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Glucocorticoid–environment relationships align with responses to environmental change in two co-occurring congeners

Talisin T. Hammond,[1,2,4] Rupert Palme,[3] and Eileen A. Lacey[2]

[1]*Department of Biological Sciences, University of Pittsburgh, 105 Clapp Hall, Pittsburgh, Pennsylvania 15260 USA*
[2]*Museum of Vertebrate Zoology, University of California Berkeley, 3101 Valley Life Sciences Building, Berkeley, California 94720-3160 USA*
[3]*Department of Biomedical Sciences, University of Veterinary Medicine, Veterinärplatz 1, 1210 Vienna, Austria*

Abstract. As more species undergo range shifts in response to climate change, it is increasingly important to understand the factors that determine an organism's realized niche. Physiological limits imposed by abiotic factors constrain the distributions of many species. Because glucocorticoids are essential to the maintenance of physiological homeostasis, identifying glucocorticoid–environment relationships may generate critical insights into both limits on species distributions and potential responses to environmental change. We explored relationships between variability in baseline glucocorticoids and sensitivity to environmental conditions in two chipmunk species characterized by divergent patterns of spatial, genetic, and morphological change over the past century. Specifically, we investigated whether the alpine chipmunk (*Tamias alpinus*), which has undergone pronounced changes, displays greater glucocorticoid sensitivity to environmental parameters than the lodgepole chipmunk (*T. speciosus*), which has exhibited little change over the same interval. From 2013 to 2015, we collected environmental data and fecal glucocorticoid metabolite (FGM) samples from these species. Using generalized linear mixed models and a model averaging approach, we examined the impacts of environmental and individual phenotypic parameters on FGMs. We found pronounced interspecific differences, with environmental parameters being better predictors of FGMs in *T. alpinus.* FGMs in this species were particularly elevated in less climatically suitable habitats and in areas with higher maximum daily temperatures. Individual phenotypic traits were not predictive of FGMs in *T. alpinus*, although they were highly predictive for *T. speciosus.* Collectively, these findings support the hypothesis that *T. alpinus* is more sensitive to environmental change. More generally, our results suggest that both phenotypic attributes and environmental conditions contribute to FGM responses but that the relative contributions of these factors differ among taxa, including among closely related species. Finally, our analyses underscore the value of glucocorticoids as bioindicators of sensitivity to environmental change in species for which the factors affecting stress physiology have been assessed.

Key words: California; chipmunks; climate change; ecological physiology; fecal glucocorticoid metabolites; noninvasive monitoring; stress.

Introduction

Many organisms have exhibited changes in their latitudinal or elevational distributions over the past century, presumably in response to changing climatic conditions (Hickling et al. 2006, Chen et al. 2011). To predict such range shifts, it is important to understand how species are affected by specific environmental parameters and how these parameters interact with intrinsic biological factors to shape the ecological niche. Physiological processes provide critical mechanisms by which organisms can respond to environmental conditions over multiple temporal scales (Wikelski and Cooke 2006, Cooke et al. 2013). On an immediate basis, physiological processes allow individuals to maintain homeostasis in the face of rapid changes in external conditions (Sapolsky et al. 2000). Over longer time periods, physiological traits should evolve to reflect a species' ecological niche (Garland and Carter 1994). Departures from typical ambient conditions are expected to produce measurable physiological responses, suggesting that analyses of physiological parameters can be used to assess the short-term impacts of environmental changes on free-living organisms (Pacifici et al. 2015, Seebacher et al. 2015, Aubin et al. 2016, Beever et al. 2016). A better understanding of interactions between physiology and environmental conditions may also elucidate why, when faced with similar environmental changes, even closely related species sometimes respond in different and unpredictable ways.

Glucocorticoid hormones (GCs) are particularly appropriate indicators of short-term physiological responses to variable environmental conditions due to their critical role in allostasis (Sapolsky et al. 2000, Wikelski and Cooke 2006, Busch and Hayward 2009). Chronically elevated GCs are usually considered maladaptive (Bonier et al. 2009), although support for this hypothesis is mixed (e.g., Thierry et al. 2013, Burtka et al. 2016, Hansen et al. 2016, Madliger and Love 2016). In general, baseline GCs are thought to reflect the social, environmental, and energetic challenges to homeostasis faced by individuals (Sapolsky et al. 2000). As averaged measures of circulating GCs over many hours to days, metabolites of GCs excreted in feces provide an appropriate, noninvasive method of characterizing an animal's baseline levels of GC production (Sheriff et al. 2011, Dantzer et al. 2014). The use of baseline GC levels, including measures of fecal GC metabolites, as proxies for response to environmental conditions requires knowledge of the factors

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[4] E-mail: talisintess@gmail.com

that affect the GC physiology of the species in question (Dantzer et al. 2014).

