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By

Talisin Tess Hammond

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requirements for the degree of

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in

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in the

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of the

University of California, Berkeley

Committee in charge:

Dr. Eileen A. Lacey, Chair
Dr. Justin S. Brashares
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Dr. Daniela Kaufer

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Abstract

Heterogeneous responses to environmental change: contrasting behavior and physiology in two California chipmunks

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Dr. Eileen A. Lacey, Chair

Biotic responses to environmental change can vary markedly, even among closely related, ecologically similar species. Such responses may be conspicuous (e.g., climate-associated range shifts) or they may be subtler and more challenging to detect. In the latter case, organisms may use individually variable mechanisms, including modifications of behavior and physiology, to cope with environmental change *in situ*. Further, in addition to providing mechanisms of response to environmental change, behavioral and physiological traits may be indicators of habitat suitability. Thus, to understand and, ideally, to predict how species will respond to environmental change, it is necessary to determine which traits are associated with vulnerability and to identify which factors constrain range limits for vulnerable species. My dissertation focuses on the behavior and physiology of the alpine chipmunk (*Tamias alpinus*) and the lodgepole chipmunk (*T. speciosus*), two co-occurring, closely related species that have been characterized by very distinct spatial responses to environmental change in Yosemite National Park, CA. Over the past century, *T. alpinus* has contracted its range upward in elevation; during the same period, *T. speciosus* has displayed no significant elevational range shift. To assess the role of behavioral and physiological variability in generating these responses, I explored interspecific differences in baseline stress hormone (glucocorticoid, GC) levels and behavioral activity budgets with the goal of identifying the environmental factors that are most important for determining range limits in the study species. First, I validated a non-invasive method to measure fecal GC metabolites (FGMs) in both study species. By exposing captive individuals to a series of controlled challenges, I also identified interspecific differences in stress reactivity, with *T. alpinus* being generally more stress-responsive. Next, I validated the use of accelerometers to remotely document the behavioral activity budgets of the study species, demonstrating that these sensors can be employed to collect behavioral data from free-living animals. I then deployed accelerometers across broader spatial and temporal scales. I used the resulting data to construct models that integrate intrinsic biological and environmental parameters to identify key predictors of activity in each species. I found that, compared to *T. alpinus*, activity in *T. speciosus* was characterized by generally greater inter-individual variance and greater variability in response to environmental parameters. Finally, I used FGM data

collected over three years and at multiple sites in and around Yosemite National Park in conjunction with data regarding multiple extrinsic (environmental) and intrinsic (life history) parameters to identify the factors that best predict FGMs in the study species. These analyses revealed FGM levels are more strongly related to environmental parameters in *T. alpinus* than in *T. speciosus*. In summary, my research indicates that *T. alpinus* is more stress-responsive to external, environmental challenges, and potentially less flexible in responding behaviorally to environmental conditions than *T. speciosus*. Overall, these results indicate that behavior and physiology are likely to be important determinants of a species' response to environmental change. These findings also suggest that individual species vary in their general sensitivity to environmental change, with some species being more change-responsive than others.

For my field assistants, who filled this work with joy,
and for the chipmunks, who tolerated it.

Table of Contents

Acknowledgements

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Chapter 1

Introduction

Organisms have always faced changing environments, but the scale and variance of such change has become greatly enhanced in the past century due to anthropogenic impacts, including climate change. Concordantly, many animals have exhibited range shifts, often moving upwards in elevation or latitude, presumably to track historical climatic niches or to follow shifting food sources (Chen et al., 2011; Hickling et al., 2006; Lenoir et al., 2008; Walther et al., 2002). While range shifts have received significant attention as part of climate change research, there are many species that have experienced significant environmental change without exhibiting any geographic shifts (e.g. Moritz et al., 2008; Tingley et al., 2009). As a result, different taxa – including closely related species that share portions of the habitat – may display dramatically different spatial responses to environmental change, for example with one species shifting its elevational range and the other showing no change in spatial distribution. This heterogeneity raises intriguing questions regarding the traits that allow a species to persist in the face of environmental change. Congeneric species that display distinct responses to changes affecting shared habitats provide a unique opportunity to study biotic response to climate change while controlling for phylogenetic and environmental differences.

The chipmunks of Yosemite National Park provide one such comparative system for evaluating responses to environmental change (Fig. 1). Over the past century, minimum temperatures in Yosemite have increased by ~3°C; simultaneously, the elevational range of the alpine chipmunk (*Tamias alpinus*) has contracted upwards while the elevational range of the lodgepole chipmunk (*T. speciosus*) has not shifted (Moritz et al., 2008; Fig. 2). Despite being closely related, the range responses of these species to the same suite of environmental changes have been strikingly different, suggesting that *T. speciosus* may have been better able to adjust *in situ*, while *T. alpinus* may have been required to move to survive. Species distribution models support temperature and vegetation as predictors of the range shift by *T. alpinus* but have failed to identify clear predictors for *T. speciosus* (Rubidge et al., 2011). Although previous work has examined the importance of abiotic and biotic factors in delimiting the elevational ranges of these species, many physiological traits – including some thermal limitations – overlap between *T. alpinus* and *T. speciosus* (Heller & Gates 1971; Heller & Poulson 1970; Heller & Poulson 1972). Behavioral studies in captivity suggests that competition between these species may constrain range edges and that *T. alpinus* may be aggressively dominant (Heller 1971); these captive experiments involved artificial arena challenges, however, and anecdotal reports of field interactions suggest the opposite dominance hierarchy (Chappell 1978). More recent work has used museum specimens to document stronger patterns of genetic, dietary, and morphological change in *T. alpinus* over the past century (Rubidge et al., 2012; Walsh et al., 2016). While, collectively, these studies suggest that *T. alpinus* is more sensitive to environmental conditions, much remains to be discovered regarding the factors are most important in determining range limits for each species. Behavior and physiology – in addition to providing flexible mechanisms for coping with rapid

environmental change – can also be reflective of environmental constraints and habitat suitability. At present, however, the roles that these processes play in shaping response to environmental change in *T. alpinus* and *T. speciosus* remain poorly understood.

My dissertation research explores the reasons for the pronounced interspecific differences in range response for these species of chipmunks. Specifically, I seek to understand why one species has undergone a significant range change over the past century while the other has not. I use tools from stress physiology and behavioral ecology to test predictions regarding the factors that determine elevational range boundaries for *T. alpinus* and *T. speciosus*. To quantify stress, I use fecal glucocorticoid metabolites (FGMs). Glucocorticoids (GCs), also known as "stress hormones," generally increase during periods of high metabolic demand or stress, but are also important metabolic hormones at baseline concentrations (Sapolsky et al., 2000). GCs have been proposed as useful bioindicators for studies requiring a physiological proxy for population health (Bonier et al., 2009; Busch & Hayward, 2009; Wikelski & Cooke, 2016). Chronically elevated GCs are generally thought to be maladaptive, although the relationship between chronic stress and fitness vary markedly among animal species (Bonier et al., 2009; Hansen et al., 2016; Burtka et al., 2016; Madliger & Love, 2016). In any system, it is critical to understand the extent to which GCs reflect natural extrinsic (environmental) and intrinsic (life history) conditions before employing these hormones as a proxy for health or fitness (Dantzer et al., 2014). Accordingly, I measured FGMs and activity in individuals from a number of sites in and around Yosemite National Park, CA (Fig. 3), specifically sampling populations at range edges and centers as well as sites of co-occurrence between the study species. To quantify behavior, I used accelerometers to characterize remotely the activity budgets of free-living members of both study species. Activity budgets are clearly tied to survival and reproductive success, but have historically been challenging to measure due to the need to collect continuous behavioral data and the logistic difficulties of doing so for cryptic or evasive species; accelerometers provide a resolution to both difficulties.

In Chapter 2, I characterize differences in the stress responses of the study species in a controlled setting and I validate the non-invasive FGM method used throughout this dissertation. By bringing *T. alpinus* and *T. speciosus* into captivity and exposing them to a series of challenges, I quantified test stress reactivity and confirmed that FGMs reliably reflect changes in circulating GC levels in these species. These analyses also revealed that, overall, *T. alpinus* tended to be more stress-reactive than *T. speciosus*. For example, stimulation with ACTH lead to the expected elevation in FGMs in both species, although response by *T. alpinus* was faster and longer-lasting. A handling challenge lead to non-significant increases in FGMs for *T. speciosus* but significant increases – comparable to the ACTH challenge – for *T. alpinus*. Finally, *T. alpinus* had a significantly stronger response to captive conditions than *T. speciosus*. Thus, collectively, these results characterize *T. alpinus* as more stress-reactive, exhibiting stronger GC increases in response to a broader range of stressors.

Chapter 3 serves to validate a novel method for remotely quantifying activity budgets in in free-living animals. While behavior is expected to be an important mechanism for

coping with rapid environmental change, behavioral activity budgets are time consuming and logistically challenging to collect, particularly for evasive animals like chipmunks. Accelerometers – tiny sensors that measure acceleration, typically in three axes of movement – have recently emerged as a useful technology for remotely detecting behavioral activity patterns in free-living animals. If patterns of acceleration can be reliably correlated with different behavioral states, then accelerometers can be used to collect behavioral data from free-living animals that are not under constant observation. In order to accomplish this, a validation dataset must be collected in which accelerometer data and behavioral observations are collected simultaneously. We brought individuals of both study species into captivity and collected accelerometer data while simultaneously filming the animals. After scoring all videos, we developed a machine learning system capable of automatically labeling raw acceleration data with behavioral categories. Finally, we deployed accelerometers on free-living animals to collect behavioral data from unobserved members of each species. This preliminary assessment of behavioral data revealed overall similarities in temporal and seasonal patterns of activity between the two study species.

In Chapter 4, I characterize activity budgets for *T. alpinus* and *T. speciosu*s over a broader spatial range, with the goal of identifying which factors are most predictive of activity in each species. I sampled populations of both species at three sites in and around Yosemite National Park. In conjunction with behavioral data obtained from accelerometers, I also collected environmental data, using ground-cover surveys for information about vegetation and iButtons for measures of daily temperatures. For each species, I constructed models for two energetically relevant categories of activity – general activity and locomotion – during all daylight hours as well as during afternoon hours when temperatures were warmest and thermal constraints are expected to be strongest. I found that activity patterns for *T. speciosus* were more variable and were better predicted by environmental parameters than activity in *T. alpinus*, potentially indicating that *T. speciosus* is better able to adjust its activity patterns to variable environmental conditions.

Finally, in Chapter 5, I explore patterns of FGM variation across multiple years and study sites to explore which factors are most predictive of baseline GC levels in each study species. In addition to the environmental data described in Chapter 4 (ground cover survey data and iButton temperature data), I employed climate-based ecological niche models to quantify habitat suitability and I used trapping data to estimate relative population densities. I then constructed models of FGM levels for each species that incorporated extrinsic (environmental) and intrinsic (individual phenotypes) data. These models demonstrate marked interspecific differences in the factors that are most predictive of FGM levels in each species. Environmental parameters, including ground cover categories, density, habitat suitability scores, and temperature all appeared to be significant predictors of FGMs for *T. alpinus*. In contrast, while ground cover categories were significant predictors of FGMs for *T. speciosus*, fewer environmental parameters were identified as important, while a greater number of intrinsic factors, including sex, body mass index, and reproductive status were found to be important predictors of FGMs.

Overall, my research suggests that there are important physiological and behavioral differences between *T. alpinus* and *T. speciosus* that may have contributed to the distinct pattern of elevational range shift detected for these co-occurring congeners. Physiologically, FGMs are more strongly influenced by extrinsic, environmental conditions in *T. alpinus,* suggesting that this species is more physiologically responsive to external stressors. Behaviorally, *T. speciosus* displays greater inter-individual variability and stronger relationships between environment and activity, suggesting that this species may make greater use of behavioral variation to adjust to short-term changes in environmental conditions. These findings add to a growing body of work suggesting that species with greater ability to cope with environmental change using phenotypic plasticity, genetic diversity, or dispersal, may be less vulnerable to environmental change.

Chapter 1 Figures

Figure 1 The alpine chipmunk (*Tamis alpinus*, A) and the lodgepole chipmunk (*T. speciosus,* B) and their current approximate geographic ranges in California (C: *T. alpinus*, blue; D: *T. speciosus*, red). Ranges are based on locality data for modern (post-1970) museum records (downloaded from the Arctos Database, http://arctos.database.museum/) and were generated with the ggmap package in R.

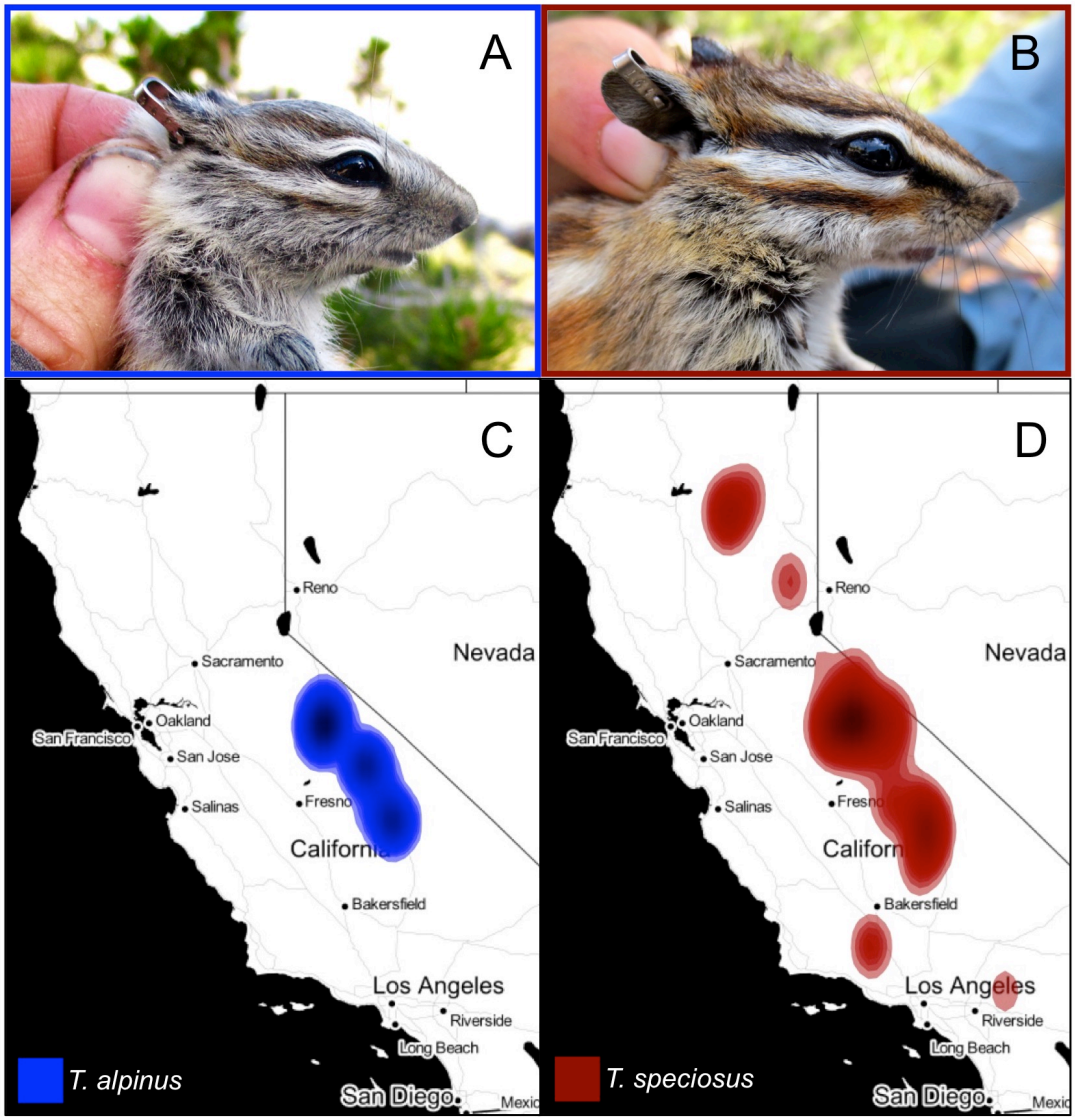


Figure 2 Elevational ranges of the co-occurring species of chipmunks *T. alpinus* (pink/purple) and *T. speciosus* (blue/purple) during historical (c.1906, left) and modern (c.2006, right) time periods in Yosemite National Park, CA (based on data from Moritz et al., 2008).

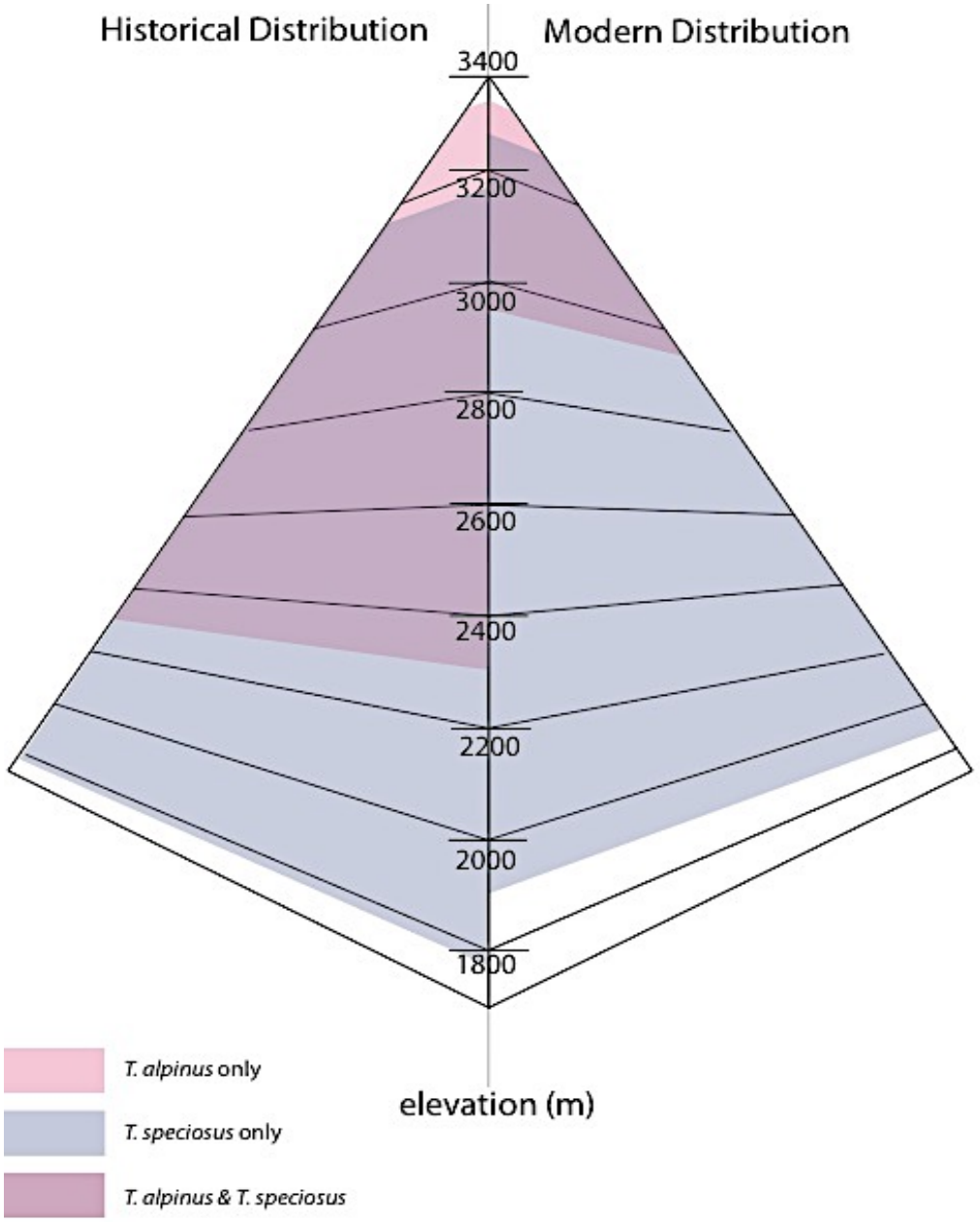
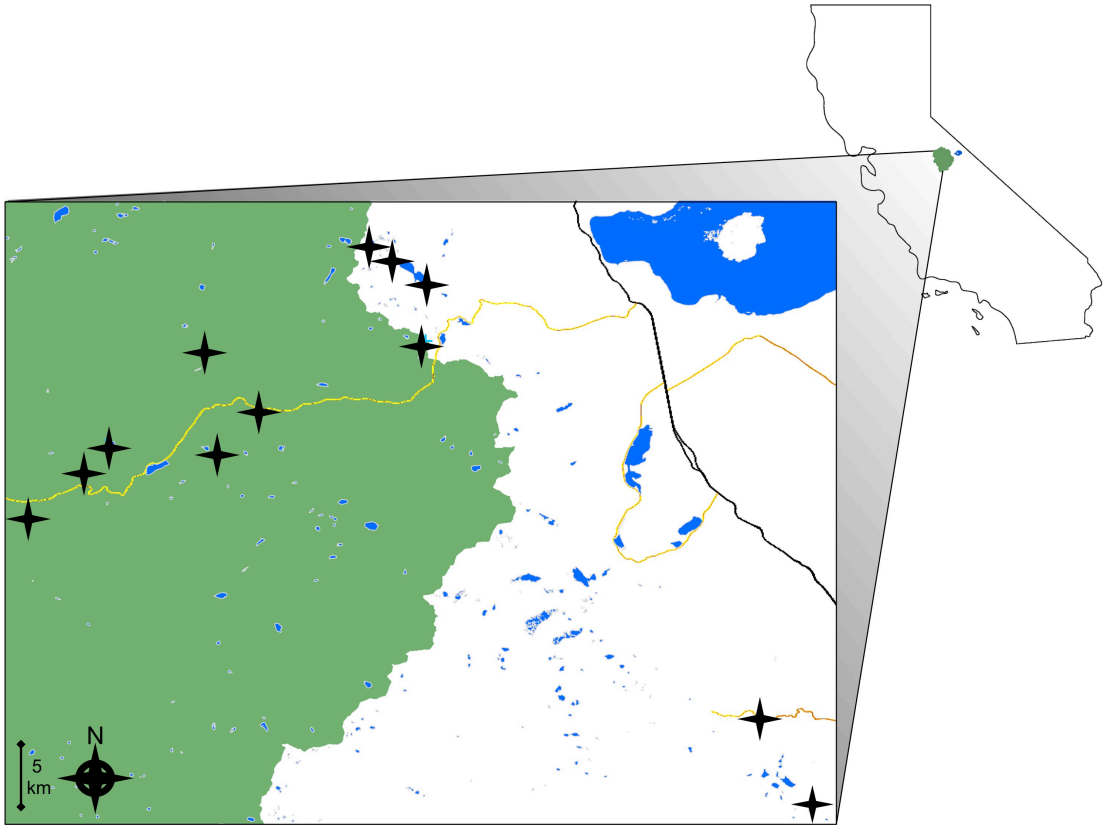


Figure 3 Map of study sites (black crosses) in and around Yosemite National Park (green), California, used in this study.



Chapter 2

Contrasting stress responses of two co-occurring chipmunk species (*Tamias alpinus* and *T. speciosus*)

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Abstract

Glucocorticoid (GC) hormones are important mediators of responses to environmental conditions. Accordingly, differences in GC physiology may contribute to interspecific variation in response to anthropogenically-induced patterns of climate change. To begin exploring this possibility, we validated the use of fecal cortisol/corticosterone metabolites (FCM) to measure baseline glucocorticoid levels in two species of co-occurring chipmunks that have exhibited markedly different patterns of response to environ- mental change. In Yosemite National Park, the alpine chipmunk (*Tamias alpinus*) has undergone a significant upward contraction of its elevational range over the past century; in contrast, the lodgepole chipmunk (*Tamias speciosus*) has experienced no significant change in elevational distribution over this period. To determine if GC levels in these species vary in response to external stimuli and to assess whether these responses differ between species, we compared FCM levels for the same individuals (1) at the time of capture in the field, (2) after a short period of captivity, and (3) after adrenocorticotropic hormone (ACTH), (4) handling, and (5) trapping challenges conducted while these animals were held in captivity. Our analyses indicate that *T. alpinus* was more responsive to several of these changes in external conditions. Although both species displayed a significant FCM response to ACTH challenge, only *T. alpinus* showed a significant response to our handling challenge and to captive housing conditions. These findings underscore the importance of species-specific validation studies and support the potential for studies of GC physiology to generate insights into interspecific differences in response to environmental change.

1. Introduction

Biotic responses to environmental challenges can vary markedly, even among closely related, ecologically similar species. The reasons for such variation are poorly understood, but physiological processes seem likely to play an integral role in generating such differences (Bernardo et al., 2007; Tingley et al., 2009; Tomanek, 2012). Among the

physiological systems that are expected to influence responses to environmental conditions are glucocorticoids (GCs), a group of metabolic hormones that help to maintain allostasis by mediating multiple systemic responses to abiotic (e.g., temperature, aridity) as well as biotic (e.g., social environment, predation) challenges (Bauer et al., 2013; Boonstra, 2004; Creel et al., 2013; Dantzer et al., 2013; Wingfield, 2013b; Woodruff et al., 2013). Due to their responsiveness to external conditions and their multiple, regulatory effects on an individual's biology, GCs have the potential to provide valuable information regarding the extent to which free-living animals are challenged by their environments (Bonier et al., 2009; Breuner et al., 2008; Romero, 2004; Wingfield et al., 1998).

While acute differences in GC levels provide information regarding responses to unexpected, short-term changes in environ- mental conditions, differences in baseline levels of GCs are more appropriate for assessing the impacts of enduring, long-term environmental challenges (Bonier et al., 2009; Busch and Hayward, 2009). Because fecal samples can be collected non-invasively and because they provide an integrated measure of hormone levels occurring over many hours, they are ideal for studies of baseline GC levels (Sheriff et al., 2011; Harper and Austad, 2000; Palme, 2012; Möstl and Palme, 2002; Touma and Palme, 2005). Prior to using fecal samples to measure GC response in a given species, however, a validation study should be completed to confirm that these samples reliably capture information regarding biologically significant changes in GC levels (Touma and Palme, 2005). Most studies employ a pharmacological challenge, in which animals are injected with adrenocorticotropic hormone (ACTH) and fecal samples are then collected at regular intervals to determine if an increase in fecal cortisol/corticosterone metabolites (FCM) occurs. Biological validation experiments can also be conducted by challenging animals with one or more relevant external stimuli (e.g., capture and handling, Bosson et al., 2012, 2013; Touma and Palme, 2005). Because stress physiology, including GC metabolism and excretion, can vary markedly among species (Boonstra and McColl, 2000; Clarke et al., 1988; Faure et al., 2003; Frisch and Anderson, 2005; Gomes et al., 2012; Juliana et al., 2014; Palme, 2005), taxon-specific validation studies are critical to measuring and interpreting data regarding FCM (Touma and Palme, 2005).

Chipmunks (genus *Tamias*) from the Sierra Nevada mountains of western North America provide an important system for exploring interactions between GC physiology and responses to environ- mental change. Two of these species – the alpine chipmunk (*Tamias alpinus*, *Ta*) and the lodgepole chipmunk (*Tamias speciosus*, *Ts*) – have been the subject of extensive study regarding interspecific differences in response to environmental conditions (Bi et al., 2012, 2013; Moritz et al., 2008; Rubidge et al., 2011, 2012, 2014). *Ta* is a small (30–50 g), high-elevation specialist that is found primarily above tree line in rocky habitats (Clawson et al., 1994). In contrast, *Ts* is a larger (50–80 g), more generalist species that occurs mainly below tree line in a variety of habitats (Best et al., 1994). Although these species co-occur at the upper end of *Ts'*s elevational distribution and the lower end of *Ta*'s elevational range, they are characterized by strikingly different responses to climate change in this region over the past century (Moritz et al., 2008). Specifically, although *Ta* has experienced an upward range con- traction of over 600 m, *Ts* has undergone no apparent change in elevational distribution (Moritz et al., 2008). While *Ta* has experienced a concordant decrease in overall genetic

diversity but increase in among-population genetic differentiation, genetic structure in *Ts* has remained stable (Rubidge et al., 2012; Bi et al., 2013). Collectively, these data suggest that *Ta* and *Ts* differ in their responses to environmental change.

As part of efforts to understand why patterns of elevational range change differ between *Ta* and *Ts,* we are exploring the role of GC physiology in mediating responses of free-living mammals to environmental conditions. The primary goals of this study are to validate the use of FCM as a measure of baseline GC response in *Ta* and *Ts* and to complete a preliminary assessment of interspecific differences in the stress physiology of these species. Using data from captive *Ta* and *Ts,* we report the results of an ACTH challenge experiment and describe circadian patterns of FCM. In addition, we characterize FCM responses of each species to both handling and trapping stressors. To relate these data to information from natural populations of chipmunks, we then compare baseline FCM levels for captive and free-living members of our study species. In addition to providing the first characterization of FCM in these chipmunk species, our data provide a critical foundation for exploring the role of GC physiology in mediating differences amongst mammalian responses to environmental change.

2. Methods

2.1 Study Animals and Sites

Lodgepole chipmunks (T. *speciosus*, TS) and alpine chipmunks (T. *alpinus*, TA) were live-trapped in the vicinity of Saddlebag Lake (37.966251, 119.265185; WGS 84), Inyo National Forest, Mono Co., CA during August and September of 2012 and 2013. By these dates, the annual breeding season was over, young-of-the-year were weaned, and no females that we trapped were lactating. All animals were captured using Sherman traps baited with peanut butter and oats. Traps were opened at dawn, checked approximately every 4–6 h, and closed at dusk. Each captured animal was weighed, its sex was determined, and standard external measurements (e.g., body length, tail length) were taken. In addition, each animal was marked for permanent identification by placing a numbered metal ear tag (1005–1, National Band and Tag, New- port, KY, USA) in each ear pinna. Adults were conservatively defined as individuals with developed testes or conspicuous (post-lactation) nipples, while juveniles were defined as animals with no testicular development or visible nipples; only adult animals were included in this study.

A subset of the animals captured (N = 15–16 per species) was transported to the Sierra Nevada Aquatic Research Laboratory (SNARL) located near Mammoth Lakes, Mono Co., CA. At SNARL, members of both study species were housed in a vivarium maintained at 10–20 °C (ambient outside temperature for the area). Lighting in the room (14L:10D) imitated natural lighting at the latitude of Saddlebag Lake during the study period. Animals were housed in commercially purchased cages (35.6 27.9 38.1 cm; Prevue Hendryx) containing screen floors and removable drop pans that allowed for non-disruptive collection of fecal samples. Each cage was provided with a plastic nest box, cotton bedding material, and a water bottle. Animals were fed daily at 0630 with ad libitum quantities of commercial rodent chow, sunflower seeds, peanut butter, oats, apples, and other fruits.

Due to spatial constraints, no more than 18 animals could be housed in this facility at the same time. As a result, all experiments were conducted twice; the first replicate was completed during August 2012 and the second during September 2012 (except for the trapping challenge, which took place in August and September of 2013). Replicates were balanced with regard to the number of individuals of each species and sex included in each round of data collection. Husbandry procedures and experimental protocols (see below) were the same for both replicates. All animal procedures were approved by the University of California, Berkeley and University of California, Santa Barbara Animal Care and Use Commit- tees and followed the guidelines of the American Society of Mammalogists for the use of wild mammals in research (Sikes and Gannon, 2011).

2.2. Sample collection and storage

In the field, fecal samples were collected from traps as soon as captured chipmunks were removed. Pellets from the same trap were placed in a cryogenic tube and then deposited in a 40 °C freezer at SNARL later the same day, within a 4–12 h period. All fecal pellets and other materials (e.g., left over bait) were removed from traps before they were reset.

In captivity, fecal samples were collected by removing the pan beneath each cage and manually transferring pellets to cryogenic tubes. Cage pans were lined with absorbent sheets to prevent extensive urine contamination and no urine-contaminated samples were collected. Tubes were then immediately frozen at 40 °C. Prior to initiation of challenge tests (see Section 2.4, below) feces were collected daily from captive individuals at 4 h intervals from approximately 0600–2200 to habituate the animals to the sample collection process. Cage pans were cleaned completely each time that samples were collected to ensure that fecal pellets were produced within a known, 4-h sampling period; pans were cleaned in a different area of the animal facility to reduce cleaning-induced stress to the study subjects. All experimental groups had their cages cleaned on the same schedule and in the same manner and thus any impact of this procedure should have been the same for all study animals.

Because freezing field-collected samples in liquid nitrogen is a logistically challenging procedure in backcountry field settings where no freezer is available, we used the captive study population to contrast the efficacy of storing pellets in ethanol at ambient temperature versus freezing samples immediately after collection. For a subset of samples collected from the captive study animals (*Ta*: 5F, 3M; *Ts*: 4F, 3M), we compared FCM data from frozen versus ethanol-stored fecal pellets collected from the same individuals. Upon collection, each pellet provided was manually split in half to ensure that the two samples were comparable, in case of heterogeneous metabolite distribution across pellets within a sample. One half of each fecal pellet was frozen immediately at 40 °C while the other half was placed in 100% ethanol and stored at ambient temperature for 1 month before freezing at 40 °C. At the end of data collection at SNARL, all samples were transported on dry ice to the to the UC Berkeley campus, where they were stored at 80 °C until analysis.

