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ORIGINAL ARTICLE

Stress hormone metabolites predict overwinter survival in yellow-bellied marmots

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Abstract Biological assays of stress, in particular noninvasive measures, are valuable tools for wildlife management. Chronic stress can have negative impacts on fitness outcomes, and an area of particular interest is if elevated baseline stress levels are predictive of survival outcomes. We examined the relationship between fecal glucocorticoid metabolites (FGMs) measured from samples collected during routine trapping and overwinter survival in a wild population of yellow-bellied marmots (*Marmota flaviventris*). In particular, we asked if elevated FGMs were associated with reduced survival probability. Both higher yearly FGM levels averaged over several months and higher late season FGM levels averaged over the several weeks before hibernation were associated with lower probability of survival. Additionally, there was an interaction between late-season FGM levels and body mass, such that the association between late-season FGMs and survival was much stronger in animals with lower body mass (i.e., in poorer condition). This study highlights the promise of using stress hormone metabolites, a noninvasive measure, for studying factors affecting survival in this and potentially other natural wildlife systems.

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Biological assays of stress provide important tools for assessing and managing free-living (Reeder and Kramer 2005) and captive animals (Möstl and Palme 2002). In response to stressors, the vertebrate hypothalamic–pituitary–adrenal (HPA) releases glucocorticoids (GCs), which can influence physiology and health in a multitude of ways (Kasl 1984; Sapolsky et al. 2000). On the short term, GC release allows animals to respond adaptively to acute stressors (Wingfield 2005), but chronically elevated levels of stress hormones can be associated with negative effects (McEwen 1998; Goymann and Wingfield 2004; Korte et al. 2005). Ecologists have diverse tools available to measure different components of the stress response at different timescales (Johnstone et al. 2012). For example, both levels of circulating GCs in blood plasma, which are typically influencing within a few minutes of an acute stressor, and levels of GC metabolites in feces, which reflect the processing and integration of GCs over a longer timescale, are now widely used measures of stress (Harper and Austad 2000; Millspaugh and Washburn 2004; Schwarzenberger 2007). Relatively noninvasive and simple methods for measuring stress are particularly desirable, and the evaluation of different stress measures and their biological interpretation in wild vertebrates continues to be an area of active research and development (Johnstone et al. 2012).

A topic of particular interest for wildlife management is if baseline levels of stress predict fitness components in natural populations. An underlying assumption is that elevated baseline GCs may indicate elevated environmental stressors, resulting in an association between elevated GCs and relatively poor condition in individuals or populations, yet currently, the overall evidence of whether GCs predict fitness is mixed (Bonier et al. 2009). Some studies have found that elevated fecal GC metabolites (FGMs) are associated with reduced survival probability at the population (Boonstra and Singleton 1993; Romero and Wikelski 2001) and individual (Rogovin et al. 2003; Pride 2005) level. However, other studies find no association (Romero and Wikelski 2010; Ebensperger et al. 2013) or even a positive association between the two (Rogovin et al. 2008). This range of results could result from underlying flaws in theory or mismatches between the hypothesized mechanisms and the patterns captured by the collected data (Bonier et al. 2009), and further empirical tests of the predictive utility of FGMs will be useful in disentangling these possible factors.

We capitalize on detailed long-term data from a population of yellow-bellied marmots, *Marmota flaviventris*, to examine the potential utility of FGMs taken during routine live trapping for predicting overwinter survival. These large sciurid rodents experience a variety of stressors that may vary by age, sex, and season. Yellow-bellied marmots typically make natal dispersal decisions when they are yearlings (1-year-olds), reach reproductive maturity when they are 2 years old, and face a short growing season followed by long yearly hibernation with potentially high mortality (Armitage 2003). Poor body condition (i.e., low relative mass) late in the year before going into hibernation is associated with lower overwinter survival in juveniles (Lenihan and Van Vuren 1996) and reproductive females (Andersen et al. 1976). Previous studies in this system have characterized patterns of plasma GCs (Armitage 1991) and FGMs (Smith et al. 2012, who also provide a laboratory validation of FGMs as indicators of a stress response).

As hibernation is energetically demanding and a major source of mortality, we hypothesized that elevated FGMs would indicate chronically stressed individuals that would be less likely to survive the winter. We tested the utility both of average FGM levels over an entire active season and of FGM levels just during the late season, closer to the time of hibernation.