To explore the utility of baseline GC levels as indicators of sensitivity to environmental change, we identified predictors of fecal glucocorticoid metabolite (FGM) levels in two species of chipmunks that occur in the Sierra Nevada Mountains of California, USA. Although the alpine chipmunk (*Tamias alpinus*) and the lodgepole chipmunk (*T. speciosus*) are partially sympatric in this region, a growing body of literature indicates that they have exhibited markedly different patterns of spatial, genetic, dietary, and morphological change over the past century (Moritz et al. 2008, Rubidge et al. 2011, Walsh et al. 2016). In all cases, *T. alpinus* has shown greater change, suggesting that this species may be more sensitive to environmental conditions than *T. speciosus.* Physiological tolerances of these species are generally similar and biotic interactions, specifically competitive exclusion by *T. alpinus*, have been proposed to limit the distribution of *T. speciosus* (Heller and Poulson 1970, 1972, Heller 1971, Heller and Gates 1971, Chappell 1978). In contrast, ecological niche models (ENMs) indicate that environmental factors such as climate and vegetation are better predictors of *T. alpinus*'s distribution (Rubidge et al. 2011). In captivity, *T. alpinus* is more GC sensitive to experimentally induced external challenges (Hammond et al. 2015). Collectively, these findings suggest that, despite their similarities, physiological responses of these species to external variables may differ in ways that inform their relative vulnerability to environmental change. Identifying the environmental factors that define physiologically suitable habitats for *T. alpinus* is especially important, as alpine species are thought to be particularly vulnerable to environmental change (Dirnböck et al. 2011, Mountain Research Initiative EDW Working Group 2015).

To explore interspecific differences in GC sensitivity, we quantified relationships between FGMs and a variety of environmental parameters experienced by *T. alpinus* and *T. speciosus* in the Yosemite region of the central Sierra Nevada Mountains (Fig. 1). Specifically, we tested the hypothesis that *T. alpinus* is more physiologically sensitive to environmental conditions, resulting in more pronounced relationships between FGM levels and environmental variables in this species. In contrast, if competitive exclusion plays a greater role in determining the distribution of *T. speciosus* (Heller 1971, Chappell 1978), we predicted that FGMs for this species should be more impacted in areas where the two study taxa co-occur. While this study focuses on only a single pair of species, it represents a critical step toward understanding the role of stress physiology as an indicator of vulnerability to environmental change. More generally, our analyses generate new insights into the relative impacts of environmental and intrinsic (individual phenotypic) factors on important bioindicators such as GC hormones.

Methods

Study species and sites

The alpine chipmunk *(Tamias alpinus)* is a small-bodied (30–50 g) species that occurs primarily at and above treeline (~3,000 m above sea level; Clawson et al. 1994). In contrast, the lodgepole chipmunk (*T. speciosus*) is larger bodied (50–80 g) and occurs primarily at and below treeline (~1,900–3,200 m above sea level; Best et al. 1994, Moritz et al. 2008). Between June and October of 2013–2015, we sampled populations of these species at 7–10 sites per year (Fig. 1, Appendix S1). Animals were captured using Sherman traps (HB Sherman Traps, Tallahassee, Florida, USA) baited with peanut butter and oats. Traps were opened from dawn to dusk and were checked every 4–6 h. Traps were set out in pairs (stations), which were arranged in grids of ~25–75 stations, with two to five grids per site. Whenever possible, grids were established to include areas that were inhabited by *T. alpinus* only, by *T. speciosus* only, or by both species. GPS localities were recorded for each station.

Upon first capture, each animal was uniquely marked using two numbered metal ear tags (Monel 100s; National Band and Tag Company, Newport, Kentucky, USA). Body mass, body length, and reproductive status were recorded. 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 2016).

FGM analyses

Fecal pellets were collected from the trap in which each animal was captured and were frozen in liquid nitrogen for up to one month before being transported to the UC Berkeley campus, where they were stored at −80°C. FGMs were extracted and assayed as described in Hammond et al. (2015). Briefly, samples were dried in an oven, crushed into a powder, extracted in methanol, and then dried in a vacuum centrifuge. After drying, samples were reconstituted in assay buffer. FGMs were quantified using a previously validated a 5α-pregnane-3β,11β,21-triol-20-one enzyme immunoassay (Touma et al. 2003, Hammond et al. 2015). Values for FGMs were log-transformed prior to analyses to meet assumptions of normality underlying our statistical models.

Quantifying environmental parameters

Ambient temperatures at each trapping grid were recorded using Thermochron iButton temperature loggers (model DS1921G; Dallas Semiconductor, Dallas, Texas, USA). Loggers were deployed within 1 m of the substrate at approximately 75% of the trap stations on each grid. Loggers were programmed to collect hourly readings. From these data, daily summary statistics for temperature were calculated for use in statistical models (see *Statistical analyses*). Data were also obtained from two nearby weather stations for use in analyses of daily and annual climatic variation (Tuolumne Meadows Ranger Station and Yosemite National Park Headquarters; data *available online*).[5]

To estimate habitat suitability, ecological niche models were constructed for each species. High-resolution data layers for nineteen bioclimatic variables were obtained from WorldClim (Hijmans et al. 2005). Data layers were clipped

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[5] https://www.ncdc.noaa.gov/

