holst, D., and De Miguel, J. (2006). Non-invasive measurement of the physiological stress response of wild rabbits to the odour of a predator. *Chemoecology*, *16*(1), 25–29. doi: 10.1007/s00049-005-0324-6

Moyer-Horner, L., & Mathewson, P. (2015). Modeling behavioral thermoregulation in a climate change sentinel. *Ecol Evol*, 5: 5810–5822. doi:10.1002/ece3.1848

Nakagawa, S., and Schielzeth, H. (2013). A general and simple method for obtaining R^2 from generalized linear mixed-effects models. *Methods Ecol Evol*, 4: 133–142. doi:10.1111/j.2041-210x.2012.00261.x

Newcomb Homan, R., Regosin, J. V., Rodrigues, D. M., Reed, J. M., Windmiller, B. S., & Romero, L. M. (2003). Impacts of varying habitat quality on the physiological stress of spotted salamanders (*Ambystoma maculatum*). *Animal Conservation, 6*(1), 11–18. doi: 10.1017/S1367943003003032

Nichols, L. (2011). Fecal pellets of American pikas (*Ochotona princep*s) provide a crude chronometer for dating patch occupancy. *Western North American Naturalist* 70(4):500-507. 2011 doi: 10.3398/064.070.0410

Nichols, L. B., Klingler, K. B., & Peacock, M. M. (2016). American pikas (*Ochotona princeps*) extirpated from the historic masonic mining district of eastern California. *Western North American Naturalist*, *76*(2), 163–171. doi: 10.3398/064.076.0203

Osborn, S. D., & Applebee, D. B. (2011). Evaluation of the petition from the Center for Biological Diversity to list the American pika (*Ochotona princeps*) as threatened. *California Department of Fish and Game*, *32*.

Ouyang, J. Q., Hau, M., & Bonier, F. (2011). Within seasons and among years: when are corticosterone levels repeatable? *Hormones and Behavior*, *60*(5), 559–564. doi: 10.1016/J.YHBEH.2011.08.004

Øverli, Ø., Sørensen, C., Pulman, K.G.T., Pottinger, T.G., Korzan, W., Summers, C.F., & Nilsson, G.E. (2007). Evolutionary background for stress-coping styles: relationships between physiological, behavioral, and cognitive traits in non-mammalian vertebrates. *Neuroscience & Biobehavioral Reviews* 31 (3), 396-412. doi: 10.1016/j.neubiorev.2006.10.006

Paetkau, D. (2003). An empirical exploration of data quality in DNA-based population inventories. *Molecular Ecology*, *12*(6), 1375–1387. doi: 10.1046/j.1365-294X.2003.01820.x

Palme, R. (2005). Measuring fecal steroids: guidelines for practical application. *Annals of the*

New York Academy of Sciences, *1046*, 75–80. doi: 10.1196/annals.1343.007

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

Parmesan, C., Ryrholm, N., Stefanescu, C., Hill, J. K., Thomas, C. D., Descimon, H., & Warren, M. (1999). Poleward shifts in geographical ranges of butterfly species associated with regional warming. *Nature*, *399*(June), 579–583. doi: 10.1038/21181

Parmesan, C., & Yohe, G. (2003). A globally coherent fingerprint of climate change impacts across natural systems. *Nature*, *421*(6918), 37–42. doi: 10.1038/nature01286

Peacock, M., & Kirchoff, V. (2002). Identification and characterization of nine polymorphic microsatellite loci in the North American pika, *Ochotona princeps*. *Molecular Ecology*. doi: 10.1046/j.1471-8286.2002.00249.x/full

Peacock, M. M., & Smith, A. T. (1997). Nonrandom mating in pikas *Ochotona princeps*: evidence for inbreeding between individuals of intermediate relatedness. *Molecular Ecology*, *6*: 801–811. doi:10.1046/j.1365-294X.1997.00249.x

Peakall, R., & Smouse, P. (2006). GENALEX 6: genetic analysis in Excel. Population genetic software for teaching and research. *Molecular Ecology Resources*.

Peakall R, & Smouse P. (2012). GenAlEx 6.5: genetic analysis in Excel. Population genetic software for teaching and research—an update. *Bioinformatics*, *28*, pp.2537-2539.

Pritchard, J. K., Stephens, M., & Donnelly, P. (2000). Inference of population structure using multilocus genotype data. *Genetics*, *155*, 945–959.

R Development Core Team (2016). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL http://www.R-project.org

Ramasamy, R. K., Ramasamy, S., Bindroo, B. B., and Naik, V. G. (2014). STRUCTURE PLOT: a program for drawing elegant STRUCTURE bar plots in user friendly interface. *SpringerPlus*, *3*(1), 431.doi: 10.1186/2193-1801-3-431

Reeder, D., & Kramer, K. (2005). Stress in free-ranging mammals: integrating physiology, ecology, and natural history. *Journal of Mammalogy, 86*(2), pp.225-235. doi: 10.1644/BHE-003.1

Reek, T., Doty, S. R., Owen, T. W., Reek, T., Doty, S. R.,& Owen, T. W. (1992). A deterministic approach to the validation of historical daily temperature and precipitation data from the cooperative network. *Bulletin of the American Meteorological Society*, 73(6), 753–762. doi: 10.1175/1520-0477(1992)073<0753:ADATTV>2.0.CO;2

Rehnus, M., Hackländer, K., & Palme, R. (2009). A non-invasive method for measuring glucocorticoid metabolites (GCM) in mountain hares (*Lepus timidus*). *European Journal of Wildlife Research*, *55*(6), 615–620. doi: 10.1007/s10344-009-0297-9

Rensel, M. A., & Schoech, S. J. (2011). Repeatability of baseline and stress-induced corticosterone levels across early life stages in the Florida scrub-jay (*Aphelocoma coerulescens*). *Hormones and Behavior, 59*(4), 497–502. doi: 10.1016/J.YHBEH.2011.01.010

Rolland, R. M., Parks, S. E., Hunt, K. E., Castellote, M., Corkeron, P. J., Nowacek, D. P., & Kraus, S. D. (2012). Evidence that ship noise increases stress in right whales. *Proceedings. Biological Sciences*, *279*(1737), 2363–8. doi: 10.1098/rspb.2011.2429

Romero, L. M. (2004). Physiological stress in ecology: lessons from biomedical research. *Trends in Ecology & Evolution*, *19*(5), 249–255. doi: 10.1016/j.tree.2004.03.008

Romero, L. M., & Reed, J. M. (2008). Repeatability of baseline corticosterone concentrations. *General and Comparative Endocrinology*, *156*(1), 27–33. doi: 10.1016/J.YGCEN.2007.10.001

Rousset, F., 2008. Genepop'007: a complete reimplementation of the Genepop software for Windows and Linux. *Mol. Ecol. Resources* 8: 103-106.

Sapolsky, R. (1992a). Cortisol concentrations and the social significance of rank instability among wild baboons. *Psychoneuroendocrinology*, *17*(6), pp.701-709. doi: 10.1016/0306-4530(92)90029-7

Sapolsky, R. (1992b). Do glucocorticoid concentrations rise with age in the rat? *Neurobiology of aging*, *13*(1), pp.171-174. doi: 10.1016/0197-4580(92)90025-S

Sapolsky, R. M. (2000). Stress hormones: good and bad. *Neurobiology of Disease*, *7*(5), 540–542. doi: 10.1006/nbdi.2000.0350

Satterlee, D.G. & Johnson, W.A. (1988). Selection of Japanese quail for contrasting blood corticosterone response to immobilization, *Poultry Science* 67 (1) 25–32, doi:10.3382/ps.0670025

Scarlata, C. D., Elias, B. A., Godwin, J. R., Powell, R. A., Shepherdson, D., Shipley, L. A., & Brown, J. L. (2011). Characterizing gonadal and adrenal activity by fecal steroid analyses in pygmy rabbits (*Brachylagus idahoensis*). *General and Comparative Endocrinology*, *171*(3), 373–380. doi: 10.1016/j.ygcen.2011.03.002

Scheuerlein, A., Van’t Hof, T., & Gwinner, E. (2001). Predators as stressors? Physiological and reproductive consequences of predation risk in tropical stonechats (*Saxicola torquata axillaris*). *Proceedings of the Royal Society B 268* (1476), 1575-1582. doi: 10.1098/rspb.2001.1691

Schielzeth, H., & Forstmeier, W. (2009). Conclusions beyond support: overconfident estimates in mixed models. *Behavioral Ecology*, *20*(2), 416–420. doi: 10.1093/beheco/arn145

Schwalm, D., Epps, C. W., Rodhouse, T. J., Monahan, W. B., Castillo, J. A., Ray, C., & Jeffress, M. R. (2016). Habitat availability and gene flow influence diverging local population trajectories under scenarios of climate change: a place-based approach. *Global Change Biology*, *22*(4), 1572–1584. doi: 10.1111/gcb.13189

Sethuraman, A. (2013). On inferring and interpreting genetic population structure-applications to conservation, and the estimation of pairwise genetic relatedness. *arXiv Preprint*, 158. doi: 10.1017/CBO9781107415324.004

Sheriff, M. J., Krebs, C. J., & Boonstra, R. (2009). The sensitive hare: sublethal effects of predator stress on reproduction in snowshoe hares. *Journal of Animal Ecology*, *78*(6), 1249–1258. doi: 10.1111/j.1365-2656.2009.01552.x

Sheriff, M. J., Dantzer, B., Delehanty, B., Palme, R., & Boonstra, R. (2011). Measuring stress in wildlife: techniques for quantifying glucocorticoids. *Oecologia 166*(40), 869-887. doi: 10.2307/41499891

Sheriff, M. J., Dantzer, B., Delehanty, B., Palme, R., & Boonstra, R. (2016). Measuring stress in wildlife: techniques for quantifying glucocorticoids *Springer in cooperation with International Association for Ecology 166*(4), 869–887.

Shutt, K. A., Setchell, J. M., & Heistermann, M. (2012). Non-invasive monitoring of physiological stress in the Western lowland gorilla (*Gorilla gorilla gorilla*): validation of a fecal glucocorticoid assay and methods for practical application in the field. *General and Comparative Endocrinology*, *179*(2), 167–177. doi: 10.1016/j.ygcen.2012.08.008

Smith, A. (1974). The distribution and dispersal of pikas: influences of behavior and climate. *Ecology*, 55: 1368–1376. doi:10.2307/1935464

Smith, A., & Ivins, B. (1983). Colonization in a pika population: dispersal vs philopatry. *Behavioral Ecology and Sociobiology*. *13*(1), pp.37-47.

Smith, A. T., and Weston, M. L. (1990). *Ochotona princeps. Mammalian Species*, (352), 1. doi: 10.2307/3504319

Stearns, S. C. (1989). Trade-offs in life-history evolution. *Functional ecology*, *3*(3), 259-268. doi: 10.2307/2389364

Stewart, J. A. E., Perrine, J. D., Nichols, L. B., Thorne, J. H., Millar, C. I., Goehring, K. E., & Wright, D. H. (2015). Revisiting the past to foretell the future: summer temperature and habitat area predict pika extirpations in California. *Journal of Biogeography*, *42*(5), 880–890. doi: 10.1111/jbi.12466

Taberlet, P. (1996). Reliable genotyping of samples with very low DNA quantities using PCR. *Nucleic Acids Research*, *24*(16), 3189–3194. doi: 10.1093/nar/24.16.3189

Taberlet, P., Waits, L. P., & Luikart, G. (1999). Noninvasive genetic sampling: look before you leap. *Tree*, *14*(8), 223–227.

Teskey-Gerstl, A., Bamberg, E., Steineck, T., & Palme, R. (2000). Excretion of corticosteroids in urine and faeces of hares (*Lepus europaeus*). *Journal of Comparative Physiology B-Biochemical Systemic and Environmental Physiology*, *170*(2), 163–168. doi: 10.1007/s003600050271

Thomas, J. A., Bourn, N. A. D., Clarke, R. T., Stewart, K. E., Simcox, D. J., Pearman, G. S., & Goodger, B. (2001). The quality and isolation of habitat patches both determine where butterflies persist in fragmented landscapes. *Proceedings of the Royal Society of London B: Biological Sciences*, *268*(1478).

Touma, C., & Palme, R. (2005). Measuring fecal glucocorticoid metabolites in mammals and birds: the importance of validation. *Annals of the New York Academy of Sciences*, *1046*(0), 54–74. doi: 10.1196/annals.1343.006

Valladares, F., Matesanz, S., Guilhaumon, F., Araújo, M. B., Balaguer, L., Benito-Garzón, M., and Zavala, M. A. (2014). The effects of phenotypic plasticity and local adaptation on forecasts of species range shifts under climate change. *Ecology Letters*, *17*(11), 1351–1364.

doi: 10.1111/ele.12348

Van Oosterhout, C., Hutchinson, W.F., wills, D.P.M., & Shipley, P. (2004). MICRO - CHECKER: software for identifying and correcting genotyping errors in microsatellite data. *Molecular Ecology Notes*, 4: 535–538. doi:10.1111/j.1471-8286.2004.00684.x

Visser, M., & Both, C. (2005). Shifts in phenology due to global climate change: the need for a yardstick. *Proceedings of the Royal Society of London B: Biological Sciences*, *272*(1581), 2561-2569. doi: 10.1098/rspb.2005.3356

Vitousek, M. N., Rubenstein, D. R., Nelson, K. N., & Wikelski, M. (2008). Are hotshots always hot? A longitudinal study of hormones, behavior, and reproductive success in male marine iguanas. *General and Comparative Endocrinology*, *157*(3), 227–232. Doi: 10.1016/J.YGCEN.2008.04.015

Vucetich, J. A., & Waite, T. A. (2003). Spatial patterns of demography and genetic processes across the species' range: null hypotheses for landscape conservation genetics. *Conservation Genetics*, *4*(5), 639–645. doi: 10.1023/A: 1025671831349

Wada, H., Salvante, K. G., Stables, C., Wagner, E., Williams, T. D., & Breuner, C. W. (2008). Adrenocortical responses in zebra finches (*Taeniopygia guttata*): individual variation, repeatability, and relationship to phenotypic quality. *Hormones and Behavior*, *53*(3), 472– doi: 10.1016/J.YHBEH.2007.11.018

Walther, G., Post, E., Convey, P., Menzel, A., & Parmesan, C. (2002). Ecological responses to recent climate change. *Nature*, *416*(6879), pp.389-395. doi:10.1038/416389a

Wasser, S. K., Bevis, K., & Hanson, E. (1997). Noninvasive physiological measures of disturbance in the northern spotted owl. *Conservation Biology : The Journal of the Society*, *11*(4), 1019. doi: 10.1046/j.1523-1739.1997.96240.x

Wasser, S.K., Hunt, K.E., Brown, J.L., Cooper, K., Crockett, C.M., Bechert, U., Millspaugh, J.J., Larson, S., & Monfort, S.L. (2000). A generalized fecal glucocorticoid assay for use in a diverse array of nondomestic mammalian and avian species. *General and Comparative Endocrinology*, *120*(3), 260–275. doi: 10.1006/GCEN.2000.7557

Weir, B., & Cockerham, C. (1984). Estimating F-statistics for the analysis of population structure. *Evolution*, 38: 1358–1370. doi:10.1111/j.1558-5646.1984.tb05657.x

Wickham, H. (2009). ggplot2: Elegant Graphics for Data Analysis. Springer-Verlag New York

Wikelski, M., & Cooke, S. J. (2006). Conservation physiology. *Trends in Ecology and Evolution*, *21*(1), 38–46. doi: 10.1016/j.tree.2005.10.018

Williams, C., Kitaysky, A., Kettle, A., & Buck, L.(2008).Corticosterone levels of tufted puffins vary with breeding stage, body condition index, and reproductive performance. G*eneral and Comparative Endocrinology 158* (1), 29-35. doi: 10.1016/j.ygcen.2008.04.018

Wilkening, J. L., & Ray, C. (2016). Characterizing predictors of survival in the American pika (*Ochotona princeps*). *Journal of Mammalogy*, (x), gyw097. doi: 10.1093/jmammal/gyw097

Wilkening, J. L., Ray, C., Beever, E. A., & Brussard, P. F. (2011). Modeling contemporary range retraction in Great Basin pikas (*Ochotona princeps*) using data on microclimate and microhabitat. *Quaternary International*, *235*(1–2), 77–88. doi:10.1016/j.quaint.2010.05.004

Wilkening, J. L., Ray, C., & Sweazea, K. L. (2013). Stress hormone concentration in Rocky Mountain populations of the American pika (*Ochotona princeps*). *Conservation Physiology*, *1*(1), 1–13. doi: 10.1093/conphys/cot027

Wilkening, J.L. (2014). Characterizing metrics and outcomes of stress in a climate-sensitive species, the American pika. University of Colorado, Boulder M.S. University of Nevada, Reno. A thesis submitted to the Faculty of the Graduate School.

Wilkening, J. L., Ray, C., & Varner, J. (2015). Relating sub-surface ice features to physiological stress in a climate sensitive mammal, the American pika (*Ochotona princeps*). *PLoS ONE*, *10*(3), 1–17. doi: 10.1371/journal.pone.0119327

Wilkening, J. L., & Ray, C. (2016). Characterizing predictors of survival in the American pika (*Ochotona princeps*). *Journal of Mammalogy*, *97*(5), 1366–1375. doi: 10.1093/jmammal/gyw097

Wingfield, J., & Vleck, C. (1992). Seasonal changes of the adrenocortical response to stress in birds of the Sonoran Desert. *J. Exp. Zool.*, 264: 419–428. doi:10.1002/jez.1402640407

Wingfield, J.C., Suydam, R., & Hunt, K. (1994). The adrenocortical responses to stress in snow buntings (*Plectrophenax nivalis*) and Lapland longspurs (*Calcarius lapponicus*) at Barrow, Alaska. *Comparative Biochemistry and Physiology Part C: Pharmacology, Toxicology and Endocrinology*, *108*(3), 299–306. doi: 10.1016/0742-8413(94)00030-E

Wingfield, J., & Romero, L. (2001). Adrenocortical responses to stress and their modulation in free-living vertebrates. *Comprehensive Physiology*. doi: 10.1002/cphy.cp070411

Winter, B. (2013). Linear models and linear mixed effects models in R with linguistic applications. *arXiv preprint arXiv:1308.5499*.

Wood, J. L. A., Belmar-Lucero, S., Hutchings, J. A., & Fraser, D. J. (2014). Relationship of habitat variability to population size in a stream fish. *Ecological Applications*, *24*(5), 1085–1100. doi: 10.1890/13-1647.1

Zuur, A., Ieno, E., Walker, N., Saveliev, A., & Smith, G. (2009). Mixed effects models and extensions in ecology with R. Gail M, Krickeberg K, Samet JM, Tsiatis A, Wong W, editors. *New York, NY: Spring Science*.

Tables

Table 1. Results of linear mixed effects modeling to determine relative importance of microclimate predictor variables in patterns of fecal GCM concentrations for the American pika (*Ochotona princeps*) population within the Pipet Tarn sampling site. The top eight microclimate-derived models are given along with their associated AICc, ΔAIC, Log(L), and pseudo R^2 values (marginal R^2= amount of variance explained by the fixed effects; conditional R^2= amount of variance explained by both fixed and random effects). Note that each of the microclimate models included sex as an additive fixed effect to account for statistically significant differences between males and females in fecal GCMs (no interactions were statistically significant). The random effects (random intercept and slope) were also kept constant for each of the models: (1 + Hindiv | Territory) + (1 | Year)

Model	Fixed effect	AIC_C	ΔAIC	BIC	LOG(L)	Marginal R^2	Conditional R^2
7	Number of days above 26°C (interstitial)	-219.3	0.00	-212.8	120.50	0.289	0.457
8	Maximum temperature in July (interstitial)	-219.2	0.03	-212.8	120.48	0.245	0.474
5	Mean temperature of the coldest quarter (surface)	-216.0	3.24	-209.6	118.88	0.156	0.439
1	Temperature seasonality (surface)	-215.9	3.40	-209.4	118.80	0.154	0.506
2	Average summer temperature (surface)	-215.0	4.25	-208.5	118.37	0.147	0.416
4	Number of days above 28°C (surface)	-214.4	4.81	-208.0	118.10	0.119	0.419
3	Number of days below -5°C (surface)	-214.4	4.82	-208.0	118.09	0.127	0.421
6	Average summer temperature (interstitial)	-213.9	5.40	-207.4	117.80	0.099	0.421

Table 2. Relative importance of predictor variables of physiological stress in pikas at Pipet Tarn given by Akaike weight, as well as direction of correlation with fecal glucocorticoid metabolite concentration and general interpretation. Akaike weights were calculated across all models tested, but each model included each fixed effect only once

Predictor Variable	Akaike weight	Direction of Correlation	Interpretation
Number of days above 26°C (interstitial)	0.37	+	As the number of days above 26°C increases, fecal GCMs increase
Maximum temperature in July (interstitial)	0.36	+	As maximum temperature in July increases, fecal GCMs increase
Mean temperature of the coldest quarter (surface)	0.07	–	As mean temperature of the coldest quarter increases, fecal GCMs decrease
Temperature seasonality (surface)	0.07	–	As seasonal variability increases, fecal GCMs decrease

Figure legends

Fig. 1. A map showing the location of two study sites, Pipet Tarn and Cabin Slope, within the Harvey Monroe Hall Research Natural Area, Mono County, CA, USA. These sites are separated by 2 km of continuous, typical talus habitat and likely represent one true population. Active territories/haypiles (yellow circles) were repeatedly visited within and across seasons during 2013-2015 for collection of fecal glucocorticoid metabolites and fecal DNA, respectively. The locations of LogTag temperature loggers (n=36) within the Pipet Tarn site only are also given (blue diamonds). The inset map provides a simplified and closer look at the active pika territories that were outfitted with LogTag temperature loggers

Fig. 2. Comparison of fecal glucocorticoid metabolites (GCMs) between and within high-elevation sites (Cabin Slope and Pipet Tarn), and sexes (as detected by molecular sexing). Note that Cabin Slope maintains significantly higher relative fecal GCM concentrations compared to Pipet Tarn ($F_{(1,266)} = 73.15$, $P = 0.000$); however only males and females within the Pipet Tarn site maintain significantly different average fecal GCM concentrations ($F_{(1, 42)} = 7.046$, $p = 0.0112$). Panel b showed a comparison of within-season variation in average fecal GCM patterns during the summer seasons of 2013-2015. All fecal GCM estimates are represented using the mean and associated standard error

Fig. 3 Average turnover rate per territory (the ratio between the total number of new captures and the total number sampling visits, represented as a percent) within both the Pipet Tarn and Cabin Slope sites and the associated average fecal glucocorticoid metabolites (GCMs) measured across three years of this study (ca. 2012-2015 (top panel, “a.”). The bottom panel (“b.”) represents individual recapture rate (represented as a percentage of the ratio between the total number of genetic recaptures and the total number of sampling visits) and the average fecal GCM concentrations measured for each individual ordered by territory. Note that the relationship between fecal GCM concentrations and average territory turnover rate or individual recapture rates varies within and between both sites. Both average turnover rates and individual recapture rates differed significantly across territories and individuals within each site ($P = 0.000$)

Fig. 4 AMOVA (top panel) showing that genetic variance is partitioned primarily within and among individuals and not populations. Principle Coordinates Analysis (PCoA) (bottom panel) showing significant overlap in PCoA space among all sampling locations: Pipet Tarn (PIP), intervening sites (INT) and Cabin Slope (CAB)

Fig. 5 Per-territory and seasonal patterns between fecal GCM concentrations and three temperature metrics within the top linear mixed effects models: a) number of days above 26°C (interstitial microclimate), b) maximum temperature in July (interstitial microclimate), and c) mean temperature of the coldest quarter (surface microclimate). Territories are named starting with a capital “H” to denote haypile identification and evidence of active occupancy. Territories differed significantly in terms of acute heat stress (da26_d) ($F_{(17, 36)} = 3.73$, $P = 0.0004$) however note that only those territories that experienced temperatures above 26°C during the years of this study were shown here. Territories also differed significantly in terms of mean temperature of the coldest quarter ($F_{(17, 36)} = 2.02$, $P = 00378$), with temperature seasonality varying significantly only between years ($F_{2, 51} = 48.91$, $P = 0.000$)

Figures

Fig. 1

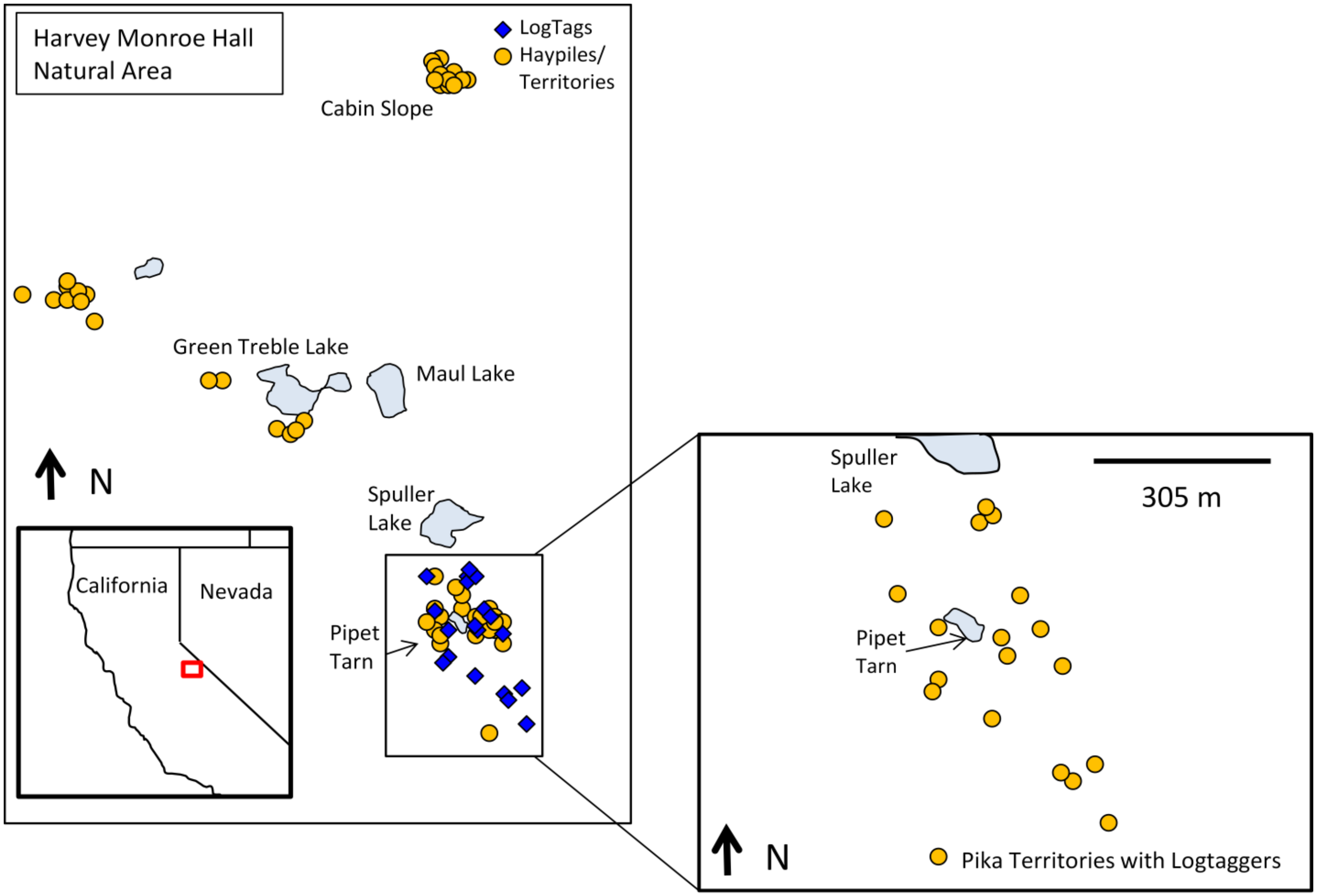


Fig. 2

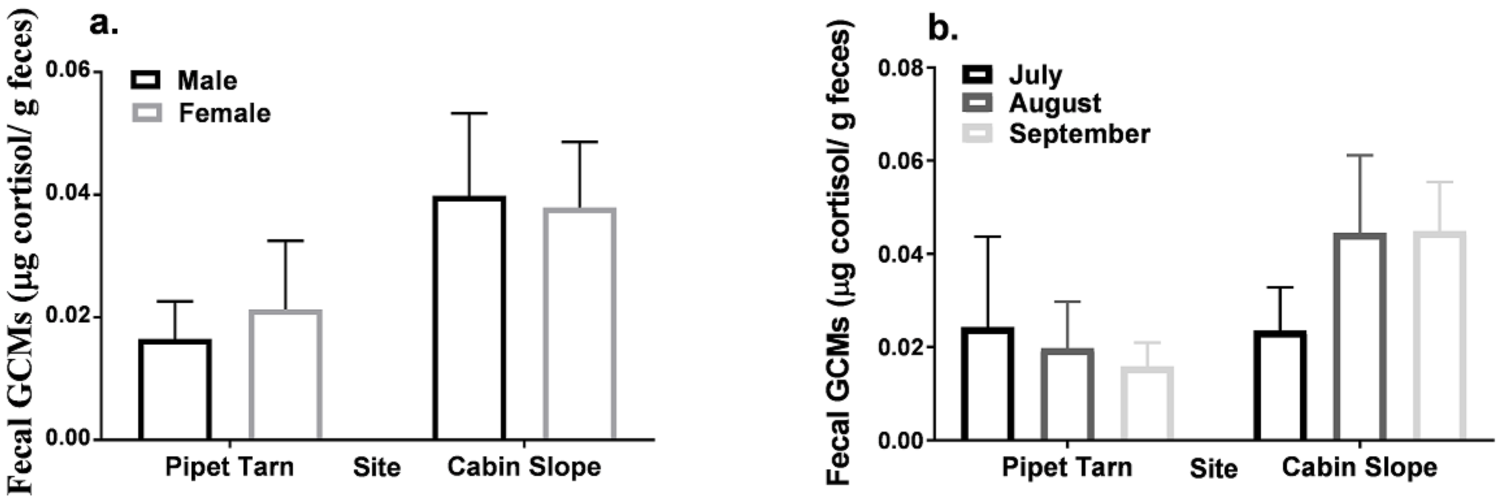

a.
Fecal GCMs (µg cortisol/ g feces)
0.06
0.04
0.02
0.00
Male
Female
Pipet Tarn
Site
Cabin Slope
b.
Fecal GCMs (µg cortisol/ g feces)
0.08
0.06
0.04
0.02
0.00
July
August
September
Pipet Tarn
Site
Cabin Slope

Fig. 3

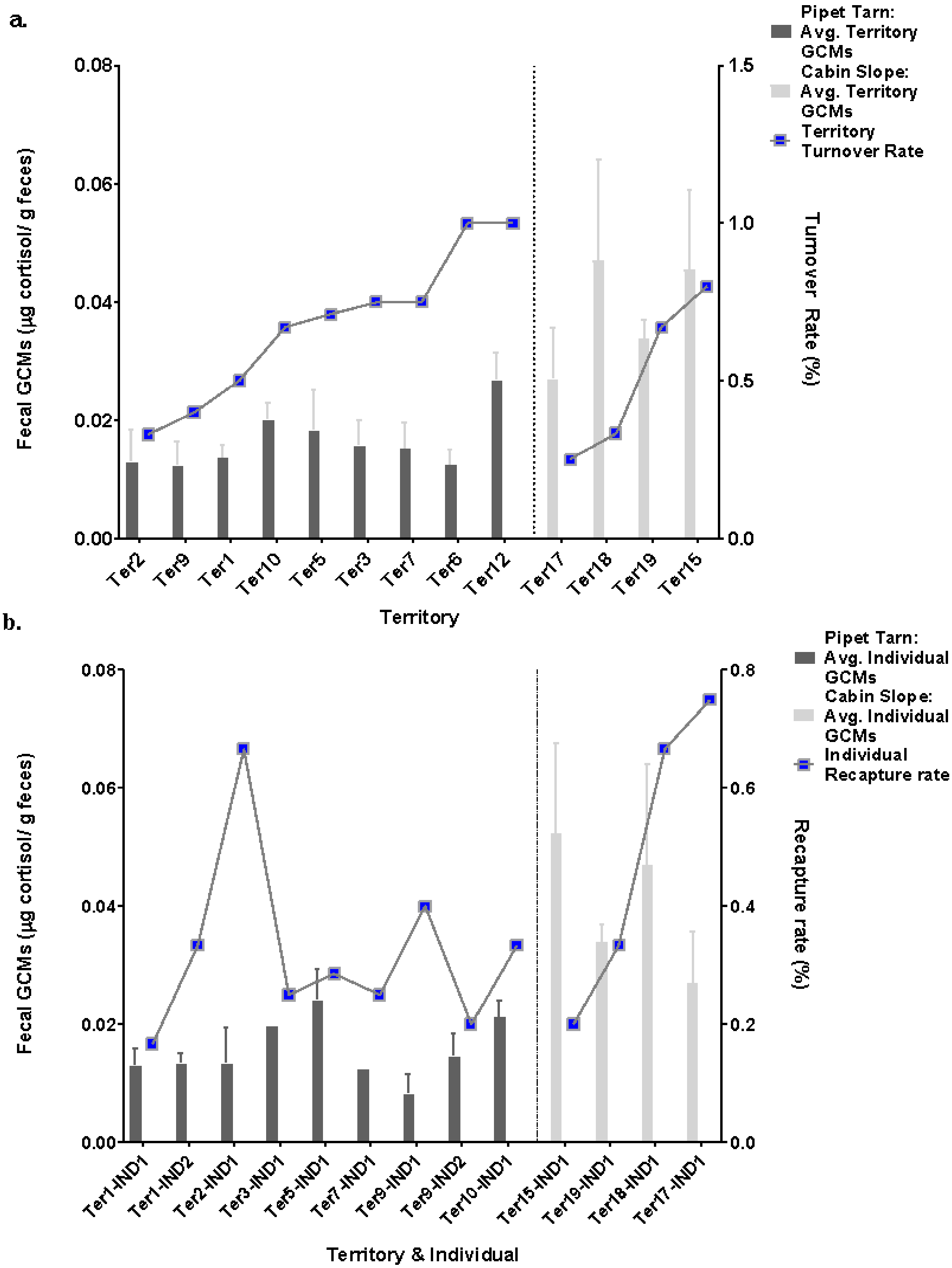
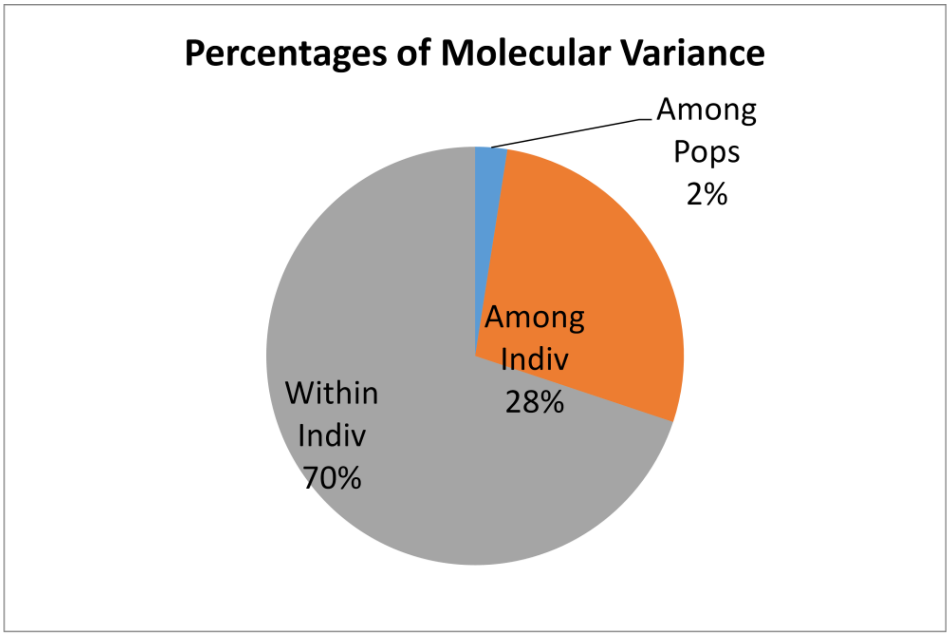

a.
Pipet Tarn:
Avg. Territory GCMs
Cabin Slope:
Avg. Territory GCMs
Territory Turnover Rate
Fecal GCMs (µg cortisol/ g feces)
Turnover Rate (%)
0.08
0.06
0.04
0.02
0.00
1.5
1.0
0.5
0.0
Ter2
Ter9
Ter1
Ter10
Ter5
Ter3
Ter7
Ter6
Ter12
Ter17
Ter18
Ter19
Ter15
Territory
b.
Pipet Tarn:
Avg. Individual GCMs
Cabin Slope:
Avg. Individual GCMs
Individual Recapture rate
Fecal GCMs (µg cortisol/ g feces)
Recapture rate (%)
0.08
0.06
0.04
0.02
0.00
0.8
0.6
0.4
0.2
0.0
Ter1-IND1
Ter1-IND2
Ter2-IND1
Ter3-IND1
Ter5-IND1
Ter7-IND1
Ter9-IND1
Ter9-IND2
Ter10-IND1
Ter15-IND1
Ter19-IND1
Ter18-IND1
Ter17-IND1
Territory & Individual