2.3. Circadian patterns of FCM excretion

In rodents, GC production typically varies throughout each 24-h period, with plasma GC levels increasing shortly before an animal becomes active and decreasing as

the animal approaches the resting phase of its circadian pattern of activity (Dickmeis, 2009; Lepschy et al. 2010). To determine whether our study species exhibit circadian fluctuations in FCM excretion, we collected fecal samples from the captive study animals (*Ta*: 8F, 7M; *Ts*: 8F, 8M) at approximately 4-h intervals for 28 h. Pellets were collected at least 5 days after capture and 5 days before any experimental manipulation of GC physiology, thereby allowing us to use these samples both to characterize circadian patterns of FCM excretion and to provide a within-subjects comparison for our ACTH challenge experiment (see Section 2.4, below). All samples were frozen at 40 °C immediately after collection and maintained at that temperature until transport to the Berkeley campus (see above, Section 2.2).

2.4. ACTH challenge

To validate our EIA procedure for quantifying GC levels and to confirm that elevated GC levels are captured by analyses of fecal samples, we performed an ACTH challenge experiment. At approximately 14:00 on day 10 after capture, individuals of both species (*Ta*: 4F, 4M; *Ts*: 4F, 4M) were injected intramuscularly with 12 IU/ kg body mass of a synthetic form of ACTH (Cortrosyn: Amphastar Pharmaceuticals Inc., Rancho Cucamonga, CA) that had been reconstituted in 0.9% saline solution. While there is no single recommended dosage for all vertebrate species, similar dosages have been used in other ACTH challenge tests for small mammals (Touma et al., 2004; Woodruff et al., 2010). Total volume injected was approximately 0.06 mL for *Ts* and 0.038 mL for TA.

In other rodent species, response times for ACTH challenge tests range from 5 to >24 h (Mateo and Cavigelli, 2005; Smith et al., 2012; Touma et al., 2004; Woodruff et al., 2010). Accordingly, we collected fecal samples from our study subjects every four hours for the first 36 h after injection, with additional collections at 48, 60, and 72 h post-injection. Samples were stored as described above (Section 2.3).

2.5. Handling challenge

To determine if the results of our ACTH challenge tests were influenced by changes in baseline FCM levels resulting from handling of captive animals, we conducted a parallel handling challenge study. Concordant with each replicate of the ACTH challenge study, a separate subset of individuals of each species (*Ts*: 4F, 4M; *Ta*: 3F, 4M) was subjected to a handling stressor, in which each animal was removed from its cage and injected with 0.9% saline solution; injection volumes and handling protocols were the same as those for ACTH-injected animals. Fecal samples collected for the study of circadian patterns of FCM excretion (see Section 2.3, above), provided a within subject, no-treatment comparison for both the ACTH and handling treatments. Assignment of individuals to ACTH or saline injection treatments was randomized and the order in which animals were handled and injected was rotated among treatments, sexes, and species.

2.6. Trapping challenge

To determine whether field-trapping protocols alter baseline FCM levels in our study species, captive individuals were subjected to a trapping stressor. During August and September 2013, *Ta* (N = 2F, 5M) and *Ts* (N = 4F, 3M) were live-trapped and transported to the SNARL vivarium; all capture, transport, and housing procedures were

the same as those used in 2012. After the animals had been held in captivity for at least 5 days, fecal samples were collected every 4 h for a total of 3 days to provide a within-subjects control set of samples. On the fourth day, the animals were placed inside Sherman traps for 4 h, which represents the typical time that individuals are held in traps in the field. The animals were then returned to their home cages and fecal samples collected every 4 h for the following 3 days.

2.7. Comparisons of baseline FCM from free-living and captive animals

Because fecal samples were collected from the same individuals at first capture and again while they were housed at SNARL, we were able to use a within-subjects design to compare FCM levels for animals living under natural versus artificial conditions. Field measures of FCM levels were based on fecal samples collected from traps (see Section 2.2, above). Traps were checked approximately every 4–6 h; cages in the lab were checked every 4 h. Thus, samples from captive and free-living animals provided information regarding baseline FCM levels over comparable time periods. Because field samples were collected at a variety of times of day, we compared the field FCM level for each individual with the temporally most similar sample (i.e. collected at the same time of day) obtained from that individual in captivity during the circadian (pre-challenge) portion of the study (see Section 2.3, above).

2.8. FCM extraction and assay

To quantify FCM levels, we first extracted GC metabolites from fecal samples following the protocol of Palme et al. (2013). In brief, samples were dried at 90 °C for 4 h, after which they were crushed using a mortar and pestle. For samples that had been stored in ethanol, the ethanol was allowed to evaporate before samples were oven-dried. Only fecal samples with dry weights P0.02 g were extracted, with actual sample weights ranging from 0.02 to 0.07 g due to natural variability in fecal sample size. For extractions, an aliquot of 80% methanol (1 mL per 0.05 g feces) was added to each sample, the sample was shaken using a multi-vortex for twenty minutes and then centrifuged for twenty minutes. The resulting supernatant was dried in a vacuum centrifuge and shipped to Vienna, Austria, where extracts were reconstituted in 80% methanol, diluted in assay buffer and assayed using a 5a-preg- nane-3b,11b,21-triol-20-one enzyme immunoassay first described for use with laboratory mice (Touma et al., 2003). This EIA measures GC metabolites with a 5a-3b,11b-diol structure. The same EIA has proven suitable for measuring FCM in other sciurid species (Bosson et al., 2009, 2013; Dantzer et al., 2010, 2013; Montiglio et al., 2012); because our assays produced meaningful measures of FCM, no other antibodies were tested. Intra- and inter-assay coefficients of variation were 9.1% and 14.0%, respectively. We tested for parallelism in our assays by comparing binding curves of serially diluted (4 times 1:2.5 each) samples from both species and both sexes (N = 4 samples total) with the binding curve of serially diluted standards.

2.9. Statistical analyses

All statistical analyses were conducted in the program R 2.15.3 (R Development Core Team, 2008). Data were not transformed prior to analysis. For two-sample analyses, the data were tested for normality (Shapiro–Wilk test) and homoscedasticity (F-test); if

these assumptions were not met, non-parametric statistical analyses were used. Welch's two sample t-tests and Wilcoxon rank sum tests were used to examine the effects of replicate and sex on FCM levels in each species. An ANCOVA was used to test for parallelism between the slope of the standard curve and the binding curve for samples from each species and sex.

Analyses of circadian patterns of FCM concentrations as well as temporal comparisons of FCM levels for ACTH, handling, and trap- ping challenge experiments were completed using general linear mixed-effect models (GLMM), implemented in the R package 'lme4' (version 1.1–6). For these analyses, FCM level was used as the response variable, with collection time and treatment (circadian pattern, ACTH, handling, trapping) as fixed effects and individual as a random effect. Significance tests were performed by comparing these models to null models that included only time and the random effect (circadian study: random effect only) using a log-likelihood ratio test (R function 'anova') as described by Rimbach et al. (2013). To corroborate the GLMM results and to examine treatment-specific FCM effects in greater detail, post hoc significance tests were used to compare specific time points that appeared to differ between treatment groups. Specifically, Welch's two-sample t-test was used if data met the associated assumptions of homoscedasticity and normality; Wilcoxon rank sum tests were used whenever these assumptions were not met. For analyses involving multiple comparisons, False Discovery Rate (FDR) adjustments (Benjamini and Hochberg, 1995) to p-values were made separately for each species. For comparisons of baseline (circadian) data versus data from ACTH and handling challenges, t- tests were used to compare FCM levels from samples collected at the same time of day.

Within-individual comparisons of mean FCM levels for field- versus vivarium-collected samples were completed using paired t-tests. Between-species comparisons of these data were completed using two-sample (unpaired) t-tests. Comparisons of frozen versus ethanol stored samples were conducted using one-sample t- tests in which the difference in FCM levels between the two portions of each sample were compared to an expected value of 0 (no difference between storage methods).

3. Results

In both study species, FCM binding curves for males and females were parallel to the standard curve for the assay (all $F < 0.51$, all $P > 0.49$, Fig. 1). Based upon comparisons of mean circadian (pre-challenge) hormone levels, we found no significant inter-sexual differences in FCM levels in either species (Welch two sample t-tests, TA: $t = 0.62$, $df = 12.95$, $P = 0.55$; TS: $t = 1.91$, $df = 12.79$, $P = 0.078$). In *Ta,* males averaged 171 ng FCM/0.05 g fecal powder (N = 7, S.D. = 66) and females averaged 193 ng FCM 0.05 g fecal powder (N = 8, S.D. = 71). Male *Ts* averaged 136 ng FCM/0.05 g fecal powder (N = 8, S.D. = 60) and females averaged 84 ng FCM/0.05 g fecal powder (N = 7, S.D. = 45). While there does appear to be some effect of sex on baseline captive FCM levels in captive *Ts,* the difference between the sexes was not significant and we do not have enough data here to make any strong conclusions. Because the distribution of males and females was balanced across experimental replicates and because all experimental manipulations employed a within-subjects design, we pooled data from males and

females of each species for subsequent analyses. When data from both sexes were combined, we found no significant differences in FCM levels between the first and second replicates of our study for any of the variables examined (circadian, handling, ACTH, and trapping challenges; two-sample t-tests, all $P > 0.08$), and thus data were from both replicates were pooled for subsequent analyses.

3.1. Comparison of storage methods

The effects of storage method on baseline FCM levels differed between the study species. In *Ta,* ethanol storage produced significantly higher GC metabolite levels than immediate freezing of samples (one sample t-test: T = 3.24, N = 5F, 3M, l = 0, P = 0.01; Fig. 2). In *Ts,* however, no significant difference was found between ethanol preserved and frozen samples (one sample t-test: T= 0.80, l=0, N=4F, 3M, P=0.46; Fig. 2). Because all samples used in the remaining analyses reported here were frozen after col- lection, this apparent interspecific difference in response to fecal pellet preservation in ethanol did not impact the analyses of FCM levels reported below.

3.2. Circadian patterns of FCM excretion

Because not all individuals provided fecal pellets during each collection period, sample sizes varied somewhat across collection time points for the circadian study (Fig. 3). For *Ta,* a full model containing time as a fixed factor and individual as a random factor outperformed a null model containing the random effect alone (v2 = 8.52, P = 0.0035), suggesting that time-of-day had some effect on FCM levels in this species. Visual inspection of these data (Fig. 3) indicated that FCM levels tended to be highest in the late morning (11:00) and early afternoon (15:00) and lower during the rest of the day. In contrast, for *Ts* the full model did not significantly out- perform the null model, although visual inspection of these data suggested that FCM production tended to be highest during the middle of the day, during the period when FCM production was lowest for *Ta* (Fig. 3; v2 = 3.54, P = 0.06). It is possible that the lack of a strong circadian pattern for either species was due to the 4-h inter-collection period, which could have masked the assay's ability to detect a more obvious rhythm. For both species, circadian samples were used as a baseline against which to compare the results of ACTH and handling challenges. While these samples do not represent a truly un-manipulated baseline because they were obtained after animals were transferred to captivity, they provide a reasonable basis for comparison with samples collected after more targeted manipulations such as our ACTH and handling challenges (see below). In all cases, comparisons were restricted to fecal pellets collected at the same time of day, thereby avoiding potential confounds resulting from any circadian variation in FCM production in either study species.

3.3. Response to ACTH and handling challenges

Injection with ACTH had a significant effect on FCM levels in both species (Fig. 3). Because not all individuals provided fecal pellets at each time point, sample sizes varied across sampling intervals. Due to limited numbers of pellets, only the following time points were assayed for each study species: 24 h (pre-injection), 0 h (injection), and 4, 16, 20, 24, 28 and 48 h post-injection. For both species, the full model (individual, time, experimental treatment) outperformed the null model containing only individual and time (TA: v2 = 39.45, P = 5.6e 8, *Ts*: v2 = 29.70, P = 5.6e 6), indicating that some time points

differed between experimental treatments. Visual inspection of the data revealed that FCM response to ACTH appeared more quickly (at +20 versus +24 h) and lasted longer (+20 through +28 h versus +24 h only) in *Ta* than in *Ts*. Averaged across individuals, peak FCM level occurred at +21.5 h for *Ta* and at +28.5 h for *Ts*. Post-hoc comparisons of specific time points revealed that for *Ta*, ACTH-injected and circadian baseline FCM values differed significantly at +20, +24, and +28 h (Fig. 3, Table 1). For *Ts,* ACTH-injected and circadian baseline FCM values were significantly different only at +24 h, although this difference disappeared when the associated p-value was corrected for multiple comparisons (Fig. 3, Table 1).

In response to handling, FCM levels for *Ta* were significantly greater than circadian (pre-challenge baseline) levels at +20, +24, and +28 h (Fig. 3, Table 1). Results of the ACTH challenge were similar, with FCM levels for ACTH-challenged animals differing significantly from circadian baseline values at +24 and +28 h (Fig. 3, Table 1). In contrast, post-handling FCM levels for *Ts* were never significantly higher than circadian (pre-challenge baseline) levels; although post-handling FCM levels in this species were significantly lower than ACTH-injected levels at +24 h, this difference dis-appeared when the associated p-value was corrected for multiple comparisons (Fig. 3, Table 1). Thus, while both species responded to injection with ACTH, only *Ta* displayed a significant response to handling.

3.4. Response to trapping challenge

Neither study species displayed a significant response to our trapping challenge. Based on the timeline for response to injection with ACTH, we assayed samples collected 72, 48, 44, 20, 0, +24, +28, and +52 h from the start of the trapping challenge to pro- vide a comprehensive series of pre- and post-challenge FCM values for each individual. A model including individual, time, and treatment did not significantly outperform the null (individual and time) model for either study species (TA: $v2 = 0.028$, $P = 0.87$, TS: $v2 = 2.78$, $P = 0.095$), suggesting that trapping did not have a significant effect on FCMs in captive animals. Further, no significant differences were found for paired t-tests between pre-challenge and post-challenge FCM levels at any sample time point (all $P > 0.16$ for unadjusted values, all $P > 0.61$ for FDR adjusted values).

3.5. Comparisons of FCM in captive and free-living animals

Within-individual comparisons of time-matched samples collected in captive versus field settings (mean difference between captive and field times of day = 96 min, range = 0–300 min, paired N = 15 for *Ta,* 15 for TS) revealed that FCM levels for *Ta* but not for *Ts* were significantly higher in captivity (paired t-tests, TA: T = 3.28, N=15,P=0.0054;TS:T=1.46,N=15,P=0.17,Fig.4). Among captive animals, mean baseline FCM levels were significantly higher in *Ta* than in *Ts* (Welch's two-sample t-test: T = 3.08, N = 15, 15, $P < 0.005$, Fig. 4). In the wild, however, there was no significant difference between mean baseline FCM levels for the study species (same individuals as those sampled in captivity; Welch's two-sample t-test: T = 1.38, N = 15, 15, P = 0.18, Fig. 4). Thus, while captivity produced a significant increase in baseline FCM levels in *Ta,* the same response to captive conditions was not detected for TS.

4. Discussion

Our analyses indicate that the EIA procedure employed was able to detect experimentally induced changes in fecal glucocorticoid metabolites in alpine and lodgepole chipmunks. Specifically, injection with synthetic ACTH induced significant increases in FCM levels in both study species beginning around 24 h after administration of this physiological challenge. With regard to the other challenges employed, *Ta* exhibited a more pronounced response than *Ts* to both handling and captivity. While multiple factors may have contributed to this difference in outcomes, one general interpretation of this finding is that *Ta* possesses a more responsive hypothalamic–pituitary–adrenal axis (HPA, the neuroendocrine pathway that regulates production of GC hormones). Future studies that examine the FCM responses of these species to a greater variety of challenges as well as studies that explore HPA function in these animals in greater detail will be valuable in revealing the extent to which the findings reported here reflect more generalized differences in endocrine response between *Ta* and TS.

4.1. Interspecific comparisons of FCM responses

The two study species displayed several intriguing differences in FCM response to the challenges administered here. First, ethanol storage resulted in significantly higher FCM levels than freezer storage for *Ta* but not for TS. While this difference did not impact our subsequent analyses, this outcome should be considered as part of future field studies that require remote storage of fecal samples for endocrine assays. Second, while *Ta* exhibited a significant increase in FCM levels after handling, a similar response was not detected for TS. It is intriguing that our handling stressor produced as marked an increase in FCM levels in *Ta* as our pharmacological (ACTH) challenge; in most species, saline-injection with handling results in lower overall increases of FCMs in comparison to ACTH challenge (Chelini et al., 2010; Sheriff et al., 2009; Touma et al., 2004; Woodruff et al., 2010). Additionally, the response to ACTH challenge in *Ta* was more rapid and more enduring, with this species exhibiting significantly higher ACTH-induced FCM levels earlier (at +20 versus +24 h) and for a longer period (at +20, +24, +28 versus only +24 h) than TS. Finally, although baseline FCM levels for free-living animals did not differ between the study species, levels for captive individuals were significantly higher for TA. Relatedly, only *Ta* displayed a significant difference in baseline FCM levels between the field and captivity. These findings suggest that *Ta* may be more responsive to some forms of seemingly diverse environmental challenges.

Several factors may have contributed to the apparent interspecific differences in FCM response reported here. For example, the sensitivity of the HPA axis may vary among species such that the same stimulus produces markedly different levels of response in different taxa. This includes potential interspecific variation in perception and evaluation of potential stressors (Malmkvist et al., 2011). At the same time, the suite of environmental conditions – the fundamental niche (Grinnell, 1914) – to which *Ta* is adapted may be more limited; *Ta* is thought to be more of a habitat specialist (Best et al., 1994; Clawson et al., 1994), with the result that homeostasis in this species may be more easily perturbed than in TS. These potential explanations are not mutually exclusive and

it seems likely that the interspecific differences in FCM responses reported here reflect a combination of causal factors.

In addition to these differences in FCM response, the study species also shared a number of important elements of GC physiology. For example, in response to ACTH challenge, both species' FCM levels peaked at approximately 24 h after injection. Across sciurids, there appears to be marked interspecific variation in the timing of this peak, with some species exhibiting significantly higher FCM only 8–12 h after injection (Bosson et al., 2009; Montiglio et al., 2012; Sheriff et al., 2012) but others requiring over 24 h to exhibit a strong response (Bosson et al., 2013; Mateo and Cavigelli, 2005; Smith et al., 2012). Such differences are generally attributed to interspecific variation in gut passage times and diet (Palme, 2005), although the specific assay methodology employed may also play a role.

Neither study species exhibited significant sex-dependent differences in FCM levels, although such differences have been reported for other chipmunk species (Montiglio et al., 2012). It is possible that our relatively small sample sizes precluded the ability to detect differences in FCM levels between males and females. Alternatively, differential effects of captivity and our experimental challenges may have served to mask typical inter-sexual differences in FCM in these animals. Sample collection during this study occurred after the end of the annual breeding season and it is possible that stronger sex differences in FCM levels would have been detected if samples had been obtained during the portion of the year when individuals are reproductively active, as has been found for other species of sciurids (Dantzer et al., 2010; Kenagy and Place, 2000).

Somewhat surprisingly, neither study species displayed a significant response to our trapping stressor. It is possible that our challenge protocol was not appropriate for eliciting a strong GC response, particularly given that in *Ta,* individuals were already exhibiting increased FCM levels as a result of being housed in captivity. Trapping-induced changes in GC levels in other small mammals have typically been measured using blood plasma samples (Bosson et al., 2012; Fletcher and Boonstra, 2006), which are likely better suited to detecting acute (i.e., rapid, short-term) changes in GC levels initiated by experiences such as our trapping challenge (Sheriff et al., 2011; but see Bosson et al., 2013). In contrast, although injection with ACTH likely also represents an acute challenge, the extreme nature of this pharmacological stimulus may make it detectable in FCM despite the more extended timeline over which GC metabolites accumulate in fecal samples. Thus, additional analyses targeting acute rather than more baseline FCM responses may be required to determine whether trapping fails to elicit a GC response in our study species or the more likely possibility that this response was simply not detectable using FCM analysis in captivity.

4.2. Implications for response to environmental change

The variation in baseline FCM levels reported here has potentially important implications for exploring the impacts of environ- mental change. Baseline GC levels may be useful as indicators of environmental challenges for several reasons (Wingfield, 2013a). Within species, differences in baseline FCM levels may provide insights into the relative severity of the environmental challenges to which individuals or populations are exposed. For example, some species undergoing elevational range shifts have exhibited varying GC responses across their distributions, with expanding populations characterized by higher

baseline and acutely stressed GCs (Addis et al., 2011; Liebl and Martin, 2012) and enhanced GC receptor frequencies (Liebl and Martin, 2013). Applying the same logic, in species undergoing range contraction it is possible that trailing-edge populations may exhibit elevated GCs; to the best of our knowledge, this aspect of range contraction has not been studied.

More generally, assays of samples collected throughout a species' distribution may reveal populations that are subject to challenges substantive enough to elicit a physiological response (Busch et al., 2011; Sheriff et al., 2012). Across species, differences in GC sensitivity may be useful in predicting which taxa are most likely to be challenged by changes in environmental conditions; for example, if increased GC sensitivity is associated with a reduced tolerance for change, then species with more responsive GC physiologies may be particularly vulnerable to anthropogenic or other sources of environmental modification (Jessop et al., 2013; Wikelski and Cooke, 2006; Wingfield, 2013a). In sum, because GCs are integrally involved in homeo- and allostasis and in moderating trade-offs between survival and reproduction (Angelier and Wingfield, 2013; Boonstra, 2004; Busch and Hayward, 2009; Wingfield, 2013a), they should provide valuable indicators of response to environmental change.

Given the complexity of the GC response, use of this physiological system as an indicator of environmental change requires basic knowledge of a species' neuroendocrine response to challenge. Pharmacological validation studies provide critical information regarding the ability of fecal GC levels to capture information regarding response to external challenges (Touma and Palme, 2005). As demonstrated here, the addition of biologically relevant challenges (e.g., trapping, handling) as well as comparisons of captive-housed and field-caught individuals can generate further insights into relationships between environmental conditions and physiological responses. Critical next steps include experimental quantification of the relative sensitivity of the GC response in our study species as well as sampling along an elevational transect of free-living chipmunk populations to determine if baseline GCs vary systematically with environmental conditions. Coupled with environmental data from localities occupied by *Ta* and *Ts,* this information will allow us to explore potential biotic and abiotic correlates of intraspecific variation in baseline FCM levels. At the same time, analyses that examine patterns of glucocorticoid and mineralocorticoid receptor activity may be important in further quantifying interspecific differences in response; these receptors – both of which mediate GC responses – have been found to differ in other species experiencing range changes (Addis et al., 2011; Liebl and Martin, 2012, 2013). Because our study species are closely allied phylogenetically and geographically yet exhibit clearly divergent responses to habitat conditions over the past century (Moritz et al., 2008; Bi et al., 2013; Rubidge et al., 2012), we believe that these animals provide a particularly important system within which to explore the potential for baseline GC responses to serve as indicators of response to environmental change.

5. Conclusions

This study documents marked differences in baseline GC physiology between two species of chipmunks, thereby emphasizing the importance of species-specific validation studies. Although congeneric and partially sympatric in the central Sierra Nevada, our

analyses indicate that *Ta* and *Ts* respond differently to several external challenges, including pharmacological administration of ACTH, captive housing conditions, and handling by humans. Specifically, *Ta* appears to be more responsive to these challenges than *Ts,* a finding that may have significant implications for understanding documented differences in range change in these species over the last century. We believe that this study confirms the importance of conducting species-specific validation studies of FCM and outlines the potential for using GC physiology to monitor

Chapter 2 Tables

Table 1. Comparisons of post-challenge FCM levels. Data are from pharmacological and handling challenge experiments conducted on alpine and lodgepole chipmunks; data from both challenges were compared to pre-challenge (circadian) data from the same individuals. Based on visual inspection of FGM levels (Fig. 2), post-hoc statistical comparisons were conducted for data collected +20, +24, and +28 hours post-challenge to test for differences between the these treatments. For each comparison, the name of the statistical test employed is given, as is the test statistic and both unadjusted and adjusted (false-discovery rate) p-values; significant contrasts are indicated in bold.

Species	Time point	ACTH vs. Circadian	Handling vs. Circadian	ACTH vs. Handling
Tamias speciosus	+20 hours	Welch, t = 3.2428, df = 8.597, **p = 0.011, FDR p = 0.020**	Wilcoxon, W = 78, **p = 0.0016, FDR p = 0.0072**	Welch, t = 1.6837, df = 8.594, p = 0.13, FDR p = 0.17
	+24 hours	Wilcoxon, W = 68, **p = 0.00099, FDR p = 0.0072**	Wilcoxon, W = 27, **p = 0.0091, FDR p = 0.020**	Welch, t = -0.117, df = 4.488, p = 0.91, FDR p = 0.91
	+28 hours	Welch, t = 3.2906, df = 9.89, **p= 0.0083, FDR p = 0.02**	Welch, t = 3.0684, df = 5.343, **p = 0.026, FDR p = 0.039**	Welch, t = 0.1273, df = 9.259, p= 0.90, FDR p = 0.91
Tamias speciosus	+20 hours	Wilcoxon, W =82, p =0.29, FDR p = 0.57	Wilcoxon, W =63, p =0.98, FDR p = 0.98	Welch, t = -0.971, df = 13.5, p= 0.35, FDR p = 0.57
	+24 hours	Wilcoxon, W = 48, **p = 0.019,** FDR p = 0.17	Welch, t = 0.566, df = 15.473, p = 0.58, FDR p = 0.65	Wilcoxon, W = 6, **p = 0.045,** FDR p = 0.20
	+28 hours	Wilcoxon, W=45, p =0.20, FDR p = 0.57	Wilcoxon, W =41, p =0.38, FDR p = 0.57	Wilcoxon, W =25, p =0.51, FDR p = 0.65

Welch = Welch two sample t-test for parametric comparisons
Wilcoxon = Wilcoxon rank-sum test for non-parametric comparisons

Chapter 2 Figures

Figure 1. Parallelism between FCM levels obtained from fecal extracts versus a serially diluted standard. Fecal extracts were assayed for one male (triangles) and one female (circles) each for both *T. alpinus* (filled symbols) and *T. speciosus* (open symbols). Slopes for FCM binding curves were not significantly different from the standard curve ($p > 0.49$).

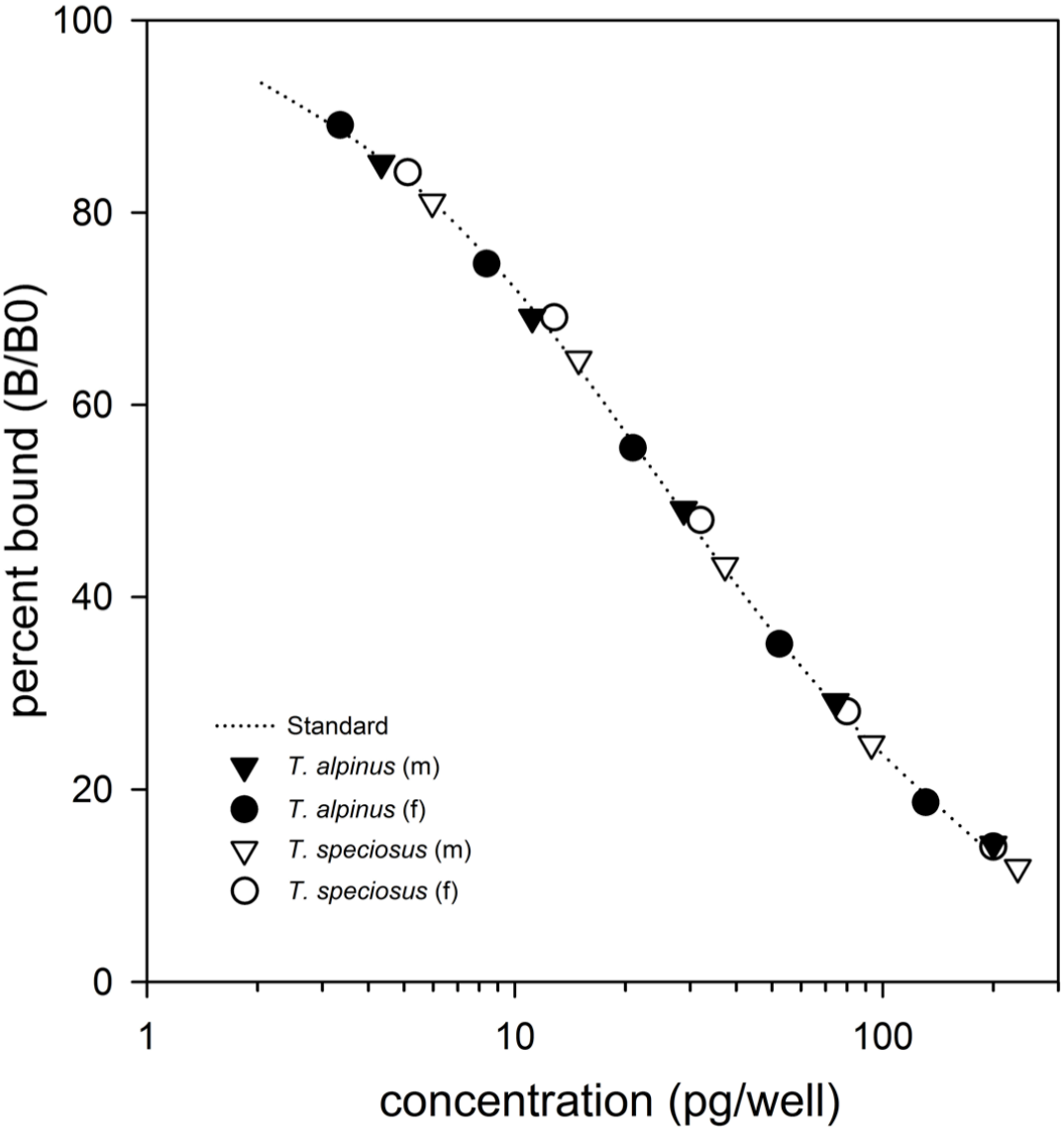


Figure 2. Comparison of baseline FCM levels for ethanol-stored versus frozen samples. Data are from 8 *TA* and 7 *TS*. For each individual, fecal samples collected in captivity were divided in half; one half was stored in 70% ethanol and the other was frozen at –80 ^{o}C immediately after collection. The difference in baseline FCM levels (EtOH – frozen) was calculated for each animal and these values compared to an expected difference of 0 (dotted line). The difference between observed values and 0 was significant for *TA* (p = 0.01) but not for *TS* (p = 0.46); statistical details are provided in the text.

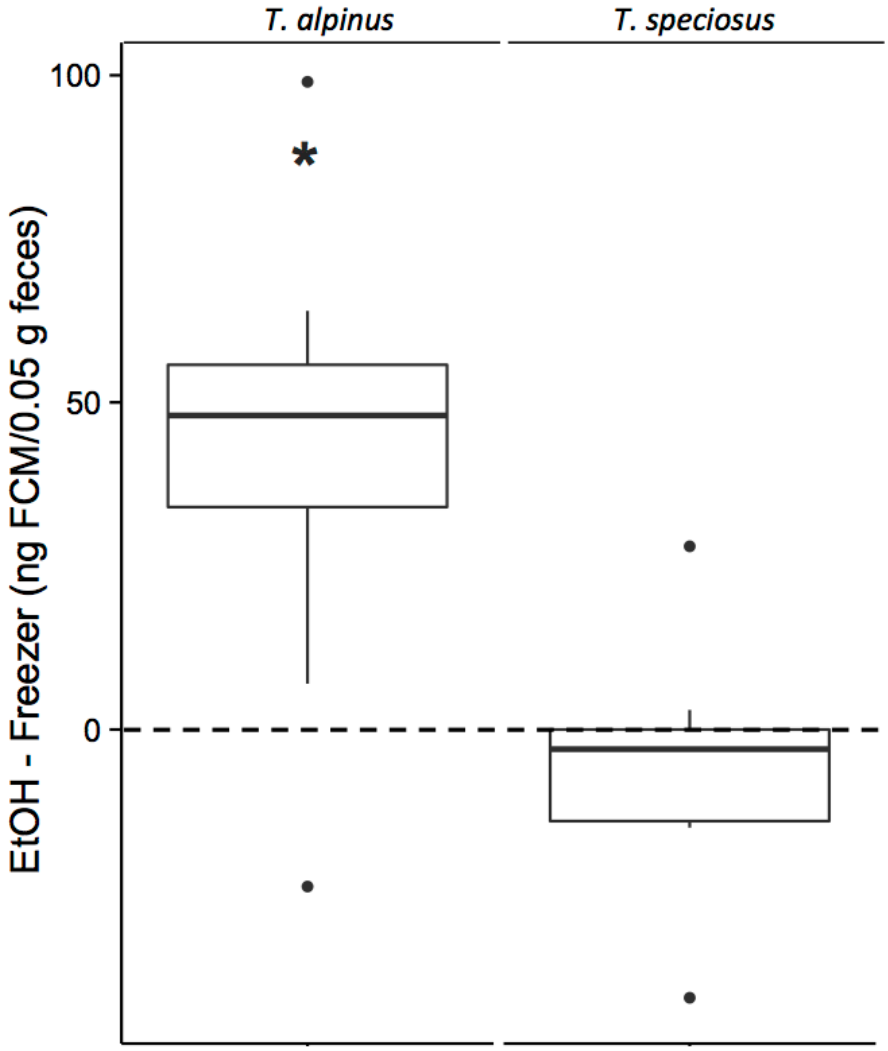


Figure 3. Comparisons of mean (± 1 SE) baseline FCM levels for circadian (no treatment, pre-stressor) samples (dashed line) and samples collected post-challenge with ACTH (dotted line) or handling (solid line). Sample sizes for each time point are indicated on the plot in the following order: ACTH, handling/saline (above curves) and circadian (below curves). Black and white bars along the bottom of the figure indicate the dark/light cycle during sample collection. Time = 0 hours occurred at approximately 14:00, the time of injection with ACTH or time of handling treatment. Circadian (no-treatment) samples were collected from 15-16 animals per species beginning five days prior to challenge and served to establish unmanipulated circadian patterns of FCM production; ACTH and handling challenges were each conducted on a subset of 7-8 of these individuals. For purposes of visual contrast, the circadian data are presented starting at hour +20, with the time (hour) of sample collection matched to the times at which post-challenge samples were collected. Asterisks denote significant contrasts between circadian (pre-challenge) and ACTH (solid symbol) or handling (open symbol) FCM levels, or between ACTH and handling/saline (triangles).