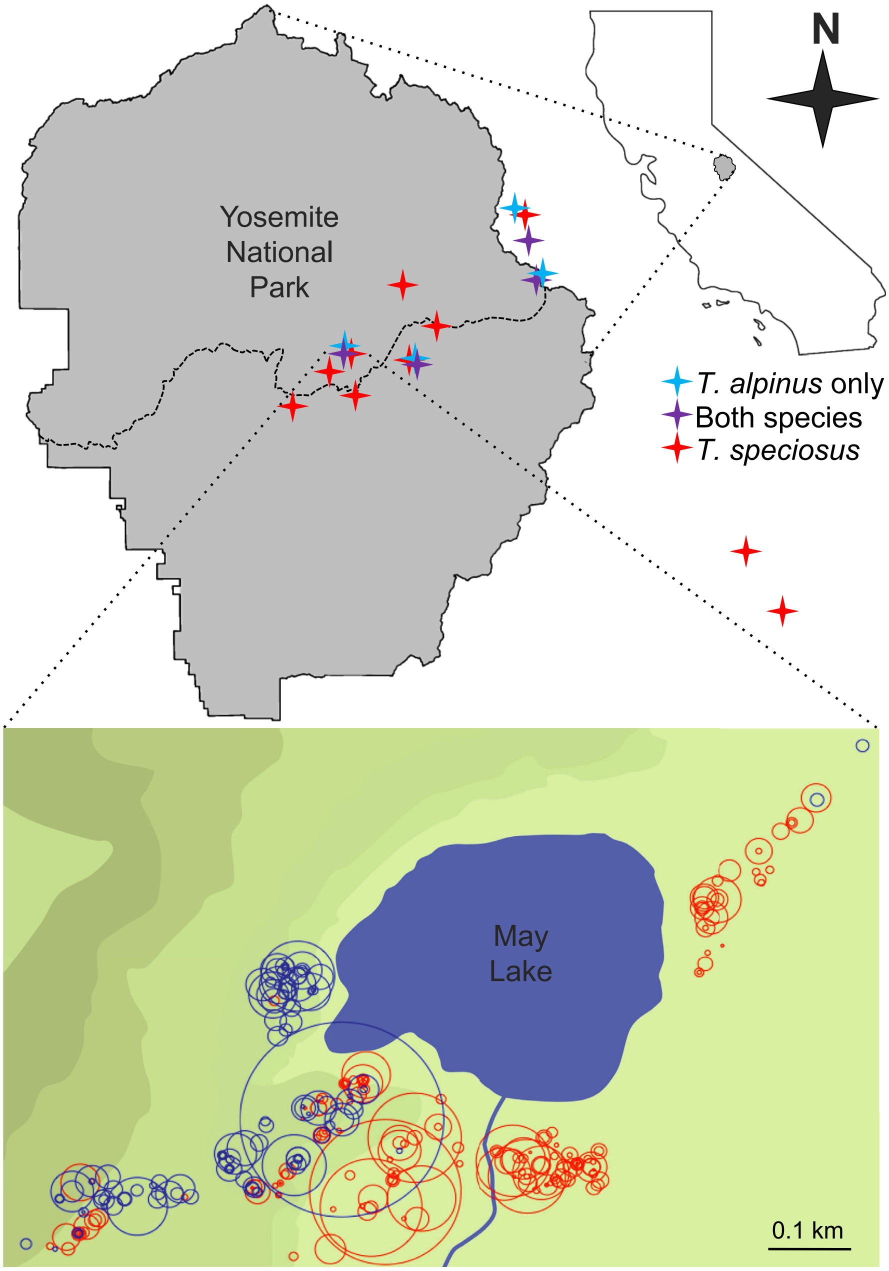

Fig. 1. Study sites and an example distribution of individuals and their fecal glucocorticoid metabolite (FGM) levels. Data were collected from multiple localities in and around Yosemite National Park, California, USA (gray). For each sampling locality, the color of the marker indicates the relative abundance of the study species (blue, *Tamias alpinus*; red, *T. speciosus*; purple, both species). Inset shows one example site, May Lake, at which each point is colored according to species and indicates an individual's first capture location, with the diameter of the surrounding circle proportional to that individual's mean FGM level.

to the geographic range of each species. Occurrence data for each species from 1980 to present (*T. alpinus* = 190 occurrences, *T. speciosus* = 793 occurrences) were obtained from the Arctos database (data *available online*).[6] Ecological niche models were generated using MaxEnt version 3.3.k (Phillips and Dudík 2008). For each species, 20 replicates were completed with cross-validation, random seed, and a maximum of 10,000 iterations; default settings were used for all other parameters. Output values from these models represented the estimated probability of occurrence of *T. alpinus* or *T. speciosus* at a given location. These values were extracted for the GPS coordinates for each trap station and used as a metric of habitat suitability in subsequent analyses (see *Statistical analyses*; Crespi et al. 2015).