Fig. 4


Percentages of Molecular Variance
Among Pops 2%
Among Indiv 28%
Within Indiv 70%

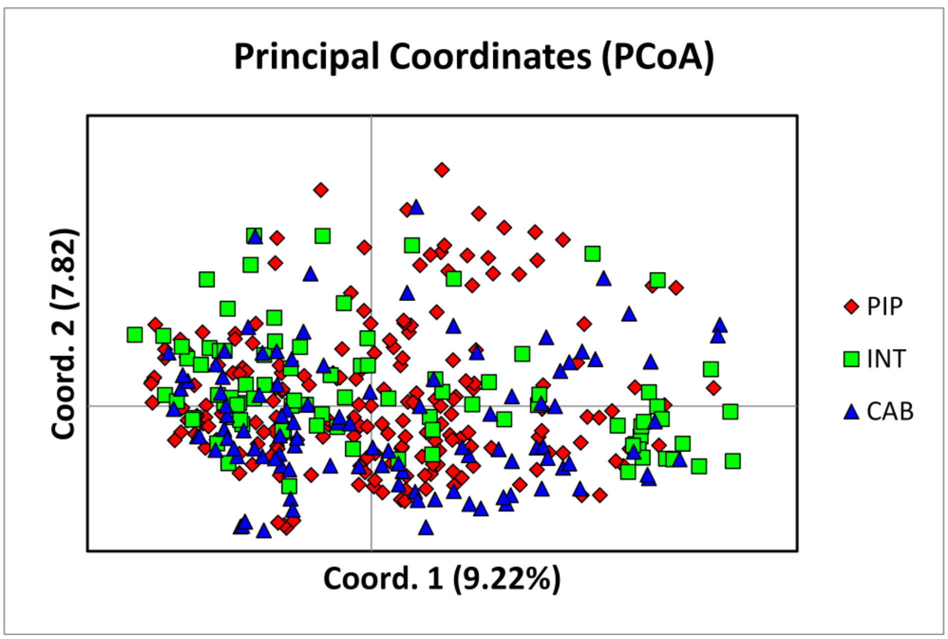
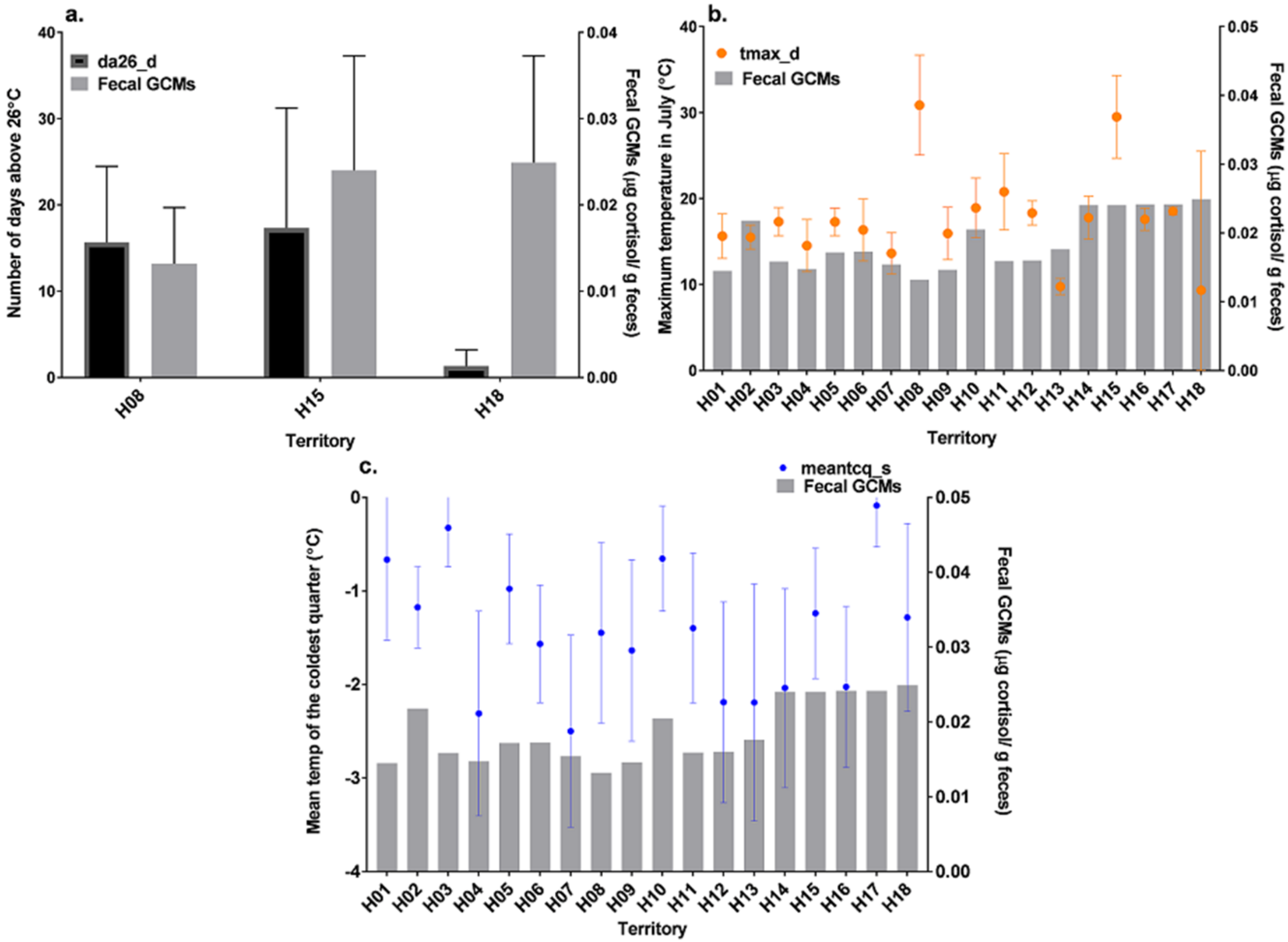

Principal Coordinates (PCoA)
Coord. 2 (7.82)
Coord. 1 (9.22%)
PIP
INT
CAB

Fig. 5


a.
da26_d
Fecal GCMs
Number of days above 26°C
Fecal GCMs (µg cortisol/ g feces)
H08
H15
H18
Territory
b.
tmax_d
Fecal GCMs
Maximum temperature in July (°C)
Fecal GCMs (µg cortisol/ g feces)
Territory
c.
meantcq_s
Fecal GCMs
Mean temp of the coldest quarter (°C)
Fecal GCMs (µg cortisol/ g feces)
Territory

Supplementary Materials

Table S1 Ten microsatellite primers developed for the American pika (*Ochotona princeps*) (Peacock et al. 2002; Peacock and Kirchoff 2009 unpublished) were optimized for PCR multiplexing (shortening fragments lengths) of fecal DNA. Product size (bp), M13 dye, product size, annealing temperature (T_A) (°C) as well as GenBank accession numbers are also provided

Locus	Primer Sequence (5'-3')	Dye	Product size (bp)	Ta (°C)	Accession No.
OCP 4b	F: GTTATTGCGCCAGGGTCTAC R: GTCCTGGCTATTGCATCCAC	FAM	170-190	70	AF487495
OCP 6b	F: GGCTTCAGATTTCCTCAACACC R:AGGAAGGGAGAAGCTAGAC	VIC	250-270	68	AF487497
OCP 7	F: ATCCTGAGCTATCTTTGCCATT R: CCCAAAACTCCTTGAGAGACA	FAM	276-300	65	AF487498
OCP 8	F: TTCCTCTGGAGTCCTCTAACCC R: CCTCGAGCAAGTTTGGTTGTT	NED	223-239	67	AF487499
OCP 9b	F: CGCAAACATCATGGCTAAAA R:GCAGGTGGCAGATTTCTCC	PET	180-200	69	AF487500
OCP 10	F:CCAATTTGGCTGTTAAGGCTAT R:CCAGTGTCTGGCAYACGGTAAGC	FAM	190-200	65	GQ461705
OCP 15	F:GGAAGCAGAGGACCTAGCCAAGC R:CCAGAGGTTTAACTTGCTATGC	VIC	180-200	65	GQ461710
OCP 16a	F:GGAAACAGCTATGACCAAGCAGTGGGCA R:GCTGACATTTAATATCGTCC	PET	240-250	65	GQ461711
OCP 17	F:CAGACACCTACTGGCCTATATGT R: CTTCAGGAGACTGACCCAACC	VIC	280-330	65	GQ461712
OCP 21	F:GTACTCACTCCTTGGCACATCTC R:GAATGGGGTTAGATGTACATTGG	FAM	160-190	65	GQ461716

Table S2 Ten candidate models for linear mixed effects modeling of fecal GCM concentrations and their hypotheses with random slope and intercept effects held constant (1 + Hindiv| Territory) (1| Year). Each of the eight temperature metrics were identified as physiologically relevant for pikas and non-collinear (variance inflation factor <6) prior to modeling and vary in terms of the microclimate type measured (i.e. surface or interstitial). The hypothesized relationship between each fixed effect and patterns of fecal GCM concentrations are given assuming that each of the variables are increasing

		Fixed effects	Hypothesized effect on fecal GCM concentrations
Individual fixed effects	1	Temperature Seasonality (surface)	↑
	2	Average summer temperature (surface)	↑
	3	Number of days below -5°C (surface)	↑
	4	Number of days above 28°C (surface)	↑
	5	Mean temperature of the coldest quarter (surface)	↓
	6	Average summer temperature (interstitial)	↑
	7	Number of days above 26°C (interstitial)	↑
	8	Maximum temperature in July (interstitial)	↑
	9	Sex	(for females) ↓
			* all hypotheses based on **increasing** fixed effects

Table S3 Summary table comparing population genetic statistics among sites including observed heterozygosity (Ho), gene diversity (He), allelic richness (A_R), inbreeding coefficient (F_{IS}) as well as pairwise F_{ST} estimates for the Pipet Tarn, Cabin Slope and the two km of intervening talus habitat. Significant FST are indicated with an asterisk p=0.016, 60 permutations

Site	Elev (m)	Sample size (n)	Ho	He	A_R	F_{IS}	Sites	Pairwise F_{ST}
Pipet Tarn	3162	214	0.612 (SE=0.065)	0.6623	6.912	0.075	PIP- CAB	0.044*
Intervening Talus	3128	93	0.6805 (SE=0.057)	0.6854	6.953	0.07	PIP-INT	0.023*
Cabin Slope	3135	122	0.621 (SE=0.087)	0.6084	5.256	-0.21	INT-CAB	0.05*

CHAPTER 2 Non-invasive monitoring and the American pika metapopulation in Bodie, California, USA: tracking population dynamics and physiological health in a fragmented habitat under climate change

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Abstract

Environmental heterogeneity often mediates the interactions between organisms and changing or unpredictable ecological conditions which can result in differing individual responses based on the physical features of the landscape surrounding them. This study investigated the role of habitat spatial structure in shaping patterns of movement and physiological response in an American pika (*Ochotona princeps*) metapopulation located in Bodie State Historic Park, CA, USA (BSHP). Using measurements of the hormonal stress response, physiological monitoring can inform predictions of population persistence over time by coupling habitat and climate to physiological state. Recent advances in measuring glucocorticoid metabolites (GCMs) excreted through the gut has enabled non-invasive quantification of physiological stress in wildlife populations. Paired with census and local weather station data collected regularly over the last 40 years, we collected fecal GCM and fecal DNA samples during 2013-2015 for habitat patches that were occupied in order to test the following predictions: (i) size and configuration of habitat patches affect levels of physiological stress and occupancy patterns, (ii) monthly and annual climate metrics are significantly correlated with average physiological stress, and (iii) annual and per-patch pika occupancy patterns are significantly associated with local climate variables. Our results reveal temporal patterns of fecal GCMs that differ between habitat patches and significantly relate to summer season climate metrics including average monthly maximum temperature as well as minimum monthly temperature. In addition to an observed decline in patch occupancy over the last 40 years and its significant association with summer heat and winter cold, the BSHP population has significantly greater average stress levels compared to other sites within the Bodie Hills region. This study highlights the tremendous potential of long-term census data and non-invasive physiological monitoring for understanding how pika populations may differentially fare in the face of changing environmental conditions.

Keywords: physiological stress, American pika, metapopulation, census, weather station data

Introduction

Habitat can be defined as the spectrum of abiotic and biotic factors that organisms interact with in explicit geographic space and time (Hall et al. 1997; Mitchell 2005; Homyack 2010). Widespread alteration and fragmentation of habitats are considered to be significant threats to the persistence of species world-wide (Wilcove et al. 1998; Wilson 1999; Cockrem 2005; Homyack 2010; Barr et al. 2015; Perkin et al. 2015; Haddad et al. 2015; Martin and Fahrig 2016). Therefore understanding how landscape level structure and quality influence population processes is essential for conservation of biodiversity (Homyack 2010; Blitzer et al. 2012; Tscharntke et al. 2012; Woltz et al. 2012; Noonburg and Byers 2016). At finer spatial scales, populations often occur across habitats of variable quality and such environmental heterogeneity can mediate fitness outcomes under changing or unpredictable ecological conditions (Walther et al. 2002; Doddington et al. 2013; Otto et al. 2014; Messer et al. 2016).

By studying habitat preferences in a structured landscape, it becomes possible to measure individual responses to both the direct and indirect effects of anthropogenic disturbance in the context of habitat quality (Fretwell and Lucas 1969; Pulliam 1988; Homyack 2010). Traditionally, habitat quality has been estimated through fitness proxies such as population growth rate; however, demographic parameters do not always provide an easily interpretable understanding of the relationship between habitat variability and population dynamics (Homyack 2010; Albano et al. 2012). In fact, the choice of demographic parameter can lead to ambiguous estimates of habitat quality if high abundance and low reproductive success are observed simultaneously within the same habitat (Knutson et al. 2006; Albano et al. 2012). Alternatively, some studies have relied on direct investigation of habitat features such as resource availability, spatial structure, or pathogen presence, but such approaches can be limited if individual level responses to such variation are not understood (Albano et al. 2012).

Conservation physiology provides an alternative framework for studying habitat quality by measuring the physiological response of individual organisms to environmental variability (Huey 1991; Carey 2005; Wikelski and Cooke 2006; Chown and Gaston 2008; Homyack 2010; Albano 2012; Bhattacharjee et al. 2015). Physiological consequences of anthropogenic disturbance, such as climate change, can then be correlated with fitness differences in growth, reproduction, or survival among individuals in the context of habitat variability (Denny and Helmuth 2009; Albano et al. 2012). It is likely that the impacts of environmental change will influence an individual's physiology before it is detected at the population level; therefore, physiological monitoring of both short and long-term effects of anthropogenic disturbance can provide context for predictions of population health and persistence over time (Blickley et al. 2012; Payne et al. 2015; Ribeiro et al. 2015; Barlow et al. 2016).

Depending on the scale and duration of monitoring, conservation physiological approaches can provide early detection of at-risk regions, identify particularly susceptible populations, and even characterize periods of greater physiological stress within and between seasons (Wasser et al. 1997; Wikelski and Cooke 2006; Thiel et al. 2008; Madliger and Love 2014; Albano et al. 2012; Bhattacharjee et al. 2015). There are a variety of physiological tools that can be used to assess both individual and population level habitat quality through an understanding of an organisms' energy budget (i.e. metabolic rates), growth and lipid storage (i.e. body condition indices), health and disease response (i.e. cell-mediated immune response), hormonal stress response (i.e. glucocorticoid measures), or diet quality (i.e. stable isotope analysis) (Homyack 2010; Albano et al. 2012). Endocrine techniques are a widely-used approach to incorporate physiological biomarkers into wildlife monitoring through measurement of stress hormones (glucocorticoids, GCs) as either baseline levels (e.g. plasma, serum, or saliva) or integrated measures (e.g. hair, and feces) (Cooke and O'Connor 2010; Madliger and Love 2014).

Considered an index of adrenocortical reactivity, these endocrine hormones reflect the interactions between an animal's health and its environment since they are released as part of the stress response for coping with negative or threatening conditions (McEwen and Wingfield 2003; Romero 2004; Korte et al. 2005; Albano et al. 2015).

The hypothalamic-pituitary-adrenal (HPA) axis regulates the release of GCs. Baseline levels of GCs are important for maintaining energetic balance and responding appropriately to predictable events within the life history of the organism such as natal dispersal or mating period (Landys et al. 2006). Under stressful conditions, the release of GCs activates changes in physiology and behavior which may increase individual survival at least in the short-term (Wingfield and Romero 2001). In response to a stressor or environmental challenge, stress hormones increase rapidly thus leading to activation of stored energy reserves, increased immune function, and subdued participation in activities that are considered non-essential to survival (Sapolsky et al. 2000; Madliger and Love 2014). This evolved stress response is partially responsible for providing vertebrates with an ability to respond appropriately to predictable daily or seasonal environmental variability in addition to stochastic events such as extreme weather, predation, or social conflict (Romero 2002; McEwen and Wingfield 2003). However, the sustained release of GCs as a result of physiological stress can lead to immunosuppression, death of nerve cells, hyperglycaemia, muscle and bone atrophy, hypertension and/or reduced reproduction, and therefore be maladaptive (Riley 1981; McEwen and Sapolsky 1995; Sapolsky et al. 2000; Wingfield and Sapolsky 2003; Engelmann et al. 2004; Romero 2004; Boonstra 2005; Korte et al. 2005; Wilkening et al. 2013; Albano et al. 2015).

Recent advances for quantifying stress in wildlife populations have focused on measurement of the glucocorticoid metabolites (GCMs) that are excreted through the gut and provide measurement of stress through animal waste products (Millspaugh and Washburn 2004;

Sheriff et al. 2010). This non-invasive approach provides an alternative to potentially biased plasma-based measurements that require stressful capture and handling of animals and likely cause increased levels of circulating hormones (Cook et al. 2000; Romero 2004). Noninvasive fecal sampling permits repeated sampling of GCMs at both spatial and temporal scales, therefore providing a more accurate representation of long-term fluctuating endocrine condition without direct disturbance to the animal (Harper and Austad 2001; Palme 2005; Wilkening et al. 2013). In fact, in terms of endocrine metrics comparisons, measures of baseline GCM concentrations are considered more closely associated with survival since fecal metabolites signify the allostatic load of the individual and may be more reflective of longer term or chronic stress (Wilkening et al. 2016). Previous vertebrate studies investigating the role of anthropogenic stressors utilized fecal GCMs to understand the effects of winter season recreation on mammal species such as elk (*Cervus elaphus*) and grey wolves (*Canis lupus*) (Creel et al. 2002). Using a proxy of snowmobile traffic, the authors found that decreased habitat quality (i.e. greater traffic) was directly related to fecal GCM concentrations for female elk and wolves in three national parks (Creel et al. 2002; Homyack 2010). Even habitat generalists, such as Carolina Chickadees (*Poecile carolinensis*), have been shown to have higher physiological stress levels and lower body mass in disturbed sites exposed to forest harvesting compared to birds from undisturbed sites (Lucas et al. 2006).

This study focused on characterizing habitat quality for an American pika (*Ochotona princeps*; hereafter, pikas) population inhabiting talus ore dump habitat in the Bodie Hills region of eastern California, through characterization of individual and population level patterns of fecal GCM levels and investigation of the relationship between endocrine condition and long-term census and local climate data. This metapopulation has been the subject of long term research focusing on ecological determinants of habitat occupancy due to its island-mainland structure and with ore dump patches separated by sagebrush steppe and varying in perimeter size from approximately 1.5 m to 280 m (Peacock and Smith 1997; Moilanen et al. 1998).

Pika populations occur in rocky substrates in alpine habitats throughout the western United States and Canada, and have been repeatedly identified as a bio-indicator species of the ecological effects of climate change (McDonald and Brown 1992; Hafner 1993; 1994; Beever et al. 2003; Grayson 2005). A non-hibernating relative of rabbits and hares, pikas are cold-adapted and rely on behavioral thermoregulation to maintain homeostasis. By accessing thermally stable microclimates available within the interstitial spaces of their rocky substrates, the thick-furred pika escapes ambient temperatures that approach their upper lethal temperature (43.1°C), and high resting body temperature (40.1°C) (Smith and Weston 1990; Beever et al. 2013). During the summer season pikas actively forage for vegetation they consume immediately and for vegetation they store in food caches known as “haypiles” on their individual territories. Pikas utilize the stored vegetation during the winter months when no fresh forage is available (Smith 1974b; Dearing 1997). Because of this species’ reliance on the climatic characteristics of their talus habitat, pika occurrence and persistence is considered to be significantly influenced by temperature especially at regional and range-wide scales (Grayson 2005; Galbreath et al. 2009). At smaller scales, the relative roles of fine-scale habitat features, such as functional connectivity (Castillo et al. 2014; Schwalm et al. 2016), habitat size, spatial configuration (Stewart et al. 2015), and vegetation cover, appear to all be important factors in constraining pika distributions (Erb et al. 2011; Jeffress et al. 2013; Varner and Dearing 2014).

A corticosterone enzyme immunoassay to measure GCMs was recently biologically validated for use with pika fecal material (Wilkening et al. 2013; Wilkening 2014). Subsequent pika endocrine studies have confirmed individual and sex differences, variation among populations, the importance of habitat features such as sub-surface ice, and an influence of temperature and precipitation regimes in influencing fecal GCM patterns (Wilkening et al. 2013; Wilkening and Ray 2015; Wilkening et al. 2016). In terms of fitness consequences, fecal GCM concentrations and flea load were found to be better stronger predictors of annual survival in pikas when compared to plasma-obtained GC samples (Wilkening and Ray 2016).

If fecal GCMs are indeed a reliable indicator of the hormonal stress response, then GCM concentrations should be consistent with regard to changes in environmental variables and relate significantly to annual survival or fecundity. However, as a broadly distributed species, pika populations occur across a diverse array of landscape types (e.g. sea level in Columbia River Gorge, non-alpine sage-scrub habitat in Bodie State Historic Park in the Sierra Nevada, alpine talus in the Northern Rocky Mountains) which vary in fine-scale factors such as habitat configuration, population connectivity, vegetation diversity and cover, substrate type, microclimate buffering capacity, and predator pressure (Moyer-Horner et al. 2015; Stewart et al. 2015; Shinderman 2015; Schwalm et al. 2016; Ray et al. 2016; Mathewson et al. 2017). As a result of both broad and fine-scale environmental heterogeneity and the effect of post-depositional environment on detected GCM concentrations, identifying a standard threshold concentration of fecal GCMs that translates into a tractable fitness consequence is unlikely to be generalizable across all range-wide populations (Washburn and Millspaugh 2002; Palme 2005; Shutt et al. 2012; Stetz et al. 2013; Wilkening et al. 2016). For example, higher temperatures in some sampling sites might increase GCM concentrations in exposed fecal samples, therefore a biologically meaningful threshold linking GCM concentrations to fitness consequences is likely to differ across the range of the species and emphasizes the need for long-term monitoring projects at much finer scales ideally within sites or regions.

Here, we establish a physiological monitoring program and characterize habitat quality for a spatially fragmented pika population in order to better understand how fecal GCM profiles vary across complex spatially structured habitat and to test whether this physiologic metric is related to a long-term patch occupancy and fine-scale climate metrics. As the first characterization of fecal GCM concentrations for pika populations within the vulnerable Great Basin lineage (*O. princeps schisticeps*), this study also enabled initial comparisons among individuals within multiple sampling sites that differ in habitat features such as rock type, and depth of talus, perimeter size, and relative isolation.

The highly fragmented habitat of this site has resulted in a variable spatial structure and a stepping stone metapopulation dynamic with habitat patches supporting 1-2 individuals and other "mainland" ore dumps or patches providing habitat for more than 20 animals (Peacock and Smith 1997; Moilanen et al. 1998; Klingler et al. *in review*). Long-term monitoring of pika occupancy at this site has recorded a consistent overall decline in the number of patches occupied since the early 1970s (Moilanen et al. 1998, Klingler et al. *in review*). Based on these previous studies, we hypothesize that there are components of habitat quality, such as unfavorable microclimates and lack of adequate forage, that are likely to be associated with elevated physiological stress in pikas leading to reduced survival and increased patch turnover, (Wingfield et al. 1998, Homyack 2010). In particular, we investigated site-level and patch-level habitat quality through physiological monitoring paired with long-term population census and local weather data to test the following predictions under a climate change hypothesis:

i. Habitat patches characterized by smaller perimeter and fewer territories will experience significantly higher turnover rates per territory and patch and occupants will have significantly greater average fecal GCM concentrations,

ii. Site level habitat quality metrics, as characterized by monthly temperature and precipitation during the summer season (i.e. June through September), will be significantly related to fecal GCM patterns,

iii. Site level habitat quality, as measured by long-term occupancy patterns over the last 40 years, will be significantly associated with the changing annual winter and summer season temperature and precipitation metrics that have occurred over this same time period, and

iv. Variation in site level habitat quality, as measured by rock type and relative isolation, among populations within the Great Basin region will be significantly correlated with fecal GCM concentrations.

Methods

Study Site

This study focused on an iconic American pika (*Ochotona princeps*) metapopulation in eastern California that has been the subject of long-term population monitoring efforts since the early 1970s (Smith 1974a, b: Smith 1980; Peacock and Smith 1997; Moilanen et al. 1998). Located within the Bodie Hills of Mono County, California, USA, this historic mining site is a designated California State Park protected from future anthropogenic development or disturbance (Fig. 1). Pika habitat in Bodie State Historic Park (BSHP) is characterized by approximately 100 ore dumps created by 19th and early 20th century hard-rock mining. The Bodie pika population has been divided into three patch networks or complexes (northern, middle, and southern) with long-term surveys focused on detecting pika occupancy through observations of fresh fecal pellets, haypiles, vocalizations, and/or visual sightings of animals (Smith 1974b; Smith and Gilpin 1997; Nichols 2011). The mining district is situated above the ghost town of Bodie and spans approximately 4 km from Bodie Bluff south to Sugarloaf Mountain and 1-1.5 km east to west across Great Basin sage-scrub habitat characterized by sagebrush (*Artemisia*), currant (*Ribes*), buckwheat (*Eriogonom*), as well as antelope bitterbrush (*Purshia tridentata*) and rubber rabbitbrush (*Chrysothamnus nauseous*) (Nichols et al. 2011). Initial colonization of this anthropogenic habitat is estimated to have occurred during the early 1900s from natural talus outcrops in the Bodie Hills and pikas have persisted in the northern portion of the district to present day despite noted population collapse across the middle and southern complexes (Smith 1974a, b; Smith 1980; Moilanen et al. 1998).

Population census surveys

Long-term population monitoring in BSHP has occurred through surveys of a sub-set of the total suitable habitat available (~79 patches) beginning in 1972 (Smith 1974a). Subsequent surveys occurred in 1977 (Smith 1980), 1989, 1991 (Smith and Gilpin 1997), 1992-2001, 2003-2006, and 2008-2016 (Lyle Nichols, unpublished data). Each patch was visited at least once per survey during the mid to late summer season (July or August) and thoroughly searched for evidence of current year occupancy indicated by either a visual sighting of an animal or latrine sites with fresh fecal pellets (Smith 1980; Moilanen et al. 1998; Nichols 2011). Fresh haypile caches are considered a secondary sign since showy surface haypiles are not particularly common within the Bodie site as they are elsewhere possible due to the relatively small rock size of the ore dumps and the potential caveat with regard to haying (i.e. the act of cutting and storing herbaceous vegetation) by other local species such as woodrats (Dixon 1919; Finley 1958; Escherich 1981; Morton and Pereyra 2008). For each patch, both the occupancy status as well the number of active territories were recorded enabling estimation of two site level metrics of pika occupancy: (i) the ratio between the total number of habitat patches occupied and the total number of patches surveyed across the entire site, and (ii) the ratio between the total number of patch extinctions and the total number of patch re-colonizations across all patches based on the previous year’s record. Survey effort was consistent within and among patches across seasons. These long-term census records provide a measure of relative site-level occupancy and turnover rates as the number of available territories per patch does not change each year only whether they happen to be occupied. The census records record demographic change over time, but because individuals were not marked they did not track individual rates of survival.

Fecal GCM sampling

To obtain a non-invasively sampled measure of physiological stress in pikas, we collected fecal pellets multiple times during June-September at occupied pika territories for approximately 20 habitat patches during 2013-2015. A minimum of 10 fresh fecal pellets (indicated by golden-green color and wet from urination) were collected for extraction of fecal GCM during each visit and stored on ice in coin envelopes until they transferred to a 20°C freezer at the University of Nevada, Reno prior to processing. In addition to BSHP, fecal GCMs were collected opportunistically during the summer of 2015 across the mix of occupied natural and ore dump habitat patches located at Bodie Bluff (~2800 m), as well as the natural talus outcrops near Masonic Mountain, and above the Chemung Mining site (~2700 m) within the Bodie Hills region (~18-20 km northwest of Bodie State Historic Park) as part of a long-term monitoring effort. For relative comparison, physiological stress data collected in the Eastern Sierra region (Pipet Tarn and Cabin Slope, 3100 m) were included to highlight population-level differences in pika stress across a range of elevations (Klingler et al. *in prep*). Statistical comparisons were made between years, sites, or sexes using one-way ANOVA, and Tukey's Honest Significant Difference test in R 3.3.2 (R Core Team, 2016) with significance assessed at α=0.05.

Extraction of hormone metabolites and enzyme immunoassays

Each sample was lyophilized and approximately six pellets were randomly chosen per sample and ground into powder using a mortar and pestle before being processed using a steroid solid extraction protocol developed by Arbor Assay Design, Inc. (Ann Arbor, MI, USA). Approximately 0.05 g of dried fecal material was immersed in 1.5 ml of 90 % aqueous ethanol and placed on a rocker for 1 hour. Rocked samples were centrifuged for 15 minutes at 5000 rpm and then 1.0 ml of supernatant was transferred to a clean screw-on lid tube. The supernatant solution was then evaporated to dryness using a nitrogen dryer and extracts were stored in -20°C freezer. Before enzyme immunoassay analysis extracts were reconstituted with 50 µl of 90 % aqueous ethanol and 950 µl of assay buffer and thoroughly mixed using a vortex mixer.

GCM levels were measured using a commercially available corticosterone enzyme immunoassay kit (Arbor Assay Design, Inc.; catalogue no. K014-H1 or K014-H5). This assay utilized a sheep poly-clonal antibody specific for corticosterone and was developed to estimate total corticosterone in many sample types including fecal extracts. Corticosterone was discovered to be the primary GC in other lagomorphs instead of cortisol, therefore corticosterone was measured in this study (Teskey-Gerstl et al. 2000; Monclus et al. 2006; Rehnus et al. 2009; Sheriff et al. 2009; Scarlata et al. 2011; Wilkening et al. 2013). Extracted samples were run in duplicate during each assay with seven known concentrations of corticosterone (5000, 2500, 1250, 625, 312.50, 156.25, and 78.125 pg/ml). A standard curve was then generated using the averaged results from these known corticosterone concentrations. The value for each extracted sample was read using a microplate reader at 420 nm. Fecal GCMs were analyzed separately by year and by patch to characterize the variation in patterns of physiological stress and movement dynamics over time and geographic space.

Fecal DNA isolation, extraction, and microsatellite genotyping

Fecal DNA samples were collected simultaneously with each fecal GCM samples to enable tracking of individuals over the course of the study using a genetic ID tag. For DNA analysis fecal pellets were identified using Nichols (2011) protocol and collected using sterilized tweezers (dipped in 70% ethanol between samples). Two to five pellets were transferred to a 2.0 ml vial of 500 µl of ATL tissue lysis buffer (DNeasy extraction kit, QIAGEN, Inc.) in the field. Then, 400 µl of supernatant from each sample were transferred to 50 µl of ice-cold proteinase K for storage until DNA extraction in the laboratory. Total genomic DNA was isolated using DNeasy96 Blood and Tissue Kits (QIAGEN, Inc.) according to the manufacturer's protocol with minor alterations specific to each sample type.

Fourteen polymorphic neutral di, tri, and tetranucleotide repeat microsatellite loci have been developed for the American pika (Peacock et al. 2002; Peacock and Kirchoff 2009; unpublished locus sequences – Genbank accession numbers GQ461714.1-461723.1). After polymerase chain reaction (PCR) optimization trials, samples were amplified with ten polymorphic co-dominant nuclear markers that consistently amplified fecal DNA and one sexing locus to identify males and females (Goode et al. 2014; Table S1). All microsatellite PCR reactions were completed on a MBS Satellite 0.2G thermal cycler (Thermo Electron Corporation) and included a 15 minute hot start at 95°C, followed by 41 cycles of 95°C for 30 seconds, a touch down annealing temperature of 65°C to 55°C for 90 seconds and an elongation step at 72°C for 30 seconds. The touch down annealing temperature protocol is as follows: 7 cycles of 65°C, 7 cycles at 61°C, 7 cycles at 58°C, and 20 cycles at 55°C. The first 21 cycles amplified the locus specific primer and the final 20 cycles amplified the labeled M13 tail and fluorescently labeled the PCR product.

All PCR products were diluted to an appropriate concentration (1:5 ratio) with deionized water when needed and 1 µl of the diluted PCR product was added to 19 µl of HiDye Formamide/LIZ500 size standard. Fragment size analysis was carried out on an Applied Biosystems 3730 DNA Genetic Analyzer (Nevada Genomics Center, http://www.ag.unr.edu/genomics/) and alleles were scored, binned, and genotyped using ABI Prism GeneScan (version 3.5.1) and GeneMapper (version 3.7) software (Applied Biosystems). To avoid genotyping and sample ID error as a result of low-quality fecal samples, individual identity was only assigned for samples that produced a consensus genotype for all 10 non-sexing microsatellite markers. Three independent PCRs were run for each multi-locus panel and a consensus genotype was assigned only if the per-locus genotype was consistent across at least two separate runs (Paetkau 2003; Ebert et al. 2012; Pederson 2012).

We identified sex per individual by amplifying Y chromosome-specific fragments or homologous fragments derived from both sex chromosomes (Fontanesi et al. 2008). In pikas, DNA obtained from hair samples has been successful amplified at the ZFX and ZFY loci for sexing (Henry et al. 2011). For this study, fragments of the ZFX/ZFY genes were amplified by fluorescently labeling the commercially available SRY microsatellite (sex determining region of Y chromosome). The SRY primer was optimized such that it could be run simultaneously with OCP 10 microsatellite locus which serves as a PCR control. Male-specific fragments amplify a homozygous peak at 117 bp, whereas females fail to amplify any fragments. In order to determine a sex for an individual, each sample was run at least twice and had to consistently amplify a homozygous peak at 117 bp during both runs to be considered a male. Individuals were scored as females if samples failed to amplify during both runs, but were consistently genotyped at OCP 10 during the same PCR reaction.

Individual genetic tagging and capture-recapture analysis

We calculated the probability of identity (*PI*) to estimate the average probability that two unrelated individuals will have the same multilocus genotype given a randomly mating population using GenAlEx 6.5 (Park et al. 2011). *PI* estimates the minimum number of loci required for reliable genetic tagging and provides information regarding the statistical power of a set of markers (Peakall and Sydes 1996; Taberlet and Luikart 1999; Park et al. 2011). Based on individual genetic profile, capture histories were constructed by defining a “recapture” as detection of the individual sampled repeatedly across distinct summer seasons. Patch turnover rate (%) was defined as the number of mortality events divided by the total number of individuals sampled within a patch per season. Individuals and the average territory turnover rates were then compared with measures of GCM concentrations at both the individual and habitat patch scales.

Local climate data using weather station records from Bodie, California

Since 1895, a weather station located in the town site of Bodie, California has recorded daily minimum temperature, maximum temperature, precipitation, snowfall, snow depth, and evapotranspiration. These long-term daily and monthly climate records were downloaded from the Utah Climate Center through the Global Historical Climatology Network (GHCN) database (https://climate.usurf.usu.edu/mapGUI/mapGUI.php). While climate records exist starting in 1895, many years contain missing data (represented as an “M” for missing data point or “S” for missing day) for climate variables especially during 1903-1963, as well as during the last five years (2011-2017). As a result, all climate data were manually checked for both: (i) presence of data point and (ii) quality or accuracy of data point using quality flags provided with the data download. Prior to using these raw temperature and precipitation data to calculate climate metrics, a 90% completeness filter was applied such that high-quality climate data was defined as 10% or less of missing data per metric per month which resulted in only a 20-year subset of the available climate data being considered adequate for use in this study. In addition, not all metrics were able to be calculated for every single 24 month period during 1972 to 2014 (Table S1).

Relationship between local climate, fecal GCMs, and pika occupancy

Multiple linear regressions were conducted using R (R core team 2016) to examine the relationship between site-level occupancy patterns, stress hormone concentrations, and local climate metrics. Specifically, we calculated the following seven climate metrics per year only for the years that had high quality climate data and corresponding census data (i.e. 1972, 1977, 1989, 1991-2001, 2003-2006, and 2008-2010): **average annual precipitation** (i.e. average of daily precipitation per year occurring during October to September to represent the water year rather than the calendar year), **average annual snow depth** (i.e. average of the integrated measure of daily snow accumulation per year during October to September), **average annual snow fall** (i.e. average of the daily amount of snow fall per year during October to September), **maximum summer temperature** (i.e. maximum temperature reading recorded for the months of June-August during year that occupancy data were collected), **average summer maximum temperature** (i.e. average maximum temperature recorded for the months of June-August during year that occupancy data were collected), **minimum winter temperature** (i.e. minimum temperature reading recorded for the month of December of previous year through February of the year that occupancy data were collected), **and average winter temperature** (i.e. average minimum temperature recorded for the month of December of previous year through February of the year that occupancy data were collected) (Table S1).