Materials and methods

Sample collection and analysis

We collected data from a population of yellow-bellied marmots studied in and around the Rocky Mountain Biological Laboratory (latitude: 38°, 57′ 29″; longitude: 106°, 59′ 06″) since 1962. The data used in this study were collected from 2005 to 2012. Marmots were trapped under research protocol ARC #2001-191-01 (approved by the UCLA Animal Research Committee on 13 May 2002 and renewed annually) and under annual permits issued by the Colorado Division of Wildlife. We focused on five geographically distinct colonies in the East Valley River basin.

From mid-April (typical emergence from hibernation) to September (preparation for immergence into hibernation), we trapped animals with Tomahawk live traps (Tomahawk Live Trap Co., Tomahawk, WI) baited with Purina Omolene 100 Horse Feed (Purina Mills, LLC, Gray Summit, MO). We set traps early in the morning or late in the afternoon and checked them within 3 h to avoid heat stress to animals. All animals received a uniquely numbered set of metal ear tags for permanent identification the first time they were trapped. Animals were also marked as needed with a distinctive dorsal marking using Nyanzol-D (Greenville Colorants, Clifton, NJ), a non-toxic black fur dye, to allow observations from a distance. During trapping, we identified each animal's sex (based on its anogenital distance) and whether it was an adult or yearling (typically based on longitudinal records of individuals who have been studied since birth, but for new subjects, based on its mass given its sex and given the date in the year). For adults, we also characterized nipple size (in females) or testes size (in males) as indicators of reproductive status. For genetic analysis, we took a hair sample from each animal the first time it was caught. To minimize stress to the animals, we handled marmots at the site of capture and released them immediately after handling. We collected fresh feces any time they were available and placed these in sealed plastic bags on ice, which were transferred to a −20 °C freezer within 2 h.

Fecal samples were transported at −20 °C, and hormone metabolites were later extracted at the San Diego Zoo's endocrinology lab or the Blumstein lab at the University of California at Los Angeles (details of the fecal GC metabolite analysis are provided in Smith et al. 2012). We confirmed if females had reproduced or not in a given year by whether they were identified as the parent of any pups in CERVUS 3.0 (Kalinowski et al. 2007), based on 12 previously developed microsatellite loci analyzed (Blumstein et al. 2010). We calculated a standardized measure of body mass for individuals using the residual of their mass from a regression of raw body mass on fixed effects of year, age, sex, and Julian day. We caught and remarked animals with dye marks at the end of the summer, after they had molted and before they went into hibernation, and overwinter survival for a year was confirmed by observation and/or trapping of individuals in following years. Individuals were considered to have died during hibernation if they were observed alive late in the season (in August or September), after dispersal would have occurred.

Statistical analysis

We fit separate generalized linear models (GLMs) to predict survival to the next year (binary outcome: yes or no) based on either average FGM levels over an entire active season or on FGM levels just during the late season (after mid-July), closer to the time of hibernation. In the first model testing average FGM levels of the entire active season, we included fixed effects of age (categorical: adult or yearling), sex (categorical: male or female), colony elevation (categorical: high or low), and average yearly within-individual FGM levels (continuous). FGM levels were log10-transformed, centered, and scaled. We also initially included two-way interactions among all categorical variables and between FGM levels and categorical variables, and simplified models in a stepwise manner until there was a significant change in the variance explained, as indicated by information criterion measures (Crawley 2007). We used a quasibinomial error distribution (to account for overdispersion) and logit link.

In the second model testing late season FGMs, we included fixed effects of age (categorical: adult or yearling), sex (categorical: male or female), colony elevation (categorical: high or low), average yearly within-individual standardized body mass during the late season, i.e., mid-July and later (continuous), and average yearly within-individual FGM levels during the late season (continuous). FGM levels were log10-transformed, and we centered and scaled continuous variables. We initially checked for two-way interactions among all categorical variables, between FGM levels and categorical variables, and between FGM levels and body mass, and simplified models in a stepwise manner until there was a significant change in the variance explained, as indicated by information criterion measures (Crawley 2007). We used a binomial error distribution and logit link.

We also fit the same models using generalized linear mixed models (GLMMs) with an additional random intercept for year. However, year did not explain significant amounts of variance (close to 0 %, $P>0.05$ in likelihood ratio tests), so we only report results for the GLMs. We fit GLMs with the "stats" package and GLMMs with the "lme4" package (Bates et al. 2013) in R statistical software, version 3.0.1 (R Development Core Team 2013; http://www.r-project.org).