Estimates of population parameters

We used mark–recapture data to generate estimates of chipmunk density at each trapping grid. Relative population density was quantified using the Schnabel Index, an

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[6] http://arctos.database.museum/

extension of the Lincoln–Peterson method that is appropriate when populations have been sampled on more than two occasions (Schnabel 1938, Napolitano et al. 2008). This 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 number of marked individuals in the population at time $t-1$, and R_t is the number of previously marked individuals captured at t. This value was calculated for each trapping grid and then divided by that grid's area to generate an estimate of relative population density; separate estimates of density were calculated for each year of the study. Grid area was calculated by establishing a circular (~20 m radius) buffer around each trapping station and calculating the total area of all buffers in the grid; overlapping buffers were merged to produce a single estimate of area for the associated trapping stations. Estimates of density were included as fixed effects in statistical models (*Statistical analyses*). A species co-occurrence score, defined as the proportion of animals captured that were conspecifics, was also calculated for each species on each trapping grid. These values were also included in models.

Statistical analyses

Predictors of FGMs.—To identify predictors of variation in FGM levels, generalized linear mixed models (GLMMs) were implemented using the lmer function in the lme4 package in R (Bates et al. 2014, R Core Team 2017). To allow for comparisons of effect sizes, all numeric variables were mean-rescaled prior to analysis. Models were validated graphically, as in Zuur et al. (2009), to ensure that they met all assumptions for GLMM analyses.

The initial, full model contained nine fixed effects (Table 1). To explore annual variation in predictors of FGMs, additional GLMMs were constructed that contained the above variables along with terms for interactions between year and each of the following variables: density, climatic habitat suitability score, and temperature. We used a model averaging approach to account for uncertainty and model selection bias (Thiele and Markussen 2012, Gruner et al. 2017, McDermott Long et al. 2017). A set of all possible models (containing all combinations of these variables) was constructed using the dredge function of the MuMIn package in R (Bartón 2015). All models with an AIC$_c$ value (the change in the Akaike information criterion adjusted for sample size) that differed by <4 from the top ranked model were included in model averaging, which was conducted using the model.avg function. Significance of random effects was assessed using a likelihood ratio test. Our presentation of the outcome of these analyses and the associated discussion focus on variables in the final, model-averaged model, particularly those that significantly improved the predictive power of the model.

Interannual differences in environment.—To determine whether FGM–environment relationships were affected by interannual differences in environmental conditions, additional GLMMs were constructed to predict annual median daily temperature and daily snow depth during the winter (October–May) preceding each field season. These variables were selected based on data availability. These models included date and year as fixed effects and weather station identity as a random effect.

Table 1. Description of fixed and random effect variables included in GLMMs.

Variable type and name	Description	Type
Temporal		
Date	day of year	integer
Year	year (2013, 2014, or 2015)	factor
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
Max Temp	maximum temperature on day of capture	continuous
ENM	output from climate-based ecological niche model for individual's capture location	continuous ($0 > x < 1$)
Intrinsic		
Sex	male or female	factor
Rep	reproductive status (adult, reproductively active adult, or juvenile)	factor
Mass	each individual's mass	continuous
Random effects		
ID	individual animal ID	factor
Site	study site of each individual	factor

Results

Interspecific comparison of FGMs

We collected 3,644 fecal samples that were used to generate FGM measurements for 270 individual *T. alpinus* and 1,028 individual *T. speciosus* (Appendix S1: Table S1). Temporally, while FGM levels for both species tended to decrease over the course of the field season, date was included as a predictor only in final models for *T. speciosus* (Table 2). For both species, year was an important predictor of FGM levels, with samples from 2015 exhibiting lower FGM levels than those from 2013 and 2014 (Table 2).

Intrinsic parameters and FGMs

Intrinsic biological parameters (sex, reproductive status, body mass) were included as predictors in final models of FGM levels for *T. speciosus*, but not for *T. alpinus* (Table 2). For example, models for *T. speciosus* suggested that males had lower FGMs than females and that juveniles had lower FGMs than either reproductive or nonreproductive adults; these relationships were not evident for *T. alpinus* (Table 2). Similarly, while FGMs increased with body mass in *T. speciosus*, this was not the case in *T. alpinus* (Table 2).

TABLE 2. Results of model-averaged GLMMs to predict FGM levels for (a) *Tamias alpinus* and (b) *T. speciosus.*

Parameter	Estimate	Adjusted SE	*z*	*P*	Var	SD
a) *T. alpinus*						
(Intercept)	4.57	0.10	47.0	**<2e-16**		
Year (2014)	0.03	0.09	0.38	0.71		
Year (2015)	−0.67	0.84	8.05	**<2e-16**		
Max Temp	0.11	0.03	3.51	0.0005		
ENM	−0.13	0.06	2.21	**0.03**		
Co-occurrence	0.07	0.03	2.06	**0.04**		
Random effects						
Site				0.77	0.02	0.15
ID				**2.7×10^{-11}**	**0.08**	**0.29**
b) *T. speciosus*						
(Intercept)	4.25	0.11	37.54	**$<2 \times 10^{-16}$**		
Year (2014)	0.13	0.07	1.88	0.06		
Year (2015)	−0.16	0.07	2.18	**0.03**		
Date	−0.30	0.03	10.57	**$<2 \times 10^{-16}$**		
Mass	0.09	0.02	4.17	**3.3×10^{-5}**		
Sex (M)	−0.33	0.04	7.62	**$<2 \times 10^{-16}$**		
Rep (Juv)	−0.19	0.05	3.54	**0.0004**		
Rep (RepAct)	−0.01	0.06	0.20	0.85		
Max Temp	−0.05	0.02	2.19	**0.03**		
Co-occurrence	0.05	0.02	2.03	**0.04**		
Random effects						
Site				**2.2×10^{-8}**	**0.10**	**0.32**
ID				**2.2×10^{-16}**	**0.15**	**0.39**

Notes: Variables listed are those included in the final, model-averaged model for each species.