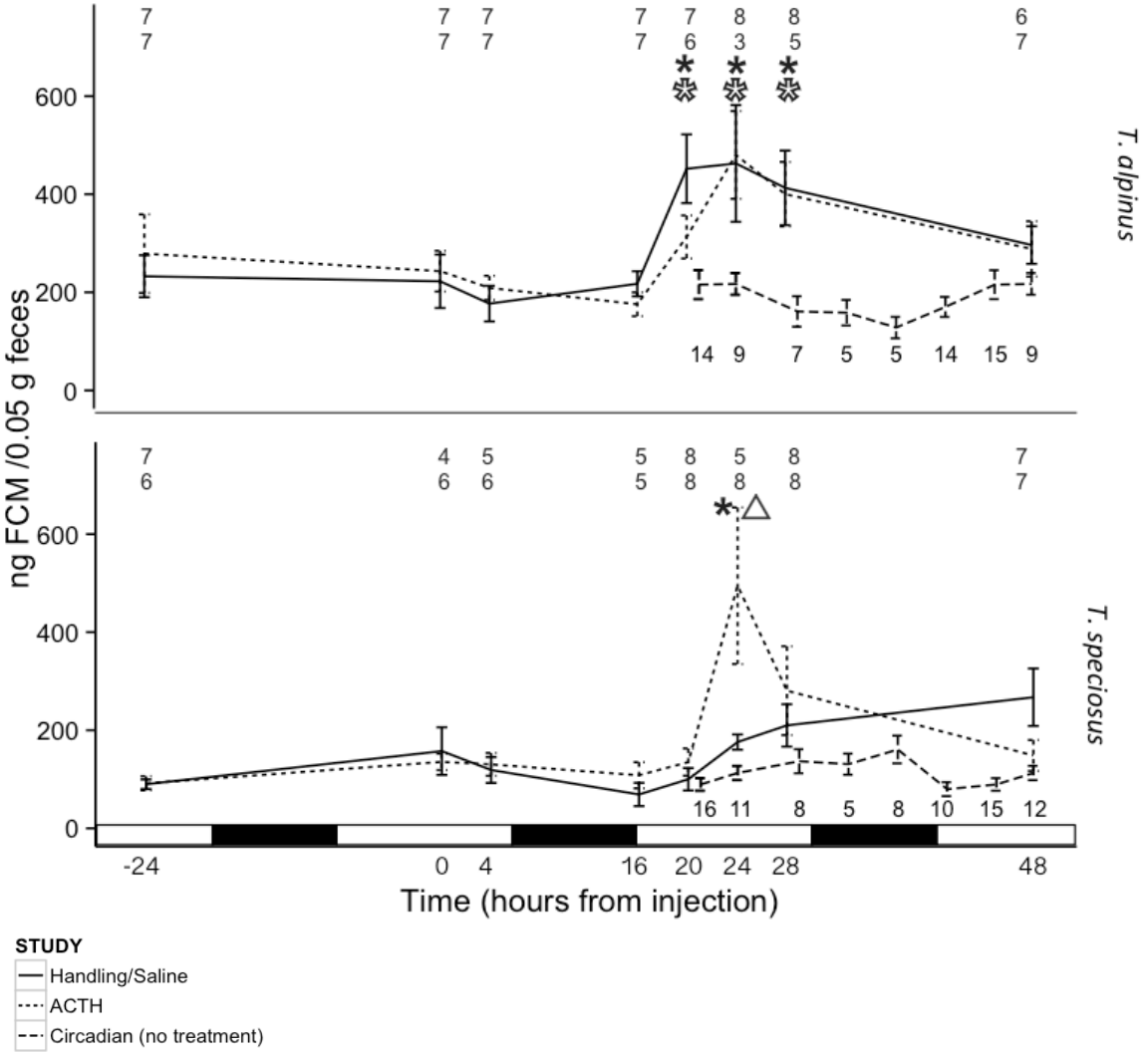
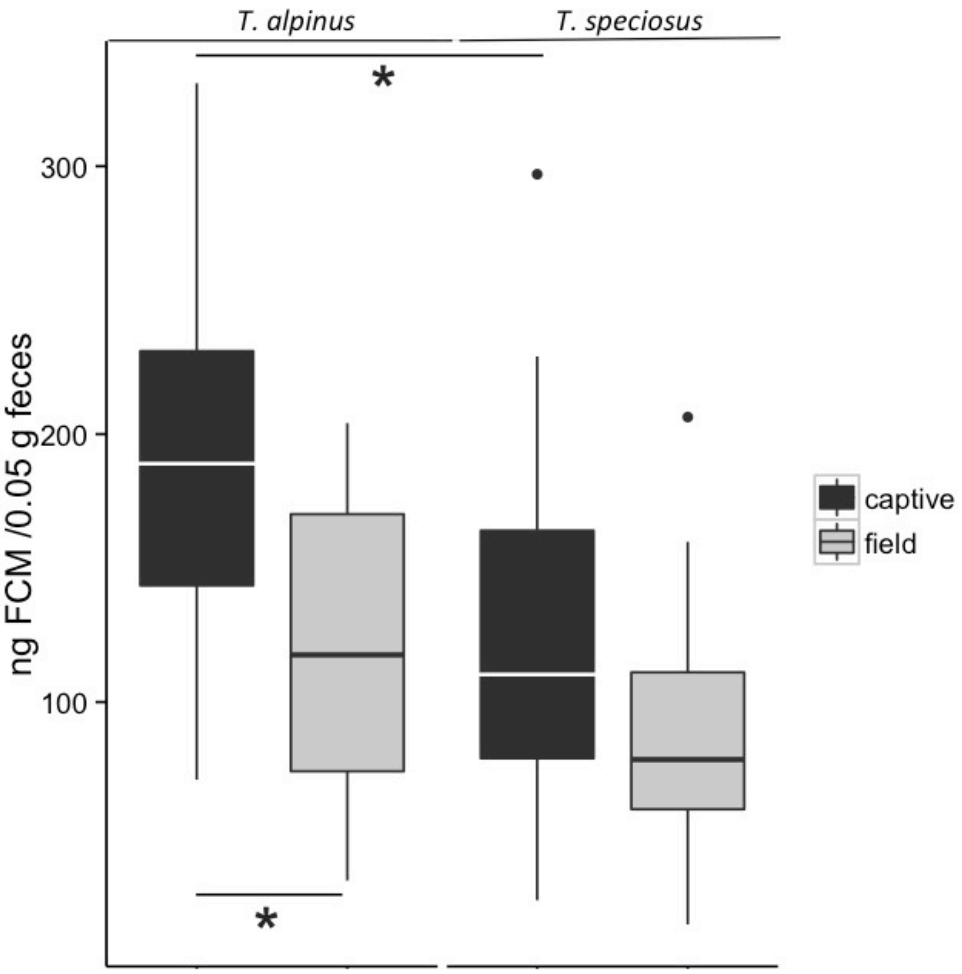


Figure 4. Comparisons of baseline FCM levels for captive versus free-living chipmunks. Data are from 15 (8F, 7M) *TA* and 15 (7F, 8M) *TS* captured in Inyo County, CA and subsequently housed in captivity. Baseline FCM levels were measured upon capture and again after at least 5 days in captivity. Significant contrasts ($p < 0.006$) within and between species are indicated; details of the statistical analyses are provided in the text.



Chapter 3

Using accelerometers to remotely and automatically characterize behavior in small animals

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Abstract

Activity budgets in wild animals are challenging to measure via direct observation because data collection is time consuming and observer effects are potentially confounding. Although tri-axial accelerometers are increasingly employed for this purpose, their application in small-bodied animals has been limited by weight restrictions. Additionally, accelerometers engender novel complications, as a system is needed to reliably map acceleration to behaviors. In this study we describe newly-developed, tiny acceleration-logging devices (1.5-2.5 grams) and use them to characterize behavior in two chipmunk species. We collected paired accelerometer readings and behavioral observations from captive individuals. We then employed techniques from machine learning to develop an automatic system for coding accelerometer readings into behavioral categories. Finally, we deployed and recovered accelerometers from free-living, wild chipmunks. This is the first time to our knowledge that accelerometers have been used to generate behavioral data for small-bodied (<100 gram), free-living mammals.

1. Introduction

Historically, constructing accurate behavioral activity budgets for wild animals has been difficult due to challenges associated with observer effects, evasive study organisms, and extensive time investment. Remote monitoring of behavior with accelerometers is an increasingly common method for measuring behavior in wild animals while mitigating these problems (Brown *et al.*, 2013; Davis *et al.*, 1999; Kays *et al*., 2015; Lagarde *et al.*, 2008; Nakamura *et al.*, 2015; Shamoun-Baranes *et al*., 2012; Weimerskirch *et al.* 2005; Wilmers *et al*., 2015). Thus far, accelerometers have mainly been deployed on larger species due to the weight limitations imposed by smaller-bodied animals (Fig.1). This technology, however, could be particularly beneficial for small-bodied species, which are often cryptic and difficult to observe in the wild.

While useful, accelerometers do engender a novel set of complications associated with the need to reliably map acceleration patterns to specific behaviors. Many simultaneous recordings of paired behavioral observations and accelerometer readings must be collected to determine the correct mapping. Machine learning techniques have successfully been used to complete this task (Carroll *et al.*, 2014; Bidder *et al.*, 2014; Escalante *et al.*, 2013; Gao *et al.*, 2013; Grünewälder *et al.*, 2012; Martiskainen et al., 2009; McClune *et al.*, 2014; Nathan *et al.*, 2012; Sakamoto *et al.*, 2009). Previously, machine-learning algorithms have not modeled sequential correlations between behaviors and have not allowed for flexible lengths of behavioral segments, two constraints that may hurt system accuracies.

In this study we use newly-engineered acceleration-logging devices of our own design to study two free-living, small-bodied species: the alpine (*Tamias alpinus, TA*) and lodgepole (*T. speciosus, TS*) chipmunks. These species are the subject of considerable research due to their divergent responses to the past century of climate change in Yosemite National Park, CA; despite being closely related and co-occurring at some sites, *TA* contracted its range significantly upwards in elevation, while *TS* did not (Moritz *et al.*, 2008). Behavior may serve as an important first-line response mechanism for responding to environmental change (Sih *et al.,* 2011). Because chipmunks are difficult to observe directly, remote monitoring technologies like accelerometers provide a potentially important tool for studying their behavior.

We conducted a validation study by collecting simultaneous video and accelerometer records of captive chipmunk behavior. We then trained a hidden semi-Markov model on this dataset and used the resulting system to predict behaviors for accelerometer logs from free-living animals. To establish biological relevance, we tested whether the data reflected rhythmicity and seasonality of behavior, predicting that animals would be day-active and more active in summer than autumn (Bahnak & Kramm, 1977; Kramm & Kramm 1980; Waters *et al*., 1992). Because *TA*'s range shift has been attributed to climate change (Moritz *et al*., 2008; Rubidge *et al*., 2010) and past work suggests that it may be particularly sensitive to dry heat (Heller & Poulson, 1972), we also hypothesized that *TA*'s behavior might be more temperature-dependent than *TS*'s, and predicted that, in contrast to *TS*, *TA* would show higher activity in the early morning and late afternoon (7:00-10:00 and 15:00-18:00) than at midday (11:00-14:00), when temperatures are typically warmer.

By combining the use of newly engineered accelerometers with the validation of behavioral data and application of new computational methods, we demonstrate that acceleration loggers can be used to remotely measure behavioral activity budgets of small-bodied species (Fig.2).

2. Materials & Methods

Animal Care and Use Committees at the Universities of California, Berkeley and Santa Barbara approved all procedures; methods followed American Society of Mammalogists guidelines (Sikes and Gannon, 2011).

2.1 Accelerometers

Transmitting accelerometers deployed in the lab (Fig.3A,C; similar to Lopes *et al.,* 2014) weighed ~1.5 grams and took constant readings at 200 Hz. For machine learning, data were down-sampled to 20 Hz/axis, the lowest sampling frequency that resulted in negligible decreases in machine learning accuracy.

Logging accelerometers, made of similar parts, were deployed in the field (Fig.3B). Devices weighed ~1.5–2.5 grams (depending on battery; mass includes battery and weather-proofing) and were composed of an ATtiny13 microcontroller (Atmel Corp.; San Jose, CA, USA), an MPU-9250 6-axis inertial measurement unit (Invensense, San Jose, CA, USA), MR25H40 magnetoresistive memory (Everspin, Chandler, AZ, USA), and a lithium-polymer battery (Powerstream, Orem, UT, USA). Including housing, tags represented ~3.5-5% of the study species' body mass. Devices were programmed to record only tri-axial acceleration at 20 Hz/axis for 10 seconds, with 15 minutes between each 10-second sample. This regime allowed for ~4.5 days of data to be collected before the memory was filled; because we expected to re-capture animals after 2-5 days (based on glue longevity), this allowed for data collection throughout the anticipated sampling period.

2.2 Captive Studies

Both chipmunk species were live-trapped in Inyo National Forest, CA, transported to the Sierra Nevada Aquatic Research Laboratory (Mammoth Lakes, CA), and housed as described in Hammond *et al.* (2015).

For each trial, a transmitting accelerometer was glued (Duo Eyelash Adhesive, American International Industries, Commerce, CA, USA) to the focal animal and activated at the same time as a camera used to film the subject's behavior. Each study animal was placed in an opaque, Plexiglas arena (~9x61x61 cm), with aspen shavings, large rocks, sticks, food, and water. A secondary, runway arena (244x30x30 cm) made of polypropylene lined with mesh flooring for traction was used to capture longer-distance running behaviors. Animals were filmed in arenas while accelerometers transmitted data. Approximately 28 hours of synchronous accelerometer and video data was collected from 7 *TA* (3F/4M) and 11 *TS* (7F/4M). Videos were scored according to an ethogram (Table 1). Behavioral scores were time-matched to accelerometer readings to generate annotated acceleration datasets.

2.3 Feature Extraction

We extracted several feature types from each behavioral segment (see Section 2.4 for definition of segment). We extracted mean, variance, min, and max from both the actual and the absolute values of each of the three accelerometer axes (separately for each axis), and from the sequence of magnitudes of the vector of all three acceleration axes (together). We extracted covariance features for each pair of axes. From the sequence of acceleration vector magnitudes we also extracted spectral features derived from the lowest eight components of an averaged sliding Fourier magnitude spectrum with a window size of 16 frames.

2.4 Hidden semi-Markov model (HSMM)

To map behaviors to accelerometer output, we used a HSMM (Levinson, 1986; Jelinek, 1998), a structured predictor that jointly scores entire sequences of behavioral labels and

segmentations. The HSMM models transitions between and lengths of individual behaviors. It is a structured extension of the support vector machine (SVM), capable of learning longer-range correlations. Let x be the full sequence of input accelerometer frames and let $x_{t:r}$ be the segment of frames between times t and r. Let $y = ((l_1, t_1, r_1), (l_2, t_2, r_2), \dots)$ be a behavioral labeling of x where l_i denotes the label of the ith segment, while t_i and r_i denote the start and end frames of the ith segment, respectively. The predicted behavioral labeling under our model is:

$$\hat{y}(x) = \operatorname{argmax}_{y \in Y(x)} [\operatorname{score}(y, x; w)]$$

$Y(x)$ denotes the set of all possible valid segmentations and labelings of x. The score our model assigns to each possible labeling is parameterized by w, a vector of weights, written as:

$$\operatorname{score}(y, x;\, w) = \overbrace{\left(\sum_{i=1}^{\operatorname{size}(y)} w^{\mathrm{T}} f(l_i, x_{t_i:r_i}) \right)}^{\text{Segment Features}} + \overbrace{\left(\sum_{i=1}^{\operatorname{size}(y)-1} w^{\mathrm{T}} g(l_i, l_{i+1}) \right)}^{\text{Transition Features}}$$

The model score decomposes into two types of potential function that score (1) individual labels assigned to individual segments or (2) transitions between neighboring labels. Each potential is a sum of weighted features. The segment feature function, f, characterizes segments of the input paired with a particular label. This differs from a Markov model, which only incorporates features on individual frames. The transition feature function, g, captures sequential dependences between behavioral labels.

Training a structured predictor involves choosing parameters w to optimize a learning objective's value on training data. We used a structured SVM objective (Taskar *et al*., 2003; Tsochantaridis *et al*., 2004) optimized with stochastic subgradient descent (Kummerfeld *et al.*, 2015). Our implementation used a structured prediction library (Kummerfeld *et al.*, 2015) available at https://github.com/tberg12/murphy.git; all models evaluated here were built on this framework (available upon request). Training was relatively fast, with the most complex model training in <15 minutes.

2.5 Compared Predictive Models

We developed two additional systems as points of comparison for the HSSM. The first was an SVM, a machine learning method that has been used in past behavioral studies (e.g. Nathan *et al.,* 2012, Campbell *et al.*, 2013). With this classifier we predicted behaviors by dividing the accelerometer output into a series of four-second fixed segments and making independent behavioral classifications for each segment. Second, as a baseline system we implemented a method that checks whether the mean acceleration norm is above a pre-defined threshold in each segment of the input signal. We set the thresholds on the training data in order to maximize training accuracy using a grid search procedure.

Hyper-parameters for all models were tuned by grid search to maximize accuracy on a held-out set consisting of all trials from a single experimental animal (e.g., SVM segment size was set to maximize held-out accuracy.) Data for this animal were not included in final evaluation.

2.6 Automated System Assessment

For consistency between the baseline models, which use pre-defined segments, and the HSMM, which predicts variably-sized segments, all predicted behavioral labels were evaluated at frame-level. Specifically, manual video annotation was used to assign a correct label to each acceleration frame (a single x, y, and z record from the accelerometer). Our model's labels were used to assign predicted labels to individual frames. Precision and recall metrics were computed by comparing the sequence of correct to predicted frame labels.

Cross-validation was used to determine system accuracy. To do this, we withheld data for one individual from the training data set, completed system training, then calculated the accuracy of the resulting system's interpretation of the removed individual's data. We repeated this process for all individuals and averaged the results, weighted by the quantity of data per individual. This procedure controlled for over-fitting the machine learning to individual-specific behaviors and thus prevented inflated accuracies. Precision and recall were calculated for each behavioral category.

2.7 Field Deployment Data Analysis

For field deployment, 30 logging accelerometers (*TA* N=15, *TS* N=15) were glued (Blink Ultra-Plus Lash Glue; Seoul, Korea) to animals in Yosemite National Park, CA (37.845041, -119.494957) between July 11-19 and September 29-October 3rd, 2015. To increase chances of recovery, accelerometers were attached to individuals that had already been captured and released at least twice. 20 functional accelerometers were recovered via re-trapping (*TA* N=4F/1M summer, 3F/1M autumn; *TS* N=5F/2M summer, 1F/3M autumn). On average, *TA* individuals weighed 38±4.8 and *TS* 56±8.0 grams.

The three-label HSMM system was applied to field data and non-parametric, two-tailed tests were used to assess data for diurnality, seasonality, and interspecific differences in activity budgets.

3. Results

3.1 Validation Study & Machine Learning

Behaviors were collapsed into 2-5 categories (Table 1). The system performed equally well for both species, thus, we pooled data for training and testing. In general the HSMM performed best, followed closely by SVM and then the baseline optimum-threshold system (Table 2).

3.2 Field Deployment

On average, field-deployed accelerometers collected data from *TA* for 58.3±17.0 hours/individual, and from *TS* for 51.1±17.8 hours/individual. Animals spent significantly more time active (not "still") during the day than the night, confirming diurnality (*TA*: 0.57±.11 day, 0.24±0.06 night; *TS*: 0.59±.09 day, 0.26±.06 night; Paired Wilcoxon signed rank test, *TA* V=45, p=0.004; *TS* V=66, p=0.0005; Fig.4). Length of the active period decreased from summer to autumn (*TA*: 13.20±1.09 hours summer, 10.75±2.63 hours autumn; *TS*: 13.14±1.46 hours summer, 10.25±2.88 hours autumn); this difference was significant when data from both species were combined for analysis (Wilcoxon rank sum test, W=82, p=0.0083) and was mainly driven by a pattern of earlier

termination of activity. Animals spent a higher proportion of time in locomotion during summer than autumn (*TA*: 0.23±0.04 summer, 0.17±0.04 autumn; *TS*: 0.22±0.05 summer, 0.17±0.04 autumn); this difference was significant when both species were combined for analyses (Wilcoxon rank sum test, W=80, p=0.012). This was true not only when averaged across all hours, but also for exclusively daylight hours (*TA*: 0.32±0.07 summer, 0.25±0.06 autumn; *TS*: 0.30±0.05 summer, 0.24±0.07 autumn; Wilcoxon rank sum test, W=74, p=0.047), suggesting that animals were spending less time active per hour of daylight. All p-values were still significant when corrected for multiple-testing using false discovery rate adjustments.

There were no significant interspecific differences in the overall average proportion of time spent active (Wilcoxon rank sum tests, all p>0.88). Both *TS* and *TA* showed patterns of spending more time active at midday (11:00-14:00) than in the morning/late-afternoon (7:00-10:00, 15:00-18:00), but this comparison was only significant for *TS* in autumn (Wilcoxon rank sum test, W=60, p=0.03). Interspecific comparisons of these time-of-day- and season-specific activity levels did not reveal any significant differences (Wilcoxon rank sum tests, all p >0.49), though visual inspection of the data did suggest a potential pattern of interspecific differences in autumn, when *TA* showed brief activity peaks around 6:00 and 14:00 in contrast to *TS*, which had higher inactivity around 6:00 and peak activity at midday.

4. Discussion

Using newly developed, low-weight, data-logging accelerometers in combination with advanced machine learning techniques we show that successful accelerometer deployment is possible for small-bodied, free-living animals. We also provide the first quantitative descriptions of activity budgets in the focal species. While the data did not support our hypothesis that, in contrast to *TS*, *TA* would spend more time active in the morning and late-afternoon than at midday, when temperature are warmer, visual inspection of activity rhythms do suggest the possibility of more fine-scale interspecific differences, and future studies with larger sample sizes can explore the impacts of various environmental variables on activity budgets in these species.

Validation Techniques

Conducting a validation study is a critical first step for using accelerometers to collect data on the activity budgets of free-living animals. Validation studies should collect time-matched, paired datasets consisting of behavioral observations and accelerometer readings. A variety of methods can determine whether accelerometer data are reliably correlated with behaviors, some of which are being developed for general-use (Gao *et al.*, 2013; Resheff *et al.*, 2014; Sakamoto *et al.*, 2009). In some cases where an independent captive study is not possible, animals may be observed in zoos or in the wild, or surrogate species may be used (Campbell *et al.*, 2013; Grünewälder *et al.*, 2012; Nathan *et al.*, 2012; Wang *et al.*, 2015). Additionally, we assessed our system's accuracy using a cross-validation method, which generated a system that was robust to individual differences in behavior.

Machine Learning & Assessment

While the HSMM did improve system performance, the enhancement was modest. However, it is possible that integrating adaptive segment length and sequential correlations – properties unique to the HSMM – into future automated accelerometer interpretation models could be useful in other study systems, particularly when constant recording is possible.

All systems were most inaccurate at identifying locomotion. This could be due to the short time scale of locomotory behaviors, particularly in captivity: average locomotory behaviors were approximately 1.8 seconds, versus 7.2 seconds for in-place movement and 18 seconds for still. This may have made it difficult to perfectly identify start and end times for locomotion. This will likely be a general challenge for using accelerometers to remotely identify behaviors of small animals, which have less inertia, meaning they can accelerate more quickly (Randall *et al.*, 2002) and are able to start and complete behaviors on shorter time scales than larger animals. Using high-speed video and/or recording data in larger arenas during validation studies could help ameliorate this problem.

Limitations & Future Directions

The methods described here are promising, but come with some limitations. First, battery life is an issue for small animals that impose limitations on weight; consequently, non-continuous recording may be necessary. Our field-recording regime was conservative but, with enough animals, sufficient for generating meaningful activity budgets. Future studies can employ adaptive programming, including logging only when movement is initiated or during specific times of interest. Second, wearing an accelerometer may alter animal behavior. Although we limited accelerometers to <5% of body weight, future studies should examine impacts of this weight. Third, our attachment method (glue-on) and data-acquisition strategy (non-transmitting) limited weight but required individuals to be recaptured relatively quickly; future work could explore the feasibility of low-weight collar or harness attachments.

Accelerometers offer important improvements over more traditional methods of monitoring animal activity, particularly for small-bodied or cryptic species that are difficult to observe directly. This technology makes data collection more efficient and machine learning can facilitate the accurate interpretation of accelerometer output. Understanding how behavior varies with season and climate could be informative for predicting and understanding responses to climate change, which is relevant to the focal species (Moritz *et al*., 2008). Although improvements to this technology will no doubt be forthcoming, use of accelerometers has the potential to generate numerous novel insights into the biology of small-bodied animals.

Chapter 3 Tables

Table 1: Behavioral Categories. Manually scored behavioral labels (left column) were collapsed into either two, three, four, or five broad behavioral categories for the machine learning training. Sample-size (#instances, #hours) for each behavior are listed.

<table>
<tr><th>Behavior</th><th>5</th><th>4</th><th>3</th><th>2</th></tr>
<tr><td>Sitting
Standing
Lying</td><td colspan="4">Still (N=2348, 12.6)</td></tr>
<tr><td>Walking
Running
Jumping
Climbing</td><td colspan="3">Locomotion (N=6364, 3.24)</td><td rowspan="5">Not still</td></tr>
<tr><td>Moving in place
Nesting
Scratching
Drinking
Digging
Rubbing</td><td colspan="3">In-place movement (N=3081, 8.5)</td></tr>
<tr><td>Eating</td><td colspan="2">Eating (N=199, 0.93)</td><td></td></tr>
<tr><td>Grooming</td><td colspan="2">Grooming (N=515, 2.72)</td><td></td></tr>
</table>

Table 2: Accuracy, Precision, and Recall for Baseline vs. Machine Learning Systems. Overall accuracy (Acc) for optimum threshold baseline (BL), support vector machine (SVM), and hidden semi-Markov model (HSMM) systems with 2-5 behavioral categories is presented in the first row, with the best performing system's numbers in bold. For each system, precision (P) and recall (R) are presented for the selected behavioral categories (S: still, L: locomotion, IM: in-place movement, E: eating, C: cleaning).

		Five Categories			Four Categories			Three Categories			Two Categories		
		BL	SVM	HSMM	BL	SVM	HSMM	BL	SVM	HSMM	BL	SVM	HSMM
Acc:		0.580	0.710	**0.733**	0.582	0.717	**0.735**	0.603	0.809	**0.816**	0.679	0.898	**0.899**
S	P:	0.638	0.885	0.901	0.638	0.899	0.916	0.638	0.915	0.944	0.638	0.949	0.972
	R:	0.876	0.894	0.901	0.876	0.884	0.891	0.876	0.870	0.853	0.876	0.846	0.827
L	P:	0.568	0.568	0.577	0.568	0.560	0.567	0.568	0.566	0.577			
	R:	0.465	0.486	0.514	0.465	0.547	0.579	0.465	0.546	0.571			
I	P:	0.361	0.520	0.540									
M	R:	0.326	0.544	0.543	0.375	0.528	0.562				0.779	0.853	0.841
E	P:	NA	0.302	0.370	0.284	0.496	0.481	0.492	0.754	0.745	0.467	0.952	0.975
	R:	0.0	0.307	0.206				0.254	0.819	0.855			
C	P:	NA	0.531	0.570	NA	0.506	0.532						
	R:	0.0	0.552	0.693	0.0	0.622	0.742						

Chapter 3 Figures

Figure 1: Plot and histograms of focal species and accelerometer masses in previous behavioral studies employing accelerometers. Non-exhaustive literature searches using Web of Science (search terms: TOPIC: (accelerometer animal behavior) AND TOPIC: (wild OR field OR "free living")) and Google Scholar (accelerometer AND "animal behavior" AND (wild OR field OR "free ranging" OR "free roaming" OR "free living") -dairy -cattle –cow) were conducted. Studies on domesticated animals and captive/trained animals housed in non-naturalistic environments were excluded. Where possible, species masses provided in each paper were used; otherwise, values from Animal Diversity Web (https://animaldiversity.ummz.umich.edu/) and peer-reviewed publications were used. Accelerometer masses listed in the paper were used when available; otherwise, if make and model were provided we located masses online if possible. In cases where the device mass included the weight of GPS or VHF technology, we did not include that study in (A) and (B). Studies that included >1 accelerometer model or species are shown a corresponding number of times. (A) Relationship between species and accelerometer masses in selected literature (N= 61 papers). (B) Histogram showing distribution of accelerometer masses in selected literature (N= 61 papers). (C) Histogram showing distribution of focal species masses in studies employing accelerometers (N= 104 papers). Species/accelerometers from this study are shown in filled circles/bars.

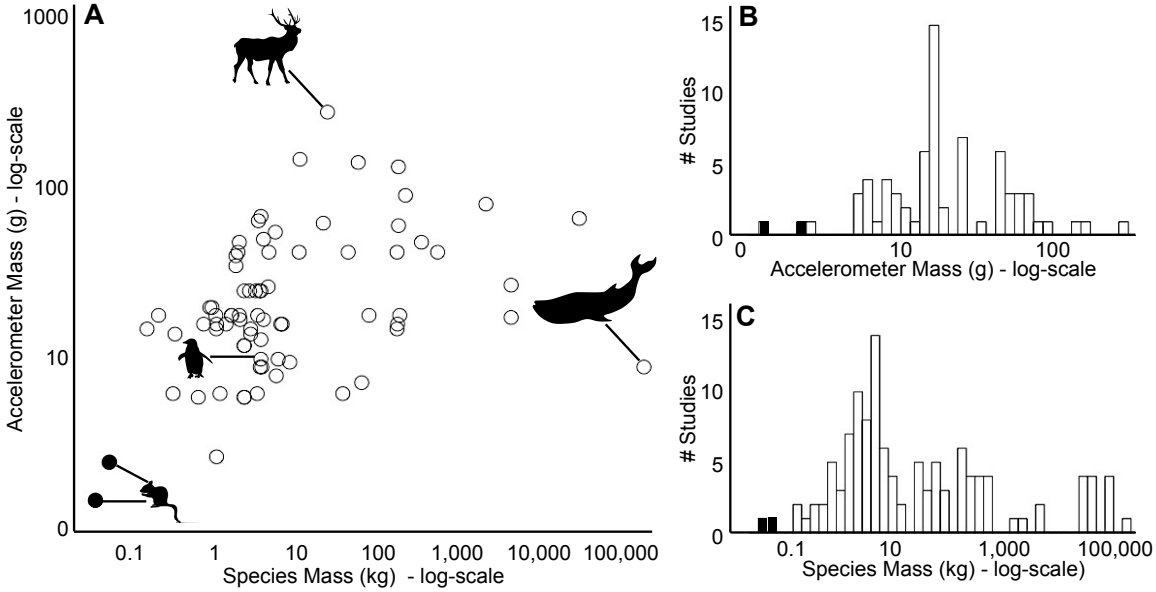


Figure 2: Flow-chart of successful method for validating and applying accelerometers. Captive animals were filmed while wearing accelerometers. After manually scoring film footage with behaviors, accelerometer and behavioral datasets were linked to generate labeled accelerometer data from which features (summary statistics, spectral features, etc.) were extracted. Machine learning trained on features for all individuals except one, and then was tested on that individual. Accuracy was calculated for that individual, and then the procedure was repeated for all individuals and an average accuracy was calculated. Once a suitably accurate system was generated, it was applied to unlabeled accelerometer data collected from unobserved wild animals to generate automatically labeled behavioral activity budgets.

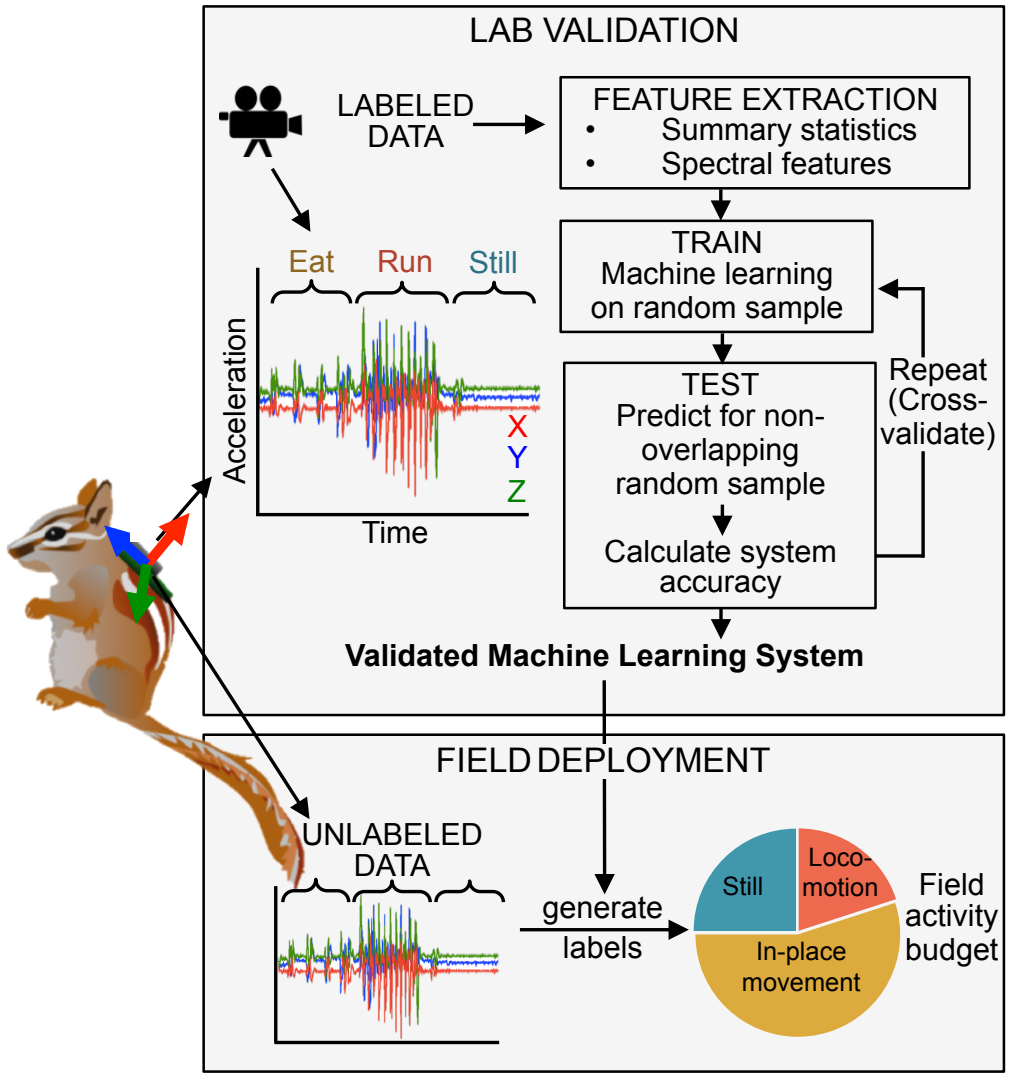


Figure 3: Transmitting and Logging Accelerometers. (A) Transmitting accelerometer used in captive studies; (B) Logging accelerometer used in field studies; (C) captive *T. speciosus* wearing an accelerometer.