Finally, to determine whether any relationship exists between fecal GCMs, and local climate metrics, we calculated **monthly total precipitation** (i.e. total daily precipitation that occurred each month)**, monthly mean temperature** (i.e. average of the daily mean temperature each month)**, average monthly maximum temperature** (i.e. average of the daily maximum temperature each month), **average monthly minimum temperature** (i.e. average of the daily minimum temperature each month)**, Tmax** (i.e. absolute maximum daily temperature recorded each month)**, and Tmin** (i.e. absolute minimum daily temperature recorded each month) for each month during June-Sept 2013-2015, for which we had overlapping stress hormone data for the same time period. Due to the quantity of missing data (i.e. greater than three days or 10% per month), we had to delete climate data from June and July 2014 from any further analysis (Table S2).

Results

During the summers of 2013-2015, 317 fecal GCM samples were collected from occupied territories on approximately 20 talus patches located in the northern section of the historic mining district at Bodie State Historic Park, Bodie Bluff, and Masonic Mountain to be compared to fecal GCM samples collected from Pipet Tarn and Cabin Slope, sites in the main Sierra Nevada (Klingler et al. 2017 *in prep*).

Long-term monitoring of patch occupancy

Census surveys indicate that an overall decline in site-level occupancy has occurred steadily since the late 1940s (Severeid 1955). Beginning with an initial crash in 1989, occupancy in the southern patch network fluctuated with an increase during 2003 (16%), and 2004 (14.8%) before declining to 3.7% occupancy in 2005 and 2006 with complete extirpation occurring by 2008 (Klingler et al. 2017 *in review*). In 2016, there was limited re-colonization of two patches in the southern patch network, one animal on the Booker Consolidated shaft and approximately five animals on the Red Cloud patch (Fig. 1). While the northern patch network has maintained consistently high patch occupancy, the middle patch network has also experienced significant extirpations among patches since the early 2000s. On a site level scale, the ratio between the number of extinctions and re-colonizations across all patches has fluctuated somewhat cyclically with the highest colonization to extinction ratios (i.e. smaller differences between numbers of colonizations and extinctions) having occurred during the 1990s (1994 to 1996).

Characterization of habitat quality within and among populations using fecal GCMs

Physiological stress in BSHP pikas varied across years ($F_{2, 314}$=10.48, P =0.000) with 2015 being significantly less stressful compared to the two previous years (2013, P =0.000; 2014, P <0.002). Within years, physiological stress tended to be significantly less during the month of June ($F_{3, 306}$=7.77, P =0.000) compared to August (P=0.000), and July (P<0.001), but not September (P=0.109). There were no significant differences between males and females in average physiological stress despite some evidence of sex differences at other sites (Wilkening et al. 2013; Klingler et al. 2017 *in prep*).

Statistically significant differences in physiological stress occurred among sites ($F_{4, 510}$=52.76, P=0.000). BSHP was not significantly more stressful than the Masonic Mountain site, but both of these anthropogenic habitats experienced higher fecal GCM concentrations compared to individuals on the natural talus at Bodie Bluff (P<0.001) (Fig. 2). BSHP was significantly more stressful than the higher elevation sites in the main Sierra Nevada, Cabin Slope (P<0.001) and Pipet Tarn (P=0.000) (Klingler et al. 2017 *in prep*.) It should be noted that we had a relatively small sample size for the natural talus patches near Masonic Mountain (n=9) due to low levels of occupancy and limited sampling time.

Talus perimeter and the number of available territories per patch were highly correlated with the extinction-re-colonization ratio such that larger patches with a greater number of territories have been more consistently occupied over the last 40 years (R^2=0.64702, P=0.000, number of territories; R^2=0.39055, P<0.002, talus perimeter). Lastly, there was no evidence to suggest that patches farther from the High Peak mainland were on average more highly stressed (R^2=-0.055, P<0.74574).

On average, fecal GCM concentrations differed significantly among patches ($F_{21, 258}$= 3.488, P =0.000) within BSHP (Fig. 3). Notably, the Midnight/Bod65 patch (part of the High Peak complex) appears to experience some of the highest fecal GCM concentrations observed in BSHP followed closely by Bodie Old Shaft (Bod 64), and Tioga Shaft (Bod 73) in contrast to our hypothesis that smaller patches would have higher average physiological stress levels due to less available habitat and fewer available territories (Table 2). Our results suggest a statistically significant and positive relationship between average physiological stress and talus perimeter (R^2=0.325, P<0.005) such that individuals on larger patches tend to have significantly higher physiological stress levels compared to individuals on smaller-sized patches. Similarly, the extinction-re-colonization ratio is significantly correlated with fecal GCMs (R^2=0.246, P=0.01). We had predicted that less consistently occupied patches over the last 40 years would be of poorer quality and therefore individuals would be more stressed physiologically. Instead, it appears that patches that are more consistently occupied tend to be patches with greater average physiological stress. However, both talus perimeter and the extinction-re-colonization ratio explain only one-third of the total variance in physiological stress observed within this study site across all the years of sampling.

Genetic ID tagging of individuals across habitat patches

A total of 72 individuals were identified from 13 habitat patches during 2013 (n=46), 2014 (n=13), and 2015 (n=11), respectively. Only 11 individuals were recaptured across years with almost all recaptures occurring on smaller sized patches. Of the total number of individuals identified only 44 genetic samples could be reliably associated with fecal GCM samples due to fecal DNA samples that did not amplify across all loci and/or fecal GCM samples that did not match to the standard curve during duplicate runs. Individuals were identified across multiple years if samples shared the same genotype at more than nine loci since the likelihood of sharing the same genotype was very low (*PI* <0.003*).* Within seasons, individuals differed significantly within and among habitat patches ($F_{1, 31}$=8.05, *P* <0.007). However, contrary to our expectation that less stressed individuals would survive through a greater number of seasons, recaptured individuals did not consistently have lower fecal GCM concentrations on the Bod70 patch ($F_{1, 2}$=2.35, *P*=0.26), BEC patch ($F_{1, 2}$=0.42, *P*=0.58) or the TGA patch ($F_{1, 1}$=0.23, *P*=0.71) compared to individuals sampled only once (either due to mortality or failure to recapture) (Fig. 4).

Habitat patches did not differ significantly in terms of turnover rate ($F_{1,10}$=1.38, *P*=0.26), however there was a trend of increasing turnover rate and significantly higher average fecal GCM concentrations as habitat patch size increases (Fig. 3). Notably, both the HOB and HPK patches appear to be outliers in terms of turnover rate, perhaps due to high relative isolation and large size. The LUC habitat patch, while retaining a fairly large total perimeter size in absolute terms, had significant amounts of rock removed for road construction, therefore this patch and its associated lower turnover rate appears to align more closely with smaller patches as a result.

Relationship between fecal GCMs, occupancy patterns, and local climate metrics

In order to determine whether long-term occupancy patterns were associated with local climate variation, multiple linear regressions were conducted using all seven climate metrics, and the number of patches surveyed/number of patches occupied during 1972-2010 for which we had highly quality climate data. In total, 14 metrics were deleted from further analysis due to greater than 10% of the data missing during relevant months for specific years mostly for average annual snow fall and average annual precipitation (Table S1). The most highly supported model explaining the variance in long-term occupancy included minimum winter temperature (β=-0.05, P=0.01), average maximum summer temperature (β =-0.24, P=0.02), and average annual snow fall (β =-0.04, P=0.08) (R^2=0.52, P=0.04) (Fig. 5). As expected, average summer maximum temperature was a statistically significant predictor variable of pika occupancy, which declined with increasing summer temperatures. In contrast, pika occupancy increased with increasingly cold minimum winter temperatures and decreasing average annual snowfall. No other precipitation-related metrics were statistically significant.

Prior to testing for an association between monthly climate metrics and average monthly fecal GCM patterns, June 2014 and July 2014 data were removed due to the quantity of missing climatic data (>10%) (Table S2). Average fecal GCM patterns were strongly related to both average monthly maximum temperature (β =0.005, P=0.003), and minimum monthly temperatures (β =-0.03, P=0.002 (R^2=0.90, P=0.004). Average fecal GCM concentrations increased in response to rising average monthly maximum temperatures as well as colder minimum monthly temperatures during the summer months (Fig. 6).

Discussion

Long-term ecological research and monitoring projects that incorporate physiological metrics can provide important baseline data and historical reference points for evaluating changes in habitat quality, especially for populations at risk of extirpation from anthropogenic mediated disturbance (Nichols and Williams 2006; Lindenmayer and Likens 2009; Lindenmayer and Likens 2010). Pikas inhabit a diversity of landscape types, which means that the ability to predict the range-wide response to environmental change for populations that differ significantly in fine-scale abiotic and biotic factors may be limited (Jeffress et al. 2013; Moyer-Horner et al. 2015; Schwalm et al. 2016; Castillo et al. 2016). The lack of human mediated physical disturbance to pika habitat does not prevent its functional degradation by climate change and therefore studies investigating patterns of habitat quality within and among populations will be useful to refine future predictions of extirpation risk.

This study benefitted from 40 years of non-invasive census surveys, local climate records, and recent advances in endocrine sampling to provide a more nuanced understanding of changing site level habitat quality. The highly fragmented, anthropogenic talus ore dump habitat within BSHP contrasts significantly with continuous or semi-continuous talus habitat found in larger mountain ranges where the majority of pika populations are located and as such presents greater environmental challenges for pikas. The long term occupancy of the BSHP site prior to the late 20th century suggests that this habitat even though fragmented, at low elevation, and in sagebrush steppe vegetation was stable. The ongoing population decline (61% of all patches surveyed were occupied in 1972, 58% in 1977, and only 32% were occupied in 2014) now suggests that the habitat has become unstable and unpredictable. Comparisons of BSHP with two other sampling sites in the Bodie Hills eco-region (Masonic Mountain, ~2700 m, and Bodie Bluff, ~2800 m; Fig. 2) indicates that ore dump habitat is not inherently low quality habitat as is often assumed due to its anthropogenic origin. Instead, it appears that fragmented ore dump patches in BSHP provide physiologically similar habitat quality compared to the natural talus outcrops at Masonic Mountain, where average fecal GCM concentrations for pikas were not significantly different from BSHP. However, recent extirpations across all other natural talus within this region suggest that other habitat features at the Masonic Mountain site are likely contributing to higher overall habitat quality since it continues to support a pika population at present. This may also be true for Bodie Bluff, a mix of natural talus and human-created ore dump habitat, which sits only 60 m above the northern mining district in BSHP well within dispersal distance of an individual pika from BSHP and whose pika occupants maintained significantly lower average fecal GCM concentrations than BSHP.

Surprisingly, compared with samples from higher quality habitat at higher elevations (~27 km distance) collected from the same sampling period in the Sierra Nevada, BSHP was only significantly more stressful than the Pipet Tarn site (~3170 m) and not Cabin Slope (~3130 m) (Klingler et al. 2017 *in prep*). Although this study did not attempt to directly investigate the effect of post-depositional environment on fecal GCM concentrations, which remain a major concern for interpretation, these results suggest that despite being located at a lower elevation, fecal GCM samples collected in BSHP were not overly biased to higher fecal GCM concentrations by warmer temperatures and increased microbial decomposition (Wilkening et al. 2016).

This study revealed that both annual and within-season environmental variation as well as the spatial configuration and size of patch habitat are important determiners of physiological stress for BSHP pikas. Larger-sized patches with a greater number of territories have been more consistently occupied over the long-term compared to smaller patches. An alternative approach for measuring occupancy patterns compares the number of colonizations with the number of extinctions per year across all patches. This metric reveals that as patches increase in size and support a greater number of territories, the annual differences between colonizations and extinctions narrow. In contrast, smaller patches or patches with fewer territories experience greater inter-annual fluctuations between the total numbers of colonizations versus extinctions.The selection of a habitat patch to disperse to appears to be extremely important considering the significant relationships between talus perimeter and the number of territories with average physiological stress and the extinction-re-colonization dynamic, respectively. Based on previous research, we hypothesized that smaller patch sizes with fewer total territories would represent lower quality habitat and therefore be less consistently occupied (Stewart et al. 2015; Schwalm et al. 2016). As hypothesized, it appears that habitat patches in BSHP differ significantly in terms of average fecal GCM concentrations however, the smaller and more isolated patches did not have the highest stress levels as predicted. Instead, the relatively large patches that are more consistently occupied over time with a greater number of territories, such as Midnight Shaft/High Peak and Tioga Shaft, had greater fecal GCM concentrations (Fig. 1). In fact, these results suggest that absolute perimeter size is not likely the best predictor of patch quality as this metric does not reflect the true quantity of suitable pika habitat within the patch (e.g. LUC). Furthermore, patch quality may be determined by multiple, interacting factors including interstitial microclimate, the availability and diversity of vegetation, and predator pressure. Indeed, large proportions of patch quality may be determined during non-summer seasons when physiological monitoring did not occur.

The differences in fecal GCM concentrations related to patch size may be a result of intraspecies competition among pikas on larger patches due to increased frequency of interactions among individuals compared to smaller patches that usually support between 1 to 2 individuals at most. In addition, larger patches, by their very nature, provide a greater amount of total habitat and likely provide suitable conditions for other species such that co-occur in close proximity with pikas (i.e. woodrats, ground squirrels) thus adding potential competitors for space and resources. Installation of camera traps at occupied territories within the study site has revealed a diversity of both predators and competitors using the ore dump habitats including mountain cottontail (*Sylvilagus nuttallii*), black-tailed jack rabbit (*Lepus californicus*), short-tailed weasels (*Mustela erminea*), long-tailed weasels (*Mustela frenata*) mule deer (*Odocoileus *hemionus*), American badger (*Taxidea taxus*), Belding's ground squirrel (*Spermophilus beldingi*) woodrat (*Neotoma cinerea*), Least chipmunk (*Tamius minimus*), and most recently, yellow-bellied marmot (*Marmota flaviventris*). As a result of use by multiple taxa, greater predation pressure may also be a factor on these patches that support a higher density of pikas (as measured by number of territories). There may be a trade-off between habitat quality and territory availability such that juvenile pikas forced to disperse from small patches with no available territories may face increased competition and greater within patch predation risk associated with larger patches despite the benefit of potentially unclaimed territories.

Using a subset of habitat patches, we then explored whether individual variation in the stress response might be responsible for the patterns in habitat quality we observed. In addition to significantly higher fecal GCM concentrations, larger patches also appear to experience relatively higher average turnover rates compared to smaller patches (Fig. 3). Individuals occupying the same patch within the same season differed significantly in average fecal GCM concentrations, which suggests that other factors such as life history stage and genetic heritability may influence the individual stress response in pikas and result in varying baseline GCM levels among individuals sharing similar habitat quality and diet type (Fig. 4; Evans et al. 2006; Williams et al. 2008; Jenkins et al. 2014; Love et al. 2014). However, recaptured individuals did not have significantly lower average fecal GCM concentrations compared to individuals that were only observed once. The lack of any substantial difference in fecal GCMs among individuals that experienced varying fates (survived, died, or failed to recapture) suggests that physiological stress itself was not a major, contributing factor in mortality events although our limited sample size of recaptures does not give us the power to directly test this. In fact, our limited number of recaptures are in accordance with results from population genetic data that suggest relatively high overwinter mortality rates occur in BSHP and create a fairly fluid movement dynamic among habitat patches from year to year (Peacock and Smith 1997; Klingler et al. 2017 *in review*).

Relationship between local weather metrics, patch occupancy, and fecal GCMs

Using the available Bodie weather station data, we also examined the relationship between local climate metrics and patterns of both fecal GCMs and long-term pika occupancy. As hypothesized, patch-level occupancy appears to be affected by changes in local climate with a statistically significant association detected between long-term pika occupancy and average maximum summer temperature, minimum winter temperature, and average annual snow fall. Perhaps not surprisingly, pika occupancy declines as average summer maximum temperatures increase and minimum winter temperatures increase (Fig. 5). These results suggest that warming temperatures during both summer and winter seasons negatively affected the number of patches occupied during each summer season with average annual snow fall playing a smaller role.

As endotherms, pikas respond to heat or cold stress by retreating to thermal refugia within favorable microclimates, hence, the importance of territory ownership. Our results indicate that increased fecal GCMs are significantly related to increasing average monthly maximum temperatures as well as minimum monthly temperature during summer months (Fig. 6). This result suggests that both warmer conditions as well as cold summer lows are physiologically stressful for pikas, a likely outcome of their vulnerability to extreme heat and cold and the critical thermoregulatory functionality that habitat heterogeneity and microclimate variability provide for this species. This significant finding supports previous pika fecal GCM research and again suggests that the physiology of these lagomorphs is indeed linked to changes in temperature (Wilkening et al. 2015; Waterhouse et al. 2017). Together, these results suggest that pikas experience individual physiologic responses to changes in temperature that eventually translate to demographic consequences at the population level in terms of patch occupancy.

Conclusions

The complexity regarding how to measure habitat quality and the resulting impacts to individual fitness across environmental gradients make it difficult to tease apart the effects of individual quality from habitat quality (Sergio et al. 2009; Germain and Arcese 2014). Although we were not able to account specifically for the intrinsic quality of individuals (e.g. differences in adaptive traits) within our study design, significant differences in fecal GCM concentrations among individuals occupying the same habitat patch within the same season indicates that the individual stress response likely plays a large role in individual fitness differences at this site. Indeed, the significant and positive association between colonization-extinction ratios among habitat patches with fecal GCMs represents the first demographic link with physiological stress in pikas. These results suggest that habitat quality can vary substantially among patches even within sites and present differing physiological challenges for the pika stress response. In fact, the significant relationship between one component of habitat quality, local climate variability, with both patch occupancy and fecal GCMs is an important finding because it represents a contributing environmental factor connecting individual physiologic response in pikas with subsequent population level demographic consequences.

Three years of stress data with over 30 years of census data may not capture the complexities and long-term patterns of demographic flux associated with changes in climate, but this study does show a relationship between climate metrics and increased stress in pikas. Both metrics of heat and cold were independently shown to be influential predictors of patch occupancy as well as fecal GCM patterns therefore highlighting pika vulnerability to extreme temperatures during both summer and winter seasons. This study also occurred during a record drought in the state of California (2013-2015) which undoubtedly impacted the average levels of physiological stress we detected. The high temperatures and lack of precipitation during recent years have combined to create the most severe drought conditions observed in the last 1200 years and are considered extraordinary relative to millennium-length paleoclimate records (Griffin et al. 2014; Belmecheri et al. 2016). It is doubtful that these extreme conditions failed to influence pika physiology and therefore our estimates of average fecal glucocorticoid concentrations may be biased high with respect to historical averages. Unfortunately, anthropogenic warming is expected to increase the future likelihood of warm-dry conditions in California such that it may become very difficult to conduct physiological monitoring during years that are not considered exceptional relative to historical conditions (Diffenbaugh et al. 2015).

The characterization of large-scale patterns of physiological stress in pikas and generalization of the relationship between population-level endocrine condition and habitat features within regions will require repeated and long-term fecal GCM collection within and among sites (Wilkening et al. 2016). Citizen science provides a research technique that is well-matched for this type of sampling especially for projects focused on characterizing regional or continental trends (Bhattacharjee 2005; Bonney et al. 2009). During the last few decades, the popularity and integration of citizen science in ecological research has grown tremendously and contributed to a number of published manuscripts (Wells et al. 1998; Hochachka et al. 1999; Cooper et al. 2007; Bonter and Harvey 2008; Bonter et al. 2009) with citizen scientists involved in data visualization and analysis steps (Bonney et al. 2009). By establishing physiological monitoring programs in pika populations that vary in specific habitat and climatic features, comparisons of physiological stress within and across seasons can be conducted within eco-regions. Not only will these monitoring data provide an early detection system for identifying vulnerable populations but if paired with non-invasive fecal DNA and microclimate temperature collection, it may be possible to start understanding the effects of habitat and climate variation on population health in pikas at a much broader scale.

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References

Albano, N. (2012). Conservation physiology tools: their role in assessing habitat quality in birds. *Ardeola*, 59(2). doi: 10.13157/arla.59.2.2012.197

Albano, N. (2015). Immunoreactive cortisone in droppings reflect stress levels, diet and growth rate of gull-billed tern chicks. *General and Comparative Endocrinology*, 213, 74–80. doi: 10.1016/J.YGCEN.2015.02.019

Baker, M. R., Gobush, K. S., & Vynne, C. H. (2013). Review of factors influencing stress hormones in fish and wildlife. *Journal for Nature Conservation, 21*(5), 309–318. doi: 10.1016/j.jnc.2013.03.003

Barlow, J., Lennox, G. D., Ferreira, J., Berenguer, E., Lees, A. C., Nally, R. Mac, ...Gardner, T. A. (2016). Anthropogenic disturbance in tropical forests can double biodiversity loss from deforestation. *Nature*, 535(7610), 144–147. doi: 10.1038/nature18326

Barr, K. R., Kus, B. E., Preston, K. L., Howell, S., Perkins, E., & Vandergast, A. G. (2015). Habitat fragmentation in coastal southern California disrupts genetic connectivity in the cactus wren (*Campylorhynchus brunneicapillus*). *Molecular Ecology*, 24(10), 2349–2363. doi: 10.1111/mec.13176

Belmecheri, S., Babst, F., Wahl, E. R., Stahle, D. W., & Trouet, V. (2016). Multi-century evaluation of Sierra Nevada snowpack. *Nature Climate Change*, *6*(1), 2-3. doi:10.1038/nclimate2809

Beever, E. A., Brussard, P. F., & Berger, J. (2003). Patterns of apparent extirpation among isolated populations of the pikas (*Ochotona princeps*) in the Great Basin. *Journal of Mammalogy*, *84*(1), 37–54. doi:10.1644/1545-1542(2003)084<0037:POAEAI>2.0.CO;2

Beever, E. A., Dobrowski, S. Z., Long, J., Mynsberge, A. R., & Piekielek, N. B. (2013). Understanding relationships among abundance, extirpation, and climate at ecoregional scales. *Ecology*, *94*(7), 1563–1571. doi: 10.1890/12-2174.1

Bhattacharjee, Y. (2005). Citizen scientists supplement work of Cornell researchers: a half-century of interaction with bird watchers has evolved into a robust and growing collaboration between volunteers and a leading ornithology lab. *Science*, *308*(5727), 1402-1404.

Bhattacharjee, S., Kumar, V., Chandrasekhar, M., Malviya, M., Ganswindt, A., Ramesh, K., & Umapathy, G. (2015). Glucocorticoid stress responses of reintroduced tigers in relation to anthropogenic disturbance in Sariska Tiger Reserve in India. *PLOS ONE,* 10(6), e0127626. doi: 10.1371/journal.pone.0127626

Blickley, J. L., Blackwood, D., & Patricelli, G. L. (2012). Experimental evidence for the effects of chronic anthropogenic noise on abundance of Greater Sage-Grouse at leks. *Conservation Biology*, 26(3), 461–471. doi:10.1111/j.1523-1739.2012.01840.x

Blitzer, E.J., Dormann, C.F., Holzschuh, A., Klein, A.M., Rand, T.A., & Tscharntke, T. Spillover of functionally important organisms between managed and natural habitats. (2012). *Agriculture, Ecosystems & Environment*, *146*(1), 34–43. doi:10.1016/J.AGEE.2011.09.005

Bonney, R., Cooper, C. B., Dickinson, J., Kelling, S., Phillips, T., Rosenberg, K. V., & Shirk, J. (2009). Citizen science: a developing tool for expanding science knowledge and scientific literacy. *BioScience*, *59*(11), 977–984. doi: 10.1525/bio.2009.59.11.9

Bonter, D., and Harvey, M. (2008). Winter survey data reveal rangewide decline in Evening Grosbeak populations. *The Condor*. doi: 10.1525/cond.2008.8463

Bonter, D., and Hochachka, W. (2009). A citizen science approach to ornithological research: twenty years of watching backyard birds. *Proceedings of the Fourth International Partners in Flight Conference*, 453-458

Boonstra, R. (2005). Equipped for life: the adaptive role of the stress axis in male mammals. *Journal of Mammalogy*, 86(2), 236–247. doi: 10.1644/BHE-001

Busch, D. S., and Hayward, L. S. (2009). Stress in a conservation context: a discussion of glucocorticoid actions and how levels change with conservation-relevant variables. *Biological Conservation*, *142*(12), 2844–2853. doi: 10.1016/j.biocon.2009.08.013

Carey, C. (2005). How physiological methods and concepts can be useful in conservation Biology. *Integrative and Comparative Biology*, *45*(1), 4–11. doi: 10.1093/icb/45.1.4

Castillo, J. A., Epps, C. W., Davis, A. R., & Cushman, S. A. (2014). Landscape effects on gene flow for a climate-sensitive montane species, the American pika. *Molecular Ecology*, *23*(4), 843–856. doi: 10.1111/mec.12650

Castillo, J. A., Epps, C. W., Jeffress, M. R., Ray, C., Rodhouse, T. J., & Schwalm, D. (2016). Replicated landscape genetic and network analyses reveal wide variation in functional connectivity for American pikas. *Ecological Applications*, *26*(6), 1660-1676. doi:10.1890/15-1452.1

Chown, S. L., and Gaston, K. J. (2008). Macrophysiology for a changing world. *Proceedings of the Royal Society of London B: Biological Sciences*, *275*(1642), 1469-1478. doi: 10.1098/rspb.2008.0137

Cockrem, J. F. (2005). Conservation and behavioral neuroendocrinology. *Hormones and Behavior*, *48*(4), 492–501. doi: 10.1016/j.yhbeh.2005.03.008

Cook, C. J., Mellor, D. J., Harris, P. J., Ingram, J. R., and Matthews, L. R. (2000). Hands-on and hands-off measurement of stress. *The biology of animal stress. CABI Publishing*, 123-46.

Cooke, S. J., and O'Connor, C. M. (2010). Making conservation physiology relevant to policy makers and conservation practitioners. *Conservation Letters*, *3*(3), 159–166. doi: 10.1111/j.1755-263X.2010.00109.x

Cooper, C. B., Dickinson, J., Phillips, T., and Bonney, R. (2007). Citizen science as a tool for conservation in residential ecosystems. *Ecology and Society*, *12*(2), art11. doi: 10.5751/ES-02197-120211

Creel, S., Spong, G., Sands, J. L., Rotella, J., Zeigle, J., Joe, L., Smith, D. (2003). Population size estimation in Yellowstone wolves with error-prone noninvasive microsatellite genotypes. *Molecular Ecology*, *12*(7), 2003–2009. doi: 10.1046/j.1365-294X.2003.01868.x

Dearing, M. D. (1997). The function of haypiles of pikas (*Ochotona princeps*). *Journal of Mammalogy*, *78*(4), 1156-1163. doi: 10.2307/1383058

Dearing, M. D. (1997). Effects of *Acomastylis rossii* tannins on a mammalian herbivore, the North American pika, *Ochotona princeps*. *Oecologia*, *109*(1), 122–131. doi: 0.1007/s004420050066

Delibes, M., Gaona, P., & Ferreras, P. (2001). Effects of an attractive sink leading into maladaptive habitat selection. *The American Naturalist*, 158(3), 277–85. doi: 10.1086/321319

Denny, M., & Helmuth, B. (2009). Confronting the physiological bottleneck: a challenge from ecomechanics. *Integrative and Comparative Biology*, 49(3), 197–201 doi:10.1093/icb/icp070

Dhondt, A.A., Kempenaers, B. and Adriaensen, F. (1992). Density-dependent clutch size caused by habitat heterogeneity. *Journal of Animal Ecology*, 61(3), 643-648. doi: 10.2307/5619

Diffenbaugh, N. S., Swain, D. L., Touma, D. (2015). Anthropogenic warming has increased drought risk in California. *Proceedings of the National Academy of Sciences*, *112*(13), 3931-3936. doi: 10.1073/pnas.1422385112

Dixon, J. 1919. Notes on the natural history of the bush-tailed wood rats of California. University of California Publications in Zoology 21:49-74.

Doddington, B. J., Bosch, J., Oliver, J. A., Grassly, N. C., Garcia, G., Schmidt, B. R., Fisher, M. C. (2013). Context-dependent amphibian host population response to an invading pathogen. *Ecology*, 94(8), 1795–1804. doi: 10.1890/12-1270.1

Dunning, J. B., Danielson, B. J., & Pulliam, H. R. (1992). Ecological processes that affect populations in complex landscapes. *Oikos*, 65(1), 169. doi: 10.2307/3544901

Ebert, C., Knauer, F., Spielberger, B., Thiele, B., & Hohmann, U. (2012). Estimating wild boar *Sus scrofa* population size using faecal DNA and capture-recapture modelling. Wildlife Biology, 18 (2), 142-152. doi: 10.2981/11-002

Engelmann, M, Landgraf, R. & Wotjak, C.T. The hypothalamic–neurohypophysial system regulates the hypothalamic–pituitary–adrenal axis under stress: An old concept revisited. *Frontiers in Neuroendocrinology*, 25 (3-4, 132-149.

Erb, L. P., Ray, C., & Guralnick, R. (2011). On the generality of a climate-mediated shift in the distribution of the American pika (*Ochotona princeps*). *Ecology*, *92*(9), 1730–1735. doi: 10.1890/11-0175.1

Evanno, G., Regnaut, S., & Goudet, J. (2005). Detecting the number of clusters of individuals using the software STRUCTURE: A simulation study. *Molecular Ecology*, *14*(8), 2611–2620. doi:10.1111/j.1365-294X.2005.02553.x

Evans, M. R., Roberts, M. L., Buchanan, K. L., & Goldsmith, A. R. (2006). Heritability of corticosterone response and changes in life history traits during selection in the zebra finch. *Journal of Evolutionary Biology*, *19*(2), 343–352. doi:10.1111/j.1420-9101.2005.01034.x

Finley Jr., R.B. 1958. The wood rats of Colorado: distribution and ecology. University of Kansas Publications Museum of Natural History 10:213-552.

Fontanesi, L., Tazzoli, M., Peccioli, E., Hauffe, H. C., Robinson, T. J., & Russo, V. (2008). Sexing European rabbits (*Oryctolagus cuniculus*), European brown hares (*Lepus europaeus*) and mountain hares (*Lepus timidus*) with *ZFX* and *ZFY* loci. *Molecular Ecology Resources*, *8*(6), 1294–1296. doi:10.1111/j.1755-0998.2008.02167.x

Fretwell, S. D., & Calver, J. S. (1969). On territorial behavior and other factors influencing habitat distribution in birds. *Acta biotheoretica*, *19*(1), 37-44.

Galbreath, K. E., Hafner, D. J., & Zamudio, K. R. (2009). When cold is better: climate-driven elevation shifts yield complex patterns of diversification and demography in an alpine specialist (American pika, *Ochotona princeps*). *Evolution*, *63*(11), 2848–2863. doi: 10.1111/j.1558-5646.2009.00803.x

Germain, R. R., & Arcese, P. (2014). Distinguishing individual quality from habitat preference and quality in a territorial passerine. *Ecology*, 95(2), 436–445. doi: 10.1890/13-0467.1

Gilbert, K., Andrew, R., Bock, D., Franklin, M., Kane, N., Moore, J., &Vin es, T. (2012). Recommendations for utilizing and reporting population genetic anlyses: the reproducibility of genetic clustering using the program STRUCTURE. *Molecular Ecology*, *21*, 4925–4930.

Goode, M. J., Beaver, J. T., Muller, L. I., Clark, J. D., Frank T. van Manen, Harper, C. A., &

Basinger, P. S. (2014). Capture—recapture of white-tailed deer using DNA from fecal pellet groups. *Wildlife Biology, 20*(5), 270-278. doi:10.2981/wlb.00050

Goudet, J. (2001). FSTAT; a program to estimate and test gene diversities and fixation indices version 2.9. 3. *Http://www. Unil. Ch/izea/softwares/fstat. Html*.

Grayson, D. K. (2005). A brief history of Great Basin pikas. *Journal of Biogeography*, *32* (12), 2103–2111. doi: 10.1111/j.1365-2699.2005.01341.x

Griffin, D., & Anchukaitis, K. J. (2014). How unusual is the 2012–2014 California drought?. *Geophysical Research Letters*, *41*(24), 9017-9023. doi:10.1002/2014GL062433.

Gunnarsson, T.G., Gill, J.A., Newton, J., Potts, P.M. & Sutherland, W.J. (2005). Seasonal matching of the habitat quality and fitness in a migratory bird. *Proceedings of the Royal Society B: Biological Sciences*, *272* (1578), 2319-2323. doi: 10.1098/rspb.2005.3214

Haddad, N. M., Brudvig, L. A., Clobert, J., Davies, K. F., Gonzalez, A., Holt, R. D., & Townshend, J. R. (2015). Habitat fragmentation and its lasting impact on Earth's ecosystems. *Science Advances*, 1(2), e1500052–e1500052. doi:10.1126/sciadv.1500052

Hadley, O. L., Corrigan, C. E., Kirchstetter, T. W., Cliff, S. S., & Ramanathan, V. (2010). Measured black carbon deposition on the Sierra Nevada snow pack and implication for snow pack retreat. *Atmospheric Chemistry and Physics*, *10*(15), 7505-7513. doi: 10.5194/acp-10-7505-2010

Hafner, D. J. (1993). North American pika (*Ochotona princeps*) as a late Quaternary biogeographic indicator species. *Quaternary Research*, *39*(3), 373-380. doi: 10.1006/qres.1993.1044

Hafner, D. J. (1994). Pikas and Permafrost: Post-Wisconsin historical zoogeography of *Ochotona* in the Southern Rocky Mountains, U.S.A. *Arctic and Alpine Research*, *26*(4), 375. doi: 10.2307/1551799

Hall, L. S., Krausman, P. R., & Morrison, M. L. (1997). The habitat concept and a plea for standard terminology. *Wildlife Society Bulletin (1973-2006), 25*, 173–182. doi: 10.2307/3783301

Harper, J. M., & Austad, S. N. (2004). Fecal corticosteroid levels in free-living populations of Deer Mice (*Peromyscus maniculatus*) and Southern Red-backed Voles (*Clethrionomys gapperi*). *The American Midland Naturalist*, 152(2), 400–409. doi: 10.1674/0003 0031(2004)152[0400:FCLIFP]2.0.CO;2

Henry, P., Sim, Z., & Russello, M. A. (2012). Genetic evidence for restricted dispersal along continuous altitudinal gradients in a climate change-sensitive mammal: the American pika. *PLoS ONE*, *7*(6), 1–10. doi: 10.1371/journal.pone.0039077

Hochachka, W. M., Wells, J. V., Rosenberg, K. V., Tessaglia-Hymes, D. L., & Dhondt, A. A. (1999). Irruptive migration of common redpolls. *Condor*, 195-204. doi: 10.2307/1369983

Holt, R. D., & Barfield, M. (2015). The influence of imperfect matching habitat choice on evolution in source–sink environments. *Evolutionary Ecology*, 29(6), 887–904. doi: 10.1007/s10682-015-9789-0

Homyack, J. A. (2010). Evaluating habitat quality of vertebrates using conservation physiology tools. *Wildlife Research*, *37*(4), 332–342. doi: 10.1071/WR08093

Huey, R. B. (1991). Physiological consequences of habitat selection. *The American Naturalist, 137*, S91–S115. doi: 10.1086/285141

Ising, M.,& Holsboer, F. (2006). Genetics of stress response and stress-related disorders. *Dialogues in Clinical Neuroscience*, *8*(4), 433–444. doi: 10.1016/j.jcin.2015.10.034

Jeffress, M. R., Rodhouse, T. J., Ray, C., Wolff, S.,& Epps, C. W. (2013). The idiosyncrasies of place: geographic variation in the climate-distribution relationships of the American pika. *Ecological Applications*, *23*(4), 864–878. doi: 10.1890/12-0979.1

Jenkins, B. R., Vitousek, M. N., Hubbard, J. K., & Safran, R. J. (2014). An experimental analysis of the heritability of variation in glucocorticoid concentrations in a wild avian population. *Proceedings. Biological Sciences*, *281*(1790), 20141302. doi: 10.1098/rspb.2014.1302

Klingler, K.B., Downs, C.J. & Peacock, M.M. (2017). The effects of site, season, and territory variability physiological stress for the American pika (*Ochotona princeps*) within high-elevation talus habitat in Mono County, California, USA. Manuscript in preparation.

Klingler, K.B., Nichols, L.B., & Peacock, M.M. (2017). Life on the edge-the American pika in Bodie, California: a 65 year case study of genetic erosin in a species of special concern. Manuscript submitted for publication.

Knutson, M. G., Hines, R. K., Powell, L. A., Friberg, M. A., & Niemi, G. J. (2009). An assessment of bird habitat quality using population growth rates. doi: 10.1650/0010-5422(2006)108[301:AAOBHQ]2.0.CO;2.