RepAct, reproductively active adult. Juv, Juvenile; M, male; Var, variance.

In (a), relative importance for Year = 1; Max Temp = 1; ENM = 0.37; and Coocc = 0.18. In (b), relative importance for Year = 1; Date = 1; Mass = 0.85; Sex = 1; Rep = 0.15; Max Temp = 0.12; and Coocc = 0.11. Boldface text indicates that the term was significant in the model ($P \le 0.05$).

Environmental parameters and FGMs

Environmental factors were more prominent in final models of FGMs for *T. alpinus* compared to *T. speciosus*. For example, in *T. alpinus,* FGMs were found to increase in less climatically suitable habitat (quantified using ecological niche models); this variable was not included in final models for *T. speciosus*. While FGMs in *T. alpinus* were higher in areas with higher daily maximum temperatures (Fig. 2A; Table 2), the converse was observed for *T. speciosus* (Fig. 2B; Table 2). In both species, FGMs were higher in areas with less interspecific overlap (Table 2). For both daily maximum temperature and interspecific overlap, the relative importance of these variables as predictors of FGMS was greater for *T. alpinus* (Table 2).

Interannual variation in FGMs and habitats

We found significant interactions between year and habitat suitability for both species and a significant interaction between year and population density for *T. speciosus*, indicating potentially important interannual differences in the parameters that were most predictive of FGMs (Table 3). Our analyses of data collected from weather stations near the study sites suggested that 2015 was the most climatically extreme year, being significantly warmer and drier than 2013 and 2014 (Fig. 3; Table 4). For *T. alpinus*, the negative relationship between habitat suitability and FGMs was strongly driven by data from this year, with data from 2013 and 2014 showing little relationship between these variables (Fig. 4A; Table 3). For *T. speciosus*, however, FGMs in 2015 were higher in areas scored as more climatically suitable; the opposite relationship was detected for data from 2013 and 2014 (Fig. 4B; Table 3). For both species, interactions between year and climatic habitat suitability scores had high relative importance in the final model-averaged model (Table 3). The relationship between population density and FGMs also differed across years for *T. speciosus.* Although no relationship with density was identified in 2013, stronger negative and positive relationships were identified in 2014 and 2015, respectively (Table 3).

DISCUSSION

Our analyses revealed that across multiple localities, these two co-occurring species of chipmunks differed markedly with regard to their physiological sensitivity to environmental conditions. Specifically, while FGM levels in *T. alpinus* were best predicted by environmental factors, those in *T. speciosus* were more closely associated with intrinsic factors such as sex, reproductive status, and body mass. Interannual differences in FGMs were better aligned with climatic variation in *T. alpinus* than in *T. speciosus*, providing further evidence that the former species may be more sensitive even to relatively short-term changes in environmental conditions. We posit that these interspecific differences in patterns of GC sensitivity are associated with the pronounced differences in elevational, morphological, and