Results

Our analysis of average yearly FGMs included 282 observations (115 deaths and 167 survivals) across 7 years. Mean FGMs of animals that died overwinter were 233.52 ng/g (standard error of the mean (SEM)=13.54), while mean FGMs of animals that survived hibernation were 177.70 ng/g (SEM=8.83). The final model only included significant effects of average FGM levels, age, and sex. Higher levels of FGMs were associated with lower survival (estimate= −0.38, $t=-2.85$, $P<0.01$; Fig. 1). Yearlings had lower survival than adults (estimate=−0.71, $t=-2.66$, $P<0.01$), and males had lower survival than females (estimate=−0.73, $t=-2.71$, $P<0.01$).

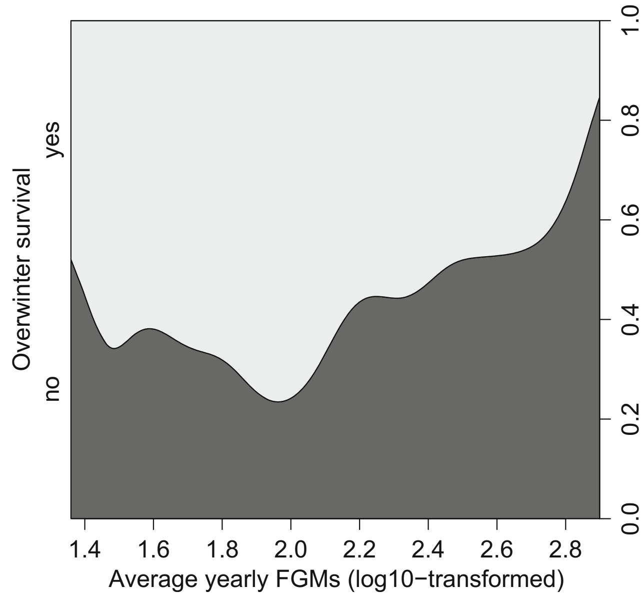

Fig. 1 Conditional density plot of overwinter survival as a function of log10-transformed fecal glucocorticoids (FGMs). *Light grey area* indicates densities for individuals that survived hibernation, and *dark grey area* indicates densities for individuals that did not survive hibernation. Probability of not surviving hibernation increased with higher average FGMs

Our analysis of late-season observations included 58 observations (18 deaths and 40 survivals) across 6 years. Mean late-season FGMs of animals that died overwinter were 215.48 ng/g (SEM=33.54), while mean late-season FGMs of animals that survived hibernation were 157.86 ng/g (SEM=15.98). The final model indicated significant effects of late-season FGM levels, a FGM by body mass interaction, and an age-by-sex interaction on survival (Table 1). Higher late-season FGM levels were associated with lower survival (estimate=− 1.86, $z=-2.48$, $P=0.01$; Fig. 2). There was a significant interaction between FGMs and standardized body mass indicating that there was less of a negative effect of FGMs in heavier animals (estimate=1.82, $z=2.34$, $P=0.02$). These two measures were not significantly correlated with each other ($r=-0.06$, $df=55$, $P=0.66$). There was also a significant interaction between sex and age such that yearling males had lower survival (estimate=−4.69, $z=-2.31$, $P=0.02$).

Discussion

We investigated the potential of fecal glucocorticoid metabolites, a stress index taken from samples readily collected during routine trapping procedures, to inform us about subsequent survival in a wild population of yellow-bellied marmots, and we found support for our hypothesis that marmots with elevated FGM levels would be less likely to survive hibernation.

Table 1 Results from a generalized linear model predicting overwinter survival

Parameter	Estimate	SEM	*z*	*P*
Intercept	*1.31*	*0.57*	*2.29*	*0.02*
Age[a]	2.34	1.36	1.72	0.09
Sex[b]	0.73	1.13	0.65	0.52
Late season mass[c]	−0.68	0.53	−1.28	0.20
Late season FGMs[d]	*−1.86*	*0.75*	*−2.48*	*0.01*
Age× sex	*−4.70*	*2.04*	*−2.31*	*0.02*
Late-season mass× late-season FGMs	*1.82*	*0.78*	*2.34*	*0.02*

Overwinter survival is binary. Error distribution is quasibinomial with logit link. N=58 (18 deaths and 40 survivals). Significant parameters are in italic