Korte, S.M, Koolhaas, J.M., Wingfield, J.C., & McEwen, B.S.The Darwinian concept of stress: benefits of allostasis and costs of allostatic load and the trade-offs in health and disease. (2005). *Neuroscience & Biobehavioral Reviews*, *29*(1), 3–38. doi: 10.1016/J.NEUBIOREV.2004.08.009

Kreuzer, M. P., & Huntly, N. J. (2003). Habitat-specific demography: evidence for source-sink population structure in a mammal, the pika. *Oecologia*, 134(3), 343–349. doi: 10.1007/s00442-002-1145-8

Landys, M. M., Ramenofsky, M., & Wingfield, J. C. (2006). Actions of glucocorticoids at a seasonal baseline as compared to stress-related levels in the regulation of periodic life processes. *General and Comparative Endocrinology*, *148*(2), 132–149. doi:10.1016/j.ygcen.2006.02.013

Lindenmayer, D. B., & Likens, G. E. (2009). Adaptive monitoring: a new paradigm for long-term research and monitoring. *Trends in Ecology and Evolution*, *24*(9), 482–486. doi: 10.1016/j.tree.2009.03.005

Love, O. P., Madliger, C. L., Bourgeon, S., Semeniuk, C. A. D., & Williams, T. D. (2014). Evidence for baseline glucocorticoids as mediators of reproductive investment in a wild bird. *General and Comparative Endocrinology*, *199*, 65–69. doi: 10.1016/J.YGCEN.2014.01.001

Lucas, J. R., Freeberg, T. M., Egbert, J., & Schwabl, H. (2006). Fecal corticosterone, body mass, and caching rates of Carolina chickadees (*Poecile carolinensis*) from disturbed and undisturbed sites. *Hormones and Behavior*, *49*(5), 634–643. doi:10.1016/j.yhbeh.2005.12.012

Madliger, C. L., & Love, O. P. (2014). The need for a predictive, context-dependent approach to the application of stress hormones in conservation. *Conservation Biology*, *28*(1), 283–287. doi: 10.1111/cobi.12185

Martin, A. E., & Fahrig, L. (2016). Reconciling contradictory relationships between mobility and extinction risk in human-altered landscapes. *Functional Ecology*, 30(9), 1558–1567. doi.org/10.1111/1365-2435.12632

Mathewson, P. D., Moyer-Horner, L., Beever, E. A., Briscoe, N. J., Kearney, M., Yahn, J. M., & Porter, W. P. (2017). Mechanistic variables can enhance predictive models of endotherm distributions: the American pika under current, past, and future climates. *Global Change Biology*, *23*(3), 1048–1064. doi: 10.1111/gcb.13454

McDonald, K. A., & Brown, J. H. (1992). Using montane mammals to model extinctions due to global change. *Conservation Biology*, *6*(3), 409–415. doi: 10.1046/j.1523-1739.1992.06030409.x

McEwen, B.S., & Sapolsky, R.M. Stress and cognitive function. (1995). *Current Opinion in Neurobiology*, *5*(2), 205–216. doi: 10.1016/0959-4388(95)80028-X

McEwen, B. S., & Wingfield, J. C. (2003). The concept of allostasis in biology and biomedicine. *Hormones and Behavior*, *43*(1), 2–15. doi:10.1016/S0018-506X(02)00024-7

Messer, P.W., Ellner, S.P., & Hairston Jr., N.G. (2016). Can population genetics adapt to rapid evolution? (2016). *Trends in Genetics,* 32(7), 408–418. doi:10.1016/J.TIG.2016.04.005

Mitchell, S. C. (2005). How useful is the concept of habitat? - a critique. *Oikos*, *110*(3), 634–638. doi: 10.1111/j.0030-1299.2005.13810.x

Mills, A.M. (2005). Can breeding habitat be sexually selected? *The Auk*, 122(2), 689-700. doi: 10.1642/0004-8038

Millspaugh, J. J., & Washburn, B. E. (2004). Use of fecal glucocorticoid metabolite measures in conservation biology research: considerations for application and interpretation. *General and Comparative Endocrinology*, 138(3), 189–199. doi: 10.1016/j.ygcen.2004.07.002

Moilanen, A., Smith, A. T., & Hanski, I. (1998). Long-term dynamics in a metapopulation of the American pika. *The American Naturalist*, *152*(4), 530-542.

Monclús, R., Rödel, H. G., Palme, R., Von Holst, D., & De Miguel, J. (2006). Non-invasive measurement of the physiological stress response of wild rabbits to the odour of a predator. *Chemoecology*, *16*(1), 25–29. doi: 10.1007/s00049-005-0324-6

Morton, M. L., & Pereyra, M. E. (2008). Haying behavior in a rodent, the bushy-tailed woodrat (*Neotoma cinerea*). doi: 10.1898/1051-1733(2008)89[113:HBIART]2.0.CO;2.

Mote, P. W. (2006). Climate-driven variability and trends in mountain snowpack in western North America. *Journal of Climate*, *19*(23), 6209-6220. doi: 10.1175/JCLI3971.1

Moyer-Horner, L., Mathewson, P. D., Jones, G. M., Kearney, M. R., & Porter, W. P. (2015). Modeling behavioral thermoregulation in a climate change sentinel. *Ecology and Evolution*, *5*(24), 5810-5822. doi:10.1002/ece3.1848.

Nichols, J. D., & Williams, B. K. (2006). Monitoring for conservation. *Trends in Ecology and Evolution*, 21(12), 668–673. doi: 10.1016/j.tree.2006.08.007

Nichols, L. B. (2010). Fecal pellets of American pikas (*Ochotona princeps)* provide a crude chronomoeter for dating patch occupancy. *Western North American Naturalist*, *70*(4), 500–507. doi: 10.3398/064.070.0410

Nicotra, A. B., Beever, E. A., Robertson, A. L., Hofmann, G. E., & O'Leary, J. (2015). Assessing the components of adaptive capacity to improve conservation and management efforts under global change. *Conservation Biology*, *29*(5), 1268–1278. doi: 10.1111/cobi.12522

Noonburg, E. G., & Byers, J. E. (2016). Bad neighbors: how spatially disjunct habitat degradation can cause system-wide population collapse. *Ecology*, 97(10), 2858–2866. https://doi.org/10.1002/ecy.1480

Otto, S. A., Diekmann, R., Flinkman, J., Kornilovs, G., & Möllmann, C. (2014). Habitat heterogeneity determines climate impact on zooplankton community structure and dynamics. *PLoS ONE*, 9(3), e90875. doi: 10.1371/journal.pone.0090875

Paetkau, D. (2003). An empirical exploration of data quality in DNA-based population inventories. *Molecular Ecology*, *12*(6), 1375–1387. doi: 10.1046/j.1365-294X.2003.01820.x

Palme, R. (2005). Measuring fecal steroids: guidelines for practical application. *Annals of the New York Academy of Sciences*, *1046*, 75–80. doi: 10.1196/annals.1343.007

Park, S. (2001). Microsatellite toolkit for Excel. *Smurfit Institute of Genetics. Trinity College. University*.

Park, H., Han, T., Kim, D, Min, M., Han, S., Kim, K. & Lee, H. (2011) Individual identification and sex determination of eurasian otters (*Lutra lutra*) in Daegu city based on genetic analysis of otter spraint. *Genes & Genomics*, 33(6), 653-657. doi: 10.1007/s13258-01100051-z.

Payne, N. L., van der Meulen, D. E., Suthers, I. M., Gray, C. A., & Taylor, M. D. (2015). Foraging intensity of wild mulloway *Argyrosomus japonicus* decreases with increasing anthropogenic disturbance. *Marine Biology*, 162(3), 539–546. doi:10.1007/s00227-014-2603-7

Peacock, M. M., Kirchoff, V. S., & Merideth, S. J. (2002). Identification and characterization of nine polymorphic microsatellite loci in the North American pika, *Ochotona princeps*. *Molecular Ecology Resources*, *2*(3), 360-362. doi:10.1046/j.1471-8286.2002.00249.x

Peacock, M. M., & Smith, A. T. (1997). The effect of habitat fragmentation on dispersal patterns in a pika (*Ochotona princeps*) metapopulation. *Oecologia*, *112*(4), 524–533.

Peakall, R., & Smouse, P. E. (2006). GENALEX 6: Genetic analysis in Excel. Population genetic software for teaching and research. *Molecular Ecology Notes*, *6*(1), 288–295. doi: 10.1111/j.1471-8286.2005.01155.x

Peakall, R., & Sydes,M.A. (1996). Defining priorities for achieiving practical outcomes from the genetic studies of rare plants.Surrey Beatty and Sons, Sydney.

Pederson, J. C., Bunnell, K. D., Conner, M. M., & McLaughlin, C. R. (2012). A robust-design analysis to estimate American black bear population parameters in Utah. *Ursus*, *23*(1), 104–

Pritchard, J. K., Stephens, M., & Donnelly, P. (2000). Inference of population structure using multilocus genotype data. *Genetics*, *155*, 945–959.

Perkin, J. S., Gido, K. B., Cooper, A. R., Turner, T. F., Osborne, M. J., Johnson, E. R., & Mayes, K. B. (2015). Fragmentation and dewatering transform Great Plains stream fish communities. *Ecological Monographs*, 85(1), 73–92. doi: 10.1890/14-0121.1

Pulliam, H. R. (1988). Sources, sinks, and population regulation. *The American Naturalist*, *132*, 652–661. doi: 10.2307/2461927

R Development Core Team (2016). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL http://www.R-project.org

Ray, C., Beever, E. A., & Rodhouse, T. J. (2016). Distribution of a climate-sensitive species at an interior range margin. *Ecosphere*, 7(6), e01379. doi: 10.1002/ecs2.1379

Rehnus, M., Hackländer, K., & Palme, R. (2009). A non-invasive method for measuring glucocorticoid metabolites (GCM) in Mountain hares (*Lepus timidus*). *European Journal of Wildlife Research*, *55*(6), 615–620. doi: 10.1007/s10344-009-0297-9

Ribeiro, E. M. S., Arroyo-Rodríguez, V., Santos, B. A., Tabarelli, M., & Leal, I. R. (2015). Chronic anthropogenic disturbance drives the biological impoverishment of the Brazilian Caatinga vegetation. *Journal of Applied Ecology*, 52(3), 611–620. doi: 10.1111/1365-2664.12420

Riley V. (1981). Psychoneuroendocrine influences on immunocompetence and neoplasia. *Science 212* (4499), 1100-1109. doi: 10.1126/science.7233204

Romero, L. (2002). Seasonal changes in plasma glucocorticoid concentrations in free-living vertebrates. *General and Comparative Endocrinology*, *128*(1), 1–24. doi:10.1016/S0016-6480(02)00064-3

Romero, L. M. (2004). Physiological stress in ecology: lessons from biomedical research. *Trends in Ecology and Evolution*, *19*(5), 249–255. doi: 10.1016/j.tree.2004.03.008

Sapolsky, R. M. (2000). Stress hormones: good and bad. *Neurobiology of Disease*, *7*(5), 540–542. doi:10.1006/nbdi.2000.0350

Scarlata, C. D., Elias, B. A., Godwin, J. R., Powell, R. A., Shepherdson, D., Shipley, L. A., & Brown, J. L. (2011). Characterizing gonadal and adrenal activity by fecal steroid analyses in pygmy rabbits (*Brachylagus idahoensis*). *General and Comparative Endocrinology*, *171*(3), 373–380. doi: 10.1016/j.ygcen.2011.03.002

Schwalm, D., Epps, C. W., Rodhouse, T. J., Monahan, W. B., Castillo, J. A., Ray, C., & Jeffress, M. R. (2016). Habitat availability and gene flow influence diverging local population trajectories under scenarios of climate change: A place-based approach. *Global Change Biology*, *22*(4), 1572–1584. doi: 10.1111/gcb.13189

Sergio, F., Bias, j. Baos, R., Forero, M.G., Donazar, J.A. & Hiraldo, R. (2009). Short-and-long-term consequences of individual and territory quality in a long-lived bird. *Oecologia*, *160*(3), 507-514. doi: 10.1007/s00442-00901314-0.

Sethuraman, A. (2013). On inferring and interpreting genetic population structure-applications to conservation, and the estimation of pairwise genetic relatedness. *arXiv Preprint*, 158. doi: 10.1017/CBO9781107415324.004

Sheriff, M. J., Krebs, C. J., & Boonstra, R. (2009). The sensitive hare: sublethal effects of predator stress on reproduction in snowshoe hares. *Journal of Animal Ecology, 78*(6), 1249–1258. doi: 10.1111/j.1365-2656.2009.01552.x

Sheriff, M. J., Dantzer, B., Delehanty, B., Palme, R., & Boonstra, R. (2011). Measuring stress in wildlife: techniques for quantifying glucocorticoids. *Oecologia*, *166*(4), 869-887

Shinderman, M. (2015). American pika in a low-elevation lava landscape: cxpanding the known distribution of a temperature-sensitive species. *Ecology and Evolution*, *5*(17), 3666–3676. doi: 10.1002/ece3.1626

Shutt, K., J. M. Setchell, & M. Heistermann. (2012). Non-invasive monitoring of physiological stress in the Western lowland gorilla (*Gorilla gorilla gorilla*): validation of a fecal glucocorticoid assay and methods for practical application in the field. *General Comparative Endocrinology.* 179,167–177. doi: 10.1016/j.ygcen.2012.08.008

Smith, A. T. (1974a). The distribution and dispersal of pikas: consequences of insular population structure. *Ecology*, *55*(5), 1112-1119. doi:10.2307/1940361

Smith, A. T. (1974b). The distribution and dispersal of pikas: influences of behavior and climate. *Ecology*, *55*(6), 1368-1376. doi:10.2307/1935464

Smith, A. T., & Gilpin, M. (1997). Spatially correlated dynamics in a pika metapopulation. *Metapopulation biology: ecology, genetics, and evolution. Academic Press, London, UK*, 407-423

Smith, A. T. (1980). Temporal changes in insular populations of the pika (*Ochotona princeps*). *Ecology*, *61*(1), 8–13. doi: 10.2307/1937147

Smith, A. T., & Weston, M. L. (1990). *Ochotona princeps*. *Mammalian Species*, (352), 1. doi: 10.2307/3504319

Smith, A.T. (1979). High local species richness of mammals at Bodie, California. *The Southwestern Naturalist, 24*(3), 553-55.

Stetz, J. B., Kendall, K. C., & Servheen, C. (2010). Evaluation of bear rub surveys to monitor Grizzly bear population trends. *Journal of Wildlife Management*, *74*(4), 860–870. doi: 10.2193/2008-435

Stewart, J. A. E., Perrine, J. D., Nichols, L. B., Thorne, J. H., Millar, C. I., Goehring, K. E., … &

Wright, D. H. (2015). Revisiting the past to foretell the future: summer temperature and habitat area predict pika extirpations in California. *Journal of Biogeography*, *42*(5), 880–890. doi: 10.1111/jbi.12466

Teskey-Gerstl, A., Bamberg, E., Steineck, T., & Palme, R. (2000). Excretion of corticosteroids in urine and faeces of hares (*Lepus europaeus*). *Journal of Comparative Physiology B-Biochemical Systemic and Environmental Physiology*, *170*(2), 163–168. doi: 10.1007/s003600050271

Thiel, D., Jenni-Eiermann, S., Braunisch, V., Palme, R., & Jenni, L. (2007). Ski tourism affects habitat use and evokes a physiological stress response in capercaillie *Tetrao urogallus*: a new methodological approach. *Journal of Applied Ecology*, *45*(3), 845–853. doi: 10.1111/j.1365-2664.2008.01465.x

Tscharntke, T., Tylianakis, J. M., Rand, T. A., Didham, R. K., Fahrig, L., Batáry, P., &Westphal, C. (2012). Landscape moderation of biodiversity patterns and processes - eight hypotheses. *Biological Reviews*, 87(3), 661–685. doi: 10.1111/j.1469-185X.2011.00216.x

Varner, J., & Dearing, M. D. (2014). Dietary plasticity in pikas as a strategy for atypical resource landscapes. *Journal of Mammalogy*, *95*(1), 72–81. doi: 10.1644/13-MAMM-A-099.1

Walther, G. R., Post, E., Convey, P., Menzel, A., Parmesan, C., Beebee, T. J., Fromentin, J.M., Hoegh-Guldberg, O. & Bairlein, F. (2002). Ecological responses to recent climate change. *Nature*, *416*(6879), 389-395. doi:10.1038/416389a

Washburn, B. E., and J. J. Millspaugh. 2002. Effects of simulated environmental conditions on glucocorticoid metabolite measurements in white-tailed deer feces. *General and Comparative Endocrinology 127*(3), 217–222. doi:10.1016/S0016-6480(02)00056-4

Wells, J. V., Rosenberg, K. V., Dunn, E. H., Tessaglia-Hymes, D. L., & Dhondt, A. A. (1998). Feeder counts as indicators of spatial and temporal variation in winter abundance of resident birds. *Journal of Field Ornithology*, *69*, 577–586. doi: 10.2307/4514362

Wikelski, M., & Cooke, S. J. (2006). Conservation physiology. *Trends in Ecology and Evolution*, *21*(1), 38–46. doi: 10.1016/j.tree.2005.10.018

Wingfield, J. C., & Sapolsky, R. M. (2003). Reproduction and resistance to stress: when and how. *Journal of Neuroendocrinology*, 15(8), 711–724. doi: 10.1046/j.1365-2826.2003.01033.x

Wilcove, D. S., Rothstein, D., Dubow, J., Phillips, A., & Losos, E. (1998). Quantifying threats to imperiled species in the United States. *BioScience*, *48*(8), 607–615. doi:10.2307/1313420

Wilkening, J. L., Ray, C., & Sweazea, K. L. (2013). Stress hormone concentration in Rocky Mountain populations of the American pika (*Ochotona princeps*). *Conservation Physiology*, *1*(1), 1–13. doi: 10.1093/conphys/cot027

Wilkening, J.L. Characterizing metrics and outcomes of a climate-sensitive species, the American pika. (2014). University of Colorado Boulder. A Ph.D Dissertation submitted to Graduate School.

Wilkening, J. L., Ray, C., & Varner, J. (2015). Relating sub-surface ice features to physiological stress in a climate sensitive mammal, the American pika (*Ochotona princeps*). *PLoS ONE*, *10*(3), 1–17. doi: 10.1371/journal.pone.0119327

Wilkening, J. L., & C. Ray. 2015. Parks, pikas, and physiological stress: implications for long-term monitoring of an NPS climate-sensitive sentinel species. Park Science 32(1):42–48.

Wilkening, J. L., Ray, C., & Varner, J. (2016). When can we measure stress noninvasively? Postdeposition effects on a fecal stress metric confound a multiregional assessment. *Ecology and Evolution*, *6*(2), 502–513. doi: 10.1002/ece3.1857

Wilkening, J. L., & Ray, C. (2016). Characterizing predictors of survival in the American pika

(*Ochotona princeps*). *Journal of Mammalogy*, *97*(5), 1366–1375. doi: 10.1093/jmammal/gyw097

Williams, C., Kitaysky, A., Kettle, A., & Buck, L.(2008).Corticosterone levels of tufted puffins vary with breeding stage, body condition index, and reproductive performance. *General and Comparative Endocrinology 158* (1), 29-35. doi: 10.1016/j.ygcen.2008.04.018

Wilson, E.O. 1999. 'The Diversity of Life.' (W.W. Norton: New York.)

Wingfield, J.C., Maney, D.L., Breuner, C.W., Jacobs, J.D., Lynn, S., Ramenofsky, M. & Richardson, R.D. (1998). Ecological bases of hormone—behavior interactions: the "Emergency Life History Stage." *American Zoologist*, *38*(1), 191–206. doi: 10.1093/icb/38.1.191

Wingfield, J. C., & Sapolsky, R. M. (2003). Reproduction and resistance to stress: when and how. *Journal of Neuroendocrinology*, 15(8), 711–724. doi:10.1046/j.1365-2826.2003.01033.x

Woltz, J.M., Isaacs, R. & Landis, D.A. (2012). Landscape structure and habitat management differentially influence insect natural enemies in an agricultural landscape. *Agriculture, Ecosystems & Environment*, 152, 40–49. doi: 10.1016/J.AGEE.2012.02.008

Tables

Table 1 Habitat patches in the northern complex of the BSHP mining district, and their associated talus perimeter (m), average fecal GCMs (physiological stress (µg cortisol/ g of feces), and colonization-extinction (CE) ratio per patch (number of colonizations/number of extinctions since 1977).

Patch	Talus Perimeter (m)	Average GCMs	CE ratio
Bechtel	4.57	0.039	0.72
BOD 55	19.05	0.037	0.73
BOD 66	22.10	0.027	0.69
BOD 70	22.10	0.04	0.65
BOD 71	24.38	0.037	0.61
Grey Mine	31.24	0.036	0.88
BOD 72	44.20	0.048	0.8
Hobart Tunnel	51.05	0.031	0.96
BOD 56	59.44	0.041	0.73
Black Hawk Shaft	60.20	0.042	0.96
BOD 68	70.87	0.03	0.84
Summit Shaft	90.68	0.04	0.92
Security Tunnel	93.73	0.055	0.92
Tioga Shaft	105.16	0.051	0.92
Sitting Bull	107.44	0.048	0.85
Midnight Shaft	120.40	0.067	0.95
Bodie Old Shaft	121.16	0.065	0.96
Vindicator Hoover	203.45	0.044	0.96
Lucky Jack/Jupiter Shaft	282.70	0.057	0.96

Figure legends

Fig. 1 A map displaying the distribution, relative size, and configuration of patch habitat within the northern complex of the Bodie State Historic Park mining district located in eastern California (Mono County)

Fig. 2 Boxplots characterizing differences in average fecal glucocorticoid metabolites (μg cortisol/g feces) per site within the Bodie Hills, and E. Sierra Nevada regions. Note that Bodie State Historic Park maintains significantly higher physiological stress concentrations compared to Bodie Bluff (P<0.001)

Fig. 3 Using a subset of the total numbers of habitat patches surveyed**,** a **l**ine plot characterizes individual turnover rate (%) per patch (i.e. number of mortality events/ number of total sampled individuals) and is ordered by increasing talus perimeter (m) for the pika population inhabiting Bodie State Historic Park, California (Mono County). All individual turnover rates were calculated per season (i.e. 2013, 2014 and 2015) and averaged for easier visualization. Per-patch average fecal glucocorticoid metabolites (μg cortisol/ g feces) are also shown in dark bar plots

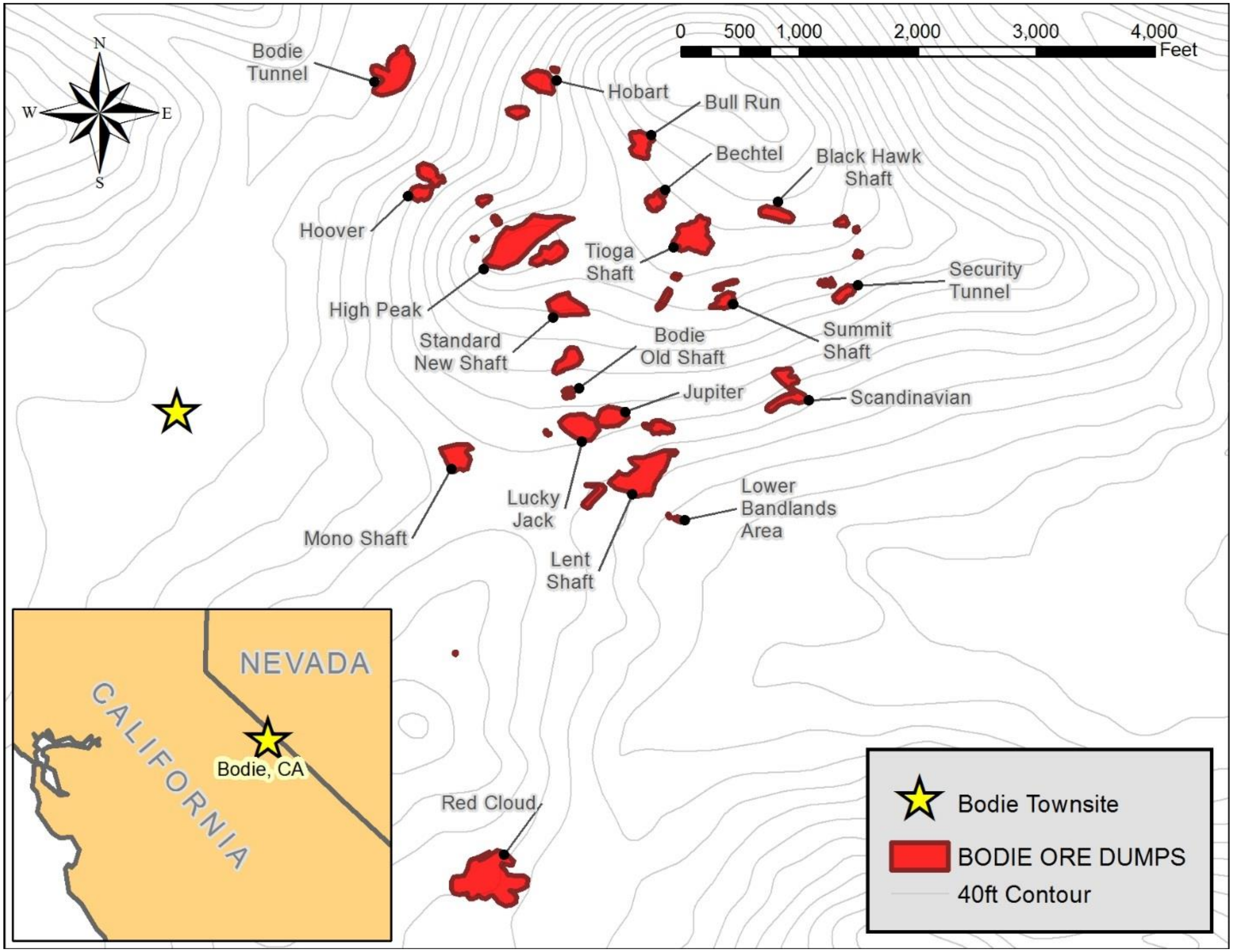
Fig. 4 A bar plot visualizing individual pika differences in fecal glucocorticoid metabolites (μg cortisol/ ug feces) collected during the 2013 summer season (Jun-Sep) across habitat patches located in Bodie State Historic Park and ordered by increasing talus perimeter (m). The individual capture history is on the X axis with the key as follows: N: new individual, NR: new/ recaptured individual (averaged fecal GCMs from 2013 and 2014 sampling seasons)

Fig. 5 Results from multiple linear regression modeling exploring the relationship between long-term pika occupancy (~1972-2014) (i.e. number of patches occupied/ number of total patches surveyed**)** and local temperature and precipitation predictor variables. The statistically significant model explaining the greatest amount of variance in pika occupancy included minimum winter temperature (β=-0.05, P=0.01), average maximum summer temperature (β =-0.24, p=0.02), and average annual snow fall (β =-0.04, P=0.08) with an overall adjusted R^2=0.52 (P=0.04) (*ggplot2*, R core team 2016)

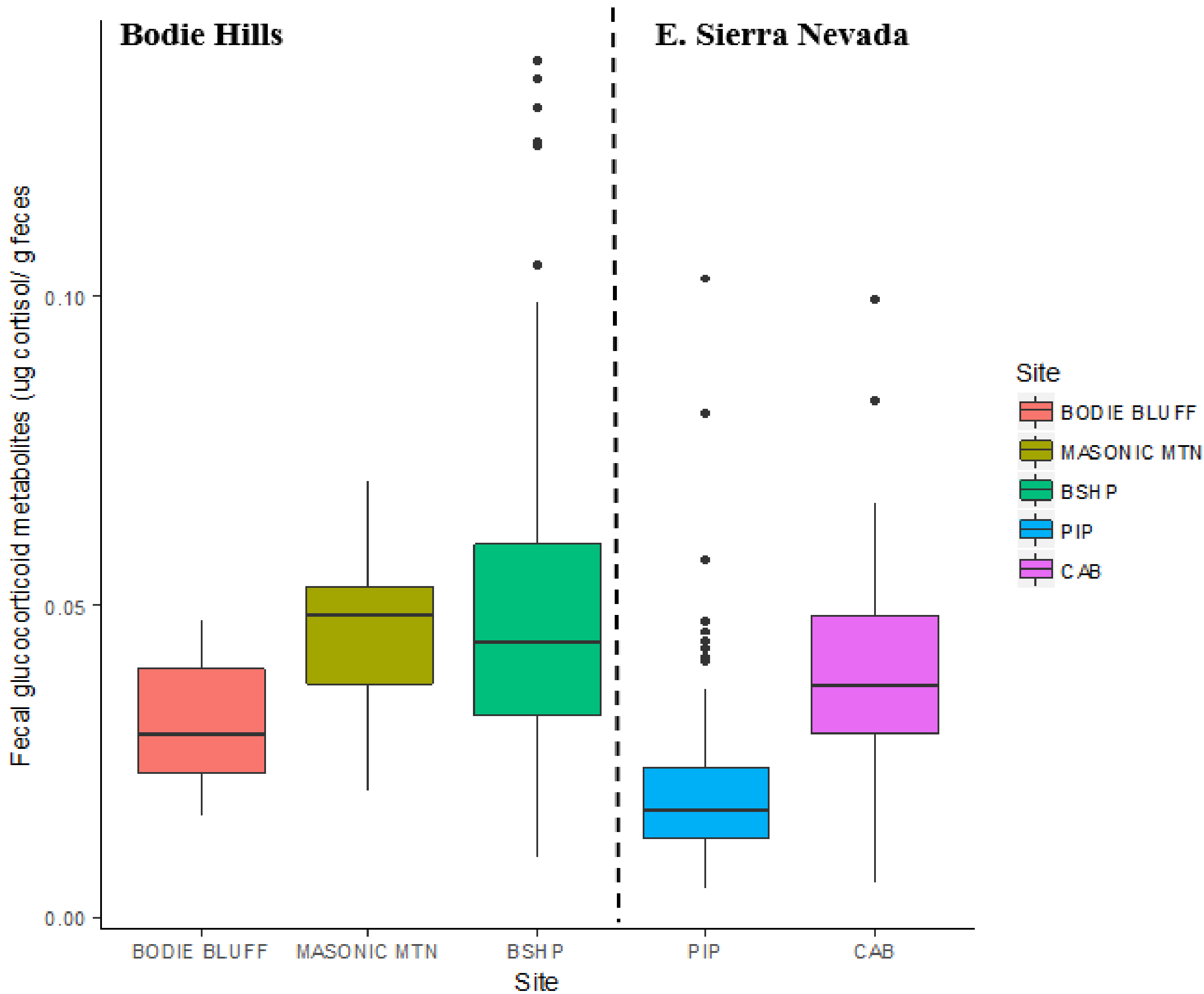
Fig. 6 Results from multiple linear regression modeling exploring the relationship between pika fecal GCM patterns during June through September of 2013-2015 and local temperature and precipitation predictor variables. The statistically significant model explaining the greatest amount of variance in fecal GCM patterns included average monthly maximum temperature (β =0.005, P=0.004), and minimum monthly temperature (β =-0.004, P=0.002) with an overall adjusted R^2=0.90 (P=0.004)

Figures

Fig 1.



3 **Fig 2.**



Bodie Hills
E. Sierra Nevada
Fecal glucocorticoid metabolites (ug cortisol/ g feces
0.10
0.05
0.00
BODIE BLUFF
MASONIC MTN
BSHP
PIP
CAB
Site
Site
BODIE BLUFF
MASONIC MTN
BSHP
PIP
CAB

4 **Fig. 3**

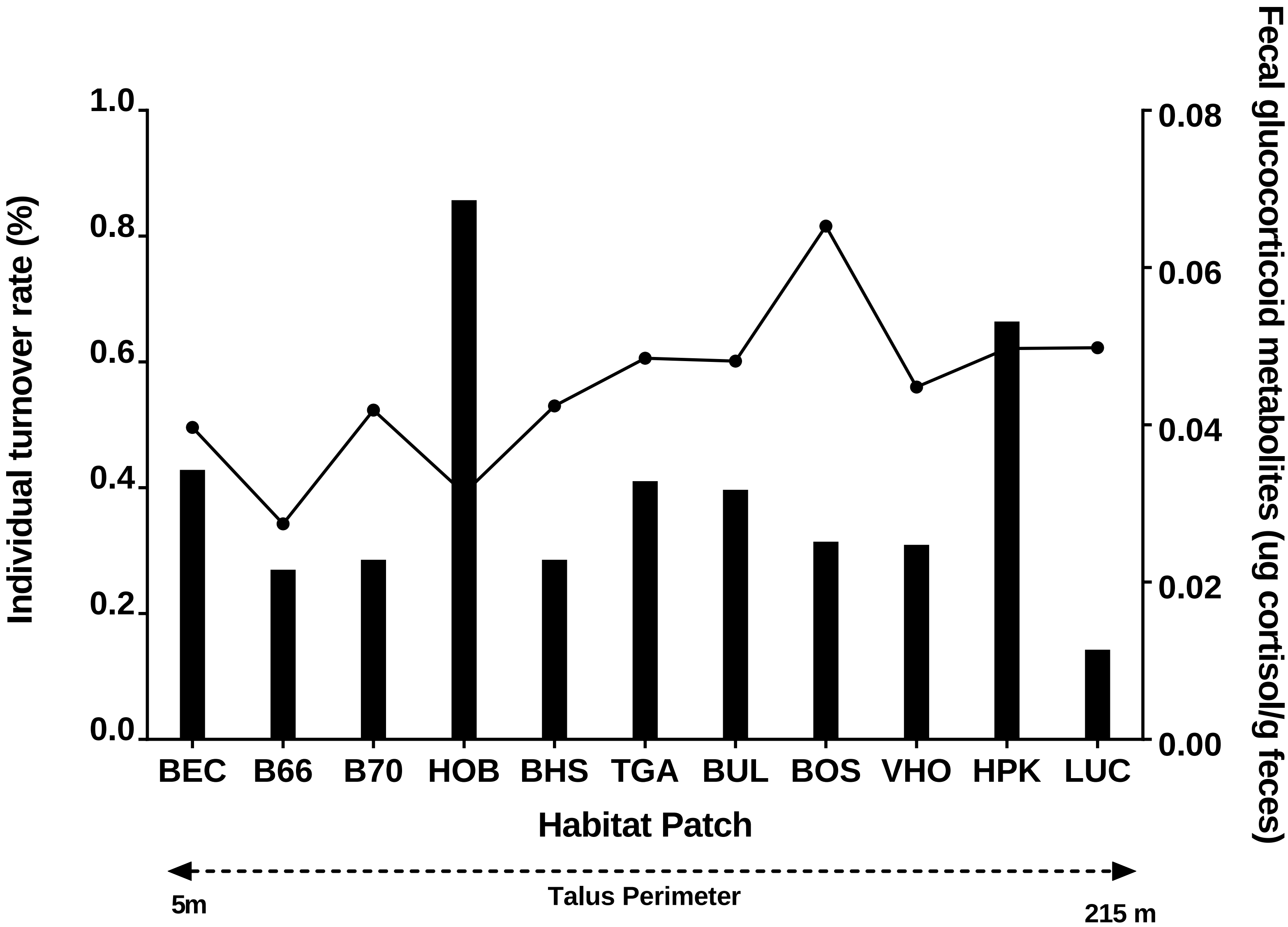
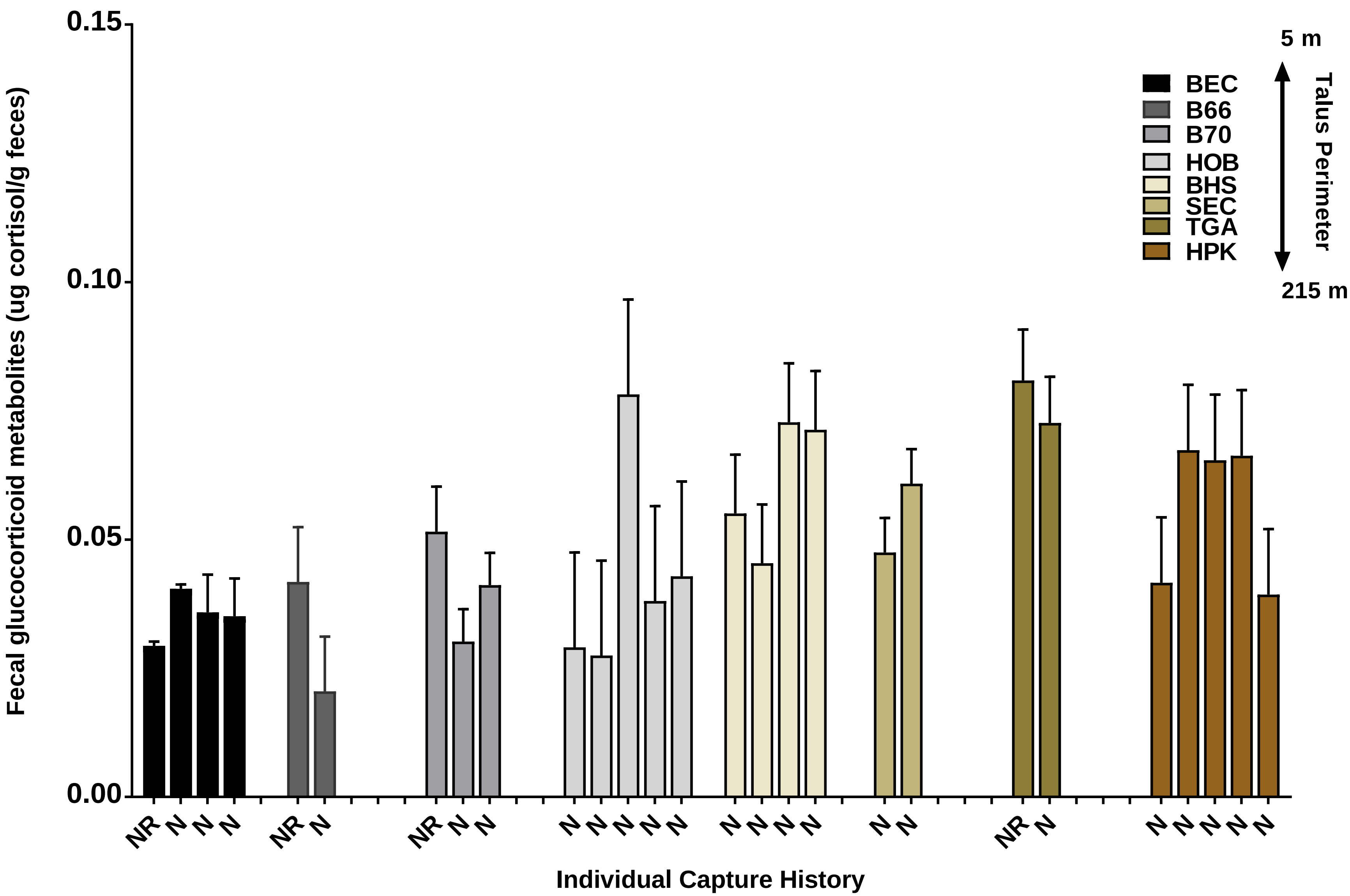
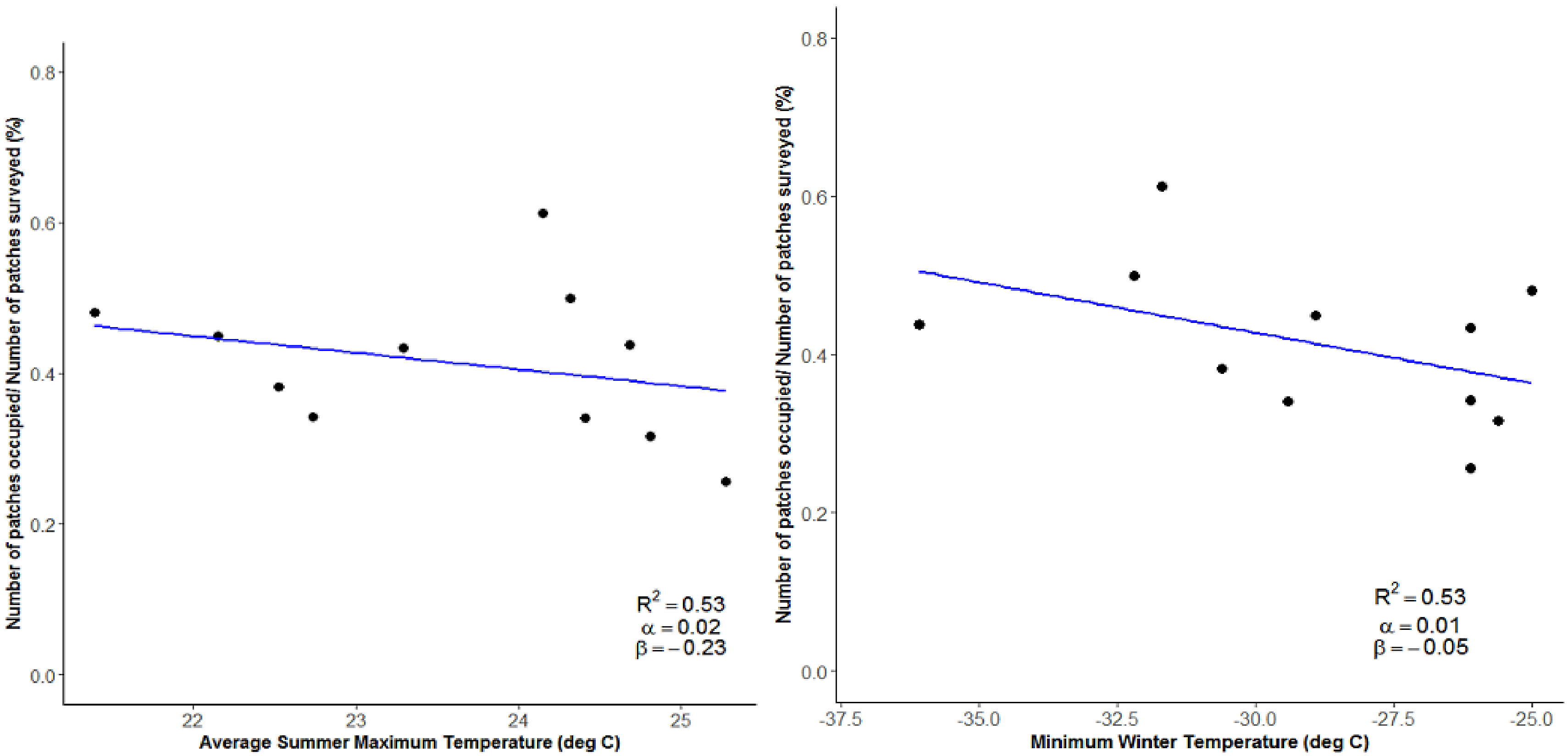