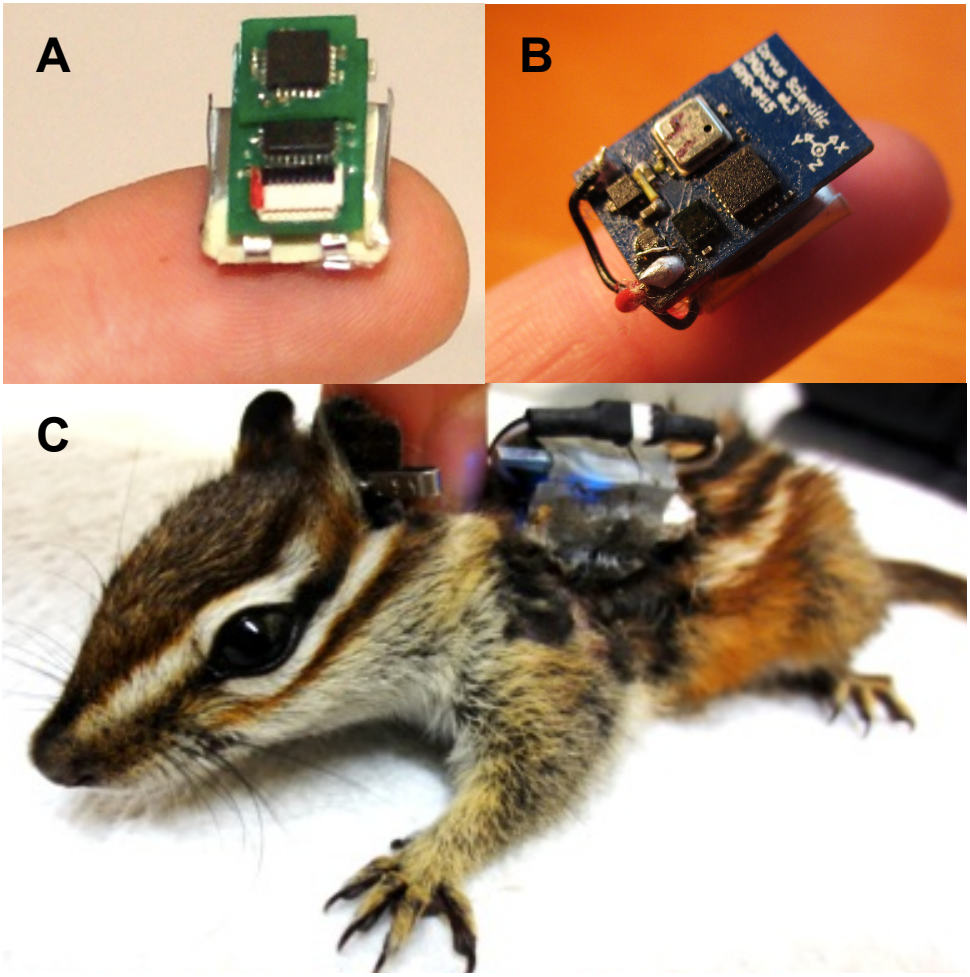
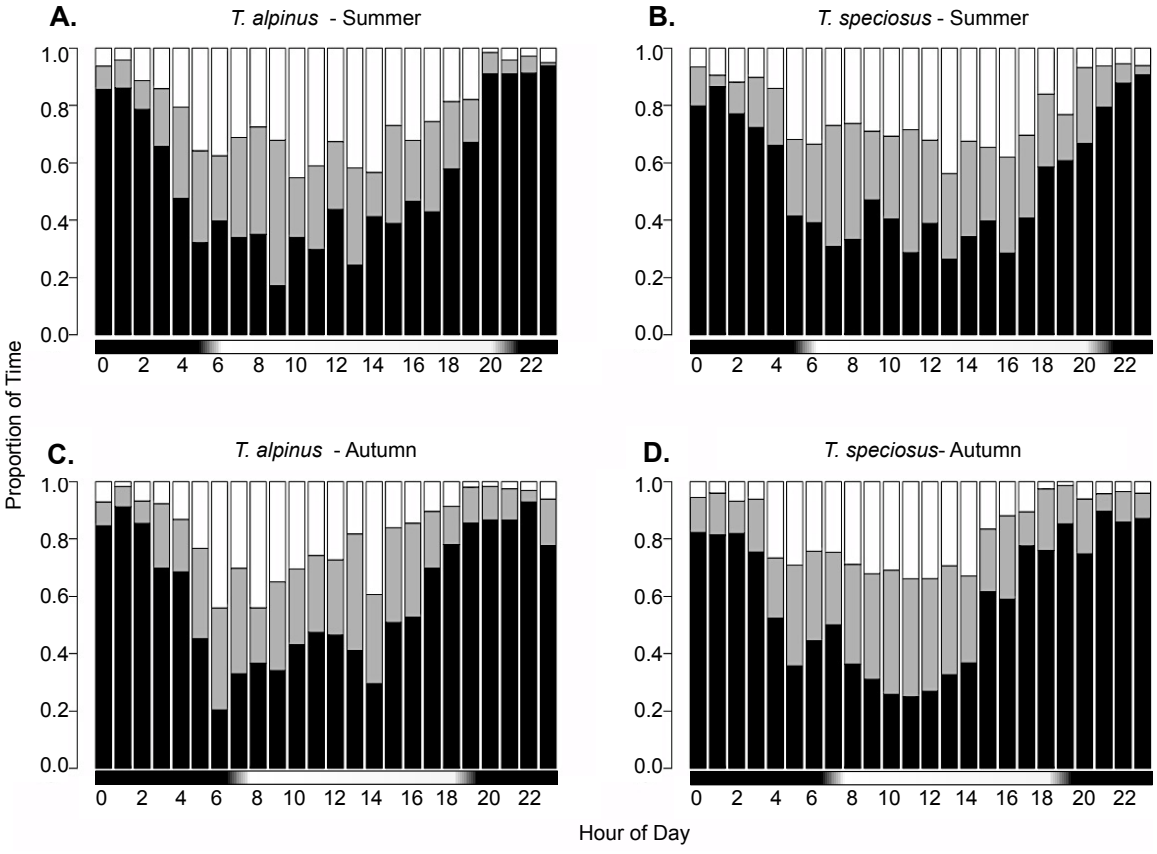


Figure 4: **Three-category HSMM labeled activity budgets for wild chipmunks in summer and autumn.** Hour of day is shown on the x-axis, mean percent time spent on each behavioral category according to the three-category HSMM (black: still, grey: in-place movement, white: locomotion), averaged across individuals, is shown on the y-axis for *T. alpinus* in (A) summer (N=5) and (C) autumn (N=4), and *T. speciosus* in (B) summer (N=7) and autumn (N=4). Black bars indicates approximate light:dark cycle.



Chapter 4

Predictors of Activity Differ Between Co-Occurring Species of Chipmunks (Genus *Tamias*)

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Abstract

Differences in spatial and temporal patterns of activity may generate marked variation in the exact environmental conditions experienced by individuals and thus may provide a critical mechanism by which animals can respond to rapidly changing environments. To explore potential relationships between patterns of activity and response to environmental conditions, we quantified activity budgets for two species of chipmunks, the alpine chipmunk (*Tamias alpinus*) and the lodgepole chipmunk (*T. speciosus*). Although populations of these species co-occur in portions of Yosemite National Park (YNP), previous research has revealed that while *T. alpinus* has experienced a significant upward elevational range contraction over the past century, the elevational range of *T. speciosus* has not changed substantially during this period. To document patterns of activity for each species, we deployed accelerometers on free-living animals at three locations in YNP at which these taxa co-occur. In addition, we quantified the ground cover and ambient temperatures present at each study site and we recorded the sex, mass, and reproductive status of each animal monitored. Analyses of fecal samples were used to measure baseline glucocorticoid levels for each study subject. Generalized linear mixed models (GLMMs) revealed that the extent to which these parameters predicted patterns of activity differed between the study species, with activity by *T. speciosus* being both more variable and more strongly associated with extrinsic factors such as temperature and ground cover. Combined with the reported differences in elevational range changes, these findings suggest that *T. speciosus* – more than *T. alpinus* – uses variability in activity patterns to mediate changes in environmental conditions.

1. Introduction

Variable behavioral traits provide an important means of responding to changes in environmental conditions, particularly changes occurring over short time scales (Sih 2013; Tuomainen & Candolin, 2011). For example, by altering spatial or temporal patterns of activity, individuals may gain access to or avoid contact with specific ambient conditions (DeGregorio et al., 2014; Longcore & Rich 2004; Moyer-Horner et al., 2015; Smith 1974). These changes in activity may be predictable, such as daily modifications that occur in species that rely on behavioral thermoregulation to achieve physiologically optimal body temperatures (Adolph 1990; Briscoe et al., 2014; Fick et al., 2009; Lagos et al., 1995). Alternatively, changes in activity may provide an immediate response to

unexpected environmental conditions that allow individuals to persist in modified habitats (e.g. Gross et al., 2010; Hoare et al., 2007; Salinas-Melgoza et al., 2013). For environmental changes that persist over extended periods of time, such changes in activity may become the foundation for adaptive (evolutionary) modifications in behavior that allow individuals to persist in modified habitats (Mery & Burns, 2010; Sih et al., 2011; Sunday et al., 2014; Tuomainen & Candolin, 2011). As a result, quantifying relationships between activity patterns and environmental parameters is critical to understanding potential responses to variable or changing environmental conditions. In addition, characterizing the ways that activity patterns are moldable to current environmental variability and the extent to which they are delineated by intrinsic biology (e.g. sex, reproductive status, mass) may yield an improved understanding of the factors that determine a species' geographic range, and ultimately can improve our understanding of organismal responses to environmental change over longer time scales.

Comparative studies of closely related, at least partially sympatric species that are characterized by different patterns of microhabitat use provide an ideal opportunity to examine the role of behavioral traits such as activity patterns in mediating impacts of environmental change. The chipmunks (genus *Tamias*) of the Sierra Nevada Mountains represent one such comparative system. Although the alpine chipmunk (*Tamias alpinus*, *Ta*; Fig. 1a) and the lodgepole chipmunk (*T. speciosus*, *Ts*; Fig. 1a) co-occur in portions of the Sierra Nevada, they have undergone very different patterns of elevational range shift over the past century (Moritz et al. 2008). Specifically, while *Ta* has undergone a significant (~ 629 m) upward range contraction, the elevational range of *Ts* has not significantly changed (Fig. 1a; Moritz et al., 2008). Subsequent studies that have explored population genetic structure (Bi *et al*., 2013; Rubidge *et al*., 2012), skull morphology, and diet (Walsh *et al*., 2016) have all revealed greater directional changes in *Ta* over the past century. Further, analyses of glucocorticoid physiology in these species have demonstrated that *Ta* is more affected by a variety of stressors (Hammond et al., 2015). Thus, *Ta* appears to be generally more responsive to the environmental changes that have taken place in the Sierra Nevada over the past century.

The lack of elevational range change by *Ts* is intriguing given that vegetation in the habitats occupied by these animals is shifting (Santos et al., 2015), as are the associated communities of vertebrates (Moritz et al., 2008; Tingley et al., 2009). It is possible that the general lack of distributional, genetic, and morphological change by *Ts* relative to *Ta* reflects Ts's greater adaptive capacity, or ability to cope with environmental change using phenotypic plasticity, dispersal, or genetic diversity (Beever et al., 2016; Wade et al., 2016). Flexibility in behavioral traits, including flexibility in activity patterns, may contribute to this increased capacity. Previous studies have demonstrated that there is substantial overlap in the fundamental niches of *Ta* and *Ts*, with these species characterized by generally similar physiological parameters, including thermal tolerance (Heller & Gates 1971; Heller & Poulson 1970; Heller & Poulson 1972). *Ts* inhabits a broader environmental and climatic niche than *Ta* and thus should experience a greater range of thermal conditions across its distribution (Walsh 2015). If this species uses flexibility in activity patterns to mediate the variation in ambient temperatures that it experiences, it seems reasonable to predict that intraspecific differences in activity patterns should be more strongly linked to temperature in *Ts* than in *Ta*.

One challenge to studying activity patterns in free-living animals is the difficulty of observing individuals throughout the day, particularly for species that are cryptic or evasive. However, the increasing use of accelerometers to measure activity remotely (Angelier et al., 2007; Brown et al., 2013; Lagarde et al., 2008; Nakamura *et al.*, 2015; Shamoun-Baranes *et al*., 2012; Weimerskirch *et al.* 2005; Wilmers *et al*., 2015) is creating new opportunities to explore the role of behavioral flexibility in responding to environmental conditions. Here, we use accelerometers previously validated for *Ta* and *Ts* (Hammond et al., 2016) to examine the role that activity patterns play in mediating responses by these species to changing ambient conditions. Specifically, we examine the effects of multiple extrinsic (ground cover, temperature, species co-occurrence) and intrinsic (sex, reproductive status, mass, baseline glucocorticoid levels) parameters on variability in the activity patterns of the focal species, exploring both general activity and locomotion, an energetically relevant behavioral category. These analyses reveal intriguing interspecific differences in predictors of activity that have important implications for understanding the capacity of a species to respond *in situ* to changing environmental conditions.

2. Methods

2.1 Study Species & Field Sites

Populations of *Tamias alpinus* (*Ta*) and *T. speciosus* (*Ts*) were studied in Yosemite National Park and adjacent Inyo National Forest, California. *Ta* is a small-bodied (30-50 g) chipmunk that lives primarily above treeline (Clawson *et al*., 1994); in the study area, it is found from 2936 to 3353 masl (Moritz *et al*., 2008; Fig. 1a). In contrast, *Ts* is larger-bodied (50-80 g) and occurs primarily at and below treeline (Best *et al*., 1994); in the study area, it is found from 1896 to 3220 masl (Moritz *et al.,* 2008; Fig. 1a). Field data were collected between 29 June and 27 August, 2015, at three locations varying in elevation from ~2700-3200 masl (Fig. 1b). These sites were selected in part because populations of *Ta* and *Ts* co-occur at each of these localities.

Members of both study species were captured using Sherman traps baited with peanut butter and oats. At each study site, traps were deployed in a grid, with 3-4 m between adjacent trap locations ("stations"); to maximize the number of captures, two traps were placed at each station. Trapping grids were established to encompass adjacent portions of the habitat that were occupied by *Ta* only, by *Ts* only, and by both species. GPS coordinates and elevation were recorded for each trapping station. Traps were opened at dawn, checked every 4-6 hours, and closed at dusk. Each animal captured was uniquely marked with two numbered metal ear tags (1005–1, National Band and Tag, New- port, KY, USA). The body, mass ($\pm$ 1 gm), sex, and reproductive status of each individual were also recorded. Fecal pellets were collected from the trap in which each animal had been captured for use in analyses of baseline glucocorticoid levels (see below); pellets were frozen in liquid nitrogen until they could be transported to the Berkeley campus, where they were stored at -80 ^{o}C until analysis. Upon completion of these procedures, each animal was released at the point of capture.

2.2 Use of Accelerometers

To quantify the activity budgets of members of our study populations, acceleration loggers, also known as accelerometers (Corvus Scientific, Albany, CA), were deployed on free-living *Ta* and *Ts* captured at each study site. Detailed specifications for these loggers are provided in Hammond *et al*. (2016). In brief, each custom-made unit included a tri-axial accelerometer, a data logger, a light sensor, and a battery. Each unit weighed 1.5-2.5 g each (<5% of body mass of individual *Ta* and *Ts*) and was affixed to an animal with eyelash glue (Blink Ultra-Plus Lash Glue; Seoul, Korea) after shaving a small patch of fur from the individual's dorsal surface. Units were programmed to turn on every 15 min and to record one light reading as well as 10 seconds of 20-Hz acceleration readings along each of the three axes of movement. Units were deployed on 8-11 individuals per species at each of the three study sites. Battery life of the loggers was approximately 4-5 days. Because it was necessary to recapture animals to retrieve accelerometers and to download the associated data, loggers were deployed only on individuals that had already been trapped at least three times; this restriction was expected to increase the probability of recapture after data collection by loggers was complete.

All procedures involving live chipmunks were approved by the Animal Care and Use Committee at the University of California, Berkeley and followed the guidelines of the American Society of Mammalogists for the use of wild mammals in research (Sikes et al. 2016).

2.3 Converting Acceleration Data to Activity Budgets

Hammond *et al*. (2016) developed a software system capable of assigning raw accelerometer readings to one of several behavioral categories. Comparisons of accelerometer data and video recordings collected simultaneously from captive *Ts* and *Ta* (Hammond et al., 2016) revealed that this system assigned accelerometer readings with ~ 82% accuracy when three behavioral categories (still, in place movement, locomotion) were used and ~ 90% accuracy when two behavioral categories (still, active) were used. Both of these categorization schemes were employed to convert the accelerometer data collected during this study to activity budgets for free-living *Ta* and *Ts*.

2.4 Glucocorticoid Analyses

To determine if use of accelerometers was stressful to the study animals and, more generally, to determine variation in activity budgets was associated with inter- or intra-specific differences in stress physiology (see section 2.6 below), we assessed fecal glucocorticoid metabolite (FGM) levels for *Ta* and *Ts* before and after individuals were fitted with data loggers. Samples were collected and preserved as described above (see section 2.1). For analysis, samples were dried and FGMs were extracted and assayed as described in Hammond *et al*. (2015). Because FGM levels in the study species do not peak until ~24 hrs after initiation of a stressor (Hammond *et al*., 2015), analyses of fecal samples collected at the time of capture do not reflect increases in FGM levels resulting from that trapping event. For pre-accelerometer FGMs, mean values were calculated for each individual based on all fecal samples collected prior to attachment of a logger. We also assessed only first-capture FGM levels to control for any effects of successive trapping on FGMs. Post-accelerometer values were based on FGM levels when animals were captured to retrieve loggers, and in a few cases when animals were captured during

accelerometer deployment. Finally, we calculated the difference between pre- and post-accelerometer FGMs as a final metric of GC reactivity in each individual.

2.5 Characterizing Environmental Conditions

Ecological niche models constructed for the study species suggest that temperature is a more important predictor of the distribution of *Ta* (Rubidge et al. 2011), raising the possibility that *Ta* and *Ts* differ in their responses to generally similar thermal environments. To characterize the thermal conditions experienced by members of our study populations, Thermochron iButton temperature loggers (model DS1921G) programmed to collect hourly temperature readings were deployed near traps within ~1m of the ground at approximately 75% of our trapping stations. Data collected by these loggers were used to generate hourly summary statistics on temperature (mean, maximum, minimum, variance) for each trapping grid for use in analyses of environmental predictors of activity patterns (see section 2.6 below).

Characterizations of the microhabitats occupied by the study species indicate that *Ta* tends to be associated with rockier, more open areas than *Ts* (Walsh 2015). To characterize the vegetative structure of our study sites, surveys of ground cover were conducted at each trapping station. Specifically, visual estimates of percent ground cover were completed for a circle with a radius of 5 m centered on the trapping station. Percent cover was assessed for each of the following categories: shrub, herbaceous, litter/duff, downed wood, bare soil, small rocks, boulders, and bedrock. Tree cover above each trapping station was also estimated visually for the same 5 m radius circle.

2.6 Statistical Analyses

Glucocorticoid data. Shapiro-Wilk tests revealed that measures of FGM levels were not normally distributed and thus non-parametric, Wilcoxon signed rank tests were used to compare pre- and post-logger FGM values for the individuals sampled (pre-logger values were averaged across all captures of the same individual prior to attachment of an accelerometer). Similarly, Wilcoxon signed rank tests were used to compare FGM levels at first capture to those measured post-logger; this analysis was undertaken to confirm that comparisons based on mean pre-logger values were not biased by potential increases in FGM levels occurring over successive captures of the same individual.

Vegetation data. For each trapping grid, we calculated the mean percent cover for each category of vegetation quantified. Mean values were compared across trapping grids using non-parametric Wilcoxon rank sum tests and false discovery rate adjusted p-values (Benjamini & Hochberg, 1995). In addition, we constructed a model of the effects of grid and elevation on vegetative cover. We first used principal component analyses (PCA) using the prcomp function in R after grouping our vegetation variables in to the following more general categories: vegetative cover (shrub or herbaceous), rocky cover (small rocks, boulders, bedrock, or bare soil), and detrital cover (litter/duff or downed wood). Models were then constructed using the used the lm function in R (R Core Team, 2016), with the first principal components axis for ground cover as the response variable and grid type (*Ta* only, *Ts* only, both species) and elevation as the predictor variables.

Light data. For each study species, data from accelerometers were used to calculate mean hourly lux values. Values for *Ta* and *Ts* were then compared using Wilcoxon signed rank tests, with separate tests conducted for all data, for data collected during the daytime (600-1900 hr, corresponding to the hours when animals were active, with sunrise defined as the median sunrise time during the data collection period and sunset defined as 19:00, by which point activity had largely ceased for both species throughout the study period), and for data collected during the afternoon (1100-1500 hr, defined as the four hottest hours of the day). In addition, mean daytime lux values were calculated for each individual, after which these values were compared between species using Wilcoxon signed rank tests to assess whether there were overall interspecific differences in lux levels. Finally, for each individual, mean lux values were calculated for accelerometer readings during which the animal was active versus inactive, after which Wilcoxon signed rank tests were used to compare these means within species

Temperature data. To compare thermal conditions on trapping grids inhabited by *Ta* only, *Ts* only or both species, we first calculated mean daytime (600-1900 hr) temperatures for each date at each grid. We then used Wilcoxon rank sum tests to examine differences among grid types. In addition, we used a generalized linear model constructed with the lm function in R to test for an effect of grid-type (*Ta* only, *Ts* only, both species) on hourly mean temperatures over the course of the 24-hour cycle.

Analyses of activity. Mean hourly activity budgets (proportion of hourly 10-sec recordings assigned to locomotion, in place movement, or still) were calculated for each individual monitored during this study. For each individual, mean values for each behavioral category were then calculated across all 24 hours of the day, for daytime (0600-1900 hrs) records only, and for afternoon (1100-1500 hrs) records only. These values were compared between species using Wilcoxon rank sum tests.

Environmental predictors of behavior. To examine the effects of different environmental parameters on the activity patterns of our study species, we used the glmer function in the lme4 package in R to construct generalized linear mixed models (GLMMs). In all models, the response variable was binomial, with 1 indicating either locomotion or activity (any behavior that was not categorized as "still") and 0 indicating the absence of locomotion or other activity. For each 10-second period that a logger was active (see section 2.2 above), response was defined as the predominant behavioral category recorded. The predictor variables examined included the measure of light intensity recorded simultaneously with each behavioral response and the concomitant measure of ambient temperature obtained from the grid on which the focal individual was active. In addition, we included date and time of behavioral data collection as well as one of the measures of individual FGM levels described above (see section 2.4). Finally, we included the fixed variables of sex, mass, reproductive status, and ground cover PCA axes for the focal individual's grid as well as the random effects of individual and trapping grid.

To control for differences in numerical scales between variables included in the model, we normalized data for each continuous predictor (Table 1) by subtracting each individual value by that variable's associated mean and dividing by its standard

deviation. Because we expected some subsets of predictor variables to exhibit co-linearity, we then conducted principal components analyses (PCA) to determine which variables should be grouped for further analysis. Separate PCAs were conducted for measures of ground cover (vegetative, rocky, detrital; see section 2.5 above), FGM levels (first, pre, post, post-pre; see section 2.4 above), and temperature (hourly mean, minimum, maximum, variance, by grid). For ground cover data, the first two PCA axes were included in the model, accounting for 100% of the variation in each species. For FGM data, the first three axes were included in the model, accounting for 100% of the variation in each species. For temperature data, only the first axis was included in the model, accounting for 90.9% of the variation in *Ta* and 89.1% in *Ts* (Table S1, S2, S3).

Our initial model contained two random effects and 12 fixed effects (Table 1); interactions between time and temperature, and between ground cover and temperature were also included in all starting models. Stepwise backwards variable selection based on AIC values was used to eliminate predictor variables that had no significant effect on model fit, resulting in final models that contained only significant predictor variables (Zuur et al., 2009). For each species, separate models were generated using data for all daytime hours (600-1900 hr) versus the subset of data from afternoon hours (1100-1500 hr). Models were run using both activity and locomotion as response variables, resulting in a total of four models per species (daytime activity, daytime locomotion, afternoon activity, and afternoon locomotion). Models with and without the random effects of site and individual identity were generated and compared using R's 'anova' function to determine whether these random effects were significant. In cases where multiple PCA axes were significant in the final model, overall directionality of the effects was calculated by multiplying the GLMM regression coefficient each axis by the variable loadings for that axis.

3. Results

3.1 Effects of Acceleration Loggers on FGM Levels

Neither study species demonstrated significant differences between baseline FGM levels measured before versus after deployment of acceleration loggers (mean number samples/individual ±S.D.: *Ta* – pre 5.1 ± 1.7, post 1.5 ± 0.7; *Ts* – pre 5.0 ± 1.6, post 1.4 ± 0.6). Specifically, no differences were found when comparing FGM values for an individual's mean pre-deployment capture to post-deployment values (paired Wilcoxon signed rank tests, *Ta*: V=49, p=0.56; *Ts*: V=158, p=0.83; Fig. 2). Similarly, no differences were found when comparing an individual's first pre-deployment and post-deployment FGM values (paired Wilcoxon signed rank tests, *Ta*: V=30, p=0.1; *Ts*: V=138, p=0.75; Fig. 2).

3.2 Interspecific Comparisons of Environmental Parameters

Grids inhabited by only *Ta* were found to have significantly more rocky cover (Wilcoxon rank sum test with FDR-adjusted p-value, W=27, p=0.01), less detrital cover (W=0, p=0.009), and less tree cover (W=0, p=0.009) than those occupied by only *Ts* or by both species (Fig. 3). Vegetative cover did not differ across grid types (all p>0.37). In general, grids inhabited by *Ta* only were more similar to grids inhabited by both species than were

grids inhabited by *Ts* only. For example, rocky cover of grids inhabited by both species differed significantly from that of grids occupied by *Ts* only (W=0, p=0.04), but did not differ from grids inhabited by *Ta* only (W=11, p=0.11).

Mean daytime (600-1900 hr) temperatures were significantly higher in areas inhabited by *Ta* only than in areas inhabited by *Ts* only or areas occupied by both species (Wilcoxon rank sum tests with FDR-adjusted p-values, *Ta* vs. *Ts*: W=2821, p=0.0012; *Ta* vs. both: W=1037, p=0.005; Ts vs. both: W=1347, p=.63; Fig. 4A). Linear models revealed that grid type (*Ta* only, *Ts* only, both species) was a significant predictor of mean daily temperature ($p<0.001$); a subsequent ANOVA indicated that including grid type significantly improved the model fit compared to a model containing only date as a predictor variable ($F=12.9$, $p<0.000007$). Visual inspection of the data revealed that the tendency for grids with *Ta* only to be characterized by higher temperatures was most pronounced in the afternoon (1100-1500 hr; Fig. 4B). In contrast, *Ta*-only habitats were characterized by lower mean nighttime temperatures than *Ts*-only habitats or habitats occupied by both species (Fig. 4B).

No differences between the study species were detected for mean lux values recorded by individual accelerometers when all time points were considered (Wilcoxon rank rum test W=195, p=0.68), when only daylight hours were considered (0600-1900 hr; Wilcoxon rank sum test W=185, p=0.9), or when only afternoon hours were considered (1100-1500 hr; Wilcoxon rank sum test W=202, p=0.54). Analyses of mean lux levels for time points when animals were active versus inactive revealed that during afternoon hours, *Ta* was more active in lower light habitats (Wilcoxon rank sum test, W=166, p=0.03; Fig. 5); similar relationships between mean lux levels and activity were not evident for the other time periods considered (all $p>0.42$; Fig. 5). No significant relationships between activity and mean lux levels were detected for *Ts* (all $p>0.35$; Fig. 5).

3.3 General Patterns of Activity

Of the 27 accelerometers deployed on *Ta* and the 29 accelerometers deployed on *Ts*, units containing usable data were recovered from 15 *Ta* (12F, 3M) and 24 *Ts* (17F, 7M). On average, these units collected data for 57.4±14 hours/individual (mean±SD) for *Ta* and 54.9±16.2 hours/individual for *Ts*.

When data from all study sites were pooled, overall patterns of daily activity were similar for both study species (Fig. 6). Both species were clearly diurnal: for both *Ta* and *Ts*, activity and light levels began increasing at ~ 0400 hr, remained relatively high until ~1500 hr, and then decreased until ~1900 hr, at which point the animals were largely still (Fig. 6). Both species exhibited comparable daily activity budgets, with no significant differences detected between the proportions of the day spent still (mean±S.D. *Ta* 0.54±0.04, *Ts* 0.55±0.07), moving in place (*Ta* 0.27±0.02, *Ts* 0.25±0.04), or locomoting (*Ta* 0.18±0.02, *Ts* 0.20±0.08); this outcome did not differ for analyses of data collected over all time points, during the daytime, or during the afternoon (Wilcoxon rank sum tests, all $p>0.41$). Thus, overall, there were no apparent differences between *Ta* and *Ts* with respect to general temporal patterns of activity.

3.4 Predictors of Daytime Activity

GLMM analyses revealed that hour of data collection was a significant predictor of daytime activity for both study species, with the percentage of individuals active decreasing over the course of the day (Fig. 6; Table 2). FGM levels were also significant predictors of daytime activity for both species, although the exact relationship between these variables differed between *Ta* and *Ts*. Specifically, while mean and first pre-accelerometer as well as post-accelerometer measures of FGM levels were negatively associated with activity in *Ts*, the difference between pre- and post-accelerometer readings was positively associated with activity (Table 2). In contrast, in *Ta*, FGM levels at first capture were positively associated with activity and the difference between pre- and post-accelerometer FGM levels was negatively related to activity (Table 2). With regard to vegetation, GLMM analyses revealed that ground cover categories were important predictors of activity for both species, although again the exact relationships between these variables differed between *Ta* and *Ts*. While higher levels of rocky cover and lower levels of detrital and vegetative cover were associated with increased activity in *Ts*, the directionality of these relationships was reversed in *Ta* (Fig. 7; Table 2). For both species, temperature was a negative predictor of activity (Table 2).

While hour, FGM levels, ground cover, and temperature were the only predictors included in the final model for daytime activity in *Ta*, the final model of daytime activity in *Ts* also included date (negative predictor) and body mass (positive predictor), indicating that heavier members of this species were generally more active. Finally, co-occurrence category was also a significant predictor for *Ts,* with members of this species being more active in sites occupied by both species than at sites occupied by *Ts* only (Fig. 8). Individual identity and site did not significantly improve model fit for either species (ANOVA Individual *Ta*: p=0.1; *Ts*: p=0.5; Site *Ta*: p=1; *Ts*: p=1). However, overall, daily patterns of activity in *Ts* appeared to reflect a more diverse suite of predictors than patterns of activity in *Ta*.

3.5 Predictors of Daytime Locomotion

Because locomotion is expected to be a particularly energetically demanding form of activity, we also examined the predictors of this behavioral category. GLMMs revealed that hour was a significant negative predictor of locomotion for both study species (Table 3). FGM levels were also significant predictors of locomotion for both species, following the same general patterns of directionality as revealed by final models for more general measures of daytime activity: for *Ts,* all measures except the difference between pre- and post-accelerometer FGM levels were negatively related to locomotion, while for *Ta* all measures except FGM levels at first capture were negative predictors of locomotion.

With regard to the other predictors considered, the final model for daytime locomotion in *Ts* was similar to that for daytime activity in including ground cover, body mass and co-occurrence with *Ta*; the directionalities of these predictors were the same in the final models for both daytime activity and daytime locomotion (see above). These models differed, however, in that temperature and date were not included in the final model for daytime locomotion, while reproductive status (reproductively active animals were less active) and individual (a random effect) significantly improved the model fit for *Ts* but not *Ta* (ANOVA Individual *Ta*: p=1, *Ts*: p=0.007; site *Ta*: p=1, *Ts*: p=0.1; Fig. S1). Differences between final models for daytime activity and daytime locomotion were more pronounced for *Ta*. Ground cover was not included in the final model for daytime

locomotion in this species, although several new predictors were identified: reproductive activity (reproductively active animals were more active), sex (females were more active), and body mass (heavier animals were less active; Table 2). Thus, for both species and in particular for *Ta*, locomotion appeared to be better predicted by intrinsic biological variables while overall activity appeared to be better predicted by extrinsic environmental variables.