FGM fecal glucocorticoid metabolite, *SEM* standard error of the mean

[a] Adult or yearling: adult is the reference group

[b] Female is the reference group

[c] Standardized mass from mid-July until September

[d] FGM levels from mid-July until September

In this study, average yearly FGMs provided a broader measure of integrated baseline stress levels over the entire active season, while late-season FGMs provided a more detailed measure of stress levels right before hibernation. The significant associations of both with overwinter survival suggest that FGMs are overall a useful indicator of stress responses that may influence an individual marmot's ability to hibernate successfully. Animals with higher average yearly FGM levels may be chronically stressed throughout the active season or in generally poor condition. The conditional density plot suggests that the pattern is driven by individuals with above-average FGM levels, making potential threshold or nonlinear effects a possibility. Importantly, these reflect patterns over the course of several months, suggesting that FGM levels earlier in the year may be associated with longer-term effects. This result needs to be interpreted with some caution as factors beyond condition, such as reproductive status and individual variation, can also influence FGMs over the course of the active season (Smith et al. 2012). The result that yearling and male had lower survival on average is consistent with other studies in this system (Armitage 1991).

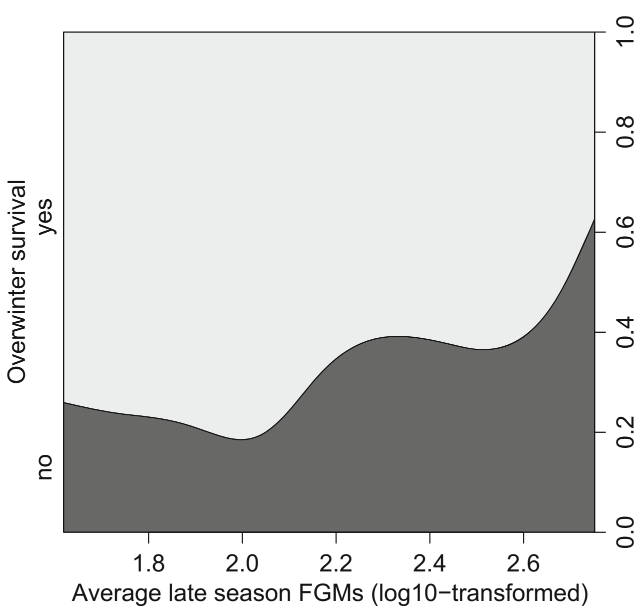

Fig. 2 Conditional density plot of overwinter survival as a function of log10-transformed late season fecal glucocorticoids (FGMs). *Light grey area* indicates densities for individuals that survived hibernation, and *dark grey area* indicates densities for individuals that did not survive hibernation. Probability of not surviving hibernation increased with higher average late season FGMs

The interaction between late-season FGMs and late-season body mass is particularly intriguing because it suggests that stressors have a stronger effect in animals that are in poor condition. Animals in better condition may have been better able to withstand environmental stressors than animals in poor condition. The mitigating effects of better late-season body condition are not due to lower stressors on animals in better condition because the two measures are not significantly correlated in the late season. Previous work suggesting that overwinter survival is largely determined by a complex interaction between body condition and length of winter (Schwartz et al. 1998) and stress hormone metabolites may indicate important physiological processes associated with preparation for hibernation. It is interesting that we found no main effect of late-season body mass on overwinter survival, given that previous work observed this effect in some age groups (Andersen et al. 1976; Lenihan and Van Vuren 1996). The inclusion of FGMs and body mass in the same model may allow for a more subtle pattern of multiple physiological mechanisms influencing hibernation. The particular role of each mechanism in determining survival is an issue that deserves more attention. The result that yearling males had lower survival than other age-sex groups may also require more follow-up to interpret properly. Most yearling males disperse; thus, any yearling males included in this analysis were the ones that had not dispersed at the end of each season and may not be representative of yearling males more generally.

Our goal was to evaluate the utility of noninvasive stress measures taken as part of a long-term study for predicting survival (a fitness correlate), and we found very promising results for this system. In yellow-bellied marmots, elevated fecal glucocorticoid metabolites were associated with lower overwinter survival. It may be that FGMs are most important for predicting survival in animals that are already in poor condition, but this suggestion requires further investigation. These results add to the set of empirical observations suggesting that stress responses reflect physiological processes that are important for survival in wild animals. The live-trapping methods used here are typical of techniques used for many mammals, and we hope it may be informative for a range of systems. Overall, especially, given the relative ease of measurement, fecal stress hormone metabolites should help us better understand how natural populations respond to a variety of environmental stressors, especially when used in combination with other measures.

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