1.0
0.8
0.6
0.4
0.2
0.0
Individual turnover rate (%)
0.08
0.06
0.04
0.02
0.00
Fecal glucocorticoid metabolites (ug cortisol/g feces)
BEC
B66
B70
HOB
BHS
TGA
BUL
BOS
VHO
HPK
LUC
Habitat Patch
Talus Perimeter
5m
215 m

Fig. 4



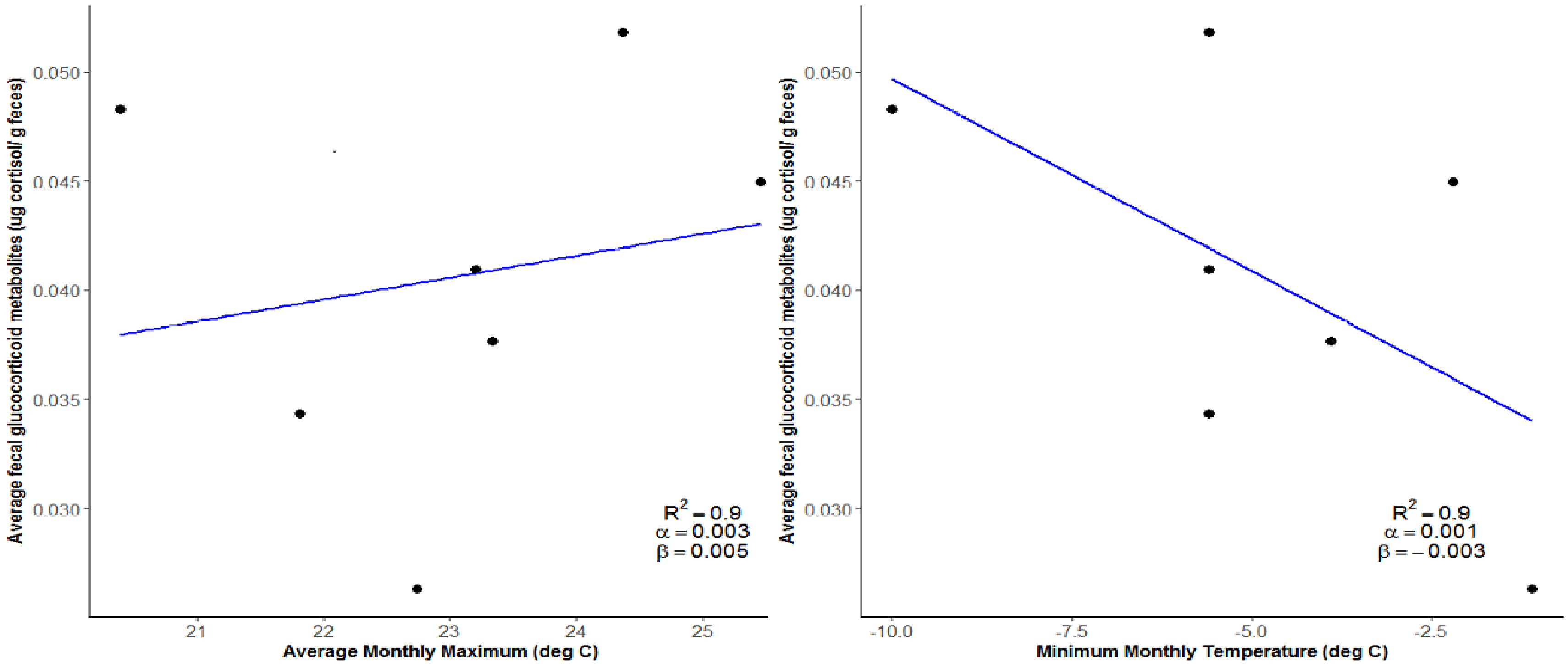
0.15
0.10
0.05
0.00
Fecal glucocorticoid metabolites (ug cortisol/g feces)
NR N N N NR N NR N N N N N N N N N N N N N NR N N N N N N
Individual Capture History
BEC
B66
B70
HOB
BHS
SEC
TGA
HPK
5 m
Talus Perimeter
215 m

Fig. 5



Number of patches occupied/ Number of patches surveyed (%)
0.0
0.2
0.4
0.6
0.8
22
23
24
25
Average Summer Maximum Temperature (deg C)
$R^2 = 0.53$
$\alpha = 0.02$
$\beta = -0.23$
Number of patches occupied/ Number of patches surveyed (%)
0.0
0.2
0.4
0.6
0.8
-37.5
-35.0
-32.5
-30.0
-27.5
-25.0
Minimum Winter Temperature (deg C)
$R^2 = 0.53$
$\alpha = 0.01$
$\beta = -0.05$

Fig 6


Average fecal glucocorticoid metabolites (ug cortisol/ g feces)
0.050
0.045
0.040
0.035
0.030
$R^2 = 0.9$
$\alpha = 0.003$
$\beta = 0.005$
21
22
23
24
25
Average Monthly Maximum (deg C)
Average fecal glucocorticoid metabolites (ug cortisol/ g feces)
0.050
0.045
0.040
0.035
0.030
$R^2 = 0.9$
$\alpha = 0.001$
$\beta = -0.003$
-10.0
-7.5
-5.0
-2.5
Minimum Monthly Temperature (deg C)

Supplementary Materials

Table S1 Table summarizing the number of months missing more than 10% of data for each raw climate variable downloaded from the Bodie COOP weather station data for comparison with long-term pika occupancy data (i.e. ~1972-2010). Please note that a 90% completeness filter was used for the raw monthly data (e.g. Precipitation, Snow Depth, Snow Fall, etc.) therefore for each of the seven specific metrics (e.g. average annual snow fall), each of the relevant months were required to contain less than 10% missing data or the metric was not included in further analysis for that year. Cells highlighted in red indicate that an associated climate metric could not be included due to low-quality data during one or more months (> 10% missing) with the relevant month (s) indicated. For all precipitation-related metrics, the climate water year was used (i.e. October of the previous year to September of the census sampling year) whereas winter season metrics used Dec to Feb and summer season metrics used Jun-Aug.

Year	**Precipitation**	**Snow Depth**	**Snow Fall**	**Min Temperature**	**Max Temperature**
Associated Climate Metric	Avg Ann. Precip	Avg. Ann. Snow Depth	Avg. Ann. Snow Fall	Min Winter Temp, Avg Winter Temp	Max Summer Temp, Avg Summer Temp
1971-1972	0	0	2	0	9
1976-1977	0	0	1 (Feb)	0	0
1988-1989	1	0	0	0	1
1990-1991	0	0	1 (Feb)	0	0
1991-1992	0	0	1	0	0
1992-1993	0	0	0	0	0
1993-1994	0	0	1 (May)	0	0
1994-1995	0	0	1	0	0
1995-1996	0	0	1	0	0
1996-1997	0	0	2 (Apr)	0	0
1997-1998	0	0	2	0	1
1998-1999	0	0	2 (Oct & Nov)	0	5
1999-2000	0	0	4	0	6
2000-2001	0	0	5 (Oct to Dec)	0	3
2002-2003	0	0	2 (Dec)	0	1
2003-2004	1	0	0	0	3
2004-2005	3 (Nov & Feb)	0	1	0	6 (June)
2005-2006	3 (March)	0	2	0	5
2007-2008	0	0	2	0	3
2008-2009	1 (Feb)	0	3 (Apr)	0	3
2009-2010	2 (Mar)	0	4 (Oct)	0	5

Table S2 Table listing the percentage of missing data for each climate metric calculated from the Bodie COOP weather station data for comparison with and the more recently collected genetic data (i.e. 2013-2015). For each year of occupancy climate metrics, the year bolded indicates the year that census data was collected with the example definition above giving the technical time period used for each metric. For all precipitation metrics, the climate water year was used (i.e. October of the previous year to September of the census sampling year). Cells highlighted in red indicates a low-quality data point that was removed prior to analysis due to the percentage of missing data being greater than 10%.

Month/ Year	Avg Monthly Maximum	Max Temp (Tmax)	Avg Monthly Minimum	Min Temp (Tmin)	Avg Monthly Mean Temp	Monthly Total Precipitation
JUNE 2013	0.00	0.00	0.00	0.00	0.00	6.67
JUNE 2014	73.33	73.33	73.33	73.33	73.33	73.33
JUNE 2015	0.00	0.00	0.00	0.00	0.00	0.00
JULY 2013	0.00	0.00	0.00	0.00	0.00	0.00
JULY 2014	12.90	12.90	12.90	12.90	12.90	12.90
JULY 2015	0.00	0.00	0.00	0.00	0.00	0.00
AUGUST 2013	9.68	9.68	9.68	9.68	9.68	9.68
AUGUST 2014	6.45	6.45	6.45	6.45	6.45	0.00
AUGUST 2015	0.00	0.00	0.00	0.00	0.00	0.00
SEPTEMBER 2013	0.00	0.00	0.00	0.00	0.00	0.00
SEPTEMBER 2014	0.00	0.00	0.00	0.00	0.00	0.00
SEPTEMBER 2015	0.00	0.00	0.00	0.00	0.00	0.00

Fig. S1 Diagnostic plots of model fit for multiple linear regression analysis of occupancy and weather station data using log transformed occupancy, and centered climate predictors: adjusted R2=0.53, p=0.042, avg ann snowfall, (beta=-0.04 p=0.0832), min winter temp (-0.05, 0.017), avg max sum temp=-0.23, 0.019)

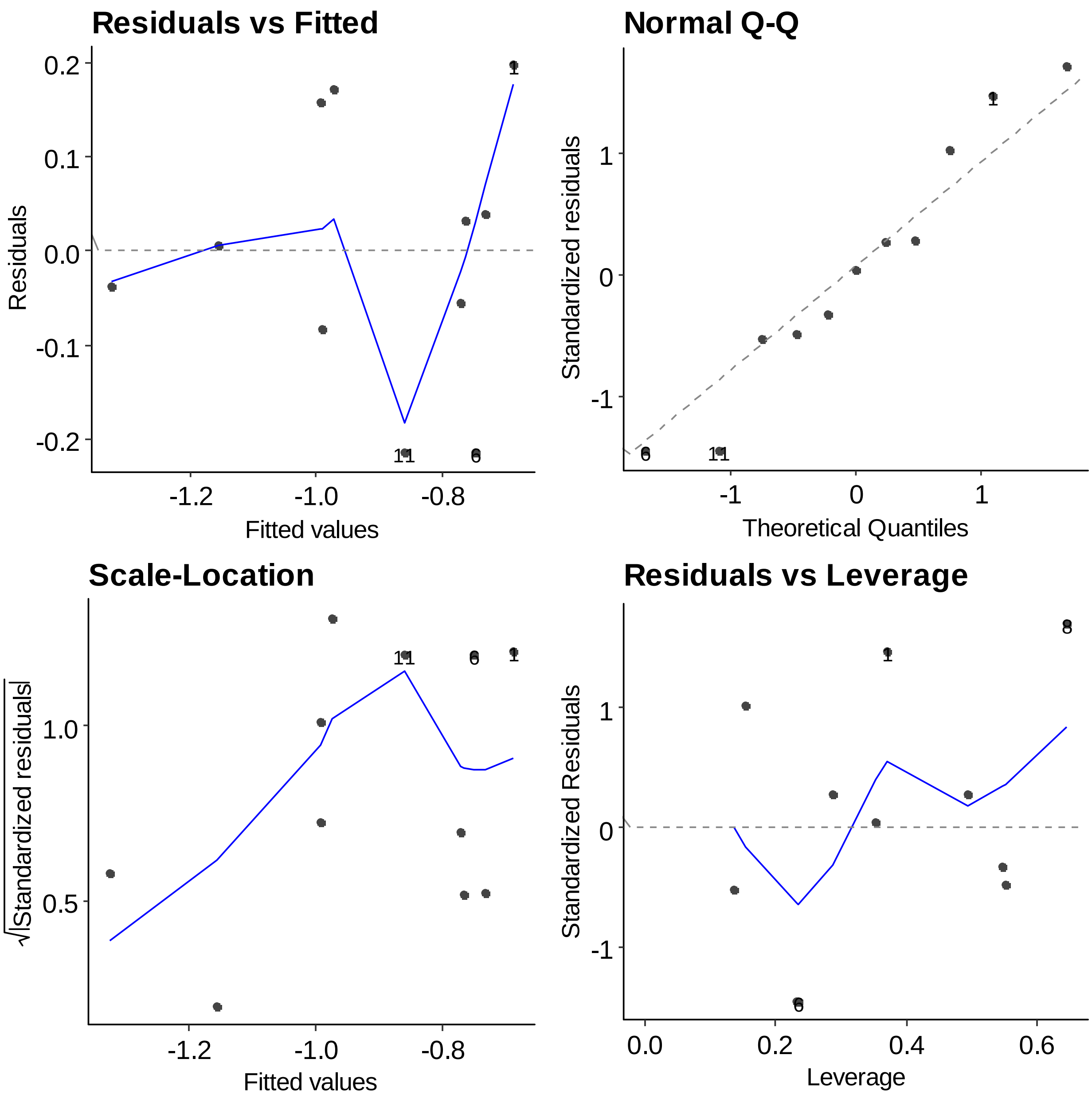
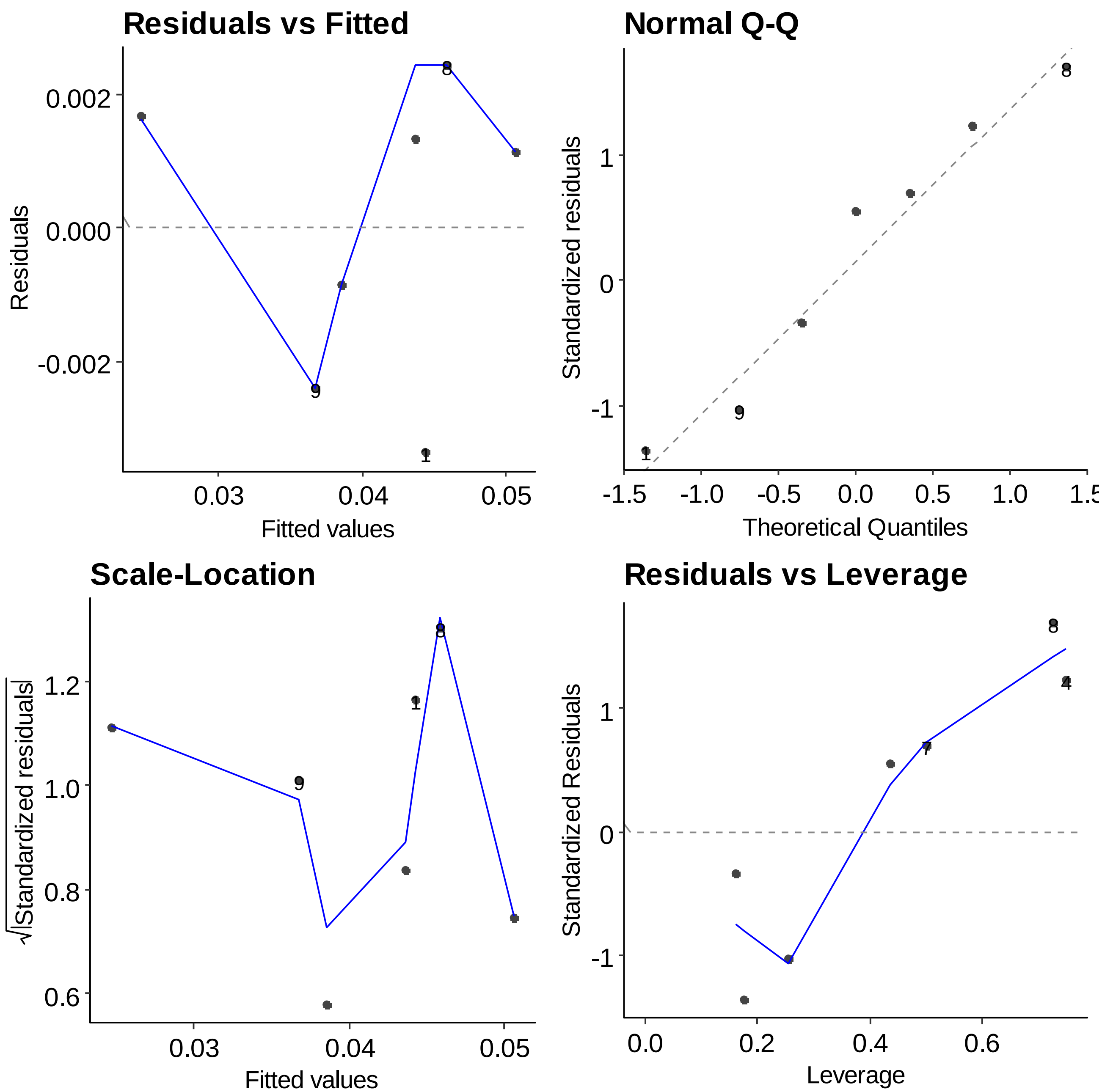


Fig. S2. Diagnostic plots of model fit for multiple linear regression analysis of fecal GCMs and weather station data climate predictors: adjusted R2=0.90, p=0.004, average monthly maximum, (beta=0.005 p=0.003), tmin (-0.003, 0.001),)



CHAPTER 3 Life on the edge - the American pika in Bodie, California: a 65 year case study of genetic erosion in a species of special concern

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Keywords: American pika, climate change, genetic monitoring, evolutionary potential, conservation biology

Abstract

Genetic monitoring enables conservation biologists to test hypotheses regarding population viability in the face of anthropogenic disturbance. In an age when wildlife populations are constantly under threat from human-mediated change, gene flow between populations and maintenance of genetic variation are critical for population persistence and evolutionary potential. Here, we document genetic changes in an iconic American pika (*Ochotona princeps*) metapopulation within Bodie State Historic Park located at the southern end of this species' range in Mono County, California over a 65 year time frame. The results of this study show declines in allelic diversity and observed heterozygosity, increased population substructure, and reductions in effective population size (N_e). It is likely that the ongoing collapse of suitable habitat due to climate change over the last 40 years, and the subsequent decline in patch occupancy, have directly contributed to these patterns of diversity loss. This study highlights the power of establishing genetic monitoring programs at local scales and provides evidence linking changes in the local climate over the last 65 years with the erosion of genetic variation. Future monitoring programs should incorporate similar protocols for a larger number of populations associated with all five subspecies of the American pika to enable greater understanding of species-wide genomic diversity and ultimately, evolutionary potential.

Introduction

Intraspecific genetic diversity provides the foundation for evolutionary response and population persistence (Hughes and Stachowicz 2004; Reusch et al. 2005; Whitham et al. 2008; Hoban et al. 2014). Wildlife populations are often under threat from a multitude of anthropogenic stressors including habitat fragmentation, emerging infectious disease, invasive species and climate change that may directly or indirectly influence maintenance of population-level genetic variation (Peacock and Dochtermann 2012; Gutiérrez-Cánovas et al. 2013; Muthukrishnan and Fong 2014; Langwig et al. 2015; Muhlfield et al. 2017; Price et al. 2017; Salo et al. 2017; Stewart et al. 2017). The loss of genetic resources is associated with lowered fitness and increased extinction vulnerability and is therefore of conservation concern (Spielman et al. 2004; Frankham 2005; Lawson et al. 2017). The characterization of genetic structure of natural populations can offer insight into local extirpation risk (Schwartz et al. 2007; Rubidge et al. 2012; Van Doornik et al. 2013; Beatty et al. 2014). Demographic and evolutionary processes underlying observed genetic structure can then be used to make predictions regarding the effects of anthropogenic stressors (Klank et al. 2012; Inostroza et al. 2016; Weiss and Leese 2016). Genetic monitoring, or the quantification of temporal changes in population genetic metrics, has therefore become an important tool to test predictions associated with the effects of such perturbations (Peacock and Dochtermann 2012; Goswami et al. 2014). The measurement of allelic diversity, observed and expected heterozygosity, and effective population size (N_e) repeatedly over time make it possible to document changes in population health within a historical context (Schwartz et al. 2007).

Historical collections can play an invaluable role in extending the genetic monitoring period to earlier in the 19th and 20th centuries. Natural history museum and university collections offer a window into the genetics of the past for many organisms (Matocq and Villablanca 2001; Mortiz et al. 2008; Arnold et al. 2017; Hekkala et al. 2017; Peacock et al. 2017; Yao et al. 2017). Retrospective monitoring can provide a record of genetic variation for populations found in the same habitats and inform conservation strategies for populations or species that are experiencing declines. Methods for the recovery of DNA from degraded material (fecal samples) or archived museum specimens (Lambert et al. 2009; Beatty et al. 2014; Hekkala et al. 2017; Hundsdoerfer et al. 2017) have become increasingly reliable and provide greater opportunity for monitoring cryptic, endangered, or hard-to-capture species.

Contemporary climate change is predicted to impact biodiversity at multiple scales ranging from organisms to ecosystems (Root et al. 2003; Parmesan 2006; Cahill et al. 2012; Hotaling et al. 2017; Lamal et al. 2017) through impacts on genetics, physiology, phenology, community-level interactions, and ecosystem services (Kiers et al. 2010; Rafferty and Ives 2010; Gilman et al. 2010, Walther 2010; Chen et al. 2011; Bellard et al. 2012). Very few studies have investigated the genetic effects of climate change, but some recent work has demonstrated that exposure to a stressor over even a few generations can lower neutral genetic diversity by increasing isolation and the rates of random genetic drift (Meyers and Bull 2002; Botkin et al. 2007; Cobben et al. 2011; Alsos et al. 2012; Pauls et al. 2013). Wildlife populations are likely impacted by changing conditions in real-time with greater overall increases in mortality and reduced rates of reproduction that create signatures of genetic decline or result in locally adapted populations that are maladapted to future climate scenarios (Davis and Shaw 2001; Jump and Penuelas 2005; Hof et al. 2011). Additionally, intraspecific genetic diversity is often harbored within cryptic evolutionary lineages. Therefore range reductions and increases in population genetic structure as a result of climate change may lead to losses of whole lineages and their unique genetic diversity (Bálint et al. 2011).

This study focuses on the American pika (*Ochotona princeps*), an alpine lagomorph distributed across mountain ranges in the Intermountain West (Smith and Weston 1990; Hafner and Sullivan 1995; Hafner and Smith 2010), where limited dispersal capacity, territorial behavior, and habitat requirements increase their vulnerability to changing environmental conditions. Observations suggest that less mobile species, such as pikas, have a particular disadvantage in tracking suitable habitat conditions compared to more vagile species that are capable of colonizing new habitat through greater migration distances (Callaghan et al. 2004; Lima et al. 2017; Pandit et al. 2017; Peacock et al. 2016; Radinger et al. 2017). Pikas tend to be philopatric and rely heavily on access to favorable microhabitat conditions within talus, lava tubes, or anthropogenic substrate to avoid extremes in ambient temperatures (MacArthur and Wang 1973; Smith 1974b; Peacock 1997; Rodhouse et al. 2010; Nichols 2011; Shinderman 2015; Stewart et al. 2017). Because pikas sustain a high resting body temperature (40.1°C) very close to their upper lethal temperature (43.1°C), the thermal consistency of interstitial habitat in talus is important for dissipation of body heat through behavioral thermoregulation. Even in relatively mild temperatures (25.5-29.4°C), pikas are at high risk of mortality if they do not have access to thermally stable substrates that buffer them from ambient conditions (MacArthur and Wang 1973; Smith 1974b).

Climate metrics (Beever et al. 2010; Calkins et al. 2012; Stewart et al. 2015), as well as habitat configuration and connectivity (Peacock and Smith 1997b; Beever et al. 2003; Stewart et al. 2015; Schwalm et al. 2016) have repeatedly been identified as good predictors of continuing pika persistence. Recently, it has become evident that fine-scale factors such as gene flow, substrate type, thermal components of talus matrices and dispersal habitat (Shinderman 2015; Millar et al. 2016), as well as intraspecific phenotypic variation (Moyer-Horner et al. 2015; Mathewson et al. 2016) play a role in the varying and regionally specific responses to changing climate conditions of this species.

Although pikas inhabit naturally fragmented talus patches throughout their range, their unique suite of life-history characteristics likely interact to elevate extinction risk under a rapidly changing climate scenario, especially in the relatively small and isolated populations that occur throughout the southwestern portion of their species distribution (Beever et al. 2003; Grayson 2005, Beever et al. 2011; Stewart et al. 2015, Nichols et al. 2016). In the hydrographic Great Basin, in particular, pikas have recently experienced widespread and rapid decline with comprehensive surveys reporting extirpation of pikas from 44.1% of historical sites recorded as extant prior to 1991 (15 of 34 sites) (Beever et al. 2016). The rate of extirpation in the Great Basin has increased over time with an additional 24% of extant sites experiencing extirpation by 1999 (Beever et al. 2003) and 40% of the remaining sites extirpated by 2008 (Beever et al. 2011). Unfortunately, current rates of extirpation, notably during 2014-2015, have increased substantially in northeastern California and in more insular regions of the Great Basin and southern Utah compared to larger, more continuous habitats in the Sierra Nevada and Rocky Mountains

(Beever et al. 2016). Within the northern Bodie Hills of Mono County, California, almost all of the natural rock outcroppings and anthropogenic mining habitat are currently unoccupied by pikas despite evidence of recent 20th and 21st century occupation (Moilanen et al. 1988; Nichols 2011; Stewart et al. 2015; Nichols et al. 2016; Nichols, unpublished data). These modern rates of extirpation and subsequent population isolation are a conservation concern because population size is clearly important to the maintenance of genetic diversity, with small populations susceptible to the effects of random genetic drift, inbreeding, and accumulation of deleterious alleles (Frankham and Ralls 1998; Frankham et al. 2003; Schwartz et al. 2005; Jang et al. 2017). The relatively recent and ongoing losses of genetic connectivity among pika populations in the Great Basin and northeastern California (Galbreath et al. 2009; 2010) will undoubtedly increase vulnerability to genetic erosion (Frankham 1996; Frankham et al. 2003; Palstra and Ruzzante, 2008; Luo et al. 2012; Cossu et al. 2017).

Here, we characterize genetic variation and population genetic structure in the American pika (*Ochotona princeps*) metapopulation within the Bodie State Historic Park (BSHP) (~2500 m), Mono County, California (Fig. 1). First reported by J.H. Severeid (Severeid 1955), BSHP was surveyed in 1972, 1977, and annually from 1989 to the present except for 2002 and 2007 (Moilanen et al. 1998; Nichols, unpublished data). Pika habitat at Bodie consists of approximately 100 various sized talus islands formed from the tailings of abandoned gold mines. The extant population is estimated to be < 100 individuals, descendants of animals that dispersed to the anthropogenically created ore dumps from natural talus outcrops in the Bodie Hills ca. 1900 (Severeid 1955; Nichols unpublished data). The BSHP population has already experienced significant reductions in population size documented through long-term census surveys (Moilanen et al. 1998; Nichols, unpublished data). Previously suitable habitat in the southern and middle sections of the district experienced population collapse beginning in the late 1970s and has never been fully recolonized by the extant population in the northern half of the mining district (Nichols, unpublished data). The increasing isolation of the BSHP pika population during recent years (ca. 2012-2015; Nichols et al. 2016) has occurred simultaneously with an exceptionally severe drought in California, with evidence of increasingly warm temperatures and low precipitation (Griffin et al. 2014). The frequency of drought years during the last two decades has increased compared to the previous 100 years and the likelihood of extremely warm, low precipitation years is projected to increase in the near future partially as a result of anthropogenic impacts (AghaKouchak et al. 2014; Diffenbaugh et al. 2015; Williams et al. 2015).

Although this species has been the focus of population genetic analyses and metapopulation and species distribution modeling efforts, our study is the first to quantify temporal changes in neutral genetic diversity over multiple generations at this site (~65 years) (Smith 1974a; Peacock 1997; Peacock and Smith 1997a, b; Smith and Gilpin 1997; Moilanen et al. 1998; Beever et al. 2011; Erb et al. 2011; Wilkening et al. 2011). Although neutral genetic variation does not represent variability in adaptive traits, and thus may not always be directly reflective of evolutionary potential in wild populations, losses of neutral genetic diversity have often been used as a surrogate for losses in adaptive variation and thus have been used to assess extinction risk for threatened taxa compared to non-threatened taxa (Spielman et al. 2004; Palstra and Ruzzante 2008; Knafler et al. 2016; Lawson et al. 2017; Marmesat et al. 2017).

We used long-term population census surveys as well as regional climate data collected during the same time periods to examine the relationship between climate and genetic parameters. Therefore we were able to investigate the genetic consequences of an observed demographic decline in this pika metapopulation by considering a combination of genetic, census, and local climate indicators in order to develope predictions for population extirpation risk over time. Thus, if this population faces an extirpation risk we expect to observe:

i. **Genetic**: a consistent decline in allelic diversity over the last 65 years as well as evidence of reductions in effective population size and observed heterozygosity,
ii. **Population census size (demographic verifier)**: a steady reduction in patch occupancy during the time period for which census data are available (1972-2014) that correlates with the decline in effective population size (N_e),
iii. **Regional context**: evidence of cryptic or distinct genetic diversity such that Bodie population represents an independent evolutionary entity compared to other sampled populations within the Bodie Hills and Sierra Nevada regions.
iv. **Local climate correlation**: support for a statistically significant association between regional temperature and/or precipitation variables with population genetic data.

Methods

Study organism

The American pika (*Ochotona princeps*) is a small, talus obligate, alpine lagomorph (~150 g) widely distributed in mountainous areas of the western United States and southwestern Canada. They are individually territorial, primarily asocial, and facultatively monogamous (Kleiman 1981; Smith and Ivins 1983; Peacock 1997). As lagomorphs, pikas do not hibernate but depend upon stored food caches (haypiles) to overwinter and differentially select plants for summer and winter diets (MacArthur and Wang 1973; Conner 1983; Smith and Ivins 1983; Huntly et al. 1986; Dearing 1997a, b; Aho et al. 1998). Pikas are thermally sensitive and depend upon the fairly consistent temperature of the talus interstitial environment to behaviorally thermoreuglate (Smith and Weston 1990). Winter snowpack acts to insulate pikas during the winter months and high summer temperatures are avoided by utilization of the talus interstitial spaces which serve as refugia due to more stable temperatures (Smith 1978; Smith et al. 2004; Kreuzer and Huntly 2003; Morrison and Hik 2007; Simpson et al. 2009; Ray et al. 2012).

Study site

Embedded within Great Basin sage-scrub habitat and isolated from the Sierra Nevada crest by approximately 35 km, the Bodie site (BSHP) is composed of ore dumps created by hard-rock mining during the late 19th and early 20th centuries (Fig.1). This anthropogenic habitat spans about 4 km north to south from Bodie Bluff south to Sugarloaf Mountain (a natural talus slope) and 1-1.5 km east to west (Fig. 1; Nichols 2011). Since at least the late 1900s, these artificial "talus" islands have provided suitable habitat for pikas.

Population census surveys

A subset of the total suitable habitat (~79 patches) was surveyed in 1972 (Smith 1974a), 1977 (Smith 1980), 1989 and 1991 (Smith and Gilpin 1997), 1992-2001, 2003-2006, and 2008-2016 (unpublished data, Nichols). Pika occupancy was determined by presence of fresh fecal pellets and/or active haypiles. The number of active territories per patch was estimated each year to characterize patch occupancy over time as well as provide a crude estimate of annual population size (Nichols 2011). Long-term survey data indicate that a steady decline in total patch occupancy has occurred since the mid-1940s when J.H. Severeid observed that "nearly every patch was occupied" (Severeid 1955; Fig. 3). The southern patch network initially crashed in 1998 and experienced a subsequent increase in occupancy in 2003 and 2004 (16% and 14.8%, respectively) before declining to 3.7% occupancy in 2005 and eventually complete extirpation by 2008. In 2016 limited recolonization was observed with five individuals found on the Red Cloud, a large patch with approximately 12 territories, and one individual at the Booker Consolidated patch, both in the southern patch network. The middle patch network has also experienced significant extirpation since the early 2000s, whereas the northern patch network has maintained consistently occupied patches.

Genetic Sample Collection

Early 20th century – Approximately 200 museum specimens collected during the mid-20th century by J.H. Severeid (1955) are curated in the mammal collections in the Museum of Vertebrate Zoology, University of California, Berkeley. We were given access to 16 specimens for tissue collection. Scissors were sterilized and forceps were submerged in a 10% bleach solution before being rinsed in water, 95% ethanol, and then subjected to a flame. A 2-5 mm square piece of dried museum pelt was cut from the ventral lip and placed in a 1.5 ml locking tube. Fourteen of these specimens were sacrificed between 1948 and 1949 through live-trapping or by gunshot and two specimens were born to wild-caught parents as the F_1 generation and maintained at UC Berkeley as part of a long-term captivity experiment from 1948 to 1951 (Severeid 1955) (Table 3).

Late-20^th^ century – Pika ear tissue was collected in BSHP as part of a metapopulation dynamics study (1988-1990; Peacock and Smith 1997b). After completion of the study the remaining DNA samples were stored in a -20°C freezer until present. A total of 115 of these preserved samples had sufficient DNA yields to be included in the present study, with the majority of those samples collected during the 1988 summer field season.