3.6 Predictors of Afternoon Activity & Locomotion

Given the difference in relationships between afternoon temperatures and lux levels detected for the study species (see section 3.2), we also used GLMMs to examine predictors of activity and locomotion during afternoon hours only. For both study species, final models for afternoon activity and afternoon locomotion tended to include fewer predictor variables than final models for daytime activity and locomotion (Table 4 & 5). For *Ts*, no fixed effects were included in final models for either measure of afternoon behavior; the only significant predictor in these models was the random effect of individual identification (Table 4 & 5), which significantly improved model fit for both of *Ts*'s models (ANOVA, activity models, individual p=0.0001, site p=0.99; locomotion models individual p=0.0007, site p=1), but for neither of *Ta*'s (all p =1). For *Ta*, the fixed effect of FGM levels was included in the final models for afternoon behavior, with FGMs at first capture being positively related to afternoon activity and locomotion. Specifically, both post-accelerometer FGM levels and the difference between pre- and post-accelerometer FGM levels were negatively related to afternoon activity and locomotion; while pre-accelerometer FGM levels were negative predictors of afternoon locomotion, they were positive predictors of daytime locomotion (Table 4 & 5). Thus, in neither species were extrinsic environmental factors identified as significant predictors of afternoon patterns of behavior.

4. Discussion

Our analyses indicate that despite the overall similarity in activity patterns documented for *Ta* and *Ts*, the factors that predict activity differed markedly between the study species. In particular, activity by *Ts* was influenced by a greater number of parameters than activity by Ta. For both species, the key predictors identified differed according to the type of activity and portion of the day examined. Specifically, while daily patterns of overall activity tended to be more strongly associated with extrinsic factors such as ground cover, daily patterns of locomotion (a subset of overall activity) were more closely tied to intrinsic variables such as FGM levels. Fewer predictors were identified for analyses of activity and locomotion occurring during the afternoon only; although this tendency was evident for both study species, only *Ta* revealed a significant tendency for afternoon activity to occur in lower lux (less illuminated) portions of the habitat. Collectively, these findings indicate that although *Ts* and *Ta* co-occur in portions of Yosemite National Park, activity by members of these species is influenced by different elements of the environment.

4.1 Impact of Accelerometers on FGM Levels

Neither study species displayed a significant elevation in FGM levels after individuals were fitted with accelerometers, suggesting that the data loggers used were not an important stressor for these animals. Similarly, other studies have failed to detect significant impacts of animal-carried technology on GC levels (Creel et al., 1997; Moll et al., 2009), suggesting that appropriately sized units (e.g., <5% of animal body mass) can be used on free-living animals without affecting the stress physiology of the study subjects. Although it is possible that accelerometers impacted aspects of the behavior and ecology of the study species that were not monitored (Brooks et al., 2008), previous research has demonstrated that FGM levels in *Ts* and, in particular, *Ta* are responsive to external challenges (Hammond et al. 2015), leading us to expect that deployment of accelerometers would have produced a change in GC levels if this technology was stressful to the study animals.

4.2 Interspecific Differences in Habitat Use

Analyses of both ambient temperature and vegetative structure revealed significant differences between the microhabitats occupied by each study species. In general, areas occupied by *Ta* were rockier and more open than those occupied by *Ts,* with areas of co-occurrence between the study species being intermediate with regard to these variables (see also Walsh 2015). These differences in ground cover were associated with warmer daytime temperatures in areas used by *Ta*, with this difference being most evident during the afternoon. Initially, this finding appears to contradict the hypothesis that the upward elevational range contraction by this species over the past century has been driven by increasing temperatures at lower elevations (Moritz et al., 2008). That microhabitats occupied by *Ta* are subject to higher afternoon temperatures, however, is not necessarily surprising given that rockier, more open substrates are often warmer due to both increased insolation (less shade) and greater reflectance (radiant heating) compared to substrates with greater vegetative cover (Geiger et al., 2009; Yuan & Bauer, 2007). Thus, while the distribution of *Ta* may be negatively related to ambient temperature at the species scale, the same does not appear to apply at the scale of individual animals. In particular, the finding that afternoon activity in *Ta* tended to be associated with lower lux values suggests that members of this species may compensate for higher afternoon temperatures by restricting activity to the shadiest portions of its habitat. Use of cooler thermal microenvironments as a mechanism of thermoregulation has been reported for other mammalian species (Briscoe et al., 2014; Fick et al., 2009; Lagos et al., 1995) and our results suggest that *Ta* may employ a similar strategy in response to the higher afternoon temperatures that it encounters relative to *Ts*.

4.3 Environmental Predictors of Activity

Our analyses indicated that, overall, activity was more strongly associated with environmental parameters in *Ts*. Given the marked differences in afternoon temperatures in the microhabitats occupied by each study species, the finding that ambient temperature was included in only one final model (daytime activity) for each species was unexpected, as was the positive directionality of this predictor, which indicated that individuals tended to be more active at warmer temperatures. While it is possible that this relationship reflects other patterns of temporal variation in behavior (individuals of both species tended to be less active later in the day, when temperatures were cooler), it is also

possible that, as argued above for *Ta*, individuals were using microhabitat selection to modify the temperatures that they experienced. Additional research is needed to explore interactions between temperature, activity, and microhabitat use in greater detail.

Habitat parameters were significant predictors of daytime activity in both *Ta* and *Ts*, although the directionality of these relationships differed between species. For example, while individual *Ta* living in less rocky areas with more detrital and vegetative cover tended to be more active, the opposite pattern was detected for *Ts*. Because models for each species were constructed using taxon-specific ground cover data, the rockiest sites for *Ts* were those at which it co-occurred with *Ta*, which were generally the least rocky sites for *Ta* (Fig.3). Thus, it is possible that variation in habitat parameters and co-occurrence of the study species were correlated in our study system. Co-occurrence was a significant predictor in models for both daytime activity and daytime locomotion in *Ts*, indicating that this species was more active in areas of co-occurrence; although co-occurrence did not appear in the comparable models for *Ta*, the small number of individuals monitored in areas also occupied by *Ts* may have limited the power of our models to detect an effect of co-occurrence on activity by *Ta*. Previous research suggests that *Ta* is aggressively dominant to *Ts* (Heller 1971, but see Chappelle 1978) and thus potential competitive interactions between the study species should also be examined as part of future studies of relationships between habitat use and activity in these animals. In general, however, our findings suggest that further comparative studies of *Ta* and *Ts* provide an important opportunity to elucidate the role of flexible behavioral traits in mediating responses to changing habitat conditions.

4.5 Intrinsic Predictors of Behavior

Measures of baseline FGM levels were the only intrinsic factor that emerged as a significant predictor in all models of daytime activity and locomotion; this outcome applied to both study species, although the exact measures of FGM levels and the directionalities of their relationships to activity differed among models. Our analyses revealed that for *Ta*, first-capture FGMs were positively associated with activity, while all other measures (which are potentially "stressed" measures, due to the effects of repeated captures and/or accelerometer application) were negatively related to activity. For *Ts*, on the other hand, all measures were negatively related to activity except for the difference between FGMs before and after accelerometer deployment, indicating that animals that were more stress responsive to the application of the accelerometer were more active. In general, glucocorticoid hormones (GCs) function to mediate interactions with the external environment, allowing individuals to maintain homeostasis (Sapolsky et al., 2000). Both baseline and acute GC levels tend to increase during periods of high energetic demand (Haase et al., 2016; Sapolsky et al., 2000; Wingfield & Kitaysky, 2002) and positive relationships between GC levels and activity have been reported for a number of vertebrate species (Angelier et al., 2007; Belthoff et al., 1998; Breuner et al., 1998; Breuner & Hahn 2003; Cash & Holberton 1999; Crossin et al., 2012; Dunn et al., 2013; Miles et al., 2007), however, results are mixed and highly context-dependent (Cottin et al., 2014; Gray et al., 1990; Øverli et al., 2002; Ricciardella et al., 2010). Consequently, the ecological significance of these interspecific differences in activity-GC relationships remains somewhat obscure; nonetheless, these results highlight the importance of considering multiple GC metrics when exploring the ecological relevance

of this physiological parameter (e.g. Voellmy et al., 2014). Because FGMs measured at first capture were arguably the most representative measure of true basline levels, it is intriguing that this measure differed from all the others for *Ta* but not *Ts*. Though non-significant, we did detect increases in GCs after capture and accelerometer application for *Ta* but not for *Ts*, further underscoring this species' increased responsivity to external challenges (Hammond et al., 2015). Future studies, including additional experimental manipulations of external stressors experienced by the study species, should help to elucidate the underlying biological significance of the patterns reported here.

Other intrinsic factors identified as important by our models included body mass, reproductive status, sex (*Ta* only) and animal identity (*Ts* only). These relationships were detected more often in models for which locomotion was the response variable, suggesting that this measure of behavior was more strongly influenced by intrinsic factors than were overall measures of daytime activity. In *Ta*, lighter individuals and reproductively active animals, particularly reproductive females, were most active; in contrast, in *Ts* lighter and reproductively active individuals were less active. Animal identity significantly improved model fits for *Ts* but not for *Ta*, indicating both greater intra-individual repeatability and greater inter-individual variation in behavior for the former species. This increased variation among individuals is consistent with the reportedly greater ecological niche width for *Ts* (Walsh 2015) and may reflect a greater capacity for behavioral flexibility in this species compared to *Ta.* These differences are intriguing given the marked interspecific differences in range response over the past century reported for the study species (Moritz et al., 2008) and lead to testable predictions regarding relationships among individual variability, ecological specialization, and tolerance for changing ambient conditions.

4.6 Behavioral Responses and Environmental Change

Behavior is a fundamental component of an organism's capacity to respond to changing environmental conditions, with implications for *in situ* responses, movement to new localities, and changes in genetic diversity (Beever et al., 2016; Wade et al., 2016). Species with flexible behaviors are expected to be better able to respond to rapidly changing environments (McCain & King 2014; Refsnider & Janzen, 2012; Snell-Rood 2013; Wong & Candolin, 2015; Wright et al., 2010) and, accordingly, behavioral parameters are increasingly being incorporated into analyses of organismal vulnerability to climate change (Huey et al., 2012; Munoz et al., 2015; Williams et al, 2008). Field studies of naturally occurring variability in behavior combined with lab studies that experimentally assess the limits of behavioral responses should be particularly effective in clarifying the role of behavioral flexibility in organismal responses to changing environmental conditions.

Understanding behavioral responses to immediate changes in environmental conditions is also important to characterizing responses occurring over longer, evolutionary time periods. For example, our analyses revealed that *Ts,* which has not experienced climate-related changes in elevational range over the past century (Moritz et al. 2008), exhibited greater individual variation in behavior and was more responsive to extrinsic impacts on activity than *Ta*. This greater flexibility in behavior may increase the range of phenotypes on which selection can act, increasing the likelihood of adaptive response to changing environmental conditions. At the same time, behavioral flexibility may itself be favored

by selection (Charmantier et al., 2008; Snell-Rood 2013), suggesting that current differences in behavioral variability between the study species may reflect differences in the historical selective pressures acting on phenotypic plasticity in these animals.

This study focused on plasticity in the temporal patterns and spatial distributions of three energetically important behavioral categories: still, in-place movement, locomotion. The timing of such behaviors during the day is likely influenced not only by the climatic parameters examined here (e.g. Aublet et al., 2009; Grant & Dunham, 1988), but also by other ecological variables that were not measured, including foraging opportunities (Welbergen 2006; Zielinski 1988), predation risk (Jacob & Brown, 2000; Lima & Bednekoff, 1999), and competition (Hayward & Slotow 2009; Richards 2002). Trade-offs between these demands presumably guide adaptive "decisions" about when organisms should be most active (Kronfeld-Schor & Dayan 2003; Welbergen 2006) and may be contribute to the evolution of behavioral plasticity. Generalist species, which are exposed to a broader range of environmental conditions both within individual lifetimes and throughout evolutionary history, are typically expected to be more plastic (Snell-Rood 2013). Although it is difficult to characterize the costs of plasticity (Sol 2009; Snell-Rod 2013; Van Buskirk & Steiner 2009), for specialist species under strong, consistent selective pressures, reducing plasticity may facilitate the evolution of more specialized phenotypes (Van Kleunen & Fischer, 2005; Van Tienderen 1991). Here, we demonstrate that *Ts*, a generalist species that has not exhibited strong responses to the past century of climatic change in its habitat, displays greater variability in behavioral activity patterns both among individuals and in response to external parameters compared to the co-occurring but more ecologically specialized *Ta*, which as experienced pronounced geographic, genetic, and morphological responses to environmental change over the same time period (Moritz et al., 2008; Rubidge et al., 2012; Walsh et al., 2016).

Behavioral flexibility alone, however, is unlikely to be sufficient for responding to long-term, large-scale environmental changes. For example, analyses of the thermal physiology and behavior of ectotherms suggest that the extent to which behavior can buffer individuals from environmental conditions is dependent on other, extrinsic factors such as habitat structure; in some environments, behavioral flexibility is expected to be insufficient to insure survival (Aubret & Shine, 2010; Kearney et al., 2009). In particular, the fitness costs of altering behavior to remain within thermoregulatory limits may not be sustainable over extended periods, meaning that behavioral flexibility is necessarily a short-term solution to changing conditions (Cunningham et al., 2015). More generally, relationships between environmental parameters and behavior are complex, making it challenging to predict the effects of specific ambient changes. As our analyses of activity patterns in *Ta* and *Ts* suggest, however, characterizing the effects of environmental parameters on basic behavioral traits such as activity may reveal important differences in phenotypic variability that allow us to better determine which species are most vulnerable to environmental change, and therefore to make more informed conservation decisions.

Chapter 4 Tables

Table 1. Description of response variables, fixed effects, and random effects included in GLMMs.

Variable Name	Description	Type
Behavior *(Response variable)*	Most common behavior each an individual at a sampling point (active/inactive, or locomotion/non-locomotion)	Binomial
Fixed effects		
Time	Time of day	Continuous
PC1_temp	First axis of PCA containing mean, maximum, minimum, and variance of hourly temperature, by grid	Continuous
PC1_cover	First axis of PCA containing ground cover variables (vegetation, rocks, detritus), by grid	Continuous
PC2_cover	Second axis of ground cover PCA	Continuous
Lux	Light levels from on-board light sensor	Continuous
Rep	Reproductive Status ("Y" if reproductively active, "N" if not reproductively active)	Categorical
Mass	Mass of each individual	Continuous
Sex	Sex of each individual	Categorical
Coocc	Grid type of each individual ("Y" if both species, "N" if single species)	Categorical
Date	Julian date	Integer
PC1_FGM	First axis of PCA containing FGM variables (first, pre, post, post-pre)	Continuous
PC2_FGM	Second axis of FGM PCA	Continuous
PC3_FGM	Third axis of FGM PCA	Continuous
Random effects		
ID (random)	Individual animal ID	Categorical
Grid (random)	Home grid of each individual	Categorical

Table 2. Final GLMM results modeling daytime activity for (a) *Ta* and (b) *Ts*.

(a) *T. alpinus*

	Estimate	SE	z-value	P-value
(Intercept)	0.41	0.09	4.6	4.47e-06
Time	-0.54	0.10	-5.6	2.16e-08
PC1_temp	-0.08	0.04	-2.0	0.044
PC1_cover	0.16	0.06	2.8	0.0059
PC2_FGM	-0.30	0.09	-3.6	0.00031
PC3_FGM	-0.62	0.23	-2.7	0.0069

Random effect	Variance (SD)
ID	0.03 (0.17)
Site	0 (0)

(b) *T. speciosus*

	Estimate	SE	z-value	P-value
(Intercept)	3.23	0.63	5.15	2.64e-07
Time	-0.56	0.08	-7.02	2.27e-12
PC1_temp	-0.10	0.03	-3.06	0.0022
PC1_cover	0.55	0.13	4.31	1.64e-05
PC2_cover	0.87	0.19	4.4	7.66e-06
Mass	0.25	0.06	4.11	3.96e-05
Sex (M)	-0.31	0.12	-2.55	0.011
Coocc	-2.14	0.49	-4.32	1.52e-05
PC1_FGM	-0.17	0.04	-3.97	7.26e-05
PC2_FGM	-0.31	0.07	-4.37	1.26e-05
PC3_FGM	-0.25	0.07	-3.28	0.0010
Date	-0.68	0.13	-5.32	1.07e-07

Random effect	Variance (SD)
ID	.01 (0.11)
Site	6.19e-08 (0.0003)

Table 3. Final GLMM results modeling daytime locomotion for (a) *Ta* and (b) *Ts*.

(a) *T. alpinus*

	Estimate	SE	z-value	P-value
(Intercept)	-0.80	0.12	-6.60	4.09e-11
Time	-0.56	0.10	-5.40	6.70e-08
Rep (N)	-0.53	0.24	-2.18	0.030
Rep (Y)	0.25	0.14	1.71	0.088
Mass	-0.27	0.11	-2.38	0.017
Sex (M)	-0.72	0.20	-3.60	0.00032
PC3_FGM	-0.76	0.18	-4.28	1.91e-05

Random effect	Variance (SD)
ID	4e-14 (2e-07)
Site	0 (0)

(b) *T. speciosus*

	Estimate	SE	z-value	P-value
(Intercept)	1.75	0.70	2.66	0.0079
Time	-0.36	0.08	-4.30	1.71e-05
PC1_cover	0.45	0.12	3.69	0.00022
PC2_cover	0.64	0.19	3.42	0.00062
Rep (N)	-0.51	0.19	-2.71	0.0068
Rep (Y)	-0.43	0.23	-1.85	0.064
Mass	0.16	0.07	2.11	0.035
Coocc	-1.71	0.49	-3.52	0.00044
PC1_FGM	-0.16	0.05	-3.21	0.0013
PC2_FGM	-0.14	0.07	-2.18	0.029
PC3_FGM	-0.16	0.07	-2.17	0.030
Date	-0.30	0.12	-2.57	0.010

Random effect	Variance (SD)
ID	7.5e-03 (8.7e-02)
Site	1.5e-09 (3.9e-05)

Table 4. Final GLMM results modeling afternoon activity for (a) *Ta* and (b) *Ts*.

(a) *T. alpinus*

	Estimate	SE	z-value	P-value
(Intercept)	0.46	0.10	4.81	1.5e-06
PC2_FGM	-0.42	0.12	-3.60	0.00032
PC3_FGM	-0.63	0.30	-2.08	0.038

Random effect	Variance (SD)
ID	0 (0)
Site	0 (0)

(b) *T. speciosus*

	Estimate	SE	z-value	P-value
(Intercept)	0.67	0.15	4.46	8.37e-06

Random effect	Variance (SD)
ID	0.35 (0.59)
Site	0 (0)

Table 5. Final GLMM results modeling afternoon locomotion for (a) *Ta* and (b) *Ts*.

(a) *T. alpinus*

	Estimate	SE	z-value	P-value
(Intercept)	-0.95	0.10	-9.14	<2e-16
PC2_FGM	-0.35	0.12	-2.85	0.0044

Random effect	Variance (SD)
ID	0 (0)
Site	0 (0)

(b) *T. speciosus*

	Estimate	SE	z-value	P-value
(Intercept)	-0.88	0.13	-6.65	3.04e-11

Random effect	Variance (SD)
ID	0.24 (0.49)
Site	3.70e-10 (1.92e-05)

Table 6. Summary of all Final GLMM results for (a) *Ta* and (b) *Ts*. For continuous variables "-" indicates a negative predictor, "+" a positive predictor (for variables included in PCAs, for each axis included in a model the estimate for that axis was multiplied by that variable's loading score for that axis of the PCA. If multiple axes were included, this was repeated and these values were summed for each variable). For categorical variables, relationships are denoted within the table. Blank cells indicate variables that had no significant effect on a model's fit and were not included in the final model. Time was never included in afternoon models. Note that for temperature, all four variables loaded in the same directionality on the first PC axis, which is the only axis that was included in GLMMs.

(a) *T. alpinus*

	Predictor	Day Act	Day Loc	PM Act	PM Loc
	Time	-	-	NA	NA
	Date				
Ground	Rocky	-			
cover	Detrital	+			
PCA	Vegetated	+			
	Coocc				
Temp PCA	Mean/Min/Max/Var	+			
	Mass		-		
	Sex		F>M		
	Rep		Y>N		
	Pre FGM	-	-	-	+
FGM	Post FGM	-	-	-	-
PCA	First FGM	+	+	+	+
	Post FGM-Pre FGM	-	-	-	-

Random
ID
Site

(b) *T. speciosus*

	Predictor	Day Act	Day Loc	PM Act	PM Loc
	Time	-	-	NA	NA
	Date	-			
Ground	Rocky	+	+		
cover	Detrital	-	-		
PCA	Vegetated	-	-		
	Coocc	Y>N	Y>N		
Temp PCA	Mean/Min/Max/Var	+			
	Mass	+	+		
	Sex				
	Rep		Y<N		
	Pre FGM	-	-		
FGM	Post FGM	-	-		

PCA	First FGM	-	-		
	Post FGM-Pre FGM	+	+		
	Random				
	ID		✓	✓	✓
	Site				

SUPPLEMENTARY TABLES

Table S1. Variable loadings for principal components of ground cover data.
a. *T. alpinus*

Variable	PC1	PC2
Rocky	-0.7471229	-0.05672371
Detrital	0.3789262	0.78224207
Vegetated	0.5460974	-0.62038678
Proportion of variance	.6	0.4

b. *T. speciosus*

Variable	PC1	PC2
Rocky	0.706634383	0.11022260
Detrital	-0.707530160	0.09847837
Vegetated	0.008301853	-0.98901617
Proportion of variance	0.66	0.34

Table S2. Variable loadings for principal components of FGM data.
a. *T. alpinus*

Variable	PC1	PC2	PC3
FGM Pre	0.4766010	-0.5965837	0.51318019
FGM Post	0.5481272	0.1892940	0.29186251
FGM First	0.5241123	-0.2747210	-0.80612326
FGM Post-FGM Pre	0.4446509	0.7299206	0.04034427
Proportion of variance	.81	0.17	0.02

b. *T. speciosus*

Variable	PC1	PC2	PC3
FGM Pre	0.2370949	0.70229863	0.55613660
FGM Post	0.7032155	-0.06465313	0.23358015
FGM First	0.3115956	0.52302087	-0.79332047
FGM Post-FGM Pre	0.5934493	-0.47858727	-0.08243193

Proportion of variance	0.48	0.34	0.18

Table S3. Variable loadings for principal components of temperature data.
a. *T. alpinus*

Variable	PC1
Mean	-0.5187121
Maximum	-0.5188670
Minimum	-0.4888851
Variance	-0.4719175
Proportion of variance	.91

b. *T. speciosus*

Variable	PC1
Mean	-0.5196856
Maximum	-0.5245939
Minimum	-0.4893422
Variance	-0.4639744
Proportion of variance	0.89

Chapter 4 Figures

Figure 1: Elevational range shifts of *Tamias alpinus* (*Ta*) and *T. speciosus* (*Ts*) and map of study locations. (a) *Ta* (upper, blue) and *Ts* (lower, red) historical (c.1906, lighter shade) and modern (c.2006, darker shade) elevational ranges; *Ta* exhibited upward range contraction while *Ts*'s range did not significantly shift (figure based on data from Moritz *et al*., 2008). (b) Map of California showing Yosemite National Park and the three main study sites, with grids inhabited only by *Ta* (blue), by both species (striped), or only by *Ts* (red).

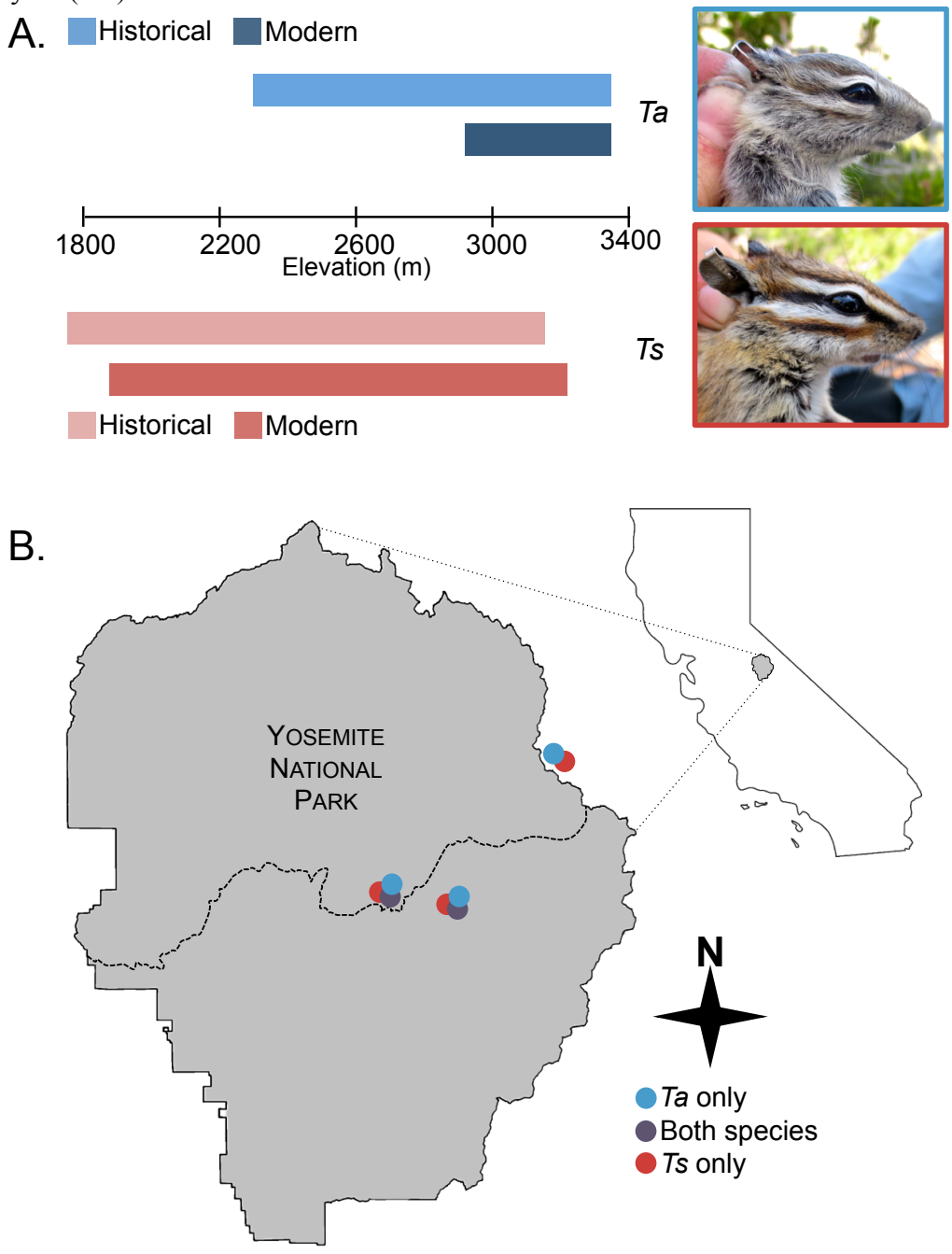


Fig. 2 First capture FGMs (white), average pre-accelerometer FGMs (grey), and average post-accelerometer FGMs (black) for each species. There were no significant differences between any categories for either *Ta* (left) or *Ts* (right).

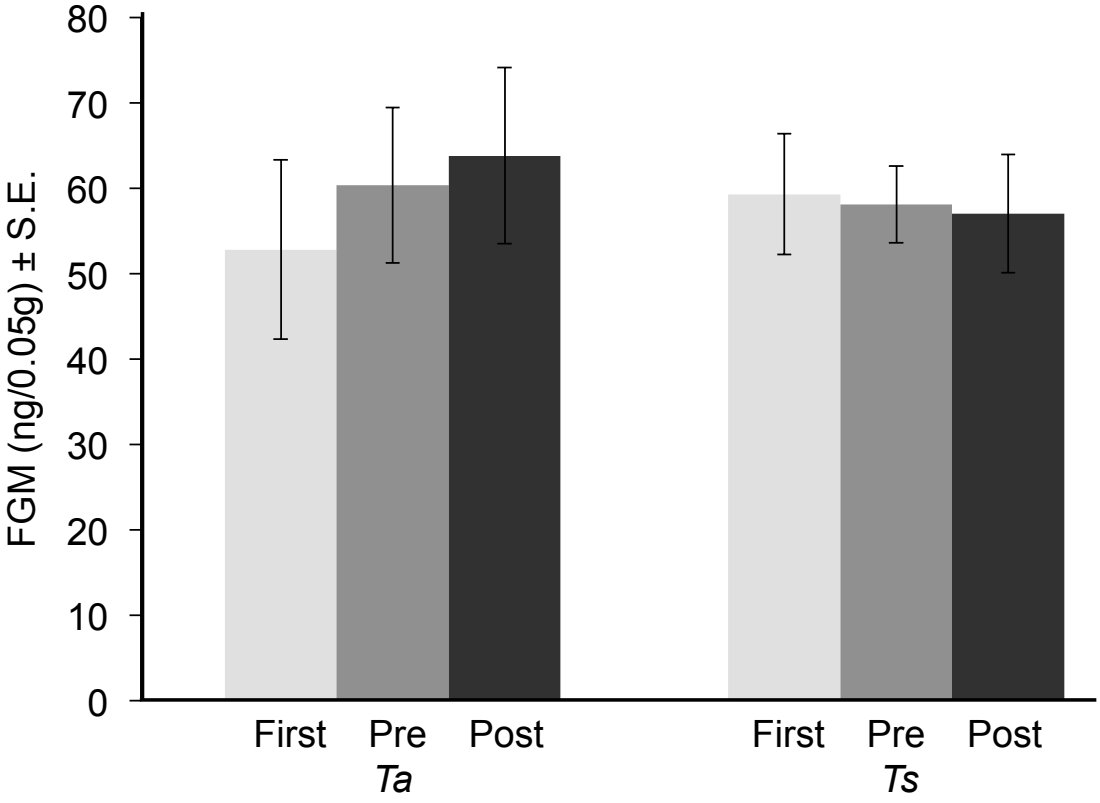


Figure 3. Ground cover data for study grids. Ground cover proportions by grid, arranged by grid type (*Ts* only, both species, *Ta* only) illustrating a gradient from less rocky and more detrital cover to more rocky and less detrital cover. Tree symbols indicate mean tree cover estimates for each grid.

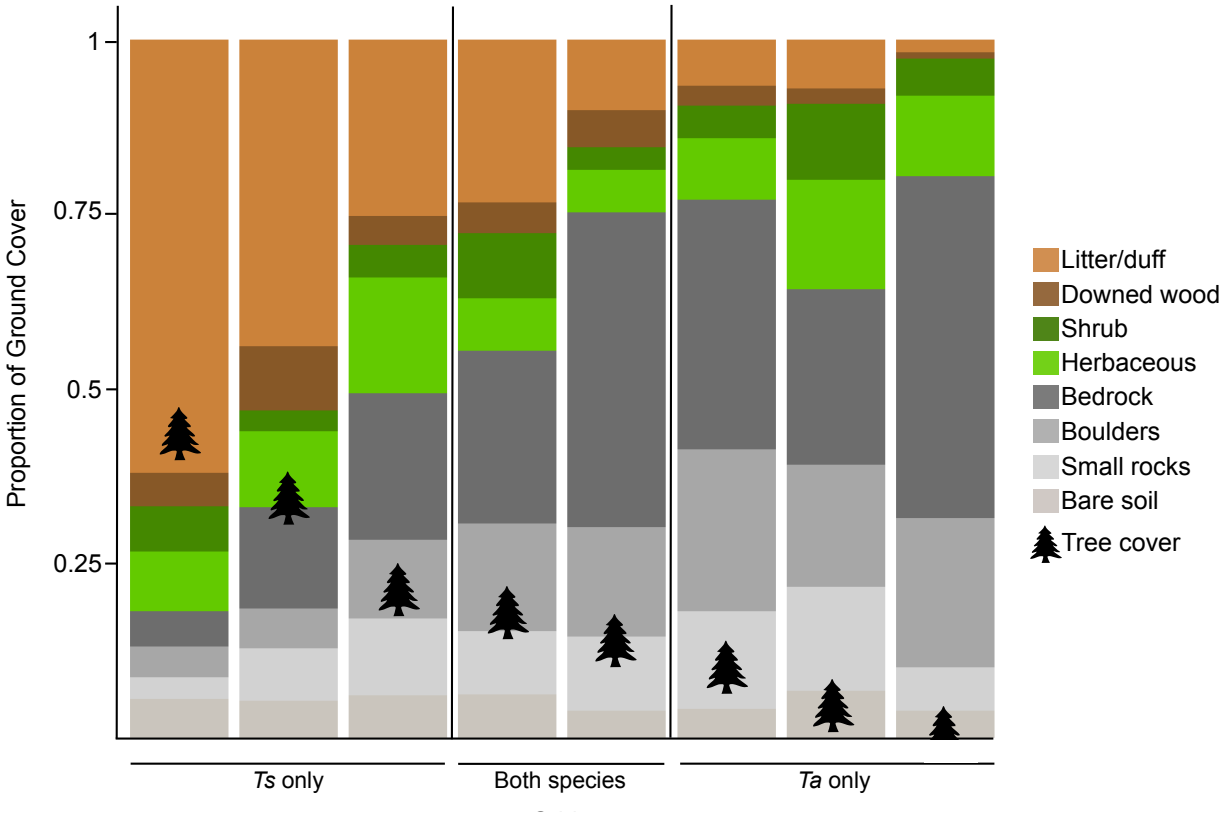


Fig. 4: Interspecific differences in average daytime temperatures and temporal patterns of temperature. (a) Habitats inhabited by only *Ta* have significantly higher daytime (600-1900 hr) temperatures than those inhabited by only *Ts* or by both species. (b) When pooling data from all study sites, temporal patterns of temperature differ over the course of the day, with *Ta* habitats being warmest at midday but cooler at night.