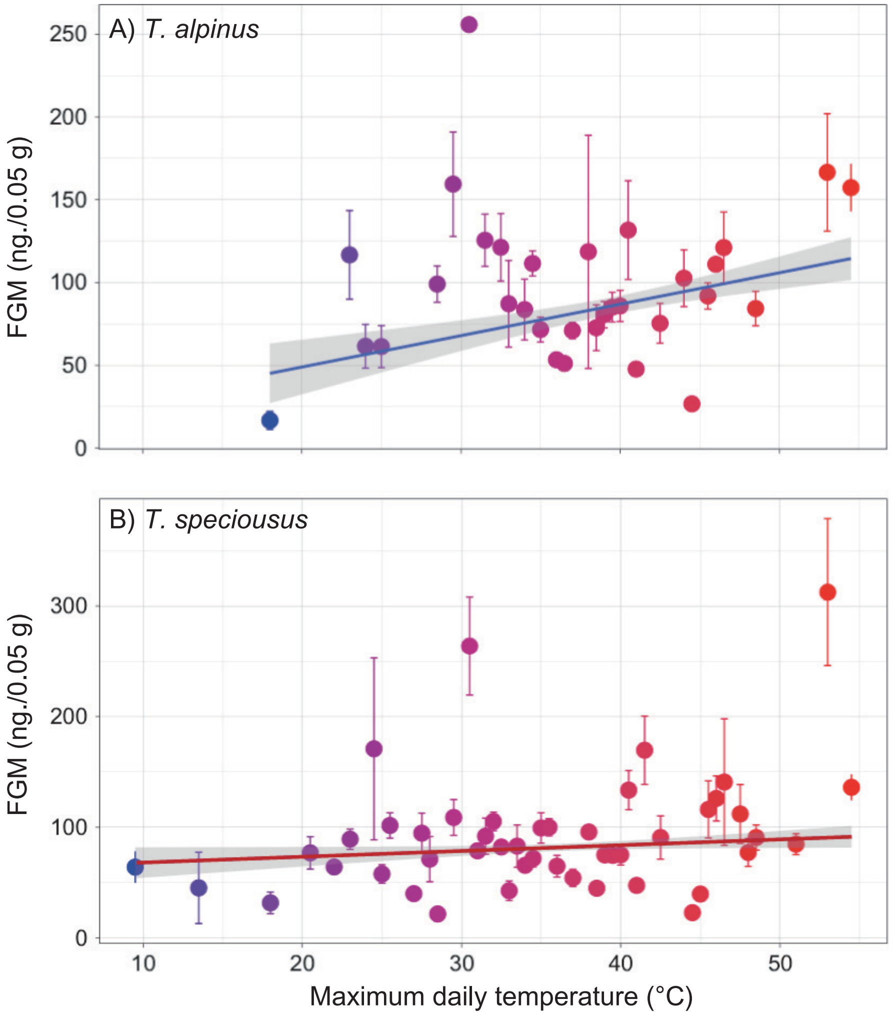

Fig. 2. Relationships between maximum daily temperature and FGMs. (A) In *T. alpinus,* FGMs were higher in areas with higher maximum daily temperatures. (B) In *T. speciosus*, this relationship was less strong (see Table 2 and *Statistical analyses*). Each point represents the mean ± SE of FGM levels across all individuals captured on days of a given maximum daily temperature. Points are colored on a scale from cooler (blue) to warmer (red) temperatures.

genetic change displayed by *T. alpinus* and *T. speciosus* over the past century. While the generalizability of our findings may be limited due to our focus on a single, congeneric pair of species, these data are nevertheless an important step toward characterizing the relationship between GC sensitivity to external conditions and vulnerability to environmental change. Our results underscore the value of documenting GC physiology in the context of environmental change as well as characterizing the impacts of both intrinsic biological attributes and environmental factors on GCs on a species-specific basis.

Intrinsic predictors of FGMs

Intrinsic biological factors were more important predictors of FGM levels in *T. speciosus* than in *T. alpinus*. Our finding that reproductively active female *T. speciosus* tended to have higher FGMs than other categories of conspecifics was not surprising given that our sampling took place primarily during lactation, which is a particularly energetically demanding life-history stage for females (Wade and Schneider 1992, Kenagy and Place 2000, Dantzer et al. 2010). More unexpected was the minimal importance of intrinsic predictors of FGM levels in *T. alpinus*; this outcome differs markedly from a large body of research on small mammals demonstrating the significance of such variables to GC physiology (e.g., Kenagy and Place 2000, Williams et al. 2008, Dantzer et al. 2010). While some intrinsic factors (e.g., reproductive status) were associated with patterns of FGM variability in *T. alpinus*, these variables were never retained in final models for this species. It is possible that the relatively greater importance of environmental variables in *T. alpinus* masked effects of intrinsic parameters on GC levels in this species but not in *T. speciosus.* Previous work has revealed that *T. alpinus* is more FGM responsive to a variety of experimental challenges (Hammond et al. 2015), underscoring the effects of external factors on the GC physiology of this species. Collectively, these data suggest that *T. alpinus* is more impacted by external conditions.

Table 3. Results of model-averaged GLMMs that included by-year interactions as predictors of FGM levels in (a) *T. alpinus* and (b) *T. speciosus.*

Parameter	Estimate	Adjusted SE	*z*	*P*	Var	SD
a) *T. alpinus*						
(Intercept)	4.53	0.12	36.3	**$<2 \times 10^{-16}$**		
Year (2014)	0.10	0.10	0.98	0.33		
Year (2015)	−0.66	0.10	6.27	**$<2 \times 10^{-16}$**		
ENM	−0.08	0.10	0.84	0.40		
Density	0.10	0.11	0.91	0.36		
Co-occurrence	0.09	0.03	2.69	**0.007**		
Year (2014) × ENM	0.13	0.10	1.33	0.18		
Year (2015) × ENM	−0.23	0.09	2.65	**0.008**		
Year (2014) × Density	0.09	0.13	0.67	0.51		
Year (2015) × Density	−0.23	0.12	1.87	0.06		
Random effects						
Site				0.07	0.05	0.22
ID				**3.5×10^{-10}**	**0.07**	**0.27**
b) *T. speciosus*						
(Intercept)	4.52	0.15	29.2	**$<2 \times 10^{-16}$**		
Year (2014)	−0.04	0.11	0.35	0.73		
Year (2015)	−0.37	0.11	3.27	**0.001**		
Date	−0.27	0.03	9.02	**$<2 \times 10^{-16}$**		
Mass	0.09	0.02	4.11	**3.9×10^{-5}**		
Sex (M)	−0.34	0.04	7.92	**$<2 \times 10^{-16}$**		
Rep (Juv)	−0.22	0.05	4.00	**6.4×10^{-5}**		
Rep (RepAct)	−0.05	0.06	0.89	0.37		
ENM	−0.19	0.08	2.53	**0.01**		
Density	0.23	0.09	2.54	**0.01**		
Co-occurrence	0.05	0.03	1.84	0.07		
Year (2014) × ENM	0.28	0.08	3.54	**0.0004**		
Year (2015) × ENM	0.33	0.08	4.32	**1.6×10^{-5}**		
Year (2014) × Density	−0.29	0.10	3.02	**0.002**		
Year (2015) × Density	−0.09	0.10	0.97	0.33		
Random effects						
Site				**5.7×10^{-5}**	**0.08**	**0.29**
ID				2.2×10^{-16}	0.14	0.37

Notes: Variables listed are those included in the final, model-averaged model for each species.