21^st^ century – Fecal DNA samples were collected from this site during June-September 2014 and 2015. Fresh fecal pellets identified using methods of Nichols (2011) were collected using sterilized tweezers (dipped in 70% ethanol between samples) and 2-5 pellets transferred to a 2.0 ml vial with 500 µl of ATL tissue lysis buffer (DNeasy96 blood and tissue kits, QIAGEN) in the field. 400 µl of supernatant from each sample were then transferred to ice-cold proteinase K for storage until DNA extraction. For herbivores, such as the pika, the ideal method for DNA extraction of fecal pellets uses only the outermost shell of the pellets which contain sloughed intestinal epithelial cells to optimize DNA yield and reduce PCR inhibitors (QIAGEN silica-binding extraction kits) (Waits and Paetkau 2005).

DNA isolation and amplification

Total genomic DNA was isolated using according to the manufacturer's protocol (DNeasy96 blood and tissue kits, QIAGEN) with minor alterations specific to each sample type. All DNA extractions and polymerase chain reactions (PCR) for early 20th century museum samples were conducted in a separate laboratory and building at the University of Nevada, Reno. Fourteen di, tri, and tetranucleotide repeat, polymorphic microsatellite loci have been developed for the American pika (Peacock et al. 2002; Peacock and Kirchoff 2009, unpublished data – Genbank accession numbers GQ461714.1-461723.1). All DNA samples were amplified with 10 polymorphic co-dominant nuclear markers after initial PCR optimization for all sample types (Table 4). PCR reactions were completed on a MBS Satellite 0.2G thermal cycler (Thermo Electron Corporation), and included a 15 minute hot start at 95°C, followed by 41 cycles of 95°C for 30 seconds, a touch down annealing temperature of 65°C to 55°C for 90 seconds and an elongation step at 72°C for 30 seconds. Seven cycles with touch down annealing temperatures of 65°C, 61°C, and 58°C each were completed, followed by 20 cycles at 55°C. The first 21 cycles amplified the locus specific primer and the final 20 cycles added a fluorescently labeled M13 tail to the PCR product. All PCR products were dilution to an appropriate concentration and 1 μl of this PCR product was transferred to 19 μl of HiDye Formamide/LIZ500 size standard (Applied Biosystems). Fragment size analysis was carried out on an Applied Biosystems 3730 DNA Genetic Analyzer at the UNR Nevada Genomics Center (http://www.ag.unr.edu/genomics/) and alleles were scored, binned, and genotyped using ABI Prism GeneScan (version 3.5.1) and GeneMapper (version 3.7) software. Details regarding modifications to DNA extraction protocol for fecal and museum genetic samples can be found in Supplementary materials (Online Resource 1).

Calculation of allelic dropout and genotyping error rates for fecal and museum samples

DNA from fecal and museum samples can lead to genotyping errors which may bias population genetic metric estimates (Murphy et al. 2000; Creel et al. 2003; McKelvey and Schwartz 2005). Common genotyping errors include allelic dropout, preferential allele amplification, and false alleles. For fecal samples, each individual was genotyped 2-4 times at all loci using separate PCR reactions. Individuals were included in genetic analyses only if a consensus genotype was reached at each locus through repeated, successful amplification ($\bar{X}$ = 2 PCR reactions/individual/locus). For museum samples, DNA was amplified in five separate PCR reactions for all loci. Individuals were included in genetic analyses only if a consensus genotype was reached at each locus for three of the five PCR reactions.

Locus-specific error rates for allelic dropout (ε_1), false alleles (ε_2), and genotyping success rate were estimated with confidence intervals using replicate genotypes per historical dataset (n=2 for fecal DNA, n=5 for museum specimens) through a maximum-likelihood based approach (program Pedant, Delphi version 7.0) (Johnson and Hayden, 2007). In addition, the programs Micro-Checker (version 2.2.3; Van Oosterhout et al. 2004) and FreeNA (Chapuis and Estoup 2007) were used to test for the presence of null alleles, and genotyping errors.

Characterization of genetic variation and population structure

To assess changes in genetic diversity over time in relation to climate – temperature, precipitation and genetic data were analyzed separately for each time period: early 20th century (1948-1949), late 20th century (1988-1990), and early 21st century (2014-2015). FSTAT (version 2.9.3.2; Goudet 2001) was used to quantify the number of alleles (N_A), to test for linkage disequilibrium and deviations from Hardy-Weinberg equilibrium (F_{IS}) per locus per population and to calculate a global θ_{ST} (Weir and Cockerham 1984; Weir 2012). If a locus exhibited systematic patterns of deviation from HWE or there was evidence of null alleles or allelic dropout it was removed from the analysis.

In order to correct for differences in sample size between the three sampling periods (early 20th century, late 20th century, and early 21st century), a hierarchical rarefaction approach was used in the program HP-RARE (version 1.0; Kalinowski 2000) to calculate estimates of allelic richness (Rs, Kalinowski 2005). The average observed (Ho) and expected (He) heterozygosities per locus per sampling era were calculated using Microsatellite Toolkit in Excel (Park 2001). Pairwise comparisons between the two historical and one modern datasets were conducted in R (R Core Development Team, 2016) using either a Welch two sample t-test or a Wilcoxon rank sum test if data were non-normal (Rubidge et al. 2012).

An analysis of molecular variance (AMOVA) was conducted in GenAlEx (6.501; Peakall and Smouse, 2006; 2012) to assess the partitioning of genetic variation for each time period. Principal coordinate analysis (PCoA, GenAlEx 6.5), Bayesian genotype clustering analysis (STRUCTURE version 2.3.4; Pritchard et al. 2000), and Euclidean hierarchical distance trees using data for each individual genotyped

(SYSTAT 8.0; SPSS 1988, IBM Corp., 2013) were used to test for population genetic structure. An admixture model was specified in STRUCTURE wherein novel genotypes can be identified and assigned to individual genotype clusters under the assumptions of HWE and gametic phase equilibrium. A 100,000 iteration burn-in followed by five 1,000,000 Markov Chain Monte Carlo (MCMC) replicates per genotype cluster (k) for k=1-10 were used to estimate posterior allelic distributions against which individual genotypes were compared and assigned to a genotype cluster. The limited sample for the mid-20th century period precluded including them in the model-based clustering methods. For the late 20th century and early 21st century periods, four replicate runs were conducted under the admixture model for k=1 to k=10, and alternating between designating correlated and uncorrelated allele frequencies, the allele frequencies were drawn independently from a Dirichlet distribution (Gilbert et al. 2012). To determine the optimal number of genotype clusters, the Δk method of Evanno et al. (2005) was used and visualized in STRUCTURE HARVESTER (version 0.6.94).

Effective population size

Effective population size (Ne) was estimated for each time period u sing the linkage disequilibrium module based on a single point sample in NeEstimator (version 1.3; Peel et al. 2004). This method is considered more appropriate for microsatellite data than the moment-based temporal method (Waples and Do 2010).

Local climate association with occupancy patterns and genetic variation

We used a canonical correlation analysis (CCA) to test whether observed reductions in genetic diversity were related to local climate conditions (Sherry and Henson 2005; Sork et al. 2010). First, we created multivariate genetic variables following Grivet et al. (2008) by transforming single locus genotypes into allelic variables. A score of 0, 0.5, or 1 was assigned to an individual based on whether a homozygous or heterozygous allele was observed at that locus (Westfall and Conkle 1992). Therefore, the number of single variables generated per locus was the number of alleles minus one, for a total of 57 allelic variables. A principal component analysis (PCA) was performed to reduce the 57 allelic variables into a smaller set of orthogonal axes.

Although there is a weather station located in Bodie, CA (https://climate.usurf.usu.edu/mapGUI/mapGUI.php; WRCC, 2013), the record contains numerous gaps during the three time periods for which we have genetic data (1946-1949, 1985-1988, and 2012-2015). Instead, temperature and precipitation data were downloaded from the United States Climate Divisional Database (https://www.ncdc.noaa.gov/cag/time-series/us). Bodie is near the border between California's Climate Division 3 (Northeast Interior Basins; CD3), and Climate Division 7 (Southeast Desert Basins; CD7), using linear regression we compared monthly temperature and precipitation from each climate division to 44 years of data from the Bodie weather station. On average, CD3 was more highly correlated to the Bodie weather station data for monthly precipitation (CD3, R^2= 50.12% vs. CD7, R^2=35.13%). Both CD datasets were correlated in terms of monthly mean temperature, however CD3 remained a better match with local conditions in Bodie overall (CD3, R^2=72.42% CD7, R^2=65.52%).

Three climate variables were selected based on known physiological thresholds of *O. princeps*: mean annual precipitation (MAP) for each water year (October to September for all four years), average maximum temperature in July (AVG TMAX), and average minimum temperature in January (AVG TMIN). These three metrics were calculated across 120 total years and checked for multicollinearity with no metric having an R^2 above 0.024. Using the Canonical Correlation Analysis (CCorA) in both XLSTAT (2017) and SPSS, we tested for correlation between multivariate genotypic scores using factor scores from the first 20 axes and three linear climate variables calculated from the CD3 data (1946-1949, 1985-1988, and 2012-2015).

Regional genetic comparisons

Fecal DNA sampling was expanded to include additional sites within the eastern central Sierra and Bodie Hills regions in order to determine whether the extant genetic variation in the BSHP population was distinct within the region. Fecal DNA was collected by K. Klingler (Bodie State Historic Park [BOD]; Harvey Monroe Hall Research Natural Area, Mono County, CA [HMH]; New York Hill/ Masonic Mining Site [MAS]) and C. Millar (Ellery Lake, Conway Uplands/ Benjamin Butte E, Bennettville Mine, N. Warner Mountains [SNE], Masonic Mountain West [BMN]). A principal coordinates analysis was conducted in GenAlEx to examine regional genetic structure across sampled populations using the associated genetic data.

Results

A total of 238 individuals were successfully genotyped from the BSHP mining district (1949, n=13; 1988, n=115; 2015, n=110; Fig. 2). For regional comparisons, after repeated amplification and genotyping fecal DNA samples, per site sample sizes were as follows: BMN, n=2, MAS, n=6, SNE n=7 and HMH, n=86.

Allelic dropout and genotyping error rates

There were no systematic deviations from HWE across loci or time periods. However, there was evidence of significant deviation from HWE at two loci in the late 20th century population, and at one locus in the mid-20th century population, therefore FreeNA was used to estimate the frequency of null alleles and their concomitant influence on estimates of genetic differentiation. The FreeNA corrected datasets provided similar estimates of pairwise genetic differentiation (F_{ST}) as the uncorrected data, which suggest the data are robust to genotyping errors despite differing sample types and associated levels of DNA degradation. The FreeNA-corrected values were used for both historical datasets (late 20th and mid-20th century samples). Finally, significant linkage disequilibrium among pairs of loci was detected across two of the three time periods (late 20th and early 21st century samples), however, these pairwise patterns varied and no locus exhibited systemic patterns.

The early 21st century fecal DNA samples had an amplification success rate of 95.6% with average rates of allelic dropout and false alleles at 3.4% and 1.5%, respectively (Table 1). Museum specimen samples from 1949-1950 displayed a lower amplification success rate, which was expected considering age and degradation of samples, whereas rates of allelic dropout (3.5%) and false alleles (3.9%) were similar to extant fecal DNA samples. The ear tissue samples collected during 1988-1990 were not repeatedly amplified due to high quality nature of these samples so rates of allelic dropout and false alleles were not calculated.

Effective population size and inbreeding

In the mid-20th century, the effective population size (Ne) was estimated at 35 individuals (95% CI 19.4-50.8), which increased to 50 individuals in 1988 (95% CI 9.2-91.8) and declined to 17.2 in 2015 (95% CI 2.9-31.5) (Table 2). Although there were significant F_{IS} values for individual loci within years, the overall F_{IS} values per year (0.055 in 1949, 0.108 in 1988 and -0.098 in 2015) were not significant (Table 2, Table 5; p=0.00185 based on 540 randomizations).

Genetic diversity and population genetic structure

Average allelic richness has significantly declined over the last 65 years (A_{R1949} = 3.92; A_{R1988}=3.65; A_{R2015}=3.25; 1949 vs. 2015, t=1.866, d.f. =16, p=0.080; Fig. 4; Table 2). A similar decline was observed in private allelic richness (the number of unique alleles in a population), especially between the mid and late 20th century sampling periods. Although observed heterozygosity has declined since the mid-20th century, estimates were not significantly different between the late-20th and early-21st samples (Ho_{1949}=0.621; Ho_{1988}=0.544; Ho_{2015}=0.607; K-W test statistic=2.374, p=0.305). However, the reduction in within sample gene diversity (Hs) between the oldest sample

(1949) and the extant population (2015) was statistically significant (Hs_{2015}= 0.552, SE± 0.058; Hs_{1949}=0.657, SE±0.037; $W_{2015-1949}$=18, p=0.050) (Fig. 4). The majority of microsatellite loci (n=6) lost at least one allele at some point during the last 65 years. However, 12 new allelic variants were also recorded in 1988, which could be due to either mutation, migration, or sampling error associated with the difference in sample size. Over the past 25 years when sample sizes were comparable, eight alleles have been lost while seven new allelic variants were observed (Fig. 4). Although corrected for significant differences in sample size, it is likely that greater sampling from the museum specimens may enable a more comprehensive understanding of the fine-scale allelic patterns that occurred in 1949.

Consistent with the collapse of the middle and southern patch networks and continuing population decline in the northern patch network over the last 65 years, greater genetic subdivision has occurred within the remaining occupied patches. Global estimates of genetic structure (θ_{ST}) show an increase in population genetic structure over the 20th and 21st centuries (1949, θst=-0.007, 95% CI: -0.131-0.124; 1988, θst=0.024, 95% CI: 0.008-0.037; 2015, θst=0.091, 95% CI: 0.062-0.125). Principal coordinate analysis (PCoA), Bayesisan genotype clustering and Euclidean hierarchical distance trees also support increased population genetic structure over time (Figs. 5, 6). The 1988 hierarchical tree topology shows fewer clades than the 2015 tree (Fig. 5). Correspondingly, the PCoA and Bayesian genotype clustering results show no per patch structuring in 1988, however in 2015 we begin to see clustering among groups of proximate patches (Figs. 4, 5). Two genotype clusters (*k*=2) was the best fit of the 1988 data (LnP (D) =-2517.32, SD±0.878; Δk=18.369; Fig 5.). In contrast, the best fit for the extant population (2015) was five genetic clusters (k=5) (LnP (D) =-1712.36, SD±13.913; ΔK=28.582; Fig. 6). AMOVA comparisons were made only between 1988 and 2015 due to the limited sample size from the museum specimens. There was greater genetic partitioning among-populations (15%) in 2015 (PhiPT=0.248, p<0.001) compared to 30 years ago when among-population variance only constituted 2% (Fig. 6). Relatedness among individuals also increased between 1988 (r=0.042) and 2015 (r=0.217).

Local climate changes

This region has experienced significantly warming temperatures over the past 120 years (AVG TMAX ($F_{1, 121}$=7.58, p=0.007) and AVG TMIN ($F_{1, 121}$=5.568, p=0.020). However, MAP has not decreased significantly ($F_{1,121}$=0.201, p=0.654).

Local climate association with genetic variation

The first 20 principal components accounted for 68.87% of the total observed genetic variation ranging from 7.28% for PC1 to 1.97% for PC20 and were used to represent the multivariate nuclear genotypes for the canonical correlation analysis. After reviewing the allele frequencies represented by each principal component, three rare alleles (Loc4-169, Loc4-181, and Loc10-192) were removed from further analysis because they were not evenly represented across all years and therefore could potentially bias the results by contributing too heavily to a particular principal component.

Correlation between genetic and climate data

A canonical correlation analysis using the three linear climate variables as predictors of the 20 multilocus genetic variables generated three functions with squared canonical correlations for each successive function of r^2_C = 0.665, 0.603, and 0.063. The third function explained less than 10% of the variance (r^2_C =6.3%), so we interpreted the first two functions only. The full model was statistically significant and the r^2 effect size was 0.876 (Wilk's λ=0.125 criterion; $F_{60, 657.19}$=11.0419, $p < 0.0001$; Table 6).

Based on the Function 1 squared structure coefficients ($F_{60,657.19}$ =11.0419, $p < 0.0001$), which indicate the amount of variance the observed variable contributes to the synthetic criterion variable, the only relevant criterion variable was PC2 (r^2=64.00). PC2 also had larger canonical function coefficients compared to the other principal components ($PC2_{coeff}$= -0.80) (Table 7). For Function 1, mean annual precipitation (MAP) (r^2=96.2%) was the dominant predictor and AVG TMAX the second (r^2=80.2%). These two predictor variables exhibited similar canonical function coefficients (MAP_{coeff}=-0.609, AVG $TMAX_{coeff}$= -2.275) with AVG TMIN (AVG $TMIN_{coeff}$=0.99). Furthermore, with the exception of MAP, all of the climate variables' structure coefficients (r_s) exhibited the same sign (i.e. negative) indicating that they are positively related to one another whereas MAP is inversely related to the other climate variables (i.e. AVG TMIN, and AVG TMAX) (Table 8). Since the structure coefficient for PC2 was negative, it was positively related to the temperature variables but inversely related to MAP.

The only criterion variable of relevance for Function 2 ($F_{33,442}$=7.42, $p<0.0001$) was PC3 (r^2=64.65%), which also had a larger canonical function coefficient compared to the other principal components ($PC3_{coeff}$= -0.804) (Table 8). For Function 2, average minimum temperature in January (AVG TMIN) (r^2=79.3%) was the dominant predictor with a relatively modest canonical function coefficient (AVG $TMIN_{coeff}$= -0.085). Again, the structure coefficients indicate that AVG TMAX and AVG TMIN are positively related to one another whereas MAP is inversely related to these temperature variables. Finally, since PC3 is negative, it was positively related to MAP and inversely related to AVG TMAX and AVG TMIN for Function 2 (Table 9).

Regional genetic comparisons

Pairwise F_{ST} estimates among populations within the Bodie Hills and among populations within the main Sierra Nevada range were not differentiated (p=0.005, 200 permutations). However the Bodie population was significantly differentiated from both the Harvey Monroe Hall Natural Area and other Sierra Nevada populations. Masonic Mountain West, also in the Bodie Hills, was significantly differentiated from Harvey Monroe Hall Natural Area (Appendix, Table 9). Small sample sizes and incomplete genotypes precluded assessing the significance of the F_{ST} estimates between Bodie and the Masonic Mountain site (n=6). In PCA space, however, the Bodie population is distinct from all other populations (Fig. 7).

Discussion

We combined environmental and genetic data to examine changes in extirpation risk under a climate change hypothesis for a pika metapopulation repeatedly censused over the 20th and 21st centuries. Despite evidence of an active metapopulation dynamic in the northern patch network, long-term census surveys have documented the collapse of the middle and southern patch complexes over the last 40 years (Moilanen et al. 1998). Our results also show a consistent decline in genetic diversity in the BSHP pika population since the mid-20th century as evidenced by reductions in allelic diversity and observed heterozygosity. Additionally, this decline in patch occupancy and reduction in population size have resulted in lower Ne estimates.

Habitat availability and connectivity are influential factors mediating interactions between organisms and climate (Stewart et al. 2015; Schwalm et al. 2016; Fourcade et al. 2017; Inoue et al. 2017; Ofori et al. 2017). Changes in movement patterns within the Bodie habitat patch network are evident as the genetic variation in this pika population is partitioned more significantly at the among-patch level now rather than within patch subpopulations as it was ~30 years ago (Peacock and Smith 1997b). These results suggest that occupied patches in Bodie are becoming more isolated due to lack of either initiated or successful dispersal among patches. It is possible that the climate buffering utility provided by habitat-specific microclimates will diminish over time if historical climate thresholds are surpassed and the substrate on individual patches no longer effectively mediates ambient conditions. This reduction in habitat quality would likely result in greater mortality and less movement among patches as offspring of remaining individuals would not need to risk dispersing from their natal patch to find available territories.

We found support for a statistically significant association between regional temperature and precipitation and multivariate genetic variation within this pika metapopulation. The dominant climate predictors associated with multivariate genetic diversity were related to both precipitation and temperature inputs. However, because the PCs represent varying frequencies of associated alleles, the directionality of the relationships with the climate predictors across the two functions also differed. In general, MAP was always inversely related to both temperature metrics, which suggests that these two types of climate inputs have distinct effects on genetic variation. During the last 120 years, this region has experienced significant warming temperatures in terms of both average TMAX and TMIN, however in contrast to our predictions, MAP has not decreased significantly although it is not possible to separate the relative influences of rainfall from snowfall for this metric.

While our three "snapshots" during the last 65 years are fairly indicative of the long-term warming trend within this region, the severe drought that recently occurred in California (Griffin and Anchukaitis, 2014; Diffenbaugh et al. 2015; Belmecheri et al. 2016) during the 2012-2015 sampling period represents an extreme outlier and may have increased the relative importance of MAP in our analysis. That being said, although the trend is not statistically significant, it is certainly negative, such that total annual precipitation has declined by approximately 1.78 mm per decade. Concomitantly, average maximum and minimum temperatures have all significantly increased by 0.1°C per decade since 1895. These patterns of warming temperatures are similar to state-wide rates of increase, however the decline in precipitation that has occurred in the Northeast Interior Basin is in significant contrast to the 0.51 mm increases across the rest of California (NOAA 2017).

Increasing physiological costs associated with changing temperature and precipitation patterns may translate into population-level declines in genetic diversity and gene flow over time via a number of possible pathways: (i) restricted diurnal foraging due to acute and chronic heat stress leading to reduced quality of forage consumed immediate and delayed (haypiles), and increasing likelihood of overwinter mortality; (ii) greater availability of unoccupied territories on natal patch due to high overwinter mortality which results in a lack gene flow among patches; and (iii) lack of long distance dispersal and gene flow among neighboring pika populations due to unsuitable dispersal conditions resulting in increased genetic drift and limited opportunity for the introduction of novel genetic variants. In addition to genetic and dispersal consequences, climate change may also indirectly impact pikas through greater exposure to the expanding distributions of novel disease vectors (e.g. plague), potential terrestrial predators (e.g. western rattlesnake, *Crotalus viridis*), and competitors for forage and habitat (e.g. Bushy-tailed woodrat, *Neotoma cinerea* or Yellow-bellied marmot (*Marmota flavivientris*) (Lowell et al. 2015; Danforth et al. 2016; Ray et al. 2016; Foley et al. 2017).

Population resilience and the role of neutral genetic variation in species' persistence

Predictions of future pika distribution using species distribution models are most reliable when they incorporate metrics such as functional connectivity and predicted surface activity time, which are highly variable across the Intermountain West, but are also associated with pika population persistence (Mathewson et al. 2016; Schwalm et al. 2016). It has become clear that the relationship between pika occurrence and climate is non-linear due to factors such as habitat configuration (Schwalm et al. 2016), rock-ice-features (Shinderman 2015; Millar and Westfall 2010), functional buffering quality of substrate type, and even phenotypic variation (Hall and Bowlus 1938; Moyer-Horner et al. 2015).

Population-level responses to local environmental conditions will ultimately determine the ability of a species to persist across its range (Davis and Shaw 2001; Yang et al. 2011; Pandit et al. 2017; Salo et al. 2017). Populations on the edge of their species range are often considered to be theoretically less capable of mounting an evolutionary response due to limited genetic variation, stressful environments, and smaller population sizes (Rogers and Peacock 2012; Bouchard et al. 2017; Ciborowski et al. 2017; Pierce et al. 2017). However, multiple studies have shown that persistence on the edge is fairly common especially when extirpation across more central parts of the range is occurring due to extrinsic factors such as naiveté to a novel disease agent (Lomolino and Channell 1995; 1998; Channell and Lomolino 2000; Gibson et al. 2009). It is also possible that peripheral populations may be more likely to harbor dispersal genotypes that are capable of colonizing new habitats because they occur in habitats similar to those just beyond the current range (Leonarduzzi et al. 2016).

Previous research investigating population genetic structure in low-elevation sites at the edge of their bioclimatic envelope in the Columbia River Gorge found that pika populations were characterized by relatively low allelic richness and heterozygosity with very little evidence of active gene flow (Robson et al. 2016). However, here we show that despite being an isolated peripheral population that is genetically differentiated from other populations in the region, the Bodie population has maintained unique allelic variants. Acknowledging that neutral genetic diversity is not directly correlated with variation at adaptive loci, this result is at least suggestive of potential adaptive differences. It is highly likely that levels of intraspecific and even subspecific lineage diversity may differ significantly between pika populations in the range core compared to sites that occur on the trailing edge or range peripheries (Lesica and Allendorf 1995; Durka 1999; Eckert et al. 2008; Robson et al. 2016; Waterhouse et al. 2017). The evidence that the genetic structure of this metapopulation is influenced by local climate, perhaps through selection on whole genotypes rather than single loci, suggests that there may be opportunity for population-level resilience through adaptation to future conditions (Sork et al. 2010). The possibility of local adaptation in these edge populations may mean that preservation of this unique genetic diversity in peripheral populations is important for species-wide adaptation potential (Leonarduzzi et al. 2016; Robson et al. 2016; David and Wright 2017).

Conclusions

Although pikas have experienced repeated fragmentation and isolation during previous periods of climate change, the current rates of warming may preclude adaptive responses (Grayson 2005; Galbreath et al. 2009; 2010). While regional cohesion has been maintained due to admixture during interglacial periods, Holocene fragmentation has generated significant genetic differentiation among populations and regions with a potential increase in extirpation risk (Galbreath et al. 2009; 2010; Robson et al. 2016). Indeed over the past 25-30 years coincident with warming temperatures, the incidence of extirpations across the species range especially in the Great Basin and within the Bodie Hills region in particular have been pronounced (Beever et al. 2003; Stewart et al. 2015; Nichols et al. 2016).

Projections suggest that in the Sierra Nevada and Great Basin, temperatures will continue to warm significantly especially during the summer season and even under lowemission scenarios (+2°C by 2100; IPCC, 2014) and severe snow losses are likely (Cayan et al. 2008; Jepsen et al. 2016; Safeeq et al. 2016). The sudden extirpation of the New York Hill pika population, located approximately 20 km from BSHP, reveals the stochastic nature of metapopulation dynamics and the potential vulnerability of these populations due to unpredictable factors (e.g. new predator pressure, extreme weather events, habitat competition with other species) especially after long-term isolation and the lack of opportunities for re-colonization and gene flow (Nichols et al. 2016). Nevertheless, variation in the realized niche for this species across its distribution suggests a range of responses to climate change are possible with both emergence of refugia in addition to extirpations (Schwalm et al. 2016).

Although the BSHP pika population does not appear to differentiated from other sampled populations within the Bodie Hills, this population is significantly differentiated from the Sierra Nevada populations and harbors unique allelic variation. Preserving intraspecific diversity at the population level is likely to improve the outlook for the species as a whole and better reflect its ancestral history and future evolutionary potential. The continued loss of genetic diversity through population extirpation or long-term genetic erosion in small, isolated populations may represent a significant evolutionary cost at the species level by reducing its capacity to respond to ongoing environmental change. This study is the first to characterize changing patterns of population genetic variation and structure in a natural metapopulation of the American pika across multiple decades. While these population-specific results have been informative, this study is limited by a lack of replication. We also have no knowledge of genetic differences or their significance among populations at adaptive trait loci. Future efforts to assess the variation in genetic diversity between core and peripheral populations of the American pika and across all five subspecies will strengthen our understanding of the adaptive capacity of this species.

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References

AghaKouchak A, Cheng L, Mazdiyasni O, Farahmand A (2014) Global warming and changes in risk of concurrent climate extremes: Insights from the 2014 California drought. Geophys Res Lett 41:8847–8852. doi: 10.1002/2014GL062308

Aho K, Huntly N, Moen J, Oksanen T (1998) Pikas (*Ochotona princeps*: Lagomorpha) as allogenic engineers in an alpine ecosystem. Oecologia 114:405–409. doi: 10.1007/s004420050463

Alsos IG, Ehrich D, Thuiller W, et al. (2012) Genetic consequences of climate change for northern plants. Proceedings Biol Sci 279:2042–51. doi: 10.1098/rspb.2011.2363

Arnold AE, Andersen EM, Taylor MJ, Steidl RJ (2017) Using cytochrome b to identify nests and museum specimens of cryptic songbirds. Conserv Genet Resour 9:451–458. doi: 10.1007/s12686-016-0680-2

Bálint M, Domisch S, Engelhardt CHM, et al. (2011) Cryptic biodiversity loss linked to global climate change. Nat Clim Chang 1:313–318. doi: 10.1038/nclimate1191

Beatty GE, Reid N, Provan J (2014) Retrospective genetic monitoring of the threatened Yellow marsh saxifrage (*Saxifraga hirculus*) reveals genetic erosion but provides valuable insights for conservation strategies. Divers Distrib 20:529–537. doi: 10.1111/ddi.12158

Beever EA, Brussard PF, Berger J (2003) Patterns of apparent extirpation among isolated populations of pikas (*Ochotona princeps*) in the Great Basin. J Mammal 84:37–54. doi: 10.1644/1545-1542(2003)084<0037:POAEAI>2.0.CO;2

Beever EA, Ray C, Mote PW, Wilkening JL (2010) Testing alternative models of climate-mediated extirpations. Ecol Appl 20:164–178. doi: 10.1890/08-1011.1

Beever E, Ray C, Wilkening J (2011) Contemporary climate change alters the pace and drivers of extinction. Glob Chang 17: 2054–2070. doi:10.1111/j.1365-2486.2010.02389.x

Beever EA, Perrine JD, Rickman T, et al. (2016) Pika (*Ochotona princeps*) losses from two isolated regions reflect temperature and water balance, but reflect habitat area in a mainland region. J Mammal 97:1495–1511. doi: 10.1093/jmammal/gyw128

Bellard C, Bertelsmeier C, Leadley P, et al. (2012) Impacts of climate change on the future of biodiversity. Ecol Lett 15:365–377. doi: 10.1111/j.1461-0248.2011.01736.x

Belmecheri S, Babst F, Wahl ER, et al. (2015) Multi-century evaluation of Sierra Nevada snowpack. Nat Clim Chang 6:2–3. doi: 10.1038/nclimate2809

Botkin DB, Saxe H, Araújo MB, et al. (2007) Forecasting the effects of global warming on biodiversity. Bioscience 57:227–236. doi: 10.1641/B570306

Bouchard JR, Fernando DD, Bailey SW, et al. (2017) Contrasting patterns of genetic variation in central and peripheral populations of *Dryopteris fragrans* (Fragrant wood fern) and implications for colonization dynamics and conservation. Int J Plant Sci 178:607–617. doi: 10.1086/693109

Cahill AE, Aiello-Lammens ME, Fisher-Reid MC, et al. (2012) How does climate change cause extinction? Proc R Soc B Biol Sci 280:20121890–20121890. doi: 10.1098/rspb.2012.1890

Calkins MT, Beever EA, Boykin KG, et al. (2012) Not-so-splendid isolation: modeling climate-mediated range collapse of a montane mammal *Ochotona princeps* across numerous ecoregions. Ecography (Cop) 35:780–791. doi: 10.1111/j.1600-0587.2011.07227.x

Callaghan T V., Björn LO, Chernov Y, et al. (2004) Responses to projected changes in climate and UV-B at the species Level. AMBIO A J Hum Environ 33:418–435. doi: 10.1579/0044-7447-33.7.418

Cayan DR, Maurer EP, Dettinger MD, et al. (2007) Climate change scenarios for the California region. Clim Change 87 (Suppl 1): 21. doi: 10.1007/s10584-007-9377-6

Channell R, Lomolino M V. (2000) Dynamic biogeography and conservation of endangered species. Nature 403:84–86. doi: 10.1038/47487

Chapuis MP, Estoup A (2007) Microsatellite null alleles and estimation of population differentiation. Mol Biol Evol 24:621–631 . doi: 10.1093/molbev/msl191

Chen I-C, Hill JK, Ohlemüller R, et al. (2011) Rapid range shifts of species associated with high levels of climate warming. Science 333:1024–6 . doi: 10.1126/science.1206432

Ciborowski K, Jordan WC, Garcia de Leaniz C, Consuegra S (2017) Temporal and spatial instability in neutral and adaptive (MHC) genetic variation in marginal salmon populations. Sci Rep 7:42416. doi: 10.1038/srep42416

Cobben MMP, Verboom J, Opdam PFM, et al. (2011) Projected climate change causes loss and redistribution of genetic diversity in a model metapopulation of a medium-good disperser. Ecography (Cop) 34:920–932. doi: 10.1111/j.1600-0587.2011.06713.x

Conner DA (1983) Response of pikas to playback of geographic variations of the short call. J Mammal 64:336–337. doi: 10.2307/1380573

Cossu P, Scarpa F, Dedola GL, et al. (2017) Surviving at the edge of a fragmented range: patterns of genetic diversity in isolated populations of the endangered giant Mediterranean limpet (*Patella ferruginea*). Mar Biol 164:41. doi: 10.1007/s00227-017-3080-6

Creel S, Spong G, Sands JL, et al. (2003) Population size estimation in Yellowstone wolves with error-prone noninvasive microsatellite genotypes. Mol Ecol 12:2003–2009. doi: 10.1046/j.1365-294X.2003.01868.x

Danforth M, Novak M, Petersen J, et al. (2016) Investigation of and response to 2 plague cases, Yosemite National Park, California, USA, 2015. Emerg Infect Dis 22:2045. doi: 10.3201/eid2212.160560

David SR, Wright JJ (2017) Genetic variation and biogeography of the spotted gar *Lepisosteus oculatus* from core and peripheral populations. J Exp Zool Part B Mol Dev Evol 328:596–606. doi: 10.1002/jez.b.22772

Davis M, Shaw R (2001) Range shifts and adaptive responses to Quaternary climate change. Science 292 (5517): 673-679. doi: 10.1126/science.292.5517.673

Dearing M (1997a) The function of haypiles of pikas (*Ochotona princeps*). J Mammal 78 (4): 1156-1163. doi: 10.2307/1383058

Dearing MD (1997b) The maniuplation of plant toxins by a food-hoarding herbivore, *Ochotona princeps*. Ecology 78:774–781. doi: 10.1890/0012-9658(1997)078[0774:TMOPTB]2.0.CO;2

Diffenbaugh NS, Swain DL, Touma D (2015) Anthropogenic warming has increased drought risk in California. Proc Natl Acad Sci U S A 112:3931–6. doi: 10.1073/pnas.1422385112

Durka W (1999) Genetic diversity in peripheral and subcentral populations of *Corrigiola litoralis* L. (Illecebraceae). Heredity (Edinb) 83:476–484. doi: 10.1038/sj.hdy.6886000

Eckert CG, Samis KE, Lougheed SC (2008) Genetic variation across species' geographical ranges: the central–marginal hypothesis and beyond. Mol Ecol 17:1170–1188. doi: 10.1111/j.1365-294X.2007.03659.x

Erb LP, Ray C, Guralnick R (2011) On the generality of a climate-mediated shift in the distribution of the American pika (*Ochotona princeps*). Ecology 92:1730–1735. doi: 10.1890/11-0175.1

Evanno G., S. Regnaut , and J. GoudetJ. 2005. Detecting the number of clusters of individuals using the software STRUCTURE, a simulation study. Molecular Ecology 14:2611–2620.

Foley P, Roth T, Foley J, Ray C (2017) Rodent–Pika Parasite Spillover in Western North America. J Med Entomol 54:1251–1257. doi: 10.1093/jme/tjx085

Fourcade Y, Ranius T, Öckinger E (2017) Temperature drives abundance fluctuations, but spatial dynamics is constrained by landscape configuration: Implications for climate-driven range shift in a butterfly. J Anim Ecol 86:1339–1351. doi: 10.1111/1365-2656.12740

Frankham R (1996) Relationship of genetic variation to population size in wildlife. Conserv Biol 10:1500–1508. doi: 10.1046/j.1523-1739.1996.10061500.x

Frankham R, Ralls K (1998) Inbreeding leads to extinction. Nature 392:441–442. doi: 10.1038/33022

Frankham R, Ballou JD, Briscoe DA (2003) Introduction to Conservation Genetics. Cambridge University Press, Cambridge, UK.

Frankham R (2005) Genetics and extinction. Biol Conserv 126:131–140. doi: 10.1016/j.biocon.2005.05.002

Galbreath KE, Hafner DJ, Zamudio KR (2009) When cold is better: climate-driven elevation shifts yield complex patterns of diversification and demography in an alpine specialist (American pika, *Ochotona princeps*). Evolution (N Y) 63:2848–2863. doi: 10.1111/j.1558-5646.2009.00803.x

Galbreath KE, Hafner DJ, Zamudio KR, Agnew K (2010) Isolation and introgression in the Intermountain West: Contrasting gene genealogies reveal the complex biogeographic history of the American pika (*Ochotona princeps*). J Biogeogr 37:344–362. doi: 10.1111/j.1365-2699.2009.02201.x

Gibson SY, Van Der Marel RC, Starzomski BM (2009) Climate change and conservation of leading-edge peripheral populations. Conserv Biol 23:1369–1373. doi: 10.1111/j.1523-1739.2009.01375.x

Gilbert K, Andrew R, Bock D, et al. (2012) Recommendations for utilizing and reporting population genetic analyses: the reproducibility of genetic clustering using the program STRUCTURE. Mol Ecol 21:4925–4930

Gilman SE, Urban MC, Tewksbury J, et al. (2010) A framework for community interactions under climate change. Trends Ecol Evol 25:325–331. doi: 10.1016/j.tree.2010.03.002

Goswami P, Thirunavukkarasu S, Godhantaraman N, Munuswamy N (2014) Monitoring of genotoxicity in marine zooplankton induced by toxic metals in Ennore estuary, Southeast coast of India. Mar Pollut Bull 88:70–80. doi: 10.1016/J.MARPOLBUL.2014.09.025

Goudet J (1995) FSTAT, Version 1.2: a computer program to calculate F statistics. J. Hered. 86:485–486.