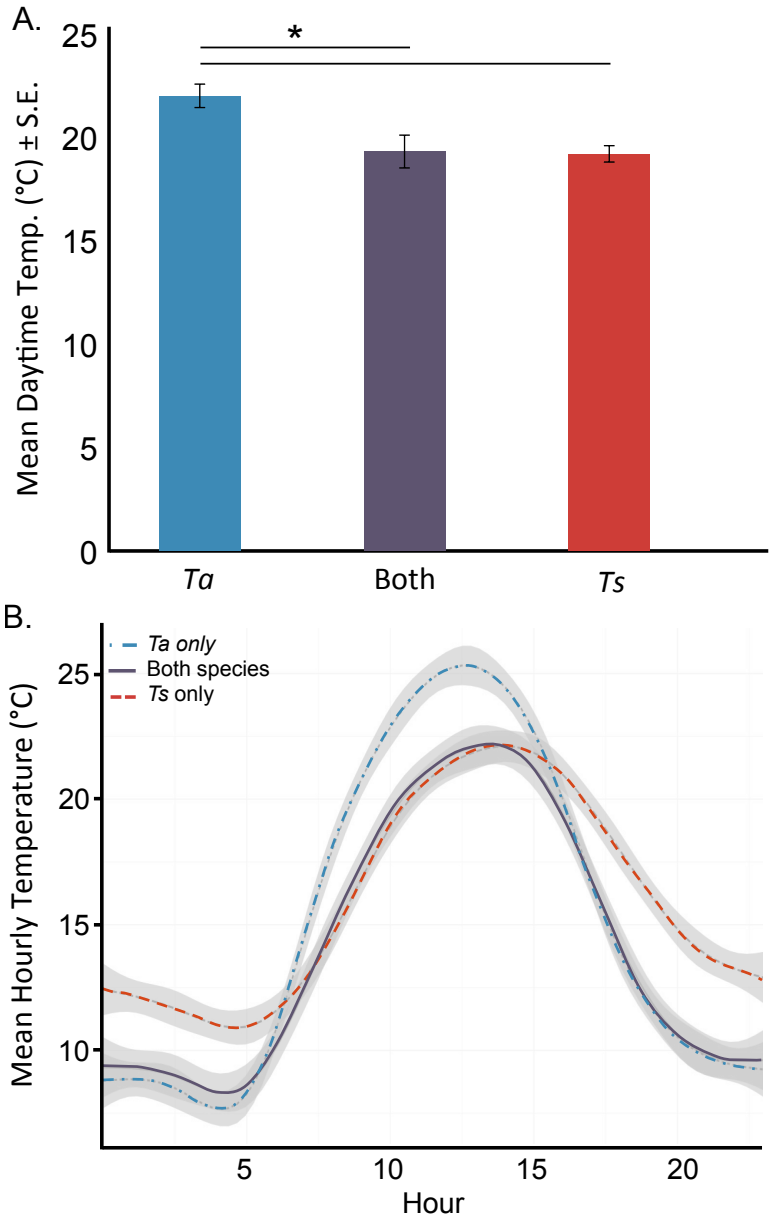


Figure 5. Relationship between lux and activity differs in the afternoon for *Ta* (left) but not *Ts* (right). Mean ± S.E. for the difference between mean lux values for inactivity and activity (as calculated for each individual). This value is shown for the afternoon (1100-1500 hr, white), when temperatures are warmest, and for other times of day (0600-1100 hr and 1500-1900 hr, grey). Values above 0 indicate that animals were more active in shadier environments (or more inactive in sunnier environments). There is a significant difference between the afternoon and other times of day for *Ta* (p=0.03) but not for *Ts*. Overall lux levels did not differ interspecifically.

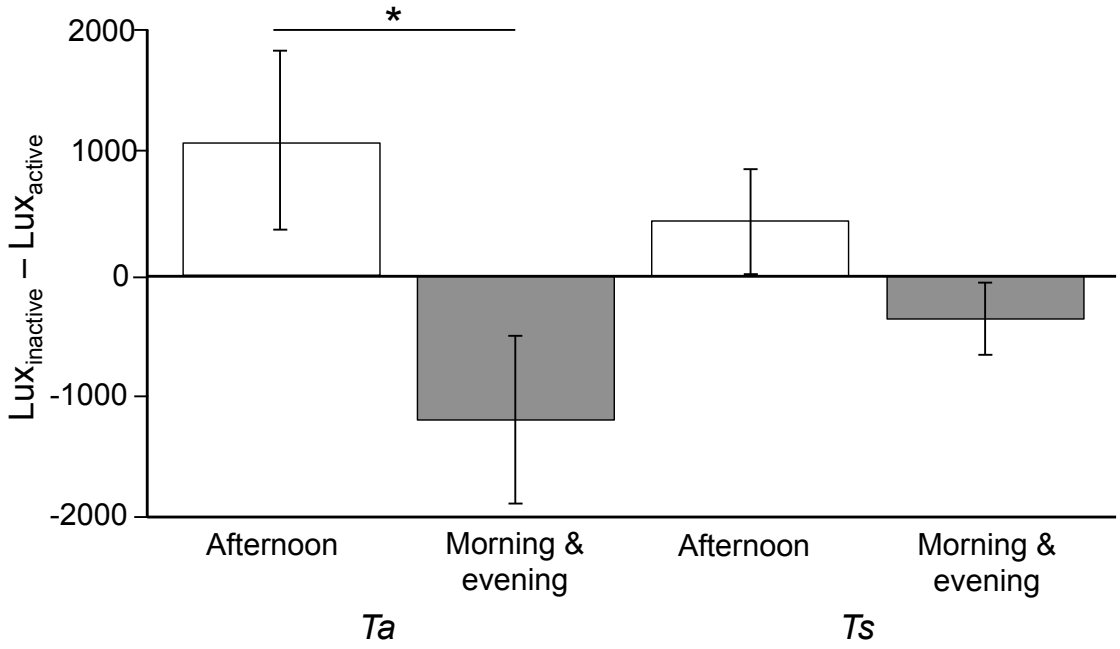


Figure 6: Overall daily activity patterns and light levels. (a) *Ts* (red) and (b) *Ta* (blue) daily activity patterns across all sites, showing proportion of each hour spent still (lightest shade), moving in place (medium shade), or in locomotion (darkest shade). Black points symbols represent average lux levels recorded in each hour.

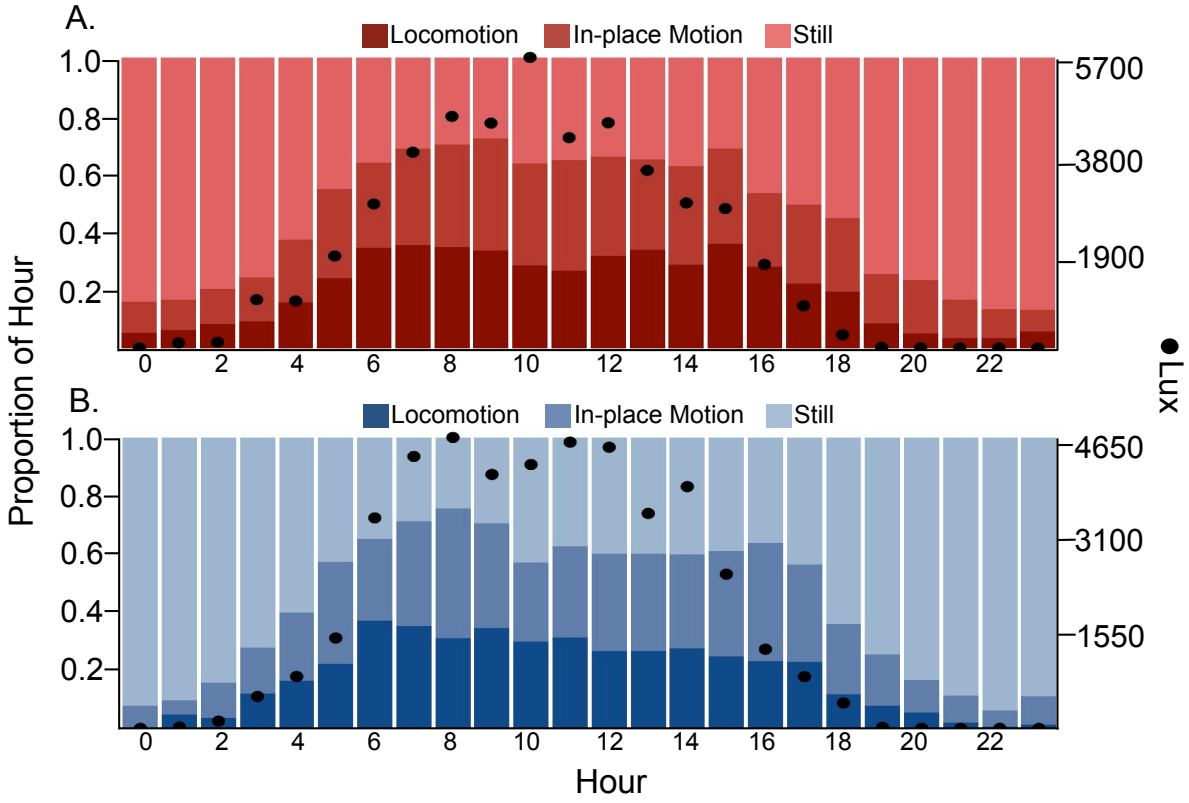


Figure 7: Daytime activity as a function of vegetation. (a) *Ts* (red) inhabiting sites with higher levels of vegetated ground cover (left, showing data from sites in approximately the top 50 percentiles of vegetated cover) exhibited lower levels of activity than *Ts* inhabiting sites with lower levels of vegetated ground cover (right, showing data from sites in approximately the bottom 50 percentiles of vegetated cover). (b) *Ta* (blue) exhibited the opposite pattern, showing higher activity at more vegetated sites (a) than at less vegetated sites (b).

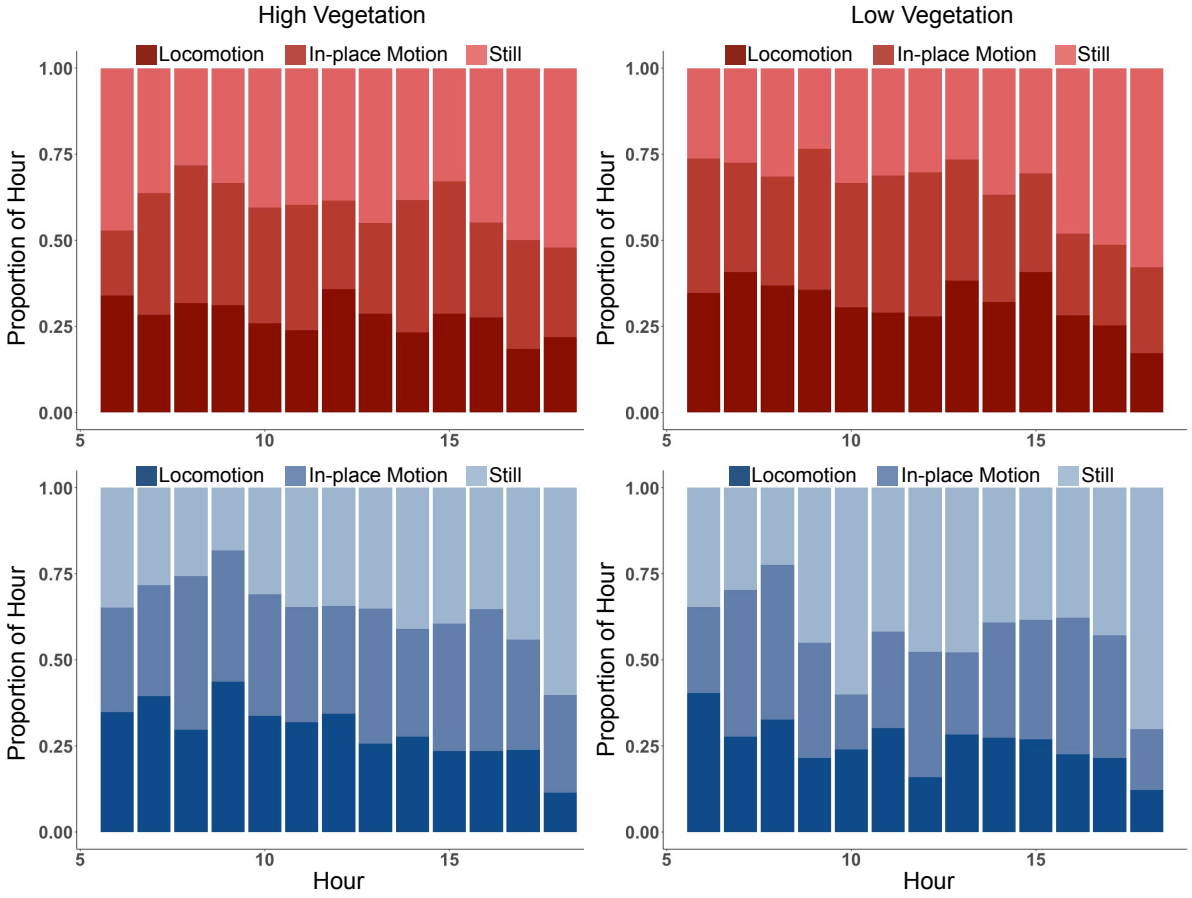


Figure 8: Daytime activity as a function of co-occurrence. (a) *Ts* (red) inhabiting sites where *Ta* did not coexist (left) exhibited lower levels of daytime activity than at sites where the two species co-occurred (right), but (b) the same pattern was not found for *Ta* (blue).

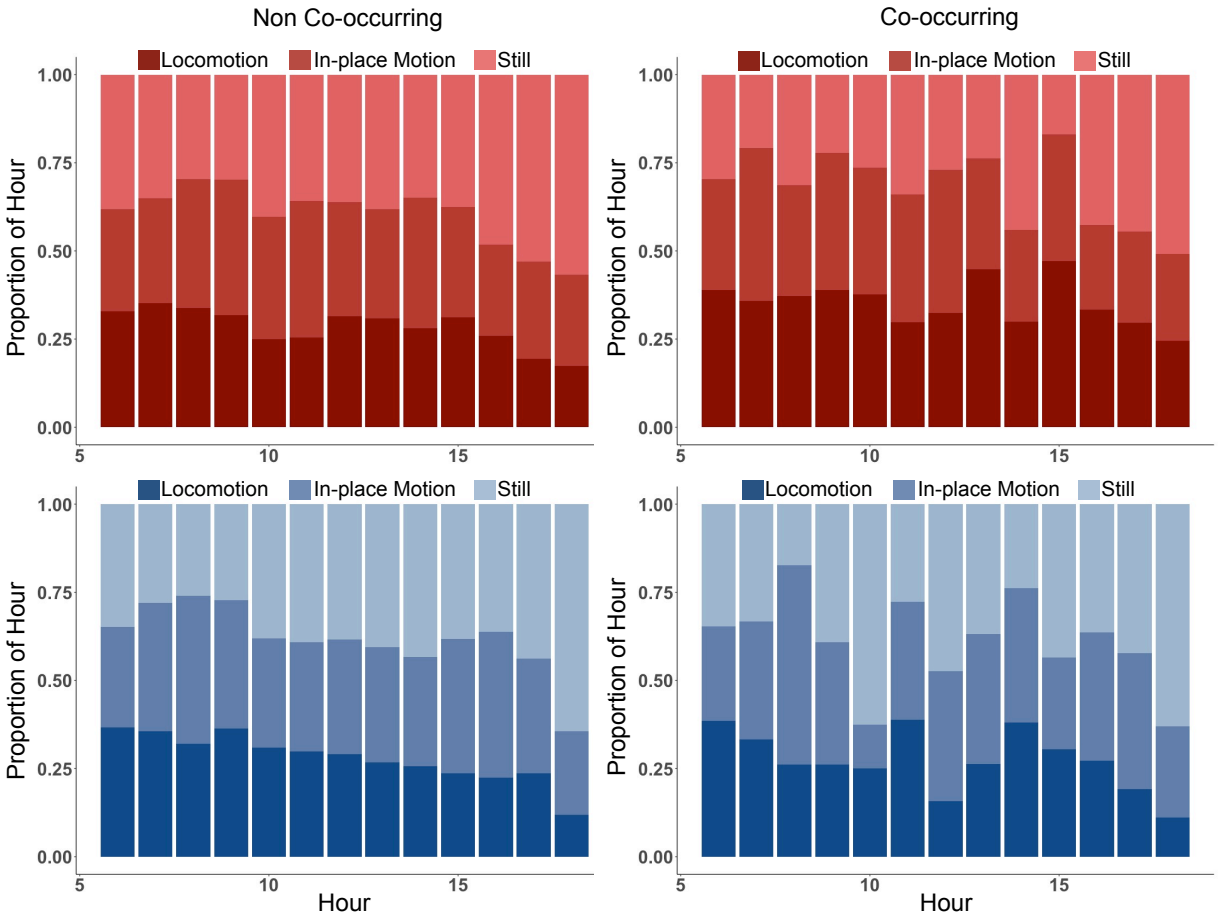
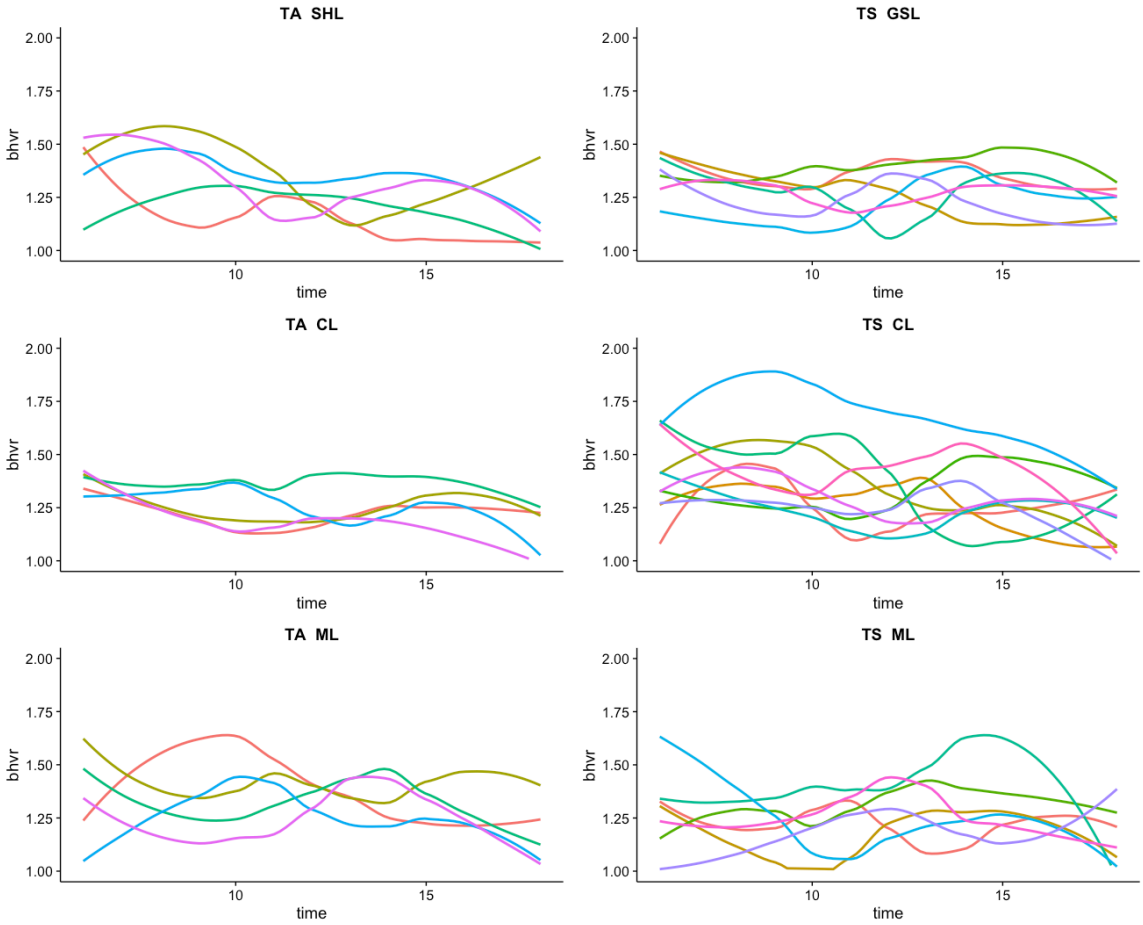


Figure S1. Daytime locomotion patterns by individual and site. Average locomotion scores for *Ta* (left) and *Ts* (right) individuals, smoothed across hours of the day. Each colored line represents a different individual, each row represents one of the three study sites.



Chapter 5

Environmental and endogenous predictors of stress in two congeners with differential responses to climate change

Anticipated co-authorship: Eileen A. Lacey, Rupert Palme

Abstract

Because many species have exhibited range shifts in response to climate change, it is becoming increasingly critical to understand which factors most constrain organisms' realized niches. Physiological limits imposed by abiotic factors, including temperature, are known to constrain range breadths for many species. Glucocorticoids – also known as "stress hormones" – have been proposed as one physiological indicator of individual and population viability. In this study we collect fecal glucocorticoid metabolite (FGM) data at multiple sites over the course of three years from two species of chipmunk that have exhibited divergent responses to the past century of environmental change: the alpine chipmunk (*Tamias alpinus*), which has exhibited substantial upwards range contraction in Yosemite National Park, and the co-occurring lodgepole chipmunk (*T. speciosus*), which has not. Combining FGM data with climate-based habitat suitability scores, estimates of relative population density, ground-cover survey data, and fine-scale temperature readings, in addition to a variety of individual traits collected upon each capture event, we construct models of FGM levels for each species. Our results show substantial interspecific differences in which factors are most predictive of FGMs. Environmental variables – including temperature, ground cover type, and habitat suitability scores – appeared in *T. alpinus*'s model of FGMs; fewer environmental variables appeared to be relevant to *T. speciosus*, whereas intrinsic biological variables like sex, body mass index, and reproductive status were significant predictors of FGMs in this species. We also found substantial interannual differences in which factors were most predictive of FGMs for each species, though the same general pattern held of environmental variables appearing to be more significant and intrinsic biological variables less significant for *T. alpinus* in contrast to *T. speciosus*. Overall, these results suggest that *T. alpinus* may be more responsive to environmental factors, potentially reflecting their sensitivity to environmental change.

1. Introduction

Geographic range shifts are a common measure of biotic response to climate change and studies of multiple taxa have demonstrated changes in the latitudinal or elevational distributions of organisms over the past century in apparent response to changing climatic

conditions (Chen et al., 2011; Hickling et al., 2006; Lenoir et al., 2008; Walther et al., 2002). To predict such range shifts, it is critical to understand how organisms are affected by specific extrinsic, environmental parameters, and how these parameters interact with intrinsic biological factors to shape an organism's ecological niche. At the same time, detailed understanding of interactions between organisms and their environments may reveal why, when faced with similar environmental changes, some species do not change their geographic distributions but, instead, appear to accommodate such changes *in situ*.

Physiological processes can reveal which abiotic factors most constrain an organism's fundamental niche, and provide a valuable means of assessing responses to environmental factors (Beever et al., 2016; Cahill et al., 2013; Kearney & Porter, 2009; Pacifici et al., 2015), particularly given that individuals may use physiology to respond to rapidly changing external conditions (Aubin et al., 2016; Seebacher et al., 2015; Wingfield 2013). Glucocorticoid hormones (GCs) are believed to be particularly useful indicators of short-term responses to environmental conditions (Bonier et al., 2009a; Busch & Hayward, 2009; Wikelski & Cooke, 2006). GCs are best known as "stress hormones" due to their importance in the fight-or-flight response, however, they are also critical for day-to-day metabolic processes (Sapolsky et al., 2000). Nevertheless, chronically elevated GCs are generally thought to be maladaptive, and the cort-fitness hypothesis posits that high GC levels are correlated with lower survival and reproductive success (Bonier et al., 2009a). While many studies have found support for this hypothesis (e.g. Hansen et al., 2016; Pride 2005; Thierry et al., 2013), results are mixed (Burtka et al., 2016; Madliger & Love, 2016) and the relationship between GCs and fitness appears to be highly context-dependent, varying with both life history (Angelier et al., 2010; Bonier et al., 2009b; Rivers et al., 2012; Smith et al., 2012) and environmental factors (Busch et al., 2009; Dantzer et al., 2016; Narayan et al., 2015). In any system it is crucial to understand the extent to which GCs reflect natural environmental and intrinsic biological conditions before employing them as a proxy for health or fitness (Dantzer et al., 2014). As averaged measures of circulating GCs over many hours to days, metabolites of glucocorticoids in feces have the potential to be more broadly reflective of an animal's overall baseline stress than point measures, like plasma GCs, and have the additional advantage of being non-invasive (Dantzer et al., 2014).

To assess the potential role of glucocorticoid physiology in mediating differential responses to a century of environmental change, we assessed predictors of baseline GCs in two partially-sympatric species of chipmunks in Yosemite National Park and adjacent regions of the Sierra Nevada mountains of eastern California. Comparisons of historical and contemporary distribution records for these species have revealed that while the alpine chipmunk (*Tamias alpinus*, *Ta*) has experienced a significant upward contraction in its elevational distribution, the lodgepole chipmunk (*T. speciosus*, *Ts*) has experienced no significant change in elevational distribution (Moritz et al., 2008). Physiological tolerances of *Ta* and *Ts* are in many cases similar (Heller & Gates 1971; Heller & Poulson 1970; Heller & Poulson 1972) and biotic interactions – specifically competitive exclusion of *Ts* from *Ta*'s habitats – have been proposed as being important for determining the degree of sympatry in these species (Chappell 1978; Heller 1971; Rubidge et al., 2011). However, certain physiological measures do differ. For example, *Ta* appears to be better adapted to arid environments (Heller & Poulson 1972) and in captivity was more stress responsive to a number of challenges (Hammond et al., 2015).

In particular, ecological niche models suggest that environmental factors like climate and vegetation are better predictors of the distribution of *Ta* than *Ts* (Rubdige et al., 2011). Over the past century *Ta* has also shown stronger genetic (Rubdige et al., 2012), morphological, and dietary shifts than *Ts* (Walsh et al., 2016). Collectively these findings suggest that *Ta* may be more impacted by environmental and abiotic factors and it is possible that this greater responsiveness has contributed to the differences in range response between *Ta* and *Ts* over the past century. In addition to experiencing more extreme climate change than other areas (Dirnbock et al., 2011; Mountain Research Initiative EDW Working Group, 2015), alpine organisms are also limited by the fact that there is a sharp upper bound to their ability to cope with climate change using range shifts. Consequently, it is especially critical to understand which factors constrain range limits for these species.

To test the prediction that *Ta* is more responsive than *Ts* to changes in environmental conditions, we use analyses of fecal glucocorticoid metabolites (FGMs) to assess the response of each study species to a variety of intrinsic and extrinsic factors. Specifically, we combine analyses of FGMs with analyses of individual phenotypes (e.g., sex, reproductive status, body mass) and the habitats (e.g., temperature, vegetative cover) in which study subjects were captured. We expect that for *Ta* GCs will be elevated in areas of lower suitability, for example, places with higher temperatures. For *Ts* we predict that GCs will be higher in areas of co-occurrence and increased chipmunk density, as biotic interactions are thought to be important in limiting the upper range boundary for this species (Chappell 1978; Heller 1971). For both species we expect that intrinsic biological factors will explain some variation in GCs, however, because *Ts* is a generalist species capable of living across a broad elevational range and because it has not shown any range shift in response to climate change, we expect that these parameters may explain a higher proportion of the variance in *Ts*'s GCs, as its physiology may be less responsive to the environmental variation within the relatively small proportion of its range that we sampled. These analyses generate new insights into potential physiological differences between the study species and suggest important differences in mediation of response to environmental change that are likely to contribute to the observed differences in range responses for alpine and lodgepole chipmunks.

2. Methods

2.1 Study Species & Sites

The alpine chipmunk *(Tamias alpinus, Ta)* is a small-bodied (30-50 g) species that occurs primarily above treeline (Clawson et al., 1994); in the Yosemite area it is found from 2936 to 3353 masl (Moritz et al., 2008). The lodgepole chipmunk (*T. speciosus, Ts*) is larger-bodied (50-80 g) and occurs primarily at and below treeline (Best et al., 1994); in the Yosemite area it is found from 896 to 3220 masl (Moritz et al., 2008). These species co-occur at multiple sites in Yosemite and adjacent areas of the Sierra Nevada Mountains; we sampled animals at 7-10 sites per year in this region (Fig. 1, Table 1) during June to October in 2013, 2014 and 2015.

Members of both species were captured using Sherman live traps baited with peanut butter and oats. Traps were opened from dawn to dusk and were checked every 4-

6 hours. Traps were set out in pairs ("stations"); trap stations were arranged in grids, typically with multiple grids established at each study site. When possible, grids were established to include areas at each site that contained *Ta* only, *Ts* only, or both species. The location of each trapping station was recorded using a hand-held GPS unit.

Upon first capture, each animal was uniquely marked using a numbered metal ear tag (Monel 100s; National Band and Tag Co., Newport, KY). In addition, we recorded the body mass, body length, and reproductive status of each individual. All procedures involving live animals were approved by the Animal Care and Use Committee at the University of California, Berkeley and adhered to the guidelines of the American Society of Mammalogists for the use of wild mammals in research (Sikes et al. 2016).

2.2 Fecal Sample Collection & Fecal Glucocorticoid Metabolite Analyses

Fecal pellets were collected from all animals captured for use in analyses of fecal glucocorticoid metabolite levels. Specifically, we removed all pellets from the trap in which an animal had been captured and placed them in a cryogenic tube that was then frozen in liquid nitrogen until it could be transported to the UC Berkeley campus and frozen at -80°C. Glucocorticoid metabolites were extracted from fecal samples and assayed as described in Hammond et al. (2015). In brief, fecal samples were dried in an oven at 90°C, crushed into a fine powder, extracted in methanol, and then dried in a vacuum centrifuge, after which they were reconstituted in assay buffer and fecal glucocorticoid metabolites (FGMs) were quantified using an enzyme immunoassay that had been validated previously for use in both the study species (Hammond et al., 2015). Because FGM values often follow a non-normal distribution, the data from our assays were log-transformed prior to statistical analyses.

2.3 Quantifying Environmental Parameters

To explore potential relationships between environmental conditions and FGM levels in our study animals, we documented multiple aspects of the habitats in which *Ta* and *Ts* were captured. Ambient temperatures at each trapping grid were recorded using iButton thermochron temperature loggers (model DS1921G) programmed to collect hourly temperature data. iButtons were deployed within one meter of the ground at approximately 75% of the trap stations in each grid. From these data, summary statistics for temperature (mean, maximum, variance) were calculated for use in analyses of environmental predictors of FGM levels (see section 2.5, below). Data regarding daily weather conditions were obtained from two weather stations in Yosemite National Park (Tuolumne Meadows Ranger Station and Yosemite National Park Headquarters); in addition, weather data for all years of the study were downloaded from NOAA for use in analyses of annual variation in climatic conditions (https://www.ncdc.noaa.gov/).

To characterize vegetative components of the habitats in which animals were captured, we surveyed the ground cover present at our trapping stations (2013: approximately one third of stations; 2014 & 2015: all stations). The percent composition of the following categories of ground cover were estimated visually for a circle with a radius of 5-m surrounding each trap station: litter/duff, downed wood, herbaceous, shrub, small rocks, bare soil, boulders, bedrock. Tree cover was also estimated visually for each 5-m radius circle.

To provide a more detailed assessment of environmental conditions and to develop ecological niche models (ENMs) for each study species, we downloaded high-resolution (30 arc s) data layers for nineteen bioclimatic variables available from WorldClim (Hijmans et al., 2005). Data layers were clipped to the geographic ranges of the focal species prior to analysis. Presence data for each study species from 1980 to present (sample sizes: *Ta* = 190 occurrences, *Ts* = 793 occurrences) were downloaded from the Arctos database (http://arctos.database.museum/). ENMs were generated using MaxEnt version 3.3.k (Phillips & Dudík, 2008). Twenty replicates were completed with cross validation, random seed, and a maximum number of iterations of 10,000; all other settings used were the default values provided by the program. ENM output values were extracted for each set of GPS coordinates (trap station location) at which an animal was captured and these values were used as a metric of habitat suitability in subsequent analyses of environmental predictors of FGM levels (see section 2.5 below; Crespi et al., 2015).

2.4 Estimates of Population Density

Because measures of FGM levels may be influenced by demographic parameters such as population density (Creel et al., 2013; Dantzer et al., 2013), we used data on recaptures of marked chipmunks to generate estimates of density for each species at each sampling site. Given that we were interested in relative rather than absolute estimates of population densities, we used the Schnabel index, an extension of the Lincoln-Peterson method for estimating population sizes; this method can be applied when populations have been sampled on more than two occasions (Napolitano et al., 2008; Schnabel et al., 1938), which was the case for all populations surveyed in this study. The Schnabel index is defined by the equation:

$$\frac{\sum(M_t C_t)}{(\sum R_t) + 1}$$

where M_t is the total number of individuals captured at time t, C_t is the total number of marked individuals in the population just before t, and R_t is the number of previously marked individuals captured at t. This index value was generated for each trapping grid in each year and then divided by the area of that grid to generate an estimate of population density. Grid area was calculated by establishing an ~20 meter buffer around each trapping station, integrating buffers for all trapping stations in the same grid, and calculating the total resulting area. These procedures did not provide an absolute metric of population abundance or density, but did allow for comparison of the relative densities across trapping grids and study sites. These values were included as fixed effects in predictive models of FGM levels (section 2.5). A species co-occurrence score, defined as the percentage of conspecifics captured in a grid, was also calculated for each species and included in these models (section 2.5).