In (a), relative importance for Year = 1; ENM = 1; Density = 0.53; and Coocc = 0.42. In (b), relative importance for Year = 1; Date = 1; Mass = 0.75; Sex = 1; Rep = 0.25; ENM = 0.86; Density = 1.0; Coocc = 0.07; Year × ENM = 0.86; and Year × Density = 1. Boldface text indicates that the term was significant in the model ($P \leq 0.05$).

Environmental predictors of FGMs

Multiple environmental parameters influenced FGM levels in both study species, although the directionality, relative importance, and temporal consistency of these relationships varied markedly. For example, while FGMs were higher for *T. alpinus* living in less climatically suitable habitats, particularly during the more climatically extreme 2015 field season, *T. speciosus* displayed lower FGMs in habitats that were less climatically suitable, even during the most climatically extreme year of our study. It is possible that although *T. speciosus* is living well within its fundamental niche and is not currently physiologically challenged by the range of environmental conditions to which it is exposed, *T. alpinus* is more frequently subject to conditions that approach its physiological limits. Temperature has been identified as a potentially important determinant of the spatial distribution of *T. alpinus* (Rubidge et al. 2011). Potentially consistent with this, we found that individual *T. alpinus* living in areas with higher maximum summer temperatures tended to exhibit increased FGMs. The stronger relationships between temperature and FGMs in *T. alpinus* suggest that this species is more affected by thermal conditions, particularly elevated temperatures. If this interpretation is correct, then predicted future increases in temperature may have more drastic consequences for *T. alpinus.*

Previous studies have suggested that interspecific competitive interactions may be more important than climatic factors in limiting the distribution of *T. speciosus* (Heller 1971, but see Chappell 1978). Consequently, we were surprised to find that FGMs in this species were higher in areas in which *T. speciosus* did not co-occur with *T. alpinus.* The effect of population density on FGMs in *T. speciosus* differed across years, suggesting that elevated FGMs in areas of lower co-occurrence were not due simply to a greater abundance of conspecifics in areas inhabited by *T. speciosus* only. These analyses indicate that relationships between co-occurrence and patterns of FGM variation in the study species are complex and are likely mediated by the presence of other ecological competitors.

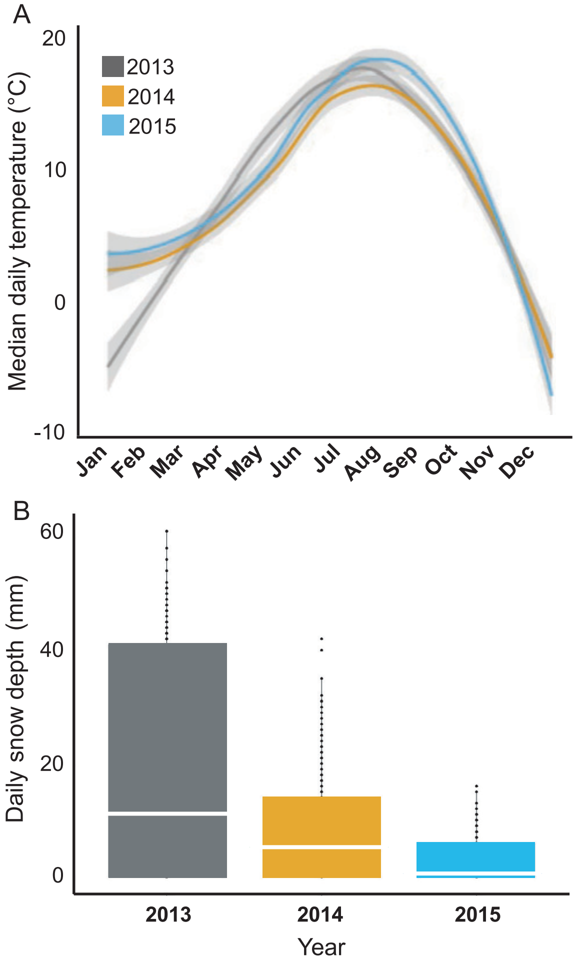

FIG. 3. Interannual differences in temperature and precipitation. (A) Median daily temperature as a function of smoothed date as recorded at weather stations in Yosemite National Park, California, USA. Temperatures varied across years of the study, with overall values for 2015 (blue) being generally higher than those for 2013 (gray) and 2014 (yellow; Table 4a). (B) Mean daily snowpack depth decreased significantly across each year of the study, with 2015 exhibiting particularly low levels (Table 4b). The midline indicates the median; box edges are the 25th (lower edge) and 75th (upper edge) percentiles. Whiskers extend to the smallest value that is at most 1.5 * interquartile range from the hinge. Outlying points beyond this range are shown individually.