Grayson DK (2005) A brief history of Great Basin pikas. J Biogeogr 32:2103–2111. doi: 10.1111/j.1365-2699.2005.01341.x

Griffin D, Anchukaitis KJ (2014) How unusual is the 2012-2014 California drought? Geophys Res Lett 41:9017–9023. doi: 10.1002/2014GL062433

Grivet D, Sork V, Westfall R, Davis F (2008) Conserving the evolutionary potential of California valley oak (*Quercus lobata* Née): a multivariate genetic approach to conservation planning. Mol Ecol 17: 139-156. doi: 10.1111/j.1365-294X.2007.03498.x

Gutiérrez-Cánovas C, Millán A, Velasco J, et al. (2013) Contrasting effects of natural and anthropogenic stressors on beta diversity in river organisms. Glob Ecol Biogeogr 22:796–805. doi: 10.1111/geb.12060

Hafner D, Sullivan R (1995) Historical and ecological biogeography of Nearctic pikas (Lagomorpha: Ochotonidae). J Mammal 76: 302-321. doi: 10.2307/1382343

Hafner DJ, Smith AT (2010) Revision of the subspecies of the American pika, *Ochotona princeps* (Lagomorpha: Ochotonidae). J Mammal 91:401–417. doi: 10.1644/09-MAMM-A-277.1

Hall E, Bowlus H (1938) A New Pika (mammalian Genus Ochotona) from southeastern Idaho: with notes on near-by subspecies. University of California Publications in Zoologyo, 42: 335-339.

Hekkala, ER, Ardema, M, Montanari, S, Norell, M, and Amato, GA. (2017) Ancient DNA solves the mystery of the extinct horned crocodile from Madagascar. Integr. Comp. Biol. 57 E287-E287 Supplement: 1

Hoban S, Arntzen JA, Bruford MW, et al. (2014) Comparative evaluation of potential indicators and temporal sampling protocols for monitoring genetic erosion. Evol Appl 7:984–998. doi: 10.1111/eva.12197

Hof C, Levinsky I, Araújo MB, Rahbek C (2011) Rethinking species' ability to cope with rapid climate change. Glob Chang Biol 17:2987–2990. doi: 10.1111/j.1365-2486.2011.02418.x

Hotaling S, Finn DS, Giersch JJ, et al. (2017) Climate change and alpine stream biology: progress, challenges, and opportunities for the future. Biol Rev 92:2024–2045. doi: 10.1111/brv.12319

Hughes AR, Stachowicz JJ (2004) Genetic diversity enhances the resistance of a seagrass ecosystem to disturbance. Proc Natl Acad Sci 101:8998–9002. doi: 10.1073/pnas.0402642101

Hundsdoerfer AK, Päckert M, Kehlmaier C, et al. (2017) Museum archives revisited: Central Asiatic hawkmoths reveal exceptionally high late Pliocene species diversification (Lepidoptera, Sphingidae). Zool Scr 46:552–570. doi: 10.1111/zsc.12235

Huntly NJ, Smith AT, Ivins BL (1986) Foraging behavior of the pika (*Ochotona princeps*), with comparisons of grazing versus haying. Source J Mammal 67:139–148. doi: 10.1644/870.1.Key

IBM Corp. Released 2013. IBM SPSS Statistics for Windows, Version 22.0. Armonk, NY: IBM Corp.

Inostroza PA, Vera-Escalona I, Wicht A-J, et al. (2016) Anthropogenic stressors shape genetic structure: insights from a model freshwater population along a land use gradient. Environ Sci Technol 50:11346–11356. doi: 10.1021/acs.est.6b04629

Inoue K, Berg DJ (2017) Predicting the effects of climate change on population connectivity and genetic diversity of an imperiled freshwater mussel, *Cumberlandia monodonta* (Bivalvia: Margaritiferidae), in riverine systems. Glob Chang Biol 23:94–107. doi: 10.1111/gcb.13369

Jang JE, Kim JH, Kang JH, et al. Genetic diversity and genetic structure of the endangered Manchurian trout, *Brachymystax lenok tsinlingensis*, at its southern range margin: conservation implications for future restoration. Conserv Genet 18:1023–1036. doi: 10.1007/s10592-017-0953-7

Jepsen SM, Harmon TC, Meadows MW, Hunsaker CT (2016) Hydrogeologic influence on changes in snowmelt runoff with climate warming: Numerical experiments on a mid-elevation catchment in the Sierra Nevada, USA. J Hydrol 533:332–342. doi: 10.1016/J.JHYDROL.2015.12.010

Johnson PCD, Haydon DT (2007) Maximum-likelihood estimation of allelic dropout and false allele error rates from microsatellite genotypes in the absence of reference data. Genetics 175:827–842. doi: 10.1534/genetics.106.064618

Jump AS, Peñuelas J (2005) Running to stand still: adaptation and the response of plants to rapid climate change. Ecol Lett 8:1010–1020. doi: 10.1111/j.1461-0248.2005.00796.x

Kalinowski ST (2005) HP-RARE 1.0: A computer program for performing rarefaction on measures of allelic richness. Mol Ecol Notes 5:187–189. doi: 10.1111/j.1471-8286.2004.00845.x

Kiers ET, Palmer TM, Ives AR, et al. (2010) Mutualisms in a changing world: an evolutionary perspective. Ecol Lett 13:1459–1474. doi: 10.1111/j.1461-0248.2010.01538.x

Klank C, Ghazoul J, Pluess AR (2012) Genetic variation and plant performance in fragmented populations of globeflowers (*Trollius europaeus*) within agricultural landscapes. Conserv Genet 13:873–884. doi: 10.1007/s10592-012-0337-y

Kleiman DG (1981) Correlations among life history characteristics of mammalian species exhibiting two extreme forms of monogamy. In: Alexander RD, Tinkle DW (eds)Natural selection and social behavior, Chiron Press, New York, pp 332–344.

Knafler GJ, Ortiz-Catedral L, Jackson B, et al. (2016) Comparison of beak and feather disease virus prevalence and immunity-associated genetic diversity over time in an island population of red-crowned parakeets. Arch Virol 161:811–820. doi: 10.1007/s00705-015-2717-3

Kreuzer MP, Huntly NJ (2003) Habitat-specific demography: evidence for source-sink population structure in a mammal, the pika. Oecologia 134:343–349. doi: 10.1007/s00442-002-1145-8

Lambert DM, Shepherd LD, Huynen L, et al. (2009) The molecular ecology of the extinct New Zealand Huia. PLoS One 4:e8019. doi: 10.1371/journal.pone.0008019

Lamsal P, Kumar L, Atreya K, Pant KP (2017) Vulnerability and impacts of climate change on forest and freshwater wetland ecosystems in Nepal: A review. Ambio 46:915–930. doi: 10.1007/s13280-017-0923-9

Langwig KE, Voyles J, Wilber MQ, et al. (2015) Context-dependent conservation responses to emerging wildlife diseases. Front Ecol Environ 13:195–202. doi: 10.1890/140241

Lawson LP, Fessl B, Hernán Vargas F, et al. (2017) Slow motion extinction: inbreeding, introgression, and loss in the critically endangered mangrove finch (*Camarhynchus heliobates*). Conserv Genet 18:159–170. doi: 10.1007/s10592-016-0890-x

Leonarduzzi C, Piotti A, Spanu I, Vendramin GG (2016) Effective gene flow in a historically fragmented area at the southern edge of silver fir (*Abies alba* Mill.) distribution. Tree Genet Genomes 12:95. doi: 10.1007/s11295-016-1053-4

Lesica P, Allendorf FW (1995) When are peripheral populations valuable for conservation? Conserv Biol 9:753–760. doi: 10.1046/j.1523-1739.1995.09040753.x

Lima JS, Ballesteros-Mejia L, Lima-Ribeiro MS, Collevatti RG (2017) Climatic changes can drive the loss of genetic diversity in a Neotropical savanna tree species. Glob Chang Biol 23:4639–4650. doi: 10.1111/gcb.13685

Lomolino M, Channell R (1998) Range Collapse, re-Introductions, and biogeographic guidelines for conservation. Conserv Biol 12: 481–484. doi:10.1111/j.1523-1739.1998.97201.x

Lomolino M V, Channell R (1995) Splendid Isolation: patterns of geographic range collapse in endangered mammals. J Mammal 76:335–347. doi: 10.2307/1382345

Lowell JL, Antolin MF, Andersen GL, et al. (2015) Single-nucleotide polymorphisms reveal spatial diversity among clones of *Yersinia pestis* during plague outbreaks in Colorado and the western United States. Vector-Borne Zoonotic Dis 15:291–302. doi: 10.1089/vbz.2014.1714

Luo MF, Pan HJ, Liu ZJ, Li M (2012) Balancing selection and genetic drift at major histocompatibility complex class II genes in isolated populations of golden snub-nosed monkey (*Rhinopithecus roxellana*). BMC Evol Biol 12:207. doi: 10.1186/1471-2148-12-207

MacArthur R, Wang L (1973) Physiology of thermoregulation in the pika, *Ochotona princeps*. Can J Zool 51: 11-16. doi: 10.1139/z73-002

Marmesat E, Schmidt K, Saveljev AP, et al. (2017) Retention of functional variation despite extreme genomic erosion: MHC allelic repertoires in the Lynx genus. BMC Evol Biol 17:158. doi: 10.1186/s12862-017-1006-z

Mathewson PD, Moyer-Horner L, Beever EA, et al. (2017) Mechanistic variables can enhance predictive models of endotherm distributions: the American pika under current, past, and future climates. Glob Chang Biol 23:1048–1064. doi: 10.1111/gcb.13454

Matocq MD, Villablanca FX (2001) Low genetic diversity in an endangered species: recent or historical pattern? Biol Cons 98:61-68. doi: 10.1016/S0006-3207(00)00142-7

McKelvey K, Schwartz M (2005) Dropout: a program to identify problem loci and samples for noninvasive genetic samples in a capture-mark-recapture framework. Mol Ecol Notes 5: 716-718. doi: 10.1111/j.1471-8286.2005.01038.x

Meyers LA, Bull JJ (2002) Fighting change with change: adaptive variation in an uncertain world. Trends Ecol Evol 17:551–557. doi: 10.1016/S0169-5347(02)02633-2

Millar CI, Westfall RD (2010) Distribution and climatic relationships of the American pika (*Ochotona princeps*) in the Sierra Nevada and Western Great Basin, U.S.A.; periglacial landforms as refugia in warming climates. Reply. Arct Antarct Alp Res 42:493–496. doi: 10.1657/1938.4246.42.4.493

Moilanen A, Smith A, Hanski I (1998) Long-term dynamics in a metapopulation of the American pika. Am Nat 152: 530-542. doi: 10.1086/286188

Moritz C, Patton J, Conroy CJ, Parra JL, White GC, Beissinger SR (2008) Impact of a century of climate change on small-mammal communities in Yosemite National Park, USA. Science 322: 261-264. doi: 10.1126/science.1163428

Morrison SF, Hik DS (2007) Demographic analysis of a declining pika *Ochotona collaris* population: linking survival to broad-scale climate patterns via spring snowmelt patterns. J Anim Ecol 76:899–907. doi: 10.1111/j.1365-2656.2007.01276.x

Moyer-Horner L, Mathewson P (2015) Modeling behavioral thermoregulation in a climate change sentinel. Ecol Evol 5: 5810–5822. doi:10.1002/ece3.1848

Muhlfeld CC, Kovach RP, Al-Chokhachy R, et al. (2017) Legacy introductions and climatic variation explain spatiotemporal patterns of invasive hybridization in a native trout. Glob Chang Biol 23:4663–4674. doi: 10.1111/gcb.13681

Murphy MA, Waits LP, Kendall C (2000) Quantitative evaluation of fecal drying methods for brown bear DNA analysis. Wildlife Soc B 28:951–957.

Muthukrishnan R, Fong P (2014) Multiple anthropogenic stressors exert complex, interactive effects on a coral reef community. Coral Reefs 33:911–921. doi: 10.1007/s00338-014-1199-1

Nichols L (2011) Fecal pellets of American pikas (*Ochotona princeps*) provide a crude chronometer for dating patch occupancy. West North Am Nat 70: 500-507. doi: 10.3398/064.070.0410

Nichols LB, Klingler KB, Peacock MM (2016) American Pikas (*Ochotona princeps*) Extirpated from the historic Masonic mining district of Eastern California. West North Am Nat 76:163–171. doi: 10.3398/064.076.0203

NOAA National Centers for Environmental information, Climate at a Glance: U.S. Time Series, published August 2017, retrieved on August 8, 2017 from http://www.ncdc.noaa.gov/cag/

Ofori BY, Stow AJ, Baumgartner JB, Beaumont LJ (2017) Combining dispersal, landscape connectivity and habitat suitability to assess climate-induced changes in the distribution of Cunningham's skink, *Egernia cunninghami*. PLoS One 12:e0184193 . doi: 10.1371/journal.pone.0184193

Palstra FP, Ruzzante DE (2008) Genetic estimates of contemporary effective population size: what can they tell us about the importance of genetic stochasticity for wild population persistence? Mol Ecol 17:3428–3447. doi: 10.1111/j.1365-294X.2008.03842.x

Pandit SN, Maitland BM, Pandit LK, et al. (2017) Climate change risks, extinction debt, and conservation implications for a threatened freshwater fish: Carmine shiner (*Notropis percobromus*). Sci Total Environ 598:1–11. doi: 10.1016/J.SCITOTENV.2017.03.228

Park S (2001) Microsatellite toolkit for Excel, v.3.1. Trinity College. University of Dublin, Dublin.

Parmesan C (2006) Ecological and evolutionary responses to recent climate change. Annu Ecol Evol Syst 37:637–669. doi: 10.2307/annurev.ecolsys.37.091305.30000024

Pauls SU, Nowak C, Bálint M, Pfenninger M (2013) The impact of global climate change on genetic diversity within populations and species. Mol Ecol 22:925–946. doi: 10.1111/mec.12152

Peacock MM (1997) Determining natal dispersal patterns in a population of North American pikas (*Ochotona princeps*) using direct mark-resight and indirect genetic methods. Behav Ecol 8: 340-350. doi: 10.1093/beheco/8.3.340

Peacock MM, Smith AT (1997b) The effect of habitat fragmentation on dispersal patterns in a pika (*Ochotona princeps*) metapopulation. Oecologia 112:524–533. doi: 10.1007/s004420050

Peacock MM, Kirchoff VS, Merideth SJ (2002) Identification and characterization of nine polymorphic microsatellite loci in the North American pika, *Ochotona princeps*. Mol Ecol Notes 2: 360-362. doi: 10.1046/j.1471-8286.2002.00249.x

Peacock MM, Dochtermann NA (2012) Evolutionary potential but not extinction risk of Lahontan cutthroat trout (*Oncorhynchus clarkii henshawi*) is associated with stream characteristics. Can J Fish Aquat Sci 69:615–626. doi: 10.1139/f2012-006

Peacock MM, Gustin MS, Kirchoff VS, et al. (2016) Native fishes in the Truckee River: are in-stream structures and patterns of population genetic structure related? Sci Total Environ 563–564:221–236. doi: 10.1016/j.scitotenv.2016.04.056

Peacock MM, Hekkala ER, Kirchoff VS, Heki LG (2017) Return of a giant: DNA from archival museum samples helps to identify a unique cutthroat trout lineage formerly thought to be extinct. R Soc Open Sci 4:171253. doi: 10.1098/rsos.171253

Peacock MM, Smith AT (1997a) Nonrandom mating in pikas *Ochotona princeps*: evidence for inbreeding between individuals of intermediate relatedness. Mol Ecol 6:801–811. doi:10.1046/j.1365-294X.1997.00249.x

Peakall R, Smouse PE (2006) GENALEX 6: Genetic analysis in Excel. Population genetic software for teaching and research. Mol Ecol Notes 6:288–295. doi: 10.1111/j.1471-8286.2005.01155.x

Peakall R, Smouse PE (2012) GenAlEx 6.5: genetic analysis in Excel. Population genetic software for teaching and research--an update. Bioinformatics 28:2537–9. doi: 10.1093/bioinformatics/bts460

Peel D, Ovenden J, Peel S (2004) NeEstimator: software for estimating effective population size, Version 1.3. Queensland Government, Department of Primary Industries and Fisheries.

Pierce AA, Gutierrez R, Rice AM, Pfennig KS (2017) Genetic variation during range expansion: effects of habitat novelty and hybridization. Proceedings Biol Sci 284:20170007. doi: 10.1098/rspb.2017.0007

Price SJ, Ariel E, Maclaine A, et al. (2017) From fish to frogs and beyond: Impact and host range of emergent ranaviruses. Virology 511:272–279. doi: 10.1016/J.VIROL.2017.08.001

Pritchard JK, Stephens M, Donnelly P (2000) Inference of population structure using Multilocus genotype data. Genetics 155: 945-959

R Development Core Team (2017) R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. https://www.R-project.org.

Radinger J, Essl F, Hölker F, et al. (2017) The future distribution of river fish: the complex interplay of climate and land use changes, species dispersal and movement barriers. Glob Chang Biol 23:4970–4986. doi: 10.1111/gcb.13760

Rafferty NE, Ives AR (2011) Effects of experimental shifts in flowering phenology on plant–pollinator interactions. Ecol Lett 14: 69–74. doi: 10.1111/j.1461-0248.2010.01557.x

Ray C, Beever EA, Rodhouse TJ (2016) Distribution of a climate-sensitive species at an interior range margin. Ecosphere 7:e01379. doi: 10.1002/ecs2.1379

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

Reusch TBH, Ehlers A, Hammerli A, Worm B (2005) Ecosystem recovery after climatic extremes enhanced by genotypic diversity. Proc Natl Acad Sci 102:2826–2831. doi: 10.1073/pnas.0500008102

Robson KM, Lamb CT, Russello MA (2016) Low genetic diversity, restricted dispersal, and elevation-specific patterns of population decline in American pikas in an atypical environment. J Mammal 97:464–472. doi: 10.1093/jmammal/gyv191

Rodhouse TJ, Beever EA, Garrett LK, Irvine KM., Munts M, Ray C, Shardlow MR. (2010) Distribution of the Lava Beds pika (*Ochotona princeps* Goldmani): conservation implications from the range periphery. J Mammal, 91 1287-1299.

Rogers SD, Peacock MM (2012) The disappearing Northern Leopard Frog (*Lithobates pipiens*): conservation genetics and implications for remnant populations in western Nevada. Ecol Evol 2:2040–2056.

Root T, Price J, Hall K, Schneider S (2003) Fingerprints of global warming on wild animals and plants. Nature 421:57–60. doi: 10.1038/nature01309.1.

Rubidge EM, Patton JL, Lim M, et al. (2012) Climate-induced range contraction drives genetic erosion in an alpine mammal. Nat Clim Chang 2:285–288. doi: 10.1038/nclimate1415

Safeeq M, Shukla S, Arismendi I, et al. (2016) Influence of winter season climate variability on snow-precipitation ratio in the western United States. Int J Climatol 36:3175–3190. doi: 10.1002/joc.4545

Salo T, Stamm C, Burdon FJ, et al. (2017) Resilience to heat waves in the aquatic snail *Lymnaea stagnalis* : Additive and interactive effects with micropollutants. Freshw Biol 62:1831–1846. doi: 10.1111/fwb.12999

Schwalm D, Epps CW, Rodhouse TJ, et al. (2016) Habitat availability and gene flow influence diverging local population trajectories under scenarios of climate change: a place-based approach. Glob Chang Biol 22:1572–1584. doi: 10.1111/gcb.13189

Schwartz MK, Ralls K, Williams DF, et al. (2005) Gene flow among San Joaquin kit fox populations in a severely changed ecosystem. Conserv Genet 6:25–37. doi: 10.1007/s10592-004-7719-8

Schwartz MK, Luikart G, Waples RS (2007) Genetic monitoring as a promising tool for conservation and management. Trends Ecol Evol 22:25–33. doi: 10.1016/j.tree.2006.08.009

Severaid JH (1955) The Natural History of the Pikas (mammalian Genus *Ochotona*) Dissertation, University of California, Berkeley

Sherry A, Henson R (2005) Conducting and interpreting canonical correlation analysis in personality research: A user-friendly primer. J Pers Assess 84: 37-48. doi: 10.1207/s15327752jpa8401_09

Shinderman M (2015) American pika in a low-elevation lava landscape: expanding the known distribution of a temperature-sensitive species. Ecol Evol 5:3666–3676. doi: 10.1002/ece3.1626

Simpson WG (2009) American pikas inhabit low-elevation sites outside the species' previously described bioclimatic envelope. West North Am Nat 69:243–250. doi: 10.3398/064.069.0213

Smith A (1974a) The distribution and dispersal of pikas: consequences of insular population structure. Ecology 55: 1112–1119. doi:10.2307/1940361

Smith A (1974b) The distribution and dispersal of pikas: influences of behavior and climate. Ecology 55: 1368–1376. doi:10.2307/1935464

Smith AT (1978) Comparative demography of pikas (*Ochotona*): effect of spatial and temporal age-specific mortality. Ecology 59:133–139. doi: 10.2307/1936639

Smith AT, Ivins BL (1983) Colonization in a pika population: dispersal vs philopatry. Behav Ecol Sociobiol 13:37–47. doi: 10.1007/BF00295074

Smith AT, Weston ML (1990) *Ochotona princeps*. Mammal Species 352:1–8

Smith AT, Gilpin ME (1997) Spatially correlated dynamics in a pika metapopulation. In Hanski IA, Gilpin ME Metapopulation Biology: ecology, genetics and evolution. Academic Press, London, pp 407-423.

Smith, AT, Weidong L, and Hik DS (2004) Pikas as harbingers of global warming. Species 41:4–5.

Sork VL, Davis FW, Westfall R, et al. (2010) Gene movement and genetic association with regional climate gradients in California valley oak (*Quercus lobata* Nee) in the face of climate change. Mol Ecol 19:3806–3823. doi: 10.1111/j.1365-294X.2010.04726.x

Spielman D, Brook BW, Frankham R (2004) Most species are not driven to extinction before genetic factors impact them. Proc Natl Acad Sci 101:15261–15264. doi: 10.1073/pnas.0403809101

Stewart JAE, Perrine JD, Nichols LB, et al. (2015) Revisiting the past to foretell the future: Summer temperature and habitat area predict pika extirpations in California. J Biogeogr 42:880–890. doi: 10.1111/jbi.12466

Stewart JAE, Wright DH, Heckman KA (2017) Apparent climate-mediated loss and fragmentation of core habitat of the American pika in the Northern Sierra Nevada, California, USA. PLoS One 12:e0181834. doi: 10.1371/journal.pone.0181834

Van Doornik DM, Eddy DL, Waples RS, et al. (2013) Genetic monitoring of threatened Chinook salmon populations: estimating introgression of nonnative hatchery stocks and temporal genetic changes. North Am J Fish Manag 33:693–706. doi: 10.1080/02755947.2013.790861

Van Oosterhout C, Hutchinson W (2004) MICRO-CHECKER: software for identifying and correcting genotyping errors in microsatellite data. Molecular Ecology Notes, 4: 535–538. doi:10.1111/j.1471-8286.2004.00684.x

Waits L, Paetkau D (2005) Noninvasive genetic sampling tools for wildlife biologists: a review of applications and recommendations for accurate data collection. J Wildl Manage 69: 1419-1433. foi: 10.2193/0022-541X(2005)69[1419:NGSTFW]2.0.CO;2

Walther GR (2010) Community and ecosystem responses to recent climate change. Philos Trans R Soc B Biol Sci 365:2019–2024. doi: 10.1098/rstb.2010.0021

Waples RS, Do C (2010) Linkage disequilibrium estimates of contemporary N-e using highly variable genetic markers: a largely untapped resource for applied conservation and evolution. Ecol Appl 3:244–262.

Waterhouse MD, Blair C, Larsen KW, Russello MA (2017) Genetic variation and fine-scale population structure in American pikas across a human-modified landscape. Conserv Genet 18:825–835. doi: 10.1007/s10592-017-0930-1

Weir B, Cockerham C (1984) Estimating F-statistics for the analysis of population structure. Evolution (NY)

Weir BS (2012) Estimating F -Statistics: a historical view. Philos Sci 79:637–643. doi: 10.1086/667904

Weiss M, Leese F (2016) Widely distributed and regionally isolated! Drivers of genetic structure in *Gammarus fossarum* in a human-impacted landscape. BMC Evol Biol 16:153. doi: 10.1186/s12862-016-0723-z

Westfall RD, Conkle MT (1992) Allozyme markers in breeding zone designation. New For 6:279–309. doi: 10.1007/BF00120649

Whitham TG, Difazio SP, Schweitzer JA, et al. (2008) Extending genomics to natural communities and ecosystems. Science 320:492–5. doi: 10.1126/science.1153918

Wilkening JL, Ray C, Beever EA, Brussard PF (2011) Modeling contemporary range retraction in Great Basin pikas (*Ochotona princeps*) using data on microclimate and microhabitat. Quat Int 235:77–88. doi: 10.1016/j.quaint.2010.05.004

Williams AP, Seager R, Abatzoglou JT, et al. (2015) Contribution of anthropogenic warming to California drought during 2012-2014. Geophys Res Lett 42:6819–6828. doi: 10.1002/2015GL064924

Yang DS, Conroy CJ, Moritz C (2011) Contrasting responses of *Peromyscus* mice of Yosemite National Park to recent climate change. Glob Chang Biol 17:2559–2566. doi: 10.1111/j.1365-2486.2011.02394.x

Yao L, Li H, Martin RD, et al. (2017) Tracing the phylogeographic history of Southeast Asian long-tailed macaques through mitogenomes of museum specimens. Mol Phylogenet Evol 116:227–238. doi: 10.1016/j.ympev.2017.08.006

Tables

Table 1 Locus-specific rates for allelic dropout (ε_1) and false alleles (ε_2) are calculated and given for two time periods of sample collection for museum and fecal DNA samples. Genotyping success rate (%) is provided per locus and averaged across all samples

	1949 Museum Sample DNA			2015 Fecal DNA		
Locus	ε_1 (allelic dropout rate)	ε_2 (false allele rate)	% success	ε_1 (allelic dropout rate)	ε_2 (false allele rate)	% success
OCP 4b	0.031	0.138	0.923	0.000	0.010	0.930
OCP 6b	0.091	0.036	0.846	0.000	0.032	0.987
OCP 7	-	-	-	0.095	0.000	0.917
OCP 8	0.050	0.050	0.969	0.006	0.000	0.974
OCP 9b	0.000	0.015	0.892	0.006	0.000	0.939
OCP 10	0.031	0.046	0.954	0.023	0.000	0.996
OCP 15	0.000	0.000	0.969	0.086	0.008	0.965
OCP 16a	0.062	0.015	0.877	0.061	0.000	0.961
OCP 17	0.015	0.000	0.877	0.052	0.070	0.956
OCP 21	0.033	0.050	0.877	0.014	0.033	0.934
Average	**0.035**	**0.039**	**0.909**	**0.034**	**0.015**	**0.956**

Table 2 Comparison of population genetic statistics across years including observed heterozygosity (corrected for sample size), allelic richness (average number of alleles), genetic differentiation θ_{st}, F_{IS} (inbreeding coefficient), and estimates of effective population size (Ne) per period of sample collection

Century	Years of Collection	Sample Type	Sample Size (n)	Avg. Gene Diversity (θ_{ST})	Obs. Het.	Unbiased Avg. Allelic Richness	Private Allelic Richness	F_{IS}	Ne (effective population size)
Mid-20th	1948-1949	Museum pelt DNA	13	0.6561 (SE:0.036)	0.6213 (SE:0.045)	3.92	0.84	0.055	35.1 (95% CI 19.4-50.8)
Late-20th	1988-1990	Tissue DNA	115	0.6106 (SE:0.037)	0.5444 (SE:0.015)	3.65	0.28	0.108	50.5 (95% CI 9.2-91.8)
Early-21st	2015	Fecal DNA	101	0.5533 (SE:0.040)	0.6073 (SE: 0.016)	3.25	0.21	-0.098	17.2 (95% CI 2.9 -31.5)

Figure Legends

Figure 1 Map of talus ore dumps in the Bodie State Historic Park (modified from Smith and Gilpin 1997)

Figure 2 Map showing orientation, area, and spatial structure of Bodie State Historic Park's ore dump patches, and locations of pika genetic sampling during mid-20th century (red patches), late-20th century (blue patches), early 21st century (yellow patches), and those patches surveyed across multiple eras (orange, and green patches respectively)

Figure 3 Patch occupancy during 1972-2014 in Bodie State Historic Park, Mono County, CA, USA. Patch occupancy is represented as the ratio between the number of patches with evidence of occupancy (fresh fecal pellets, fresh haypiles, and/or visual observation of animal) and the total number of patches censused. All census data were collected by L. Nichols

Figure 4 Observed changes in parameters of population genetic diversity and local climate shown for three time periods (e.g. mid-20th century, late-20th century, and early 21st century) for an American pika (*Ochotona princeps*) population in Bodie State Historic Park through box plots or bar plots of a) numbers of lost and new alleles, b) corrected allelic richness, c) N_e (i.e. effective population size), and d) changes to average maximum temperature in July (°C) and average mean annual precipitation (mm), respectively.

Figure 5 Principal component analyses (PCA) and Euclidean distance hierarchical trees of all genotyped individuals for 1988 and 2015. Individuals in the PCA analyses are color coded to match the color coded clades in the Euclidean distance trees. Note the increased genetic structure observed in 2015 compared to 1988

Figure 6 Bayesian genotype clustering and AMOVA results for 1988 and 2015. Two genotype clusters was the best fit of the data in 1988 (LnP (D) =-2517.32, SD±0.878; Δk=18.369) and k = 5 for 2015 (LnP (D) =-1712.36, SD±13.913; Δk=28.582)The AMOVA results show an increase in among population variance in 2015 compared to 1988

Figure 7 A principal component analysis (PCA) of spatial population genetic variation based on axes of genetic distance among American pika (*Ochotona princeps*) populations located in the Sierra Nevada (SNE, HMH) and Bodie Hills regions (BMN, BOD, and MAS) (program GenAlEx) Map indicates the geographic location of sampling sites with site abbreviations as follows: BMN (Masonic Mountain West), BOD (Bodie State Historic Park), MAS (New York Hill, Masonic Mining district), SNE (Conway Uplands, Ellery Lake, Bennettville Mine, and Silverpine Lake), and HMH (Harvey Monroe Hall Research Natural Area)

Figures
Figure 1

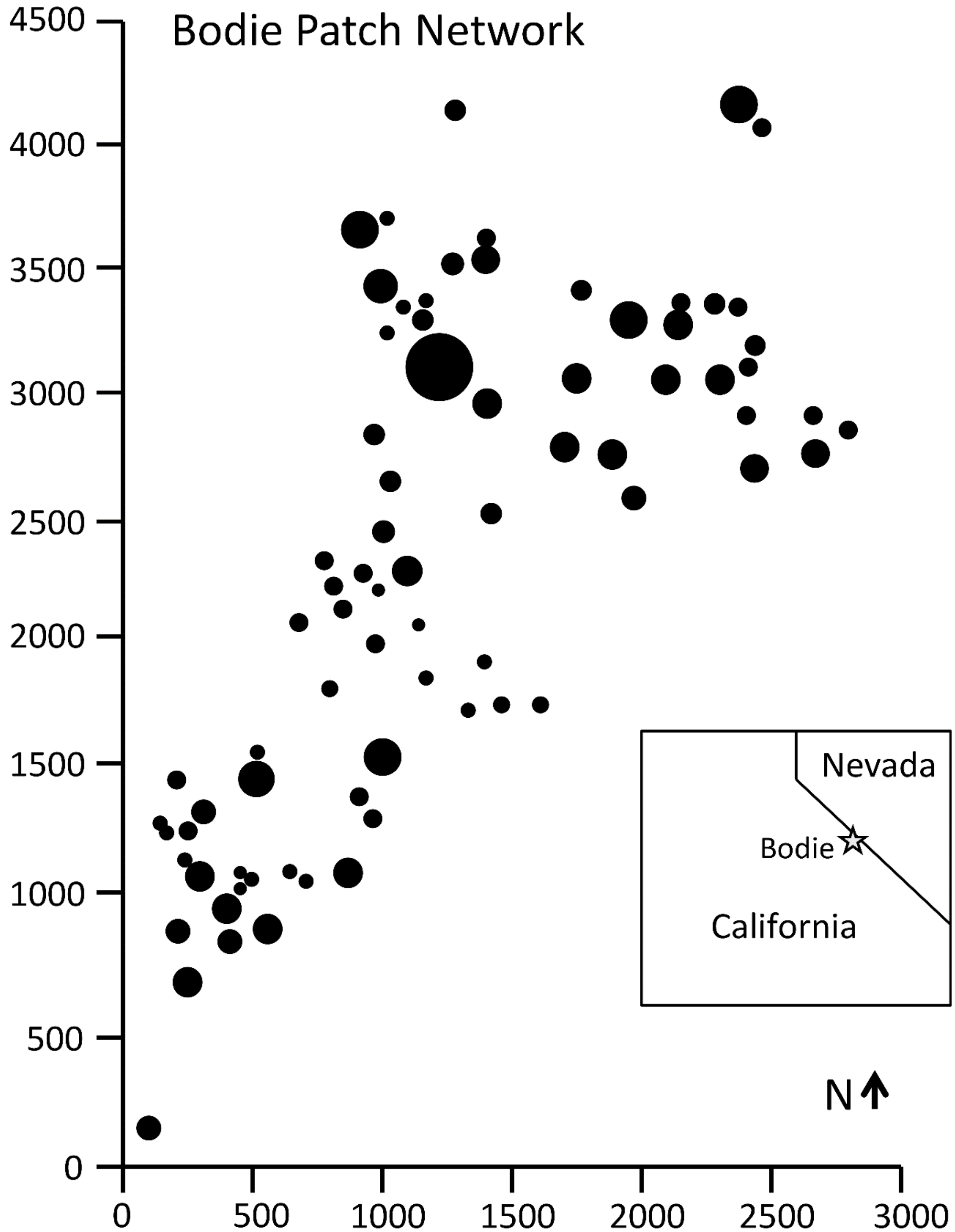
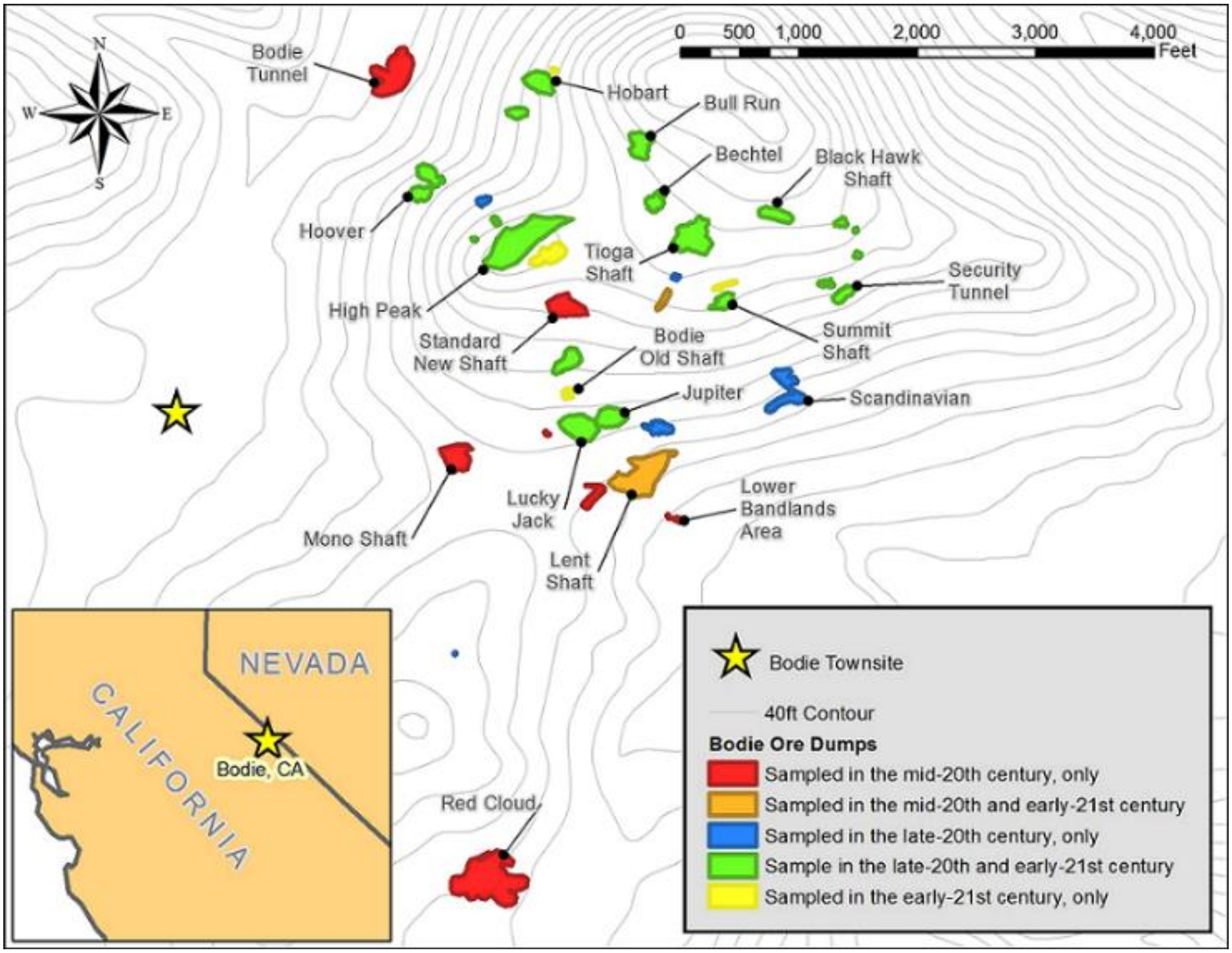