2.5 Statistical Analyses

Principal Components Analyses (PCAs). To address collinearity among environmental parameters and to reduce the number of predictor variables used in model construction, we conducted separate PCA analyses for measures of temperature and ground cover. For

ground cover data, bare soil and small rocks were combined into a single category ("rocky"), and litter/duff and downed wood were combined into a single category ("detrital"). Based on these analyses, the first three PCA axes for ground cover measures (accounting for 83.2% and 82.2% of variation for *Ta* and TS, respectively) and the first two PCA axes for temperature (accounting for 87.4% and 91.3% of variation for *Ta* and *Ts,* were included as fixed effects in predictive models of FGM variation (Tables 2 & 3).

Relationships Between Temperature and Vegetation. To examine potential relationships between mean daily temperature (as measured by iButtons) and measures of habitat conditions, a linear mixed model was constructed using the lm function in R (R Core Team, 201), with year, species co-occurrence score, and date as fixed effects and site as a random effect. A separate linear model was constructed to examine interannual differences in median daily temperatures (as measured from weather station data); this model included only date, year, and station ID. Finally, a linear mixed model was constructed to assess the fixed effects of year, co-occurrence score and date and the random effect of site on the first PCA axis of the vegetation data; this model was applied to the subset of sites that were visited in all three years of the study. Wilcoxon rank sum tests were used to compare snow depths and herbaceous cover across years.

Generalized Linear Mixed Models (GLMMs). To identify predictors of variation in FGM levels within and between the study species*,* GLMMs were implemented using the lmer function in the lme4 package in R. The initial, full model contained sixteen fixed effects and five interaction terms as well as two random effects (Table 4); these predictors were selected from a larger number of potential variables after substantial preliminary visual exploration of the data set. The GLMM for assessing interannual differences in predictors of FGMs contained these same variables as well as interaction terms between year and co-occurrence score, density, habitat suitability score, temperature, vegetation, and date. The significance of each fixed effect was assessed using Satterthwate's approximation based p-values as calculated using the lmerTest package in R; non-significant predictor variables were eliminated in a stepwise fashion until the model contained only significant fixed effects. Models with and without the random effects of site and individual identity were generated and compared using R's anova function to determine if these random effects were significant. This function was also used to compare models for each species containing only environmental versus only biological variables. All numeric variables (Table 4) were rescaled prior to analysis in order to prevent problems associated with model convergence. Models were validated graphically, as in Zuur et al., (2009), to ensure that they met all assumptions for GLMMs.

In cases in which the final model included multiple significant PCA axes, the directionalities of these effects were calculated by multiplying the GLMM regression coefficient for each axis by the variable loadings for that axis. To visualize relationships between individual fixed effects and FGMs while controlling for the other fixed effects included in the final model, partial residuals plots were used, in which all values of an individual fixed effect are plotted against all values resulting from the following sum: $\hat{\beta}_i X_i + r$, where $\hat{\beta}_i$ is the regression coefficient for the individual fixed effect of interest, X_i is the fixed effect of interest, and r are the residuals from the full model. For some analyses, post-hoc tests were used to examine specific relationships between predictor

variables and FGMs. Non-parametric tests (Wilcoxon signed rank/rank sum tests) were used if data did not meet the assumptions for standard parametric tests; for post-hoc analyses involving multiple comparisons, False Discovery Rate adjustments (Benjamini & Hochberg, 1995) to p-values were made.

3. Results

3.1 Interspecific and Interannual Comparisons of Environmental Parameters

Our analyses confirmed results from previous studies indicating significant habitat differences between areas occupied by *Ta* only, by *Ts* only, or by both species (Hammond et al., 2017; Walsh 2015). Localities at which predominantly *Ta* was captured had higher mean daytime temperatures and co-occurrence of the study species was a significant predictor of mean daytime temperatures (Table 5; Fig. 2). Areas occupied by *Ta* also tended to be rockier and to have less litter, duff, and downed wood than areas occupied by *Ts* (Fig. 1 & 3); species co-occurrence was also a significant predictor of ground cover composition (Table 6).

Temperature and ground cover differed significantly between years of the study. Year was a significant predictor of median daily temperatures recorded by weather stations in Yosemite, with 2015 generally being warmer than 2013 or 2014 (Table 7; Fig. 4A). Notably, all three years of this study took place during a progressively severe period of drought in California (Berg & Hall, 2017) and mean recorded snow depth in the Sierra Nevada decreased significantly during each year of the study (Wilcoxon rank sum tests with FDR-adjusted p values: 2013 vs. 2014 W=40757, p =0.0000005; 2014 vs. 2015 W=28048, p=0.00000001; Fig. 4B). Ground cover also differed between years when comparing sites that were visited in all three years of the study. These analyses revealed that 2014 was distinct from the other two years of this study (Table 6); for example, herbaceous cover was significantly greater in 2014 than in 2013 or 2015 (Wilcoxon rank sum tests with FDR-adjusted p-values: 2013 W=38, p = 0.027; 2015 W=248, p = 0.027; Fig. 5). Thus, the localities sampled were subject to potentially important interannual differences in biotic and abiotic conditions.

3.2 Interspecific Comparison of FGMs

Fecal samples were collected for a total of 929 capture events representing 270 total individuals for *Ta,* and 2,715 capture events representing 1,028 individuals for *Ts* (Table 1). Among adults, FGM levels for females were higher in *Ts* than *Ta;* the reverse pattern was detected for FGM levels for males (Table 8). No significant differences between species were found for FGM levels in juveniles (Table 8). In both species, the random effect variables – individual and site – significantly improved the fit of all models (ANOVA individual: *Ta* p=2.3e-11; *Ts* p=2.2e-16; site: *Ta* p=0.01; *Ts* p=0.0000008). Temporally, FGM levels for *Ts* tended to decrease over the course of the day and over the course of the season; no significant temporal variation in FGMs was detected for *Ta* (Table 9).

3.3 *Intrinsic Biological Parameters & FGMs*

Intrinsic biological parameters were identified as significant predictors of FGM levels in *Ts* but not in *Ta.* Specifically, in *Ts,* males had lower FGMs than females (Tables 8 & 9); non-reproductive adults had significantly higher FGMs than juveniles but significantly lower FGMs than reproductive adults (Table 9). Body mass index (BMI) was a significant positive predictor of FGM levels (Table 9, Fig. 6). In addition, there was a significant interaction between reproductive status and BMI, with the positive relationship between these variables being stronger for adults than for juveniles. There was also a significant interaction between reproductive status and sex, with reproductive individuals displaying a greater tendency for males to have lower FGMs than females (Table 9). Finally, there was a significant negative relationship between the total number of times individual *Ts* were recaptured and FGM levels (Table 9). In contrast, none of these intrinsic biological parameters were retained in the final model for *Ta* (Table 9).

3.4 Environmental Parameters & FGMs

Environmental factors appeared in *Ta*'s models of FGMs more than *Ts*'s. Habitat suitability, (measured from climatic niche models) was a negative predictor of FGMs for *Ta* (Fig. 7), as were mean and maximum daily temperatures; in contrast, variance in daily temperatures was positively associated with FGMs in *Ta* (Table 9). PCA scores for ground cover were also significantly associated with FGMs in *Ta*; when all three PCA axes were considered, measures of ground cover, tree cover, detrital cover, herbaceous cover, and boulders were negatively associated with FGMs, while measures of bedrock, small rocky cover, and shrubby cover were positively associated with FGMs, with these relationships being strongest for detrital, tree, bedrock, and rocky cover (Fig.8). FGMs in *Ta* were negatively associated with population density (Table 9). In addition, there were significant interactions between ground cover and population density, with the effect of ground cover on FGMs being less apparent at higher population densities (Table 9; Fig. 9).

In contrast, in *Ts,* only ground cover PCA scores and the proportion of conspecifics on the trapping grid were significantly associated with FGMs (Fig. 8); no other environmental variables were included in the final model for this species (Table 9). Further, some of the relationships between specific ground cover categories and FGMs differed between the study species. In *Ts,* herbaceous, shrubby and detrital cover were positively associated FGMs, while bedrock, boulder, rocky, and tree cover were negatively associated with FGMs; these relationships were strongest for shrub, herbaceous, and bedrock cover (Fig. 8). The relatively greater power of extrinsic over intrinsic parameters to predict FGM levels in *Ta* was secondarily confirmed by comparisons of models containing only intrinsic biological (including intrinsic interaction terms; Table 4) or only environmental variables (including environmental interaction terms; Table 4); the performance of the environment-only model was significantly better for this species (Table 10). For *Ts,* the same comparison failed to reveal any significant difference between models based on extrinsic versus intrinsic parameters (Table 10).

3.5 Interannual Differences in FGMs

Including interactions between year and environmental variables in our models revealed significant inter-annual differences in the factors that were most predictive of FGM levels in each study species (Table 11). In *Ts,* relationships between FGMs and co-occurrence

score, population density, date, and ground cover scores differed significantly among years of the study (Table 11; Fig. 10). Specifically, in 2013 FGMs were more strongly positively associated with higher proportions of conspecifics; in 2015 density was more strongly positively associated with FGMs; and date was positively associated with FGMs in 2013, but negatively associated in 2014 and 2015 (Table 11). Ground cover scores differed between all three years: in 2013, FGMs were most strongly positively associated with bedrock, boulder, and herbaceous cover, and were most negatively associated with tree and detrital cover, in 2014 these relationships were similar but less strong, and in 2015 their directionalities were reversed (Table 11, Fig. 10). In *Ta,* the only predictors of FGMs that varied among years were habitat suitability and ground cover scores. Specifically, habitat suitability was significantly negatively associated with FGMs in 2015 more so than other years (Table 11). Ground cover scores, on the other hand, were more strongly associated with FGMs in 2014 than in other years; specifically the same relationships reported above (section 3.4) were stronger in 2014 than in other years (Table 11, Fig. 10).

4. Discussion

Overall, our analyses suggest that *Ta* is more physiologically responsive to extrinsic, environmental factors than *Ts.* For example, while ground cover, habitat suitability, and temperature emerged as significant predictors of FGM levels in *Ta,* only ground cover was identified as a significant predictor of FGMs in *Ts*. In contrast, FGM levels in *Ts* appeared to be more strongly predicted by differences in intrinsic parameters such as sex, reproductive status, and BMI. Thus, the physiological responses of the study species differed markedly, providing a potential mechanism by which exposure to the same general environments may have generated pronounced interspecific differences in range response over the past century.

4.1 Intrinsic Predictors of FGMs

Intrinsic biological factors explained significant variation in Ts's but not Ta's FGMs. For example, reproductively active, female *Ts* had the highest FGMs, which was expected because much of our sampling took place during lactation. While patterns are somewhat taxon-dependent (Romero et al., 2002), lactation is energetically demanding (Wade & Schneider, 1992) and many previous studies have documented increased baseline GCs in females during gestation and lactation (e.g. Boonstra et al., 2001; Boswell et al., 1994; Dantzer et al., 2010; Kenagy & Place, 2000). *Ts* individuals with higher BMIs also had higher FGMs (this pattern was also found for individuals with higher masses), which diverged from previous work that has documented elevated FGMs in individuals with lower body condition scores (Sockman & Schwabl 2001; Waye & Mason 2008; Williams et al., 2008); again, this result points to the importance of verifying GC relationships on a species-specific basis. Hour and date were also significant predictors of FGMs for *Ts* but not *Ta,* again suggesting the increased importance of intrinsic biology – specifically, circadian and seasonal rhythms – to this species. On the whole, a huge body of work points to the importance of life history variables to GCs, thus, it was surprising that, when controlling for environmental predictors, these factors did not explain significant

additional variation in *Ta*'s FGMs. Previous work suggests that *Ta* is broadly more stress-responsive to external challenges than *Ts* (Hammond et al., 2015), and together these results highlight the significance of environmental factors to *Ta,* potentially suggesting that this species may be more vulnerable to changing environments.

Individual identity, which was included as a random effect in all models, was a significant predictor of FGMs for both species, suggesting that there is some degree of inter-individual difference and intra-individual repeatability in FGMs. These measures were possible because we had numerous recaptures for many individuals, and future studies will use this dataset to explore the extent to which within- and between-season repeatability in FGMs exists in this system.

4.2 Environmental Predictors of FGMs

Many environmental parameters were predictive of FGMs. Ground cover variables were important predictors for both species. In most years both species generally exhibited higher FGMs in environments with more bedrock and boulder cover, and lower FGMs in areas with more trees and detrital cover (Fig. 10). These results align with a number of previous studies that have found higher GCs in high-elevation, alpine habitats, which are often associated with higher levels of bedrock and boulder and lower tree and detrital cover (Addis et al., 2011; Graham et al., 2013; Sheriff et al., 2012; but see Hik et al., 2001). Vegetation has changed over the past century in Yosemite (Santos et al., 2015), and these results more broadly suggest that further change in vegetation has the potential to impact the stress physiology of both *Ta* and *Ts.*

As expected, *Ta* individuals living in more climatically suitable habitats exhibited lower FGMs. Unexpectedly, however, when controlling for habitat suitability and other environmental variables that were included in the final model, daytime temperatures during the sample collection period were negatively associated with FGMs. This aligns with previous work showing that *Ta*'s activity does not decrease in high daytime temperatures (Hammond et al., 2016; Hammond et al., 2017), and suggests that climatic factors at other times of year may be more limiting. Variables such as temperature of the coldest month and quarter were included in the niche models that generated habitat suitability scores, and changes in winter climatic parameters can have substantial fitness consequences for hibernating, terrestrial animals in temperate areas (Humphries et al., 2002; Lane et al., 2012; Williams et al., 2015). However, it is worth noting that daily temperatures and habitat suitability scores were often correlated, which makes interpreting these GLMM results complicated; there was a strong positive relationship between FGMs and daytime temperatures in a simple, single linear regression analysis. Consequently, while our results support the relative importance of climate to *Ta* over *Ts,* it is difficult to say for certain whether or not high summer temperatures are physiologically limiting to this species. Moreover, site, which was included as a random effect in all models, also explained a significant component of the variation in FGMs for both species, suggesting that there are differences not explained by environmental and climatic parameters alone. This could be explained by parameters we didn't measure, for example, predation risk or human impacts.

As predicted, species co-occurrence score was a strong predictor of FGMs for *Ts* and not for *Ta,* although not in the direction expected. Specifically, our analyses revealed that FGMs were highest in areas where *Ts* did not co-occur with *Ta,* which did not

support previous hypotheses that *Ts* is biotically limited by competition with *Ta.* Moreover, population density was not a significant predictor of FGMs in this species. For *Ta,* on the other hand, areas with higher density were associated with lower FGMs and with a reduced significance of ground-cover parameters. This first finding is also unexpected, as in other species GCs are often elevated in high-density areas (Dantzer et al., 2013; Sheriff et al., 2012), though it could be explained by the fact that areas with higher densities may tend to have higher-quality habitat. The modulating effect of density on vegetation in more understandable. Altogether, population-related parameters were not as significant to *Ts* as we had hypothesized.

4.3 Interannual differences in predictors of FGMs

There were significant interactive effects between year and environmental predictors for both of the focal species, pointing towards inter-annual differences in which factors were most predictive of FGMs. For *Ta,* individuals had much lower FGMs in more climatically suitable habitats in 2015 in contrast to the other years. According to both iButton and weather station data, 2015 was the warmest year of this study, and was also the most extreme year of the drought in California (Berg & Hall, 2017). These climatic data may shed light on the interannual differences we saw: FGMs may have been tied to climate-based habitat suitability scores in 2015 more so than the other years due to the fact that 2015 was the most climatically extreme year, when animals may have been pushed closest to their physiological limits. The relationship between ground-cover and *Ta*'s FGMs was stronger in 2014 than in other years. 2014 was the coolest of the three years, and showed significantly higher herbaceous cover. Consequently, the fact that ground cover was more predictive of FGMs in this year – including herbaceous cover being increasingly associated with lower FGMS - makes sense. *Ta* are known to depend more on herbaceous food sources (e.g. sedges and grasses) than *Ts* (Grinnell & Storer, 1924, Best et al., 1994; Clawson et al., 1994; Walsh et al., 2016), and previous work has identified negative relationships between GCs and food availability (Bauer et al., 2013; Jenni-Eiermann et al., 2008; Jessop et al., 2013; Kitaysky et al., 1999; Stabach et al., 2015). Altogether, the interannual differences we found for *Ta* aligned with this species' overall tendency to show directional responsivity to environmental parameters, and the interactive effects we saw aligned with the interannual climate and habitat differences we documented.

Ts showed different patterns. In 2013 their FGMs were elevated in areas with higher proportions of conspecifics and later in the season in contrast to other years; in 2015 FGMs were elevated in high-density areas, in contrast to low-density areas in 2014 (and little relationship in 2013); and in 2015 FGMs were elevated in areas with more trees and less bedrock, boulder and herbaceous cover in contrast to other years. These interannual differences – which include complete reversals of directionality in the relationship between an environmental variable and FGMs (e.g. Fig.10) – are not easy to tease apart. Possibly biotic variables that we did not measure (e.g. predation risk) could explain some of this variation. *Ts*'s responses, however, have generally been less predictable than *Ta*'s, and in some ways our results match up with previous research that has found it difficult to strictly tie *Ts*'s biology to environmental parameters. For

example, vegetation and climate-based models were unable to predict *Ts*'s lack of elevational range shift in the central Sierras from ~1900-2000 (Rubidge et al., 2011).

Regardless of the specific patterns of change from year to year, our data confirm the importance of controlling for study year when interpreting FGM results, even after controlling for environmental and biological variables (Foltz et al., 2015). Moreover, given the variation in FGM patterns from year to year, our data highlight the importance of collecting data across multiple years whenever possible.

4.4 Glucocorticoids as bioindicators

Glucocorticoids have frequently been employed as bioindicators to inform the conservation and management of wildlife (Busch & Hayward, 2009; Millspaugh & Washburn, 2004). The Cort-fitness hypothesis (Bonier et al., 2009) suggests that elevated baseline GCs are generally correlated with lower survival and reproductive success. While many studies have documented relationships supporting this notion (e.g. Hansen et al., 2016; Pride 2005; Thierry et al., 2013), significant work has contested its generalizability (Bonier et al., 2009; Burtka et al., 2016; Madliger & Love 2016). It is important to be conservative and cautious when using GCs – particularly fecal GCs – as a proxy for individual or population health and fitness; in addition to requiring a substantial methodological validation (Touma & Palme, 2005), significant work demonstrates important context dependencies of GC-fitness relationships (Angelier et al., 2010; Madliger & Love, 2016; Rivers et al., 2012). Here we document links between GCs and intrinsic biological factors, external environmental parameters, and year to begin to characterize such relationships. Future work will more explicitly explore ties between fitness and FGMs in the focal species.

Our results provide compelling and novel insights into the connections between GCs and environmental parameters. Previous work exploring GC-environment patterns has been mixed, with some studies describing strong correlations (Davies et al., 2013; Ozella et al., 2015; Spercoski et al., 2012), and others finding weak or absent relationships (Corlatti et al., 2014; Crespi et al., 2015; Madliger et al., 2015). When is an organism expected to show a strong physiological response to environmental parameters? Species and populations living in more extreme or variable environments, including near range edges, tend to be more physiologically limited (Busch et al., 2011; Sorte & Hofmann, 2004), and thus more responsive to environmental variables. More specialized species are also thought to be more vulnerable to climate change (Foden et al., 2009; Thuiller et al., 2005), and in some cases they have adapted tolerance to life at climatic extremes at the cost of lowering their acclimation capacity, or ability to adjust to even relatively small changes in climatic parameters (Seebacher et al., 2015; Stillman 2003). *Ta,* an alpine specialist that has exhibited increased responsivity to climate change along not only spatial (Moritz et al., 2008), but also genetic (Rubdige et al., 2012) and morphological metrics (Walsh et al., 2016) may be one such species. Our results suggest that FGMs of *Ta* are more strongly related to environmental parameters than those of *Ts.*

In the context of climate change, it is important to understand which factors determine an organism's fundamental and realized niches (Cooke et al., 2013; Holt 2009; Jankowski et al., 2013; Wikelski & Cooke 2006). In some cases, animals may be prevented from inhabiting certain areas due to hard, physiological limits, however, often certain habitats – though not physiologically uninhabitable –may simply confer lower

competitive ability or other fitness costs (Cooke et al., 2013). We expect that GCs may be a signal of this latter scenario, and that elevated FGMs could be reflective of sub-optimal habitats, particularly for species with more narrow ecological niches. In this study we documented elevated FGMs in climatically less suitable habitats for a species that has responded strongly to the past century of climate change. Understanding which specific environmental factors define subpar habitats for this species is increasingly important, given the limited ability of this organism to adjust to future climate change with further upwards elevational shifts (due to the upper bound set by mountaintops). More broadly, characterizing relationships between physiological traits and environmental variables for organisms living at range edges that may be most susceptible to the effects of future climate change will improve our ability to predict how an when organisms will respond to environmental change.

Chapter 5 Tables

Table 1. Spatial coordinates, elevations, visit dates, and sample sizes for each study site. Sample sizes are presented for each species as the total number of individuals, followed by the total number of captures in parenthesis.

Site	Lat	Lon	Elev (m)	Visit Years	Visit Dates	*Ta*	*Ts*
AL	37.58252	-118.9799	2850-3020	2014 2015	11-14 Aug 12-21 Jun	-	109(219) 107(349)
CL	37.83915	-119.4183	2800-3060	2013 2014 2015	3-10 Jul 27 Jun-5 Jul 15-27 Aug	22(55) 39(101) 43(209)	16(38) 50(103) 45(194)
GA	37.91219	-119.4207	2380-2435	2013	16-22 Jun	-	16(25)
GL	37.91595	-119.2664	3095-3230	2014 2015	17-23 Jun 3-6 Sep	18(37) 11(17)	10(31) 16(19)
HC	37.82916	-119.5134	2585-2660	2013	24-28 Jul	-	12(18)
MA	37.6498	-119.0106	2570-2710	2013 2014 2015	30 Aug 18 Sep 26 May-2 Jun 16-17 Aug 8-14 Sep	-	16(17) 103(161) 103(341)
ML	37.84419	-119.4951	2690-2995	2013 2014 2015	27 Jul-10 Aug 10-19 Jul 30 Jun-19 Jul 24 Sep-4 Oct	29(61) 43(79) 46(189)	55(123) 80(126) 61(294)
PC	37.79501	-119.5504	2330-2460	2013	16-21 Jul	-	18(32)
SL	37.96497	-119.2686	2945-3230	2013 2014 2015	16-18 Aug 7-11 Sep 26-29 Jul 22-23 Sep	27(36) 11(14) 2(2)	31(45) 27(33) 5(6)
SLN	37.98362	-119.2939	2955-3210	2013 2014 2015	17-18 Aug 31 Jul-4 Aug 23-29 Aug 25 Jul-6 Aug	3(3) 16(37) 22(89)	6(6) 46(144) 51(231)
RD	37.87431	-119.3717	2545-2690	2013 2014	27 Jun-1 Jul 5-11 Jun 6-8 Aug	-	16(27) 80(125)
VR	37.62836	-118.9975	2485-2495	2013	4-5 Sep	-	8(8)
All sites				2013 2014 2015		81(155) 127(268) 114(506)	194(339) 505(942) 387(1434)
Total						270(929)	1028(2715)

Table 2. Variable loadings for principal components of ground cover/vegetation data.

a. *T. alpinus*

Variable	PC1	PC2	PC3
Detrital	-0.5182968	0.2295427	-0.2317310
Tree	-0.4244610	0.4356104	-0.1321606
Shrub	-0.2393992	-0.5277958	0.1593050
Herbaceous	-0.1263366	-0.5089710	-0.4892741
Bedrock	0.5325326	0.1692200	0.2557227
Boulder	0.3714252	-0.1897356	-0.5709516
Rocky	-0.2374461	-0.3940797	0.5221947
Percent of Variance	39.3	30.3	13.6

b. *T. speciosus*

Variable	PC1	PC2	PC3
Detrital	0.5017350	-0.02965969	0.1710783
Tree	0.3991220	0.23536735	-0.0996272
Shrub	-0.1989567	-0.24278549	0.8326217
Herbaceous	-0.3507442	0.38037663	0.3061842
Bedrock	-0.4409203	0.15922528	-0.3456123
Boulder	-0.4546650	0.12439921	-0.0887389
Rocky	-0.1588312	-0.83621976	-0.2155839
Percent of Variance	52.2	15.9	14.1

Table 3. Variable loadings for principal components of temperature data.

a. *T. alpinus*

Variable	PC1	PC2
Mean	-0.5768722	0.5813367
Maximum	-0.6082570	0.1631569
Variance	-0.5451989	-0.7971371
Percent of Variance	68.2	19.2

b. *T. speciosus*

Variable	PC1	PC2
Mean	-0.6761660	0.3152150
Maximum	-0.7129906	-0.0522809
Variance	-0.1855907	-0.9475791
Percent of Variance	57.0	34.3

Table 4. Description of fixed and random effects included in GLMMs.

Variable Type	Variable Name	Description	Type
Temporal	Date	Julian date	Integer
	Year	Year (2013, 2014, or 2015)	Factor
	Hour	Hour of capture	Integer
Environmental	Coocc	Proportion of captures that were of conspecifics (vs. heterospecifics) in a grid	Continuous (0>x<1)
	Density	Schnabel index of population estimate divided by area for each grid	Continuous
	PC1_cover	First axis of PCA containing ground cover variables (vegetation, rocks, detritus), by grid	Continuous
	PC2_cover	Second axis of ground cover PCA	Continuous
	PC3_cover	Third axis of ground cover PCA	Continuous
	PC1_temp	First axis of PCA containing temperature variables	Continuous
	PC2_temp	Second axis of temperature PCA	Continuous
	Elev	Elevation of the capture location	Continuous
	ENM	Habitat suitability score (output from climate-based ecological niche model) of the capture location	Continuous (0>x<1)
Intrinsic	#recap	Number of times an individual was re-captured in a visit	Integer
	Sex	Male or female	Factor
	Rep	Reproductive Status (adult, reproductively active adult, or juvenile)	Factor
	BMI	Each individual's mass divided by its body length	Continuous
	Interaction Terms		
	Density x PC1_cover; Density x PC2_cover; Density x PC3_cover Rep x Sex; Rep x BMI		
	Random effects		
	ID (random)	Individual animal ID	Factor
	Site (random)	Study site of each individual	Factor

Table 5. GLMM results modeling mean daily daytime temperatures across all sites.

	Estimate	SE	df	t-value	p-value
(Intercept)	29.94	1.71	106.9	17.47	<2.e-16
Co-occurrence	-1.55	0.51	663.8	-3.02	0.00264
Year (2014)	-1.21	0.52	650.8	-2.33	0.02
Year (2015)	0.20	0.50	654.7	0.39	0.70
Date	-0.05	0.007	660.3	-7.69	5.6e-14

Random effect	Variance (SD)
Site	8.17 (4.27)

Table 6. GLMM results modeling first PCA axis of ground cover data from sites visited during all three years of the study

	Estimate	SE	df	t-value	p-value
(Intercept)	-2.67	1.63	36.95	-1.64	0.109
Coocc	3.48	0.42	38.62	8.26	4.6e-10
Year (2014)	-1.59	0.44	39.14	-3.63	0.0008
Year (2015)	-0.84	0.43	36.11	-1.96	0.056
Date	0.004	0.007	40.96	0.66	0.515

Random effect	Variance (SD)
Site	0.53 (0.73)

Table 7. Linear model of weather station based daily median temperature data (January 2012-December 2015).

	Estimate	SE	t-value	p-value
(Intercept)	35.89	0.82	43.9	<2e-16
Year (2014)	0.01	0.77	0.02	0.99
Year (2015)	1.84	0.77	2.38	0.02
Date	0.02	0.003	6.60	5.3e-11
Station	16.2	0.62	26.01	<2e-16

Table 8. Mean, standard error, and Wilcoxon rank sum test results (with FDR-adjusted p-values) comparing interspecific differences in FGMs between sex/age classes.

	Ta		*Ts*			
	Mean	S.E.	Mean	S.E.	W	p-value
Adult Females	97.3	6.7	123.0	5.4	14262	0.001
Juvenile Females	90.1	10.1	88.6	9.3	7207	0.20
Adult Males	115.6	8.0	69.1	4.7	15563	3.2e-11
Juvenile Males	75.6	6.5	68.0	3.7	9844	0.09

Table 9. GLMM results modeling FGMs for (a) *Ta* and (b) *Ts.*

(a) *T. alpinus*

	Estimate	SE	df	t-value	p-value
(Intercept)	4.74	0.17	10.6	28.2	2.6e-11
Year (2014)	0.04	0.11	667.7	0.31	0.75
Year (2015)	-0.93	0.15	592.5	-6.17	1.3e-09
PC1_cover	0.11	0.03	69.2	3.88	0.0002
PC2_cover	-0.07	0.04	408.4	-1.96	0.05
PC3_cover	0.22	0.06	590.6	3.65	0.00005
Density	-0.16	0.04	319.5	-4.12	0.0003
ENM	-0.19	0.06	150.2	-3.16	0.002
PC1_temp	-0.09	0.04	556.6	-2.43	0.02
PC2_cover* Density	-0.09	0.03	615.7	-3.56	0.0004

Random effect	Variance (SD)
ID	0.08(0.28)
Site	0.07(0.26)

(b) *T. speciosus*

	Estimate	SE	df	t-value	p-value
(Intercept)	4.18	0.11	12.3	39.6	5.1e-14
Hour	-0.03	0.01	2201.7	-2.26	0.02
Date	-0.18	0.03	1238.5	-6.99	4.5e-12
Cocc	0.05	0.02	1207.1	2.17	0.03
PC3_cover	0.08	0.02	1000.9	3.71	0.0002
#recap	-0.10	0.03	486.6	-3.78	0.0002
Rep (Juv)	-0.20	0.07	928.4	-2.89	0.004
Rep (RepAct)	0.30	0.09	807.8	3.19	0.001
BMI	0.15	0.05	948.6	3.06	0.002
Sex(M)	-0.38	0.13	710.4	-2.90	0.004
Rep (Juv)*BMI	-0.17	0.06	1047.0	-2.92	0.004
Rep(RepAct)*BMI	-0.08	0.07	921.6	-1.17	0.24
Rep (Juv)*Sex(M)	0.22	0.14	791.0	1.53	0.13
Rep(RepAct)*Sex(M)	-0.39	0.15	749.8	-2.59	0.01

Random effect	Variance (SD)
ID	0.15(0.39)
Site	0.42(0.65)

Table 10: Results from an ANOVA comparing models containing only environmental *versus* only biological variables for (a) *Ta* and (b) *Ts*

(a) *T. alpinus*

Model Type	Df	AIC	BIC	logLik	Deviance	Chisq	p-value
Environmental	14	1823.4	1889.6	-897.68	1795.4		
Intrinsic Biological	16	1758.9	1834.5	-863.43	1726.9	68.5	1.3e-15

(a) *T. speciosus*

Model Type	Df	AIC	BIC	logLik	Deviance	Chisq	p-value
Environmental	16	5454.1	5546.3	-2711	5422.1		
Intrinsic Biological	13	5282.7	5357.7	-2628.4	5256.7	0	1

Table 11. GLMM results including by-year interactions modeling FGMs for (a) *Ta* and (b) *Ts*

(a) *T. alpinus*

	Estimate	SE	df	t-value	p-value
(Intercept)	4.56	0.12	5.7	37.2	4.6e-8
ENM	-0.07	0.08	202.2	-0.93	0.35
PC2_temp	-0.09	0.04	724.5	-2.35	0.02
Year (2014)	0.12	0.08	711.5	1.44	0.15
Year (2015)	-0.65	0.08	509.1	-7.69	7.8e-14
PC1_cover	0.02	0.03	632.2	0.67	0.50
Year (2014) * PC1_cov	0.11	0.04	500.3	2.45	0.01
Year (2015) * PC1_cov	0.01	0.04	578.4	0.30	0.77
Year (2014) * ENM	0.04	0.07	834.9	0.52	0.61
Year (2015) * ENM	-0.28	0.07	476.6	-3.78	0.0002

Random effect	Variance (SD)
ID	0.08(0.28)
Site	0.04(0.20)

(a) *T. speciosus*

	Estimate	SE	df	t-value	p-value
(Intercept)	4.04	0.14	172.0	28.3	2.2e-16
Hour	-0.03	0.01	2195.3	-2.06	0.04
Date	0.37	0.09	342.5	4.12	0.00005
Year (2014)	0.34	0.13	866.6	2.65	0.008
Year (2015)	0.09	0.13	690.5	0.68	0.49
Coocc	0.21	0.07	867.1	3.07	0.002
Density	-0.19	0.11	1196.9	-1.83	0.07
PC1_cover	-0.10	0.03	501.8	-3.66	0.0003
#recap	-0.05	0.03	489.4	-1.90	0.06
Rep (Juv)	-0.25	0.07	842.6	-3.61	0.0003
Rep (RepAct)	0.24	0.09	766.1	2.62	0.009
BMI	0.15	0.05	881.1	2.86	0.004
Sex(M)	-0.41	0.13	623.8	-3.21	0.001
Year(2014)*Date	-0.55	0.10	523.3	-5.82	1.0e-8
Year(2015)*Date	-0.61	0.10	331.8	-6.28	1.0e-9
Year(2014)*Coocc	-0.17	0.08	1089.7	-2.21	0.03
Year(2015)*Coocc	-0.24	0.08	737.0	-2.97	0.003
Year(2014)*Density	0.14	0.11	1184.4	1.27	0.20
Year(2015)*Density	0.34	0.11	1278.6	3.03	0.010.003
Year(2014)*PC1_cove	0.10	0.04	229.7	2.42	0.770.02
Year(2015)*PC1_cove	0.19	0.04	271.3	4.70	0.614.2e-6
Rep (Juv)*BMI	-0.17	0.06	951.8	-2.86	0.004

Rep(RepAct)*BMI	-0.12	0.07	884.6	-1.68	0.09
Rep (Juv)*Sex(M)	0.26	0.14	702.8	-1.87	0.06
Rep(RepAct)*Sex(M)	-0.32	0.15	666.0	-2.13	0.03

Random effect	Variance (SD)
ID	0.13(0.36)
Site	0.02(0.15)

Chapter 5 Figures

Figure 1 Map of study sites and their ground cover characteristics. Pie charts represent proportion of ground-cover types for each study site, separated by grid co-occurrence type and colored accordingly (blue outline: >80% *Ta*; purple outline: both species; red outline: >80% *Ts*).