TABLE 4. GLMM for (a) year-round median daily temperature and (b) daily snow depth for the winter (October–May) preceding each field season.

Parameter	Estimate	SE	df	*t*	*P*
a) Median temperature					
(Intercept)	44.0	8.14	1	5.41	0.11
Year (2014)	0.01	0.77	1,858	0.01	0.99
Year (2015)	1.84	0.77	1,858	0.0	**0.02**
Date	0.02	0.003	1,858	6.60	**5.2×10^{-11}**
b) Daily snow depth					
(Intercept)	21.6	7.74	1	2.79	0.22
Year (2014)	−13.97	0.83	727	−16.87	**$<2 \times 10^{-16}$**
Year (2015)	−18.14	0.83	727	−21.82	**$<2 \times 10^{-16}$**
Date	−0.01	0.003	727	−3.89	**0.0001**

Notes: In (a), random effect: weather station; variance 131.3, SD 11.5. In (b), random effect: weather station; variance 119.0, SD 10.9. Boldface text indicates that the term was significant in the model ($P \leq 0.05$).

Interannual variation in predictors of FGMs

Our analyses revealed significant interannual differences in the factors that were most predictive of FGMs. For *T. alpinus,* variation in FGM–environment relationships was consistent with interannual variation in climatic and habitat conditions. For example, the tendency for this species to show increased FGMs in less climatically suitable habitats was strongest in 2015, the warmest year, and driest year of the study (Berg and Hall 2017). In contrast, in *T. speciosus*, relationships between FGMs and several predictor variables (e.g., population density) were not clearly associated with annual habitat conditions and in some cases reversed directionality across years. It is possible that factors not monitored during this study contributed to some of this variation. In general, however, genotypic and phenotypic changes in *T. speciosus* over the past century have been less consistent and more difficult to relate to specific environmental factors than those in *T. alpinus* (Moritz et al. 2008, Rubidge et al. 2011, Walsh et al. 2016). These findings underscore the conclusion that environmental factors are more consistent predictors of response in *T. alpinus* compared to *T. speciosus.*

Glucocorticoids as bioindicators

Previous efforts to characterize GC–environment relationships have produced mixed results, with some studies describing strong correlations (Davies et al. 2013, Ozella et al. 2017) and others reporting weak or no relationships (Corlatti et al. 2014, Crespi et al. 2015, Madliger et al. 2015). This variability in outcomes raises questions regarding the circumstances under which organisms will display strong, consistent physiological responses to environmental conditions. In general, organisms living in more extreme or more variable environments, including range edges, may be more likely to encounter physiological limitations (Sorte and Hofmann 2004, Busch et al. 2011) and thus may be more sensitive to environmental conditions. Concomitantly, more ecologically specialized species may be more affected by environmental change (Thuiller et al. 2005, Foden et al. 2009) because the evolution of traits that increase success in specific habitats may come at the expense of a reduced ability to accommodate even relatively small changes in habitat parameters (Stillman 2003, Seebacher et al. 2015). Among our study species, *T. alpinus* is more ecologically specialized and is characterized by greater spatial (Moritz et al. 2008), genetic (Rubidge et al. 2012), and morphological (Walsh et al. 2016) change over the past century. We suggest that our results regarding FGMs provide additional evidence of the greater sensitivity of *T. alpinus* to environmental perturbations.

In the context of assessing responses to environmental change, it is critical to understand which factors determine an organism's fundamental and realized niches (Holt 2009, Cooke et al. 2013, Jankowski et al. 2013). Although organisms may be prevented from inhabiting certain areas by hard, physiological limits, it is also possible that habitats occurring within physiological tolerances are not occupied because they confer lower competitive ability or other increased fitness costs (Cooke et al. 2013). This tendency

Fig. 4. Interannual differences in relationships between FGMs and climatic habitat suitability scores. (A) In *T. alpinus,* the negative relationship between climatic habitat suitability scores and FGMs was driven primarily by 2015 (blue), while 2013 (gray) and 2014 (yellow) showed no strong relationships between these variables. (B) In *T. speciosus*, the directionality of the relationship between climatic habitat suitability scores and FGMs differed across years, with 2013 and 2014 showing negative patterns, and 2015 showing a strong positive relationship (see Table 3 and *Statistical analyses*). Each point in the figure represents the mean ± SE of FGM levels across all individuals from locations of a given climatic habitat suitability score.

may be particularly pronounced for species with narrower ecological niches, since a more limited niche space should result in a greater probability of exposure to extreme conditions, particularly in changing environments. Our findings for FGMs in *T. alpinus* and *T. speciosus* are consistent with this expectation and underscore the importance of exploring environmental and intrinsic impacts on GC physiology on a species-specific level. Further, our results suggest that the use of biologically relevant, noninvasive measures of physiology to assess spatial and temporal responses to external conditions may facilitate efforts to determine which taxa are most susceptible to the effects of environmental change.

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Supporting Information

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Data Availability

Data is available on the Dryad Digital Repository at https://doi.org/10.5061/dryad.8s444dr.