Figure 2


0
500
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Hoover
Tioga Shaft
Security Tunnel
High Peak
Standard New Shaft
Bodie Old Shaft
Summit Shaft
Jupiter
Scandinavian
Lucky Jack
Lower Bandlands Area
Mono Shaft
Lent Shaft
NEVADA
CALIFORNIA
Bodie, CA
Red Cloud
Bodie Townsite
40ft Contour
Bodie Ore Dumps
Sampled in the mid-20th century, only
Sampled in the mid-20th and early-21st century
Sampled in the late-20th century, only
Sample in the late-20th and early-21st century
Sampled in the early-21st century, only

Figure 3

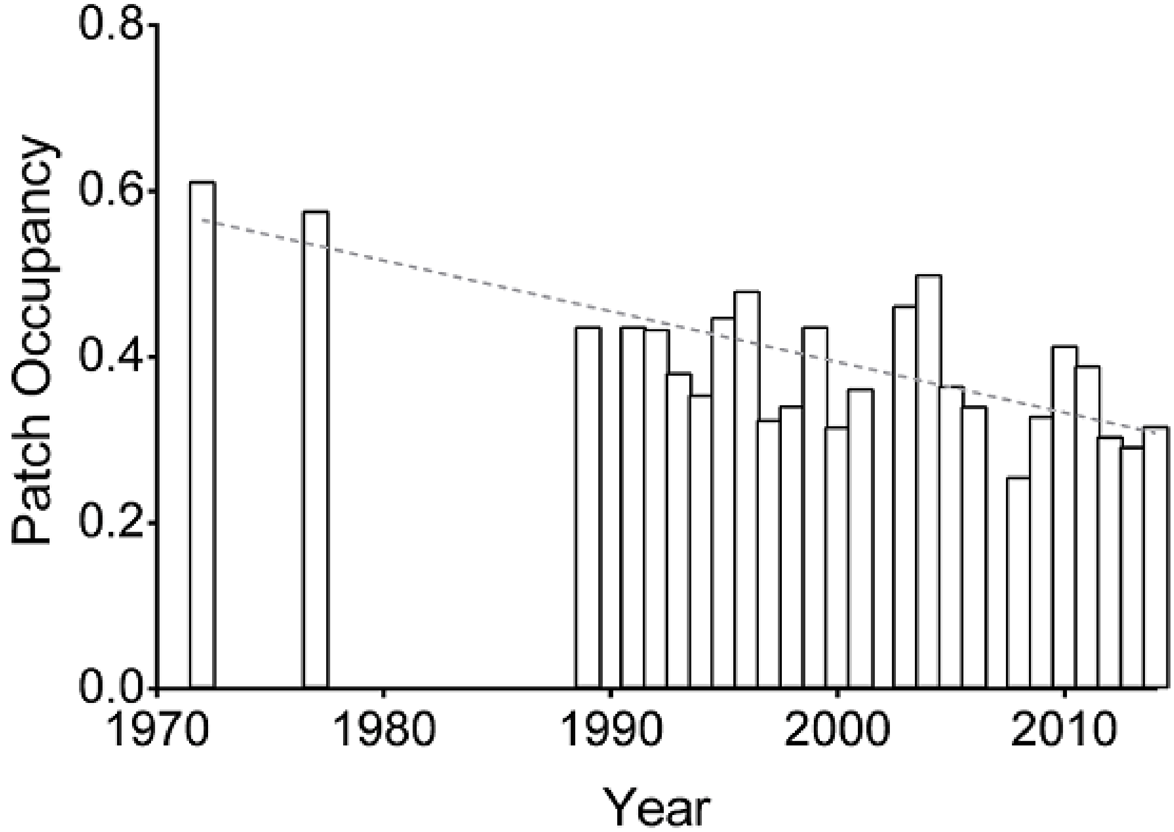


Figure 4

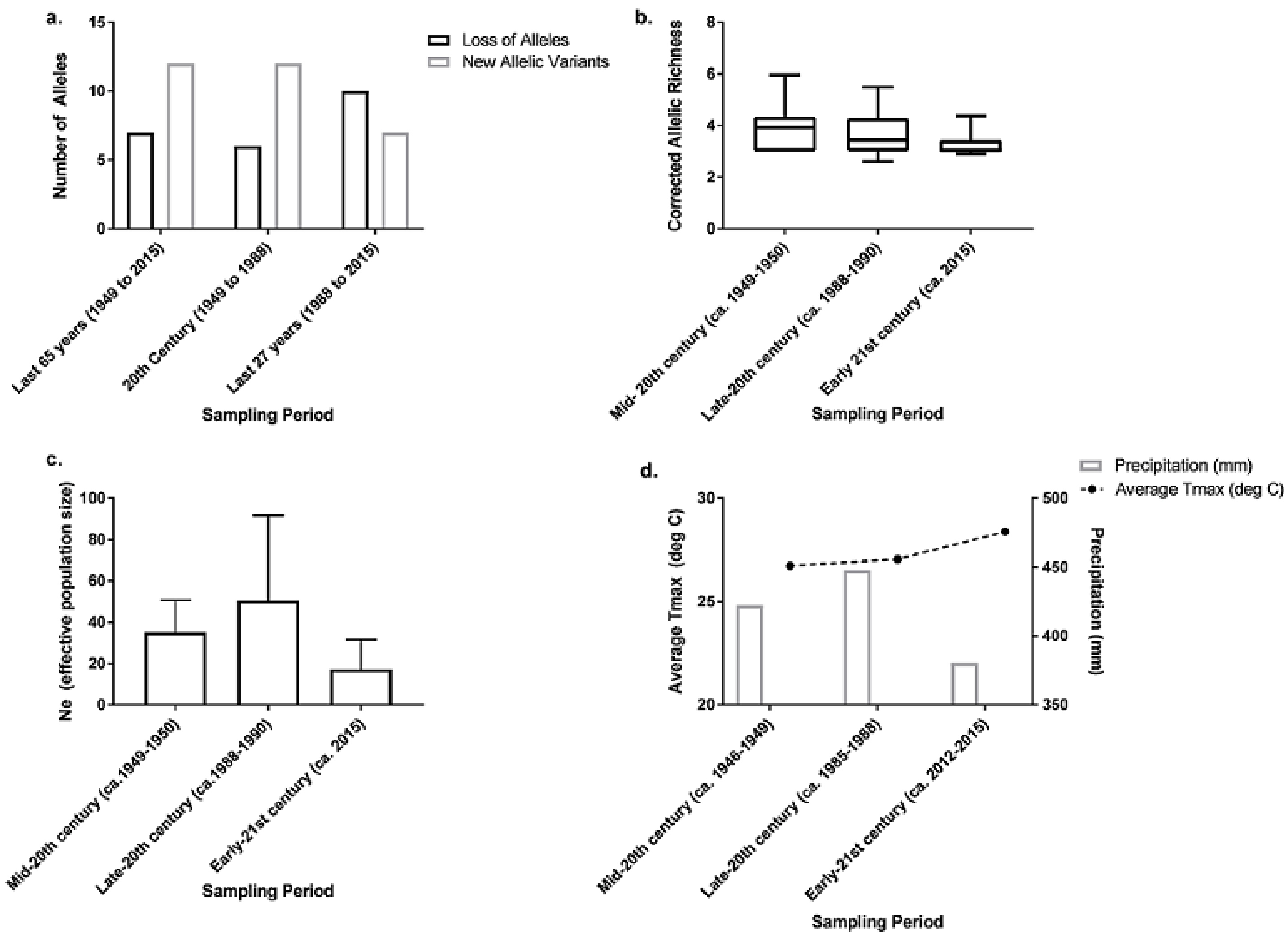

a.
Number of Alleles
Loss of Alleles
New Allelic Variants
Last 65 years (1949 to 2015)
20th Century (1949 to 1988)
Last 27 years (1988 to 2015)
Sampling Period
b.
Corrected Allelic Richness
Mid- 20th century (ca. 1949-1950)
Late-20th century (ca. 1988-1990)
Early 21st century (ca. 2015)
Sampling Period
c.
Ne (effective population size)
Mid-20th century (ca.1949-1950)
Late-20th century (ca.1988-1990)
Early-21st century (ca. 2015)
Sampling Period
d.
Precipitation (mm)
Average Tmax (deg C)
Average Tmax (deg C)
Precipitation (mm)
Mid-20th century (ca. 1946-1949)
Late-20th century (ca. 1985-1988)
Early-21st century (ca. 2012-2015)
Sampling Period

Figure 5

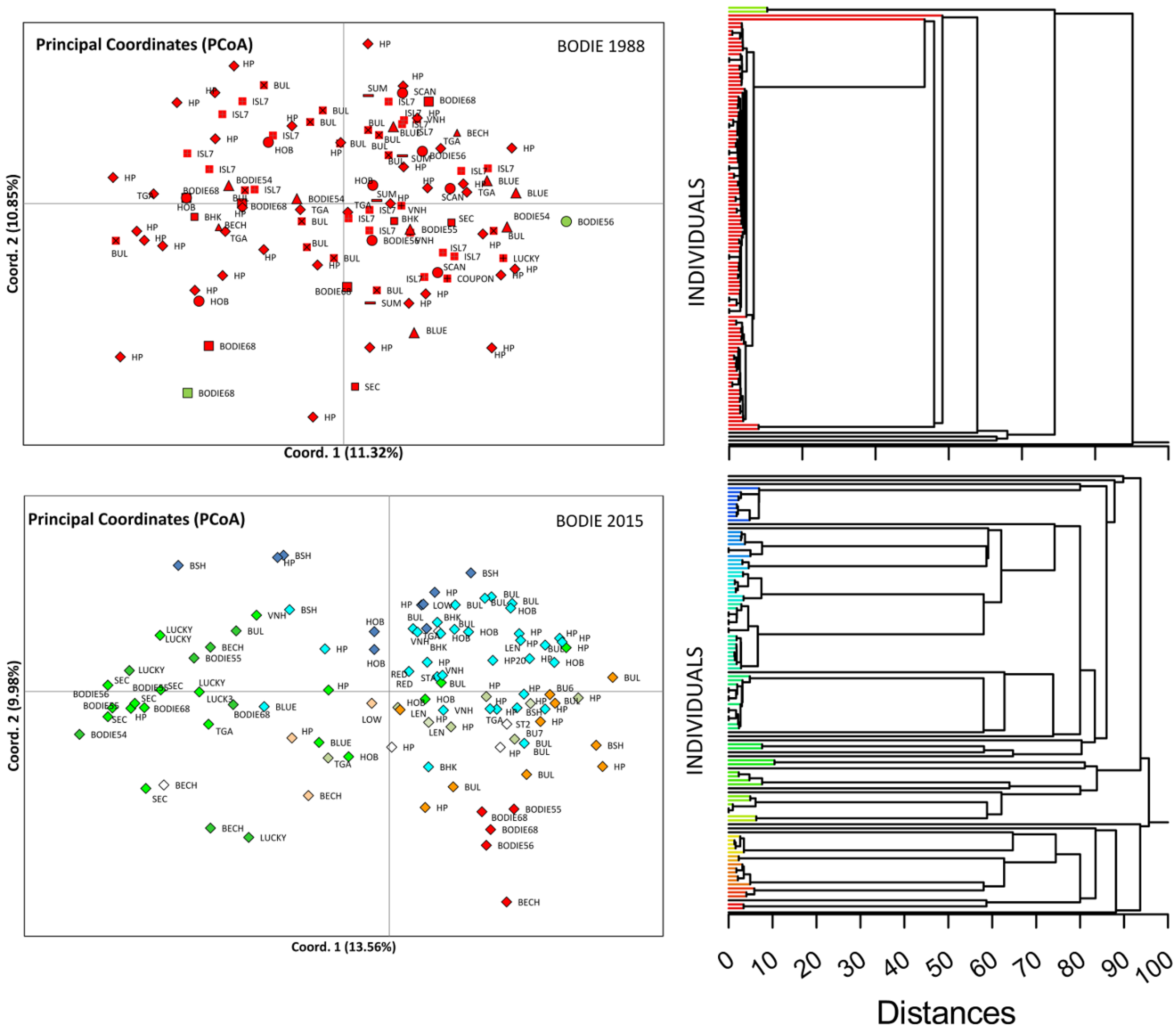

Principal Coordinates (PCoA)
BODIE 1988
Coord. 2 (10.85%)
Coord. 1 (11.32%)
INDIVIDUALS
Principal Coordinates (PCoA)
BODIE 2015
Coord. 2 (9.98%)
Coord. 1 (13.56%)
INDIVIDUALS
0
10
20
30
40
50
60
70
80
90
100
Distances

Figure 6

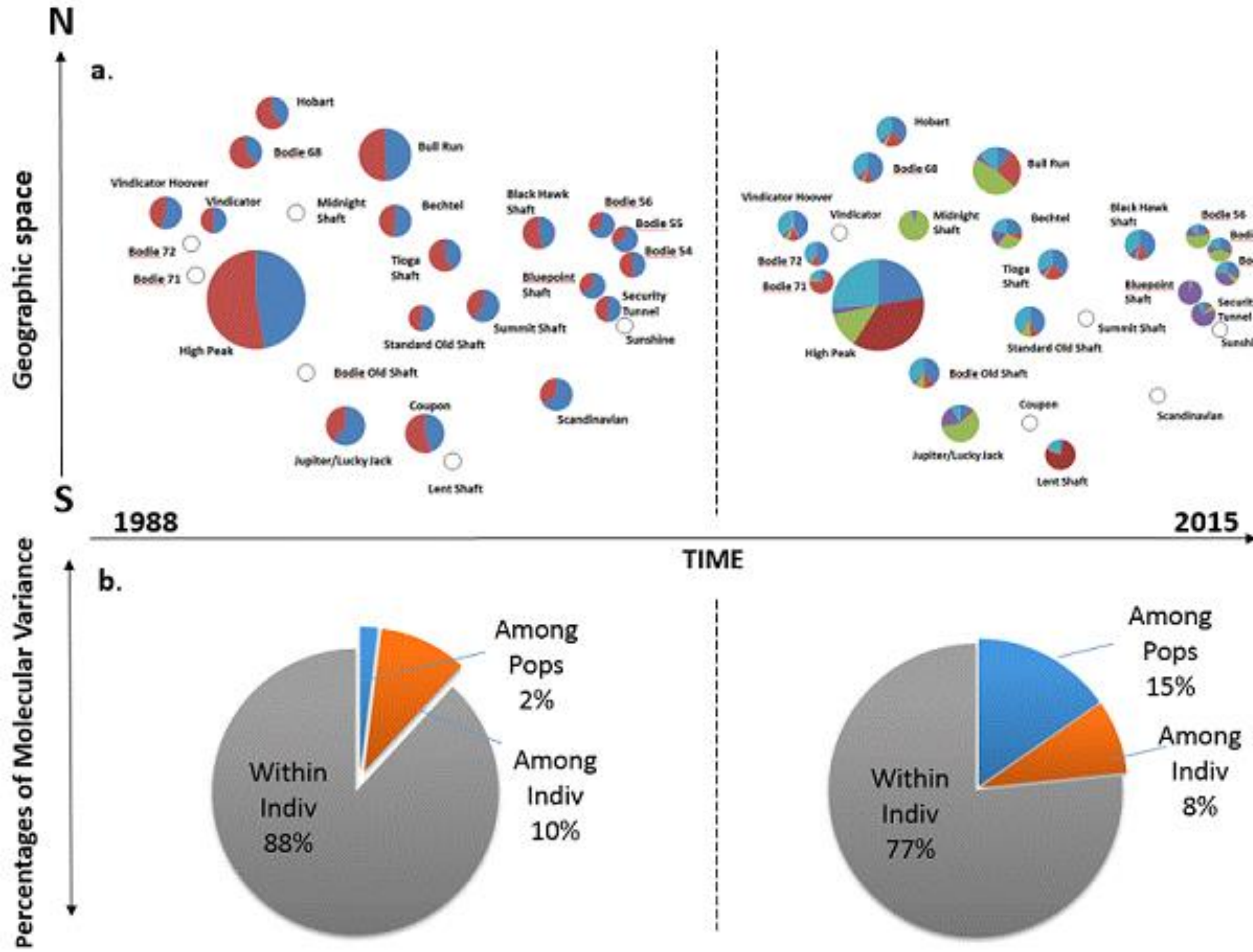
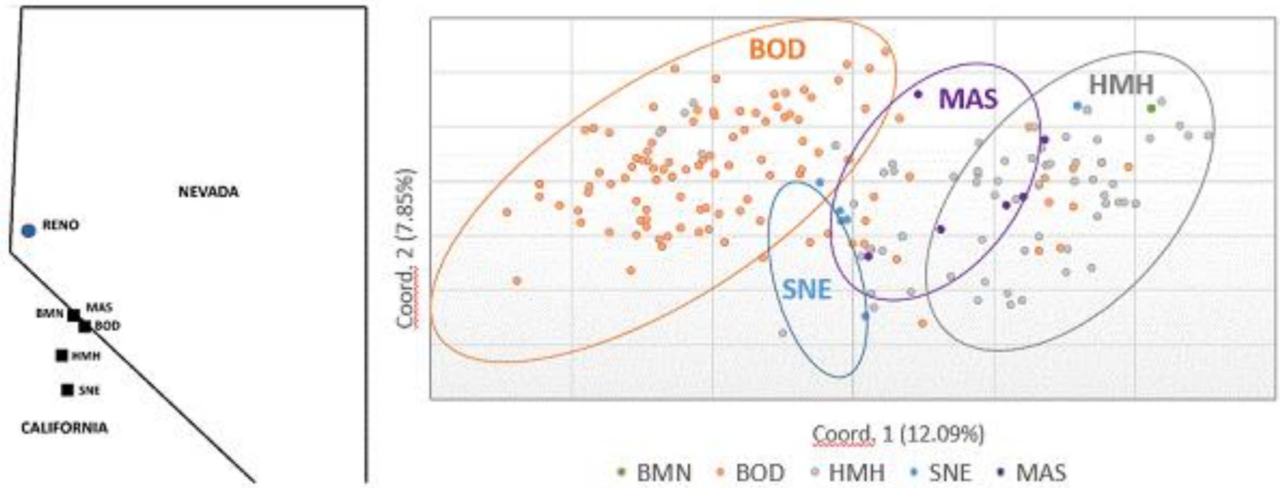

N
S
a.
Geographic space
1988
2015
TIME
b.
Percentages of Molecular Variance
Among Pops 2%
Among Indiv 10%
Within Indiv 88%
Among Pops 15%
Among Indiv 8%
Within Indiv 77%

Figure 7


NEVADA
RENO
BMN
MAS
BOD
HMH
SNE
CALIFORNIA
BOD
MAS
HMH
SNE
Coord. 2 (7.85%)
Coord. 1 (12.09%)
BMN BOD HMH SNE MAS

Supplementary Materials
Chapter 3

Life on the edge - the American pika in Bodie, California: a 65 year case study of genetic erosion in a species of special concern

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Table S1 List of 16 *O. princeps* museum specimens loaned from UC Berkeley Museum of Vertebrate Zoology for Genomic DNA extraction and isolation. These individuals represent collections of JH Severeid (1955) and were either sacrificed in the field, or part of the F1 generation of his captive breeding experiments

MVZ GUID	JHS 4-digit ID	Ear tag No.	Date caught/born	Date of Death	Sex	Sta. No.	Trap No.	Patch Location
135140	1013	x	8-Aug-48	8-Aug-48	M	96	6	Mono Mine. Dead in trap
135147	1078	#10	23-May- 49	24-May-49	M	136	9	SE edge of Lent Mine Dumps
135148	1084	#15	29-May-49	29-May-49	M	141	5	Burned Mill/ Roseklip Mine
135162	1111	#36	24-Jun-49	25-Jun-49	M	162	9	Upper Badlands area
135150	1078	#10	23-May- 49	24-May-49	M	136	9	SE edge of Lent Mine Dumps
135149	1088	#12	10-May-49	30-May-49	M	137	1	East facing rock ravine of Lent Shaft.
135216	1241	#38	29-Jun-49	16-Feb-51	M	171	7	Mid Central/ E of Old Excavator
135225	1316	#5	8-May-49	9-Feb-52	M	92	4	Bodie Tunnel
135220	1305	#27	18-Jun-49	13-Dec-51	M	x	x	Pregnant mother was wild-caught under loading shoot on South side of Red Cloud dump.
135151	1089	#18	1-Jun-1949	1-Jun-49	M	85	14	S. Boulder Dump/ East exposure
135206	1203	#24	4-Jun-1949	17-Apr-50	F	x	x	Mother collected from Burned Mill/Roseklip Mine
135142	x	x	x	28-Apr-48	M	x	x	x

Table S2 Ten microsatellite primers developed for the American pika (*Ochotona princeps*) (Peacock et al. 2002; Peacock and Kirchoff 2009 unpublished) were optimized for PCR multiplexing (shortening fragments lengths) of fecal DNA. Product size (bp), and number of alleles (N_A) per time period are given. Note that M13 dye, product size, annealing temperature (T_A) (°C) as well as GenBank accession numbers are also provided

Locus	Primer Sequence (5'-3')	Dye	Product size (bp)	Ta (°C)	N_A Mid-20th	N_A Late-20th	N_A Early 21st	Accession No.
OCP 4b	F: GTTATTGCGCCAGGGTCTAC R: GTCCTGGCTATTGCATCCAC	FAM	170-190	70	6	5	4	AF487495
OCP 6b	F: GGCTTCAGATTTCCTCAACACC R:AGGAAGGGAGAAGCTAGAC	VIC	250-270	68	4	3	3	AF487497
OCP 7	F: ATCCTGAGCTATCTTTGCCATT R: CCCAAAACTCCTTGAGAGACA	FAM	276-300	65	--	5	6	AF487498
OCP 8	F: TTCCTCTGGAGTCCTCTAACCC R: CCTCGAGCAAGTTTGGTTGTT	NED	223-239	67	4	6	7	AF487499
OCP 9b	F: CGCAAACATCATGGCTAAAA R:GCAGGTGGCAGATTTCTCC	PET	180-200	69	5	4	4	AF487500
OCP 10	F:CCAATTTGGCTGTTAAGGCTAT R:CCAGTGTCTGGCAYACGGTAAGC	FAM	190-200	65	3	4	4	GQ461705
OCP 15	F:GGAAGCAGAGGACCTAGCCAAGC R:CCAGAGGTTTAACTTGCTATGC	VIC	180-200	65	3	4	5	GQ461710
OCP 16a	F:GGAAACAGCTATGACCAAGCAGTGGGCA R:GCTGACATTTAATATCGTCC	PET	240-250	65	3	5	3	GQ461711
OCP 17	F:CAGACACCTACTGGCCTATATGT R: CTTCAGGAGACTGACCCAACC	VIC	280-330	65	4	6	6	GQ461712
OCP 21	F:GTACTCACTCCTTGGCACATCTC R:GAATGGGGTTAGATGTACATTGG	FAM	160-190	65	4	5	5	GQ461716

Table S3 F_{IS} per locus per population, bolded F_{IS} values are lower than randomization values, bolded and italicized are larger than randomization values, (p=0.00185 based on 540 randomizations)

	2015	1988	1949
OCP 4	***-0.293***	-0.044	-0.228
OCP 6	-0.125	-0.086	0.079
OCP 8	-0.067	0.056	-0.076
OCP 9	-0.148	0.139	-0.274
OCP 10	0.268	0.467	0.458
OCP 15	0.099	0.468	1.00
OCP 16a	0.014	-0.022	-0.194
OCP 17	-0.046	-0.081	-0.226
OCP 21	***-0.39***	0.193	0.049
All	-0.098	0.108	0.055

Table S4 Summary of the statistical tests of canonical correlation analysis for genetic variables and three linear climate variables (Mean Annual Precipitation, Average Tmax (maximum temperature in July), Average Tmin (minimum temperature in January) using Wilk's **λ.** Test

Canonical axes	Canonical correlation	Sq. Corr	Wilks L (λ)	F	Hypoth DF	Error DF	Pr > F
Function 1	0.815	0.665	0.125	11.04	60	657.19	<0.0001
Function 2	0.776	0.603	0.372	7.42	38	442	<0.0001

Table S5 Canonical solution for local climate variables predicting population genetic variation for the dependent/criterion variables of Functions 1 and Function 2. Structure coefficients (r_s) greater than 0.45 are bolded. Communality coefficients (h^2) greater than 45% are underlined. Coef=standardized canonical function coefficient; r_s=structure coefficient; r^2s=squared structure coefficient; h^2=communality coefficient.

Function 1				Function 2				
var	coef	r_s	r^2	var	coef	r_s	r^2	h^2
PC1	0.28	0.28	7.89	PC1	-0.20	-0.20	0.04	7.93
PC2	-0.80	**-0.80**	64.00	PC2	0.01	0.01	0.00	<u>64.00</u>
PC3	0.12	0.12	1.37	PC3	-0.80	**-0.80**	64.65	<u>66.02</u>
PC4	0.04	0.04	0.12	PC4	0.24	0.24	5.54	5.66
PC5	0.10	0.10	0.93	PC5	0.06	0.06	0.41	1.33
PC6	0.30	0.30	9.29	PC6	0.29	0.29	8.59	17.87
PC7	0.10	0.10	1.04	PC7	-0.05	-0.05	0.26	1.30
PC8	-0.02	-0.02	0.06	PC8	-0.15	-0.15	2.11	2.17
PC9	-0.24	-0.24	5.62	PC9	-0.17	-0.17	2.91	8.54
PC10	0.10	0.10	1.01	PC10	0.13	0.13	1.78	2.79
PC11	0.13	0.13	1.79	PC11	-0.17	-0.17	2.76	4.55
PC12	0.13	0.13	1.81	PC12	0.22	0.22	4.99	6.80
PC13	0.04	0.04	0.14	PC13	-0.07	-0.07	0.56	0.70
PC14	0.18	0.18	3.12	PC14	-0.04	-0.04	0.20	3.32
PC15	-0.03	-0.03	0.08	PC15	0.03	0.03	0.06	0.14
PC16	-0.08	-0.08	0.58	PC16	-0.08	-0.08	0.64	1.22
PC17	-0.02	-0.02	0.03	PC17	0.04	0.04	0.12	0.15
PC18	-0.09	-0.09	0.80	PC18	-0.01	-0.01	0.00	0.80
PC19	-0.01	-0.01	0.01	PC19	-0.06	-0.06	0.38	0.39
PC20	0.06	0.06	0.31	PC20	-0.03	-0.03	0.11	0.41

Table S6 Canonical solutions for local climate predicting genetic variation for the independent/covariate variables of Functions 1 and 2 with standardized canonical function coefficients (coef), structure coefficients (r_s), and squared structure coefficients (r^2) given. Communality coefficients (h^2) (sum of two r^2 variables) are also provided and give an indication of how useful the variable was for the solution (Sherry and Hensen 2005) Variable abbreviations are as follows: MAP (mean annual precipitation), AVG TMAX (average maximum temperature in July), and AVG TMIN (average minimum temperature in Jan)

Function 1				**Function 2**			
var	**coef**	**r_s**	**r^2**	**var**	**coef**	**r_s**	**r^2**
MAP	-0.61	0.98	96.24	MAP	3.50	-0.18	3.22
AVGTMAX	-2.28	-0.90	80.23	AVG TMAX	3.88	0.44	19.30
AVGTMIN	1.00	-0.44	19.41	AVG TMIN	-0.09	0.89	79.34

Table S7 Pairwise F_{ST} comparisons between regional sampling locations obtained after 200 permutations. Significant F_{ST} values are bolded and marked with an asterisk (p=0.005) Site abbreviations are as follows: BOD (Bodie State Historic Park, Mono County, CA, Bodie Hills), BMN (Masonic Mountain West, Bodie Hills), HMH (Harvey Monroe Hall RNA, Sierra Nevada), and SNE (Conway Uplands, Ellery Lake, Bennettville Mine, and Silverpine Lake, Sierra Nevada), and MAS (New York Hill, Masonic Mining site, Bodie Hills) We were unable to test for significance for the MAS F_{ST} estimates due to small sample size

Population	**BMN**	**BOD**	**HMH**	**SNE**
BOD	0.302			
HMH	**0.177***	**0.102***		
SNE	0.303	**0.121***	0.078	
MAS	*0.431*	*0.167*	*0.105*	*0.237*

Figure S1

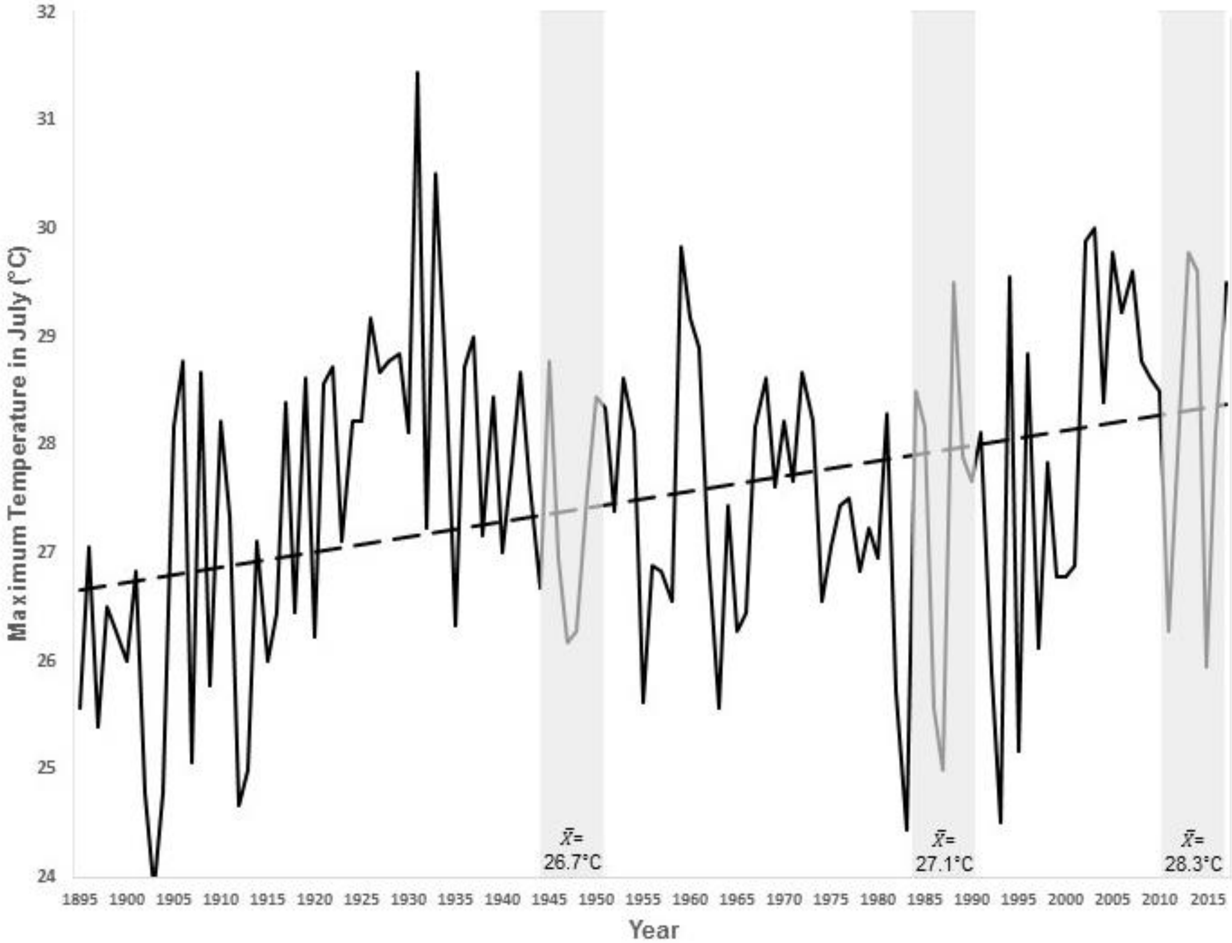

Figure S1 Patterns of increasing (adjusted $R^2 = 0.05$) maximum temperature in July (°C) (Tmax) over time (1895 to 2015) within Climate Division 3 (Northeast Interior Basins) data downloaded from NOAA National Centers for Environmental information, Climate at a Glance (https://www.ncdc.noaa.gov/cag/time-series/) Each of the three time periods investigated within this study (i.e. 1946-1949, 1985-1988, and 2012-2015) are highlighted in gray boxes with the associated average Tmax indicated in text and the linear trend line plotted (y = 0.01x + 7.9724**)** There is a statically significant pattern of increasing Tmax ($F(1, 121) = 7.58$, $p = 0.007$) for the entire time period during1895 through 2015; however, the difference in Tmax between each of the three time periods is not statistically significant ($F(1, 10) = 1.96$, $p = 0.191$)

Figure S2

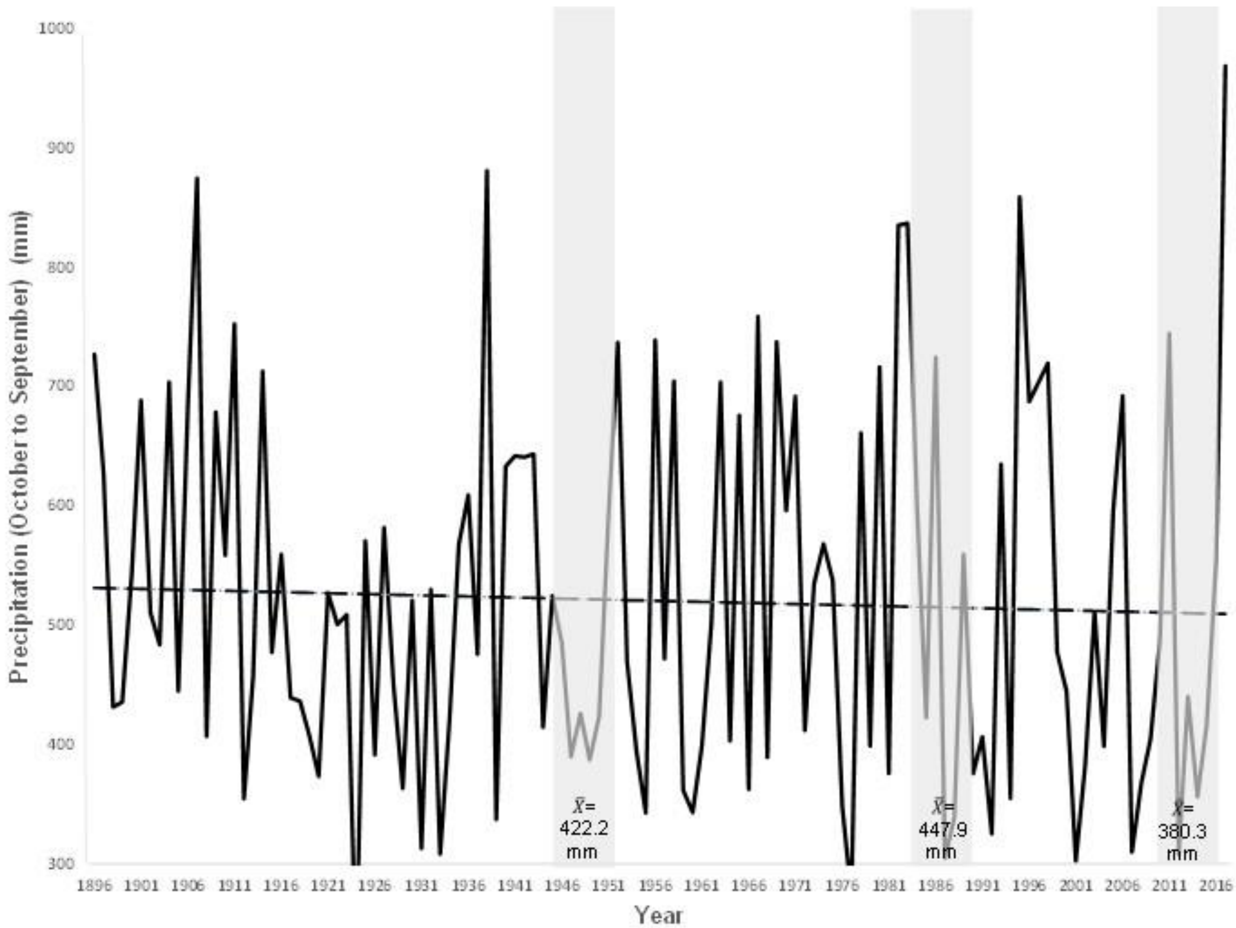

Figure S2 Patterns of precipitation (cm) (adjusted R^2= -0.007) over time (October to September) (1895 to 2015) within Climate Division 3 (Northeast Interior Basins) data downloaded from NOAA National Centers for Environmental information, Climate at a Glance (https://www.ncdc.noaa.gov/cag/time-series/) Each of the three time periods investigated within this study (i.e. 1946-1949, 1985-1988, and 2012-2015) are highlighted in gray boxes with the associated average precipitation indicated in text and the linear trend line plotted ($y = -0.1814x + 875.59$**)** Precipitation has not declined significantly ($F (1,120) =0.201$, $p=0.654$) for the entire time period during 1895 through 2015 nor for the three sampling periods