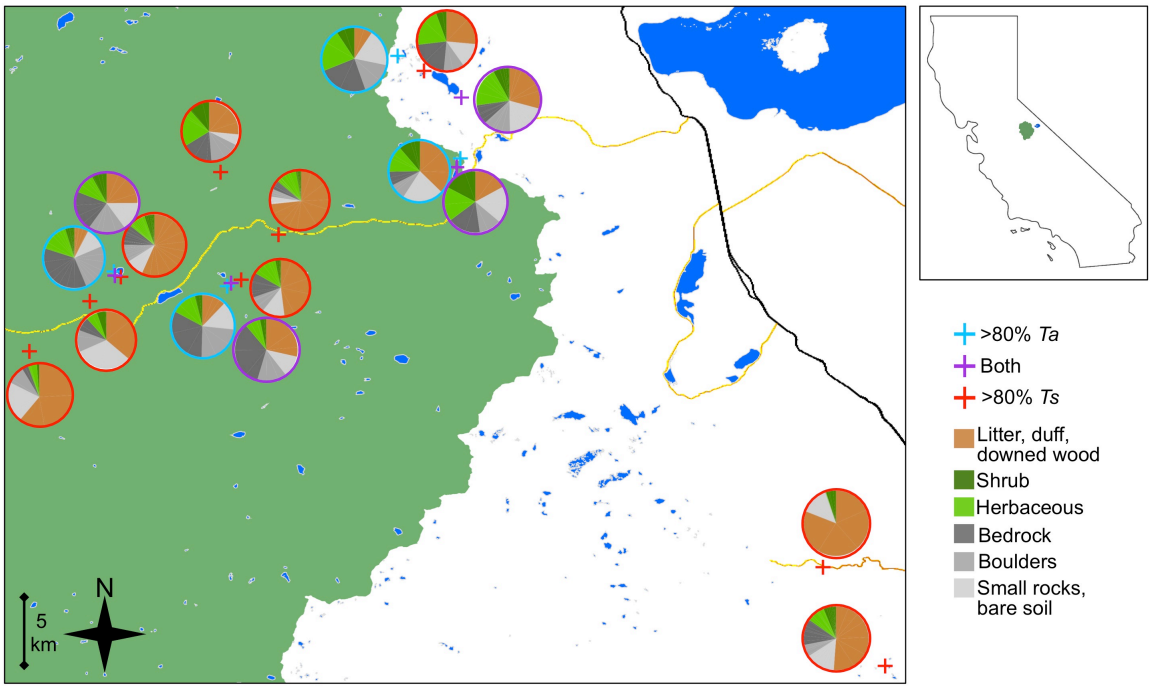


Figure 2 Relationship between co-occurrence score and mean daily daytime temperature. Models demonstrated a strong negative relationship between the proportion of *Ts* in a grid and mean daily temperature during daylight hours, as recorded by iButtons (*Ta*ble 5).

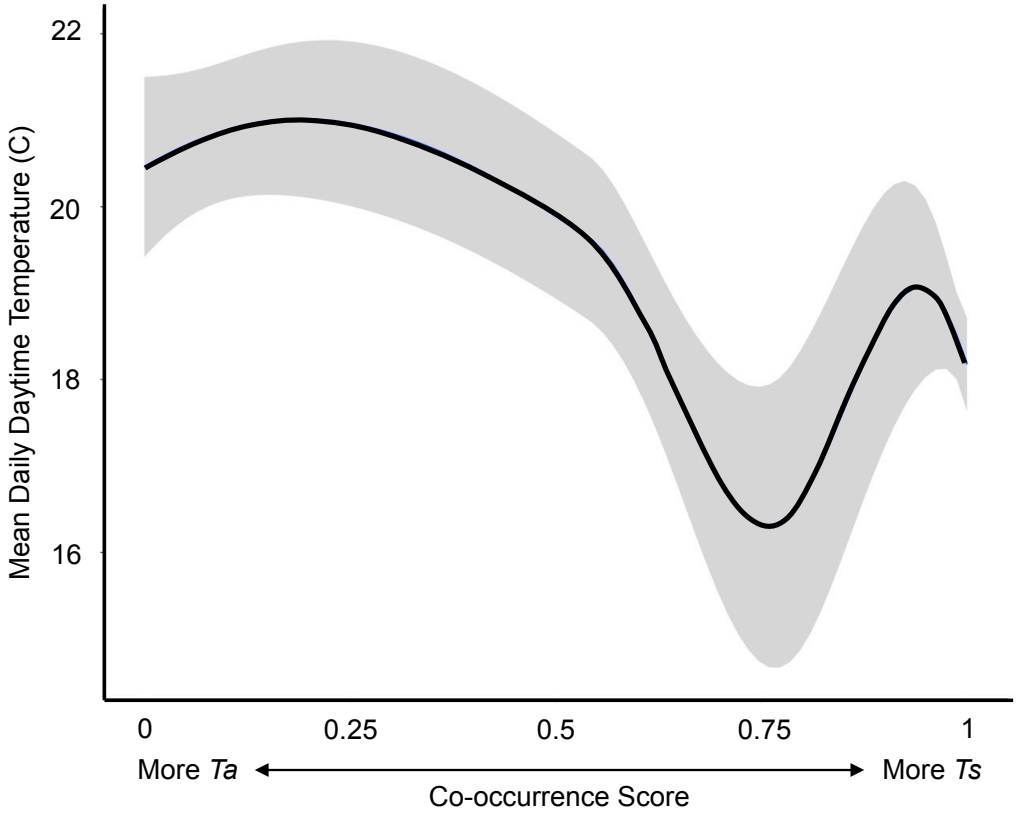


Figure 3 Relationships between ground cover types and co-occurrence scores. Models identified a strong effect of the proportion of *Ts* vs. *Ta* in a grid and ground cover types (*Ta*ble 6). As the percent of *Ts* increased, litter, duff, and downed wood (detrital cover) increased, and rocky cover decreased.

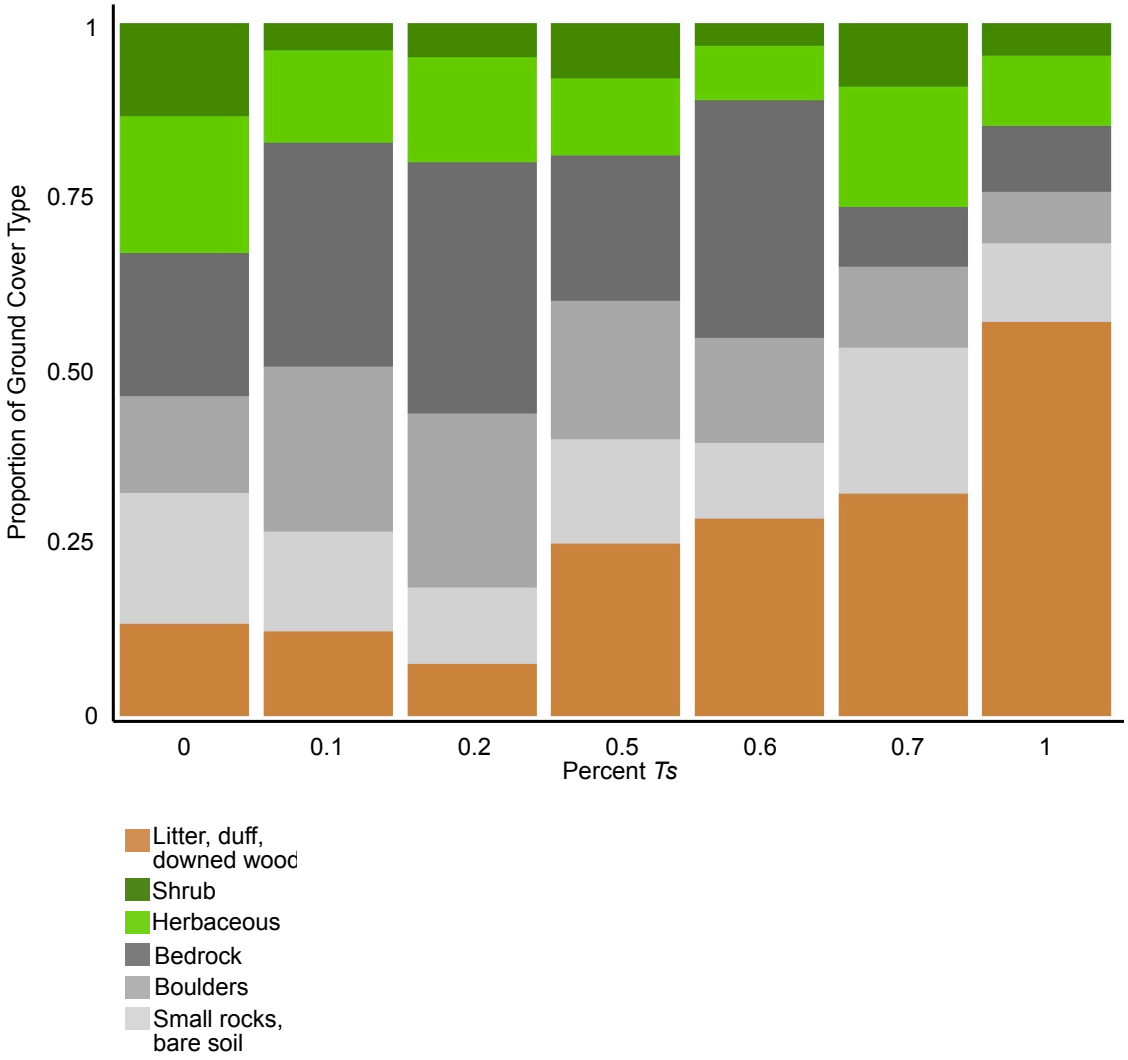


Figure 4 Interannual differences in climatic parameters. (A) Median daily temperature as recorded by weather stations in Yosemite National Park was dependent on year, with 2015 showing significantly higher values than other years (*Ta*ble 7). (B) Snowpack decreased significantly from 2013 to 2014, and from 2014 to 2015 (Wilcoxon rank sum tests with FDR-adjusted p values: 2013 vs. 2014 W=40757, p =0.0000005; 2014 vs. 2015 W=28048, p=0.00000001).

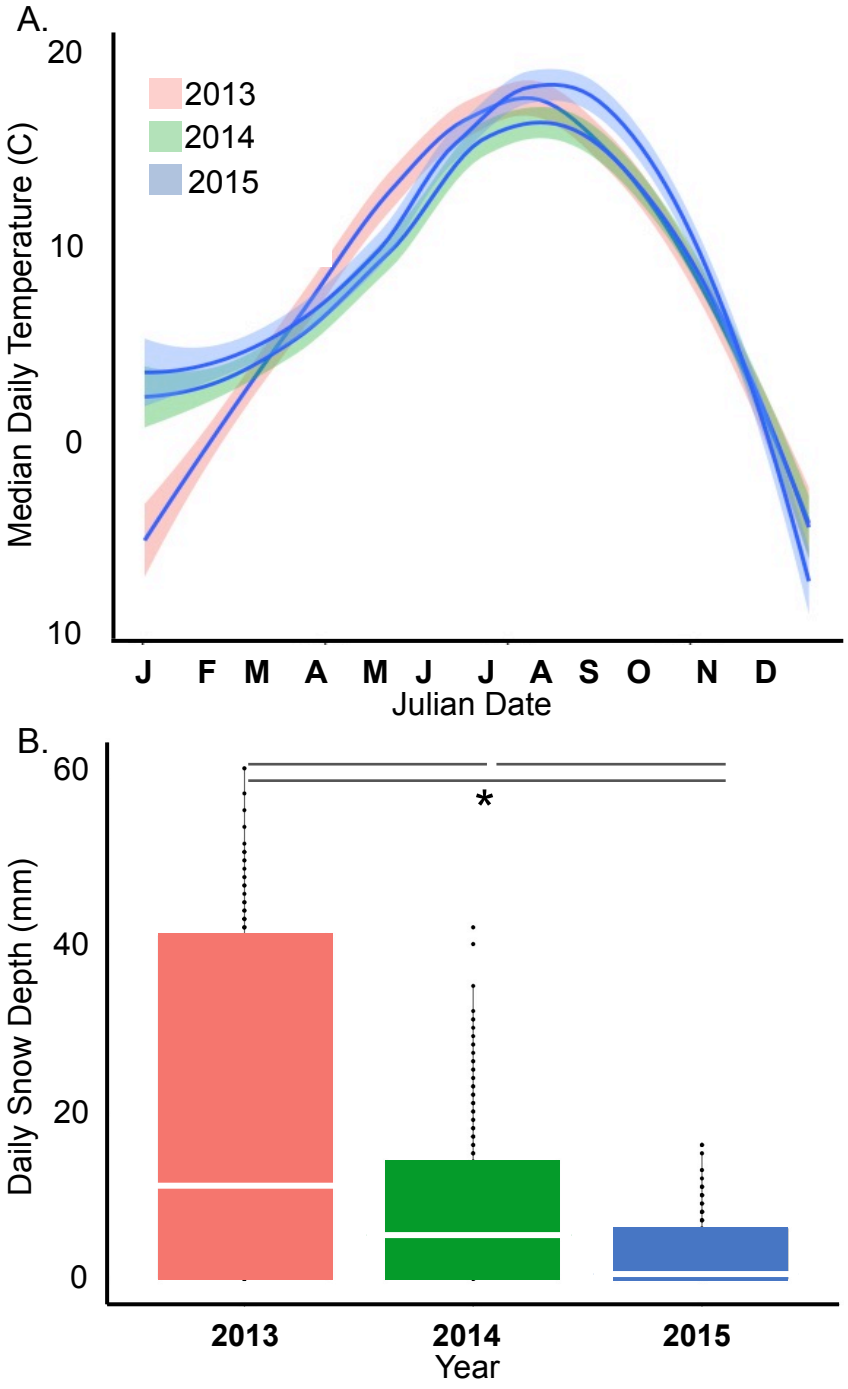


Figure 5 Interannual differences in ground cover. Year was a strong predictor of ground cover (*Ta*ble 6), with 2014 showing more differences from other years, including significantly higher proportions of herbaceous cover (Wilcoxon rank sum tests with FDR-adjusted p-values: 2013 W=38, p = 0.027; 2015 W=248, p = 0.027).

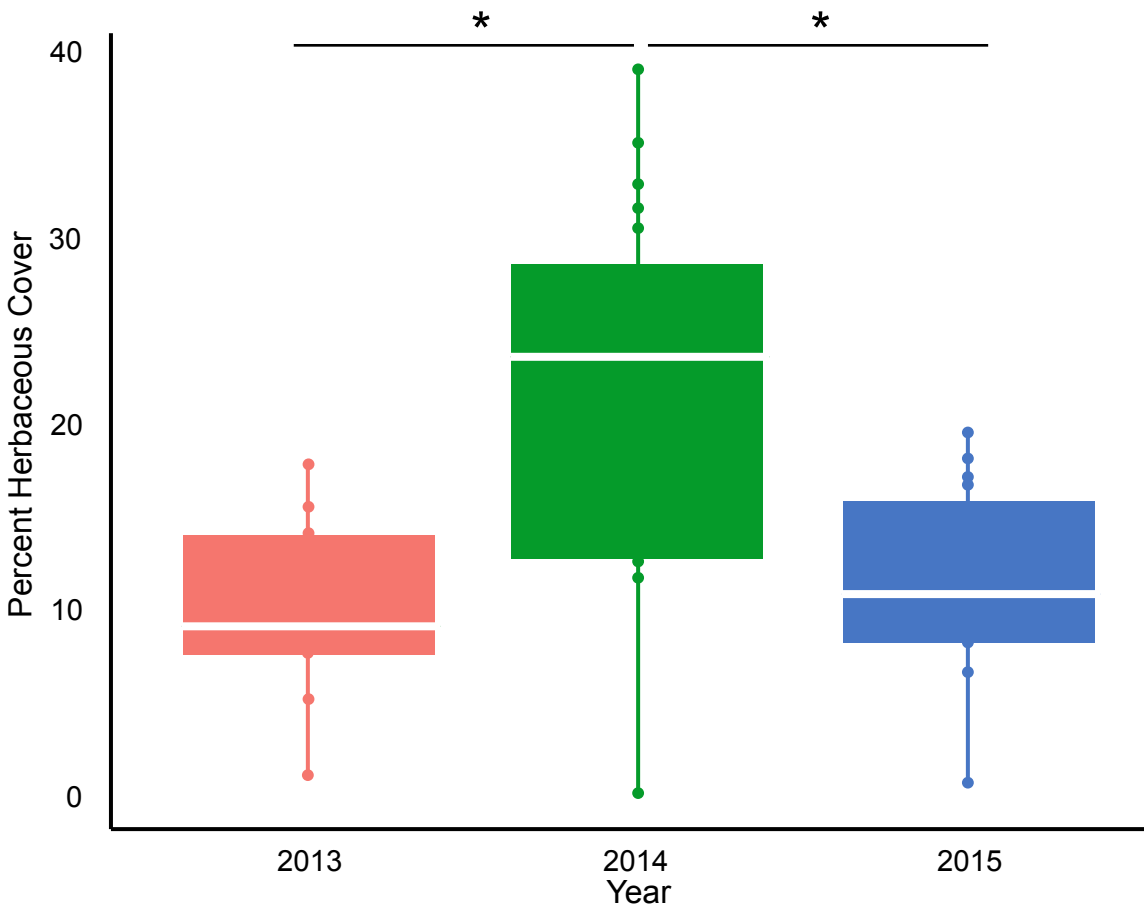


Figure 6 Partial residuals plot for fixed effect of BMI in a model predicting FGMs for *Ts*. GLMMs revealed a positive relationship between BMI and FGMs in *Ts* (*Ta*ble 9).

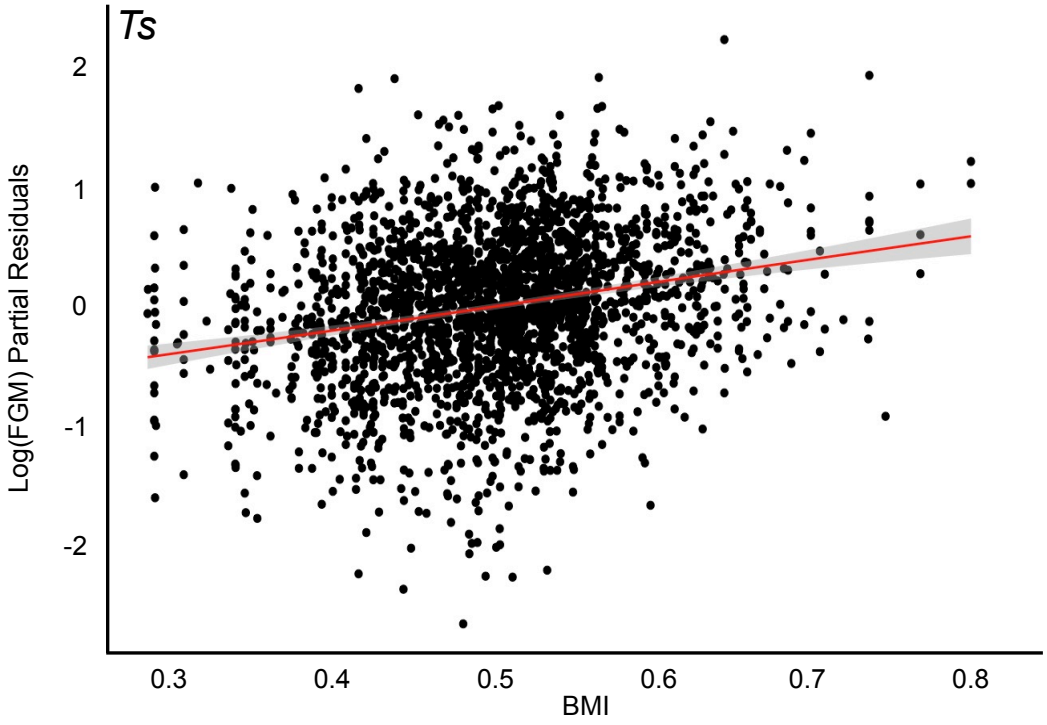


Figure 7 Partial residuals plot for fixed effect of habitat suitability score in a model predicting FGMs for *Ta.* GLMMs revealed a negative relationship between habitat suitability score (defined as the output from a climate-based ecological niche model) and FGMs in *Ta,* such that individuals living in more suitable habitats had lower FGMs (*Ta*ble 9).

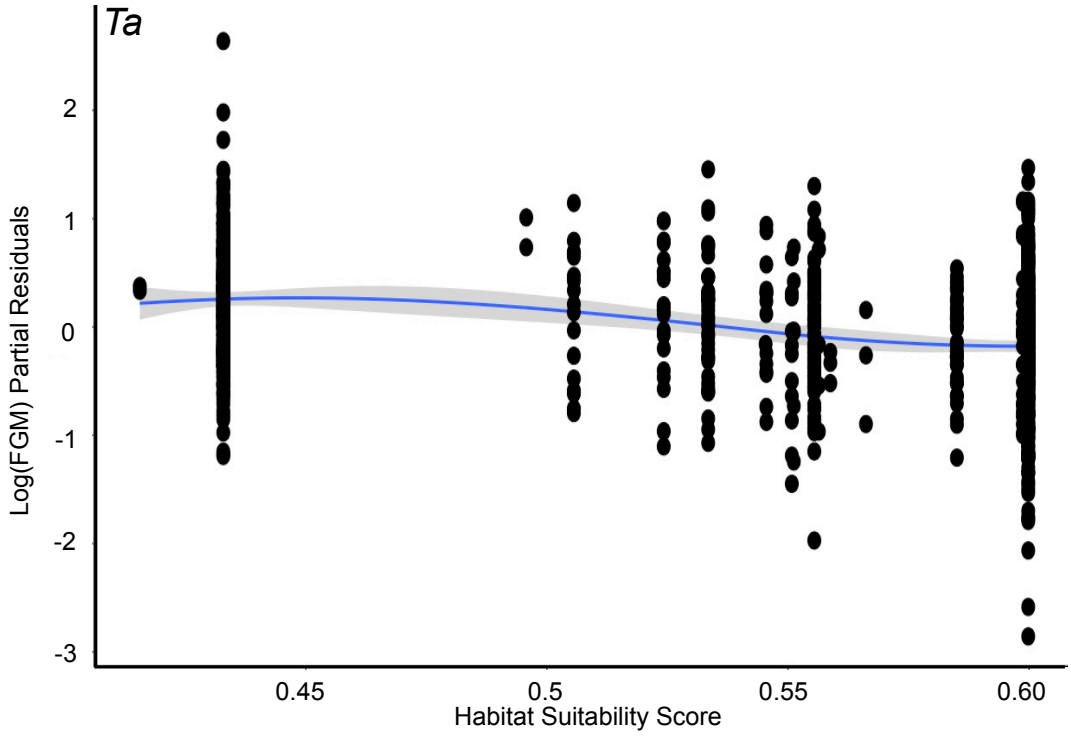


Figure 8 Partial residuals plots for fixed effects of ground cover categories in models predicting FGMs for (A) *Ta* and (B) *Ts.* GLMMs revealed significant relationship between FGMs and PCA axes of ground cover categories for both species (*Ta*ble 9). The loadings of ground-cover variables onto the relevant PCA axis for each species are listed below each axis; ground-cover categories in grey loaded more weakly (<|0.4|) onto the axis. For *Ts,* the inverse of the third PCA axis of ground cover has been presented in order to better align the axis directionality in parts (A) and (B) of this figure.

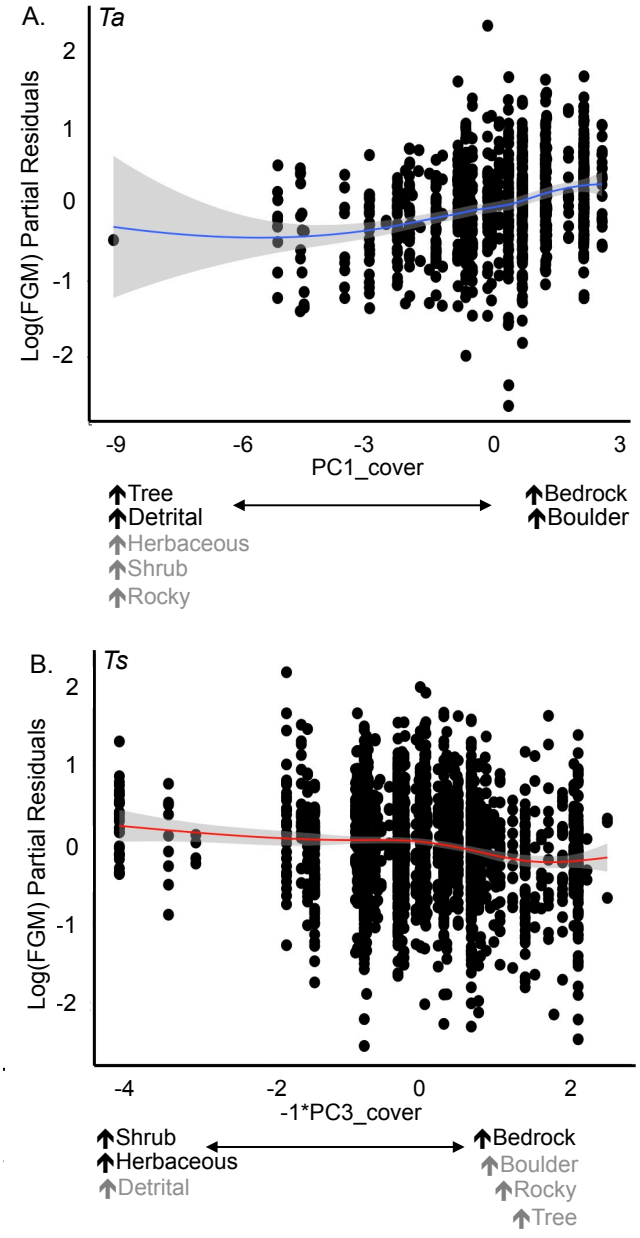


Figure 9 Partial residuals plot for fixed effect of the interaction between density and ground cover categories in a model predicting FGMs for *Ta.* GLMMs revealed a density-dependent effect of ground cover category on FGMs for *Ta* (*Ta*ble 9). Specifically, at high population density scores, the effects of ground cover categories on vegetation became negligible. The loadings of ground-cover variables onto the relevant axis are listed below the x-axis; ground-cover categories in grey loaded more weakly (<|0.4|).

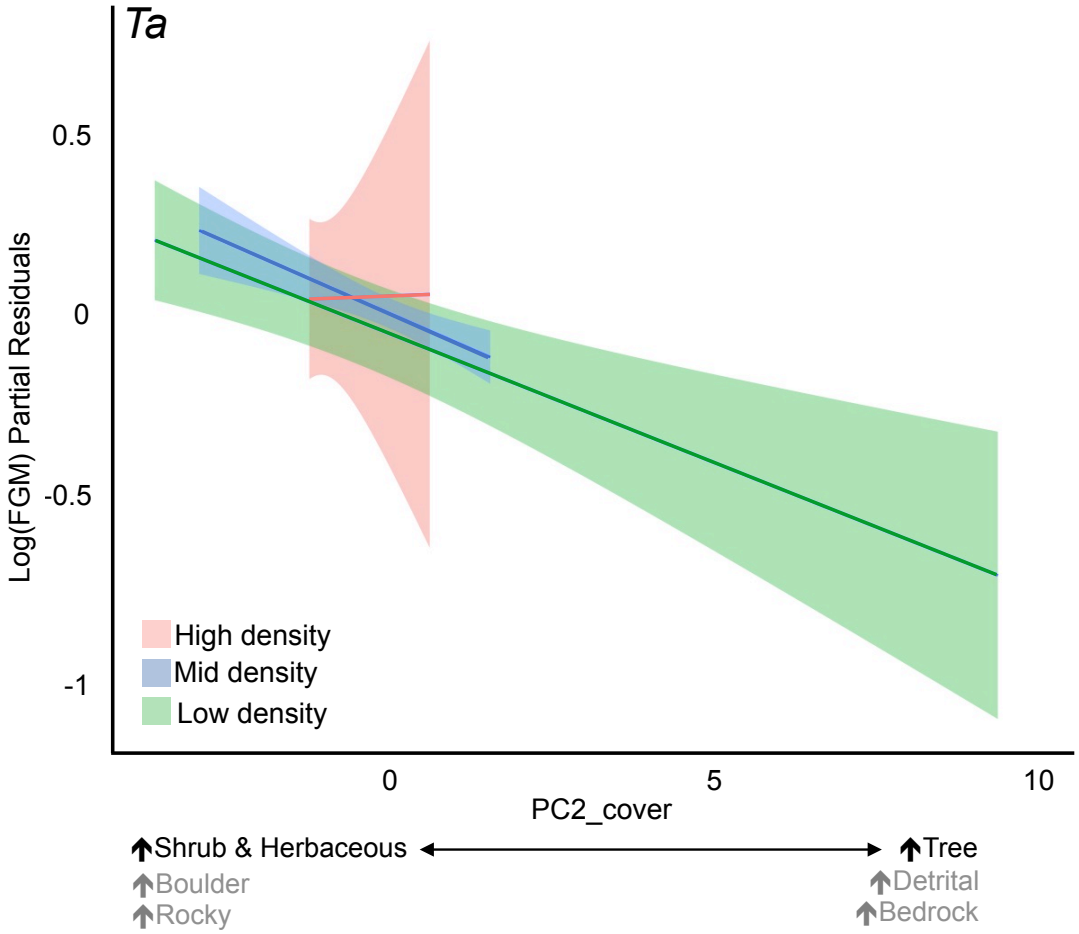
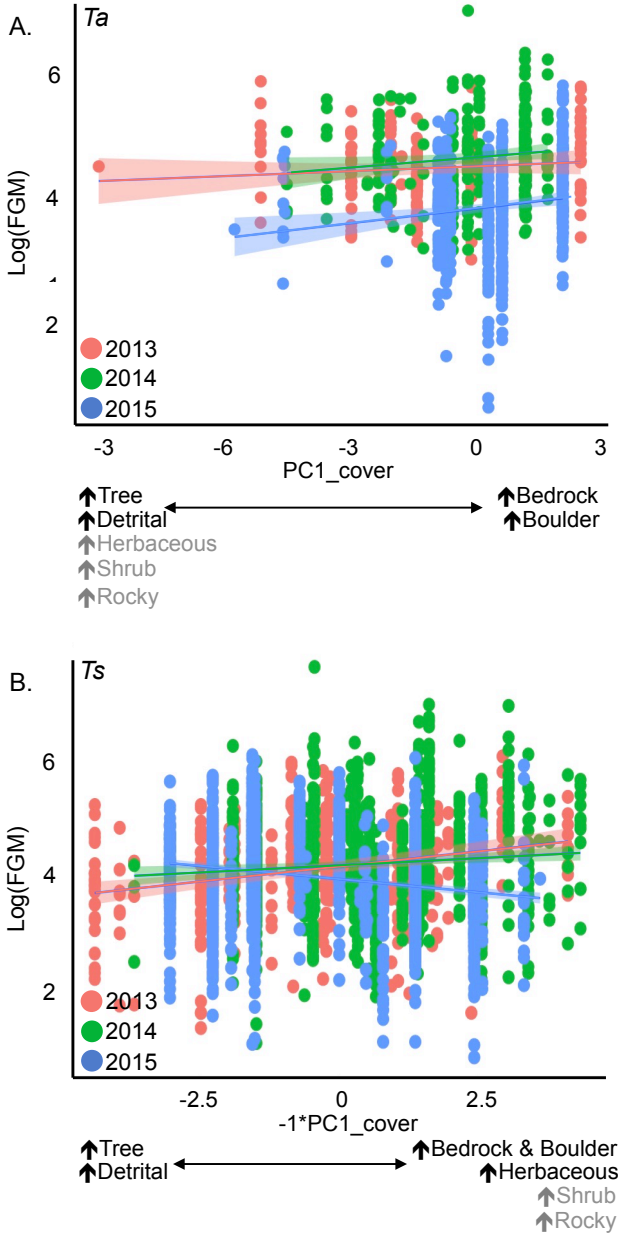


Figure 10 Interannual differences in the relationships between ground cover categories and FGMs for (A) *Ta* and (B) *Ts*. Both species showed significant interannual differences in the relationships between ground-cover categories and FGMs (*Ta*ble 11). The loadings of ground-cover variables onto the relevant PCA axis for each species are listed below each axis; ground-cover categories in grey loaded more weakly (<|0.4|) onto the axis. For *Ts,* the inverse of the third PCA axis of ground cover has been presented in order to better align the axis directionality in parts (A) and (B) of this figure. (A) For *Ta* 2014 showed a slightly stronger relationship between the first PCA axis of ground cover and FGMs than other years. (B) For *Ts* 2015 showed a reversed directionality of the relationship between the first PCA axis of vegetation cover and FGMs in contrast to 2013 and 2014



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