Received: 4 January 2018 | Accepted: 19 February 2018

DOI: 10.1111/1365-2664.13140

RESEARCH ARTICLE

Journal of Applied Ecology | BRITISH ECOLOGICAL SOCIETY

The importance of individual heterogeneity for interpreting faecal glucocorticoid metabolite levels in wildlife studies

Joy Coppes[1] | Jim-Lino Kämmerle[1,2] | Mirjam Willert[1] | Annette Kohnen[1] | Rupert Palme[3] | Veronika Braunisch[1,4]

[1]Wildlife Ecology, Forest Research Institute of Baden-Wuerttemberg FVA, Freiburg, Germany

[2]Wildlife Ecology and Wildlife Management, University of Freiburg, Freiburg, Germany

[3]Department of Biomedical Sciences, University of Veterinary Medicine, Vienna, Austria

[4]Conservation Biology, Institute of Ecology and Evolution, University of Bern, Bern, Switzerland

Correspondence
Joy Coppes
Email: joy.coppes@forst.bwl.de

Handling Editor: Marc-André Villard

Abstract

1. As a non-invasive and inexpensive method, the use of faecal glucocorticoid metabolites (FGM) analysis in wildlife research is increasing. Various environmental factors have been shown to influence FGM levels, or faecal corticosteroid metabolites (FCM) levels in birds, but most studies do not account for inter-individual variance, which we hypothesized may substantially affect results.
2. We combined FCM analysis with genetic analysis to identify the sex and individual's identity in samples collected in three consecutive winters; with repeated samples per individual, across the entire range of an endangered population of capercaillie *Tetrao urogallus* in south-western Germany. Using generalized additive mixed models, we modelled FCM levels as a function of sex, season and environmental covariates at two spatial scales: location and home range. We compared two models: one including information on the individual animal and the other excluding this information (i.e. naïve model) to assess the influence of individual heterogeneity on the results obtained.
3. Models accounting for inter-individual differences explained 44.0% and 45.1% (at the location and home-range scale respectively), while only very little (4.0% and 5.1%, respectively) was explained by the environmental predictors. When ignoring individual effects, the model results changed considerably with other, previously non-informative predictors, becoming significant.
4. In the full models, accounting for inter-individual variance, weather conditions had no effect at either scale. FCM levels were negatively correlated with habitat quality at the sampling location, while human recreation at the home-range scale led to elevated FCM levels. In the naïve models, two additional predictors appeared significant: one weather variable at the local scales and two at the home-range scale. In all models, seasonal FCM patterns differed significantly between males and females.
5. *Synthesis and applications*. By combining faecal corticosteroid metabolites (FCM) analysis with genetic individual assessment, we demonstrate that individual heterogeneity can explain most of the variance in faecal corticosteroid metabolites levels and that ignoring this information can lead to erroneous conclusions when testing for environmental stressors. We therefore stress the importance of identifying individuals when studying faecal corticosteroid metabolites in wildlife and

recommend combining faecal corticosteroid metabolites analyses with genetic analyses to adequately address this issue.

KEYWORDS
Capercaillie, corticosterone levels, environmental stressors, faecal corticosterone metabolites, faecal glucocorticoid metabolites, genetic individual assessment, human disturbance, individual heterogeneity

1 | INTRODUCTION

If confronted with actual or perceived threats, animals elicit stress responses which help them adjust to changes in their environment (Cockrem, 2007). One frequently studied stress response in vertebrate ecology is the change in glucocorticoid (cortisol or corticosterone) levels (Möstl & Palme, 2002; Sheriff, Dantzer, Delehanty, Palme, & Boonstra, 2011). Those stress hormones with their pleiotropic role within the organisms are recognized as mediators of allostasis that help maintain homeostasis of bodily functions (McEwen & Wingfield, 2003; Sapolsky, Romero, & Minck, 2000). Although it is natural that corticosteroid levels fluctuate (e.g. due to time of day, season, food availability, social status, reproductive status, age or sex) (Broom & Johnson, 1993; Moberg & Mench, 2000), prolonged exposure to high levels can reduce growth (Sapolsky, 2002), suppress the immune system (Cyr, Earle, Tam, & Romero, 2007; Stier et al., 2009) or inhibit the reproductive system (Sapolsky, 2002), a condition known as allostatic overload (McEwen & Wingfield, 2003). This in turn may affect fitness (Boonstra, Hik, Singleton, & Tinnikov, 1998; Rangel-Negrın, Alfaro, Valdez, Roman, & Serio-Silva, 2009; Sheriff, Krebs, & Boonstra, 2009; Thierry, Ropert-Coudert, & Raclot, 2013), making it a relevant conservation issue for threatened species. Glucocorticoids are frequently measured to evaluate the response of organisms to various stressors (Goymann, 2012; Touma & Palme, 2005). In wildlife research, they are often assessed indirectly and non-invasively by analysing their metabolites in faecal samples (Möstl, Maggs, Schrötter, Besenfelder, & Palme, 2002; Thiel, Jenni-Eiermann, & Palme, 2005), so as to avoid additional stress by capturing or handling the animal, biasing the results (Buehler et al., 2008; Goymann, 2012; Sheriff et al., 2011). It is also important to recognize that the faecal metabolites represent an integrated measure of adrenocortical activity at a certain time before the faecal excretion (Palme, 2005; Touma & Palme, 2005).

Previous studies showed that many endogenous and exogenous factors can affect the concentration of faecal corticosteroid metabolites (hereafter referred to as FCM levels) (Hadinger, Haymerle, Knauer, Schwarzenberger, & Walzer, 2015). In free-living animals, FCM levels can be affected by food availability (Jenni-Eiermann, Glaus, Grübler, Schabl, & Jenni, 2008; Schoech, Bowman, Bridge, & Boughton, 2007), with increased food availability being associated with lower FCM levels (Jenni-Eiermann et al., 2008). Habitat conditions, related to cover or foraging conditions are also suggested to affect FCM levels (Rangel-Negrın et al., 2009). For several species, an effect of the ambient temperature has been found, with higher FCM levels during the cold season (Corlatti, Palme, Frey-Roos, & Hackländer, 2011; Frigerio, Dittami, Möstl, & Kotrschal, 2004). Predators have been found to increase corticosterone levels in birds (Cockrem & Silverin, 2002), and indirect predator effects, such as mere predator presence or elevated densities (Monclús, Palomares, Tablado, Martínez-Fontúrbel, & Palme, 2009; Sheriff et al., 2009), can be as important as direct ones (Preisser, Bolnick, & Benard, 2005; Schmitz, Beckerman, & O'Brien, 1997). Similarly, human recreational activities have been linked to elevated FCM levels in a variety of bird species (Arlettaz et al., 2007; Thiel, Jenni-Eiermann, Palme, & Jenni, 2011). Sex-specific (Rangel-Negrın et al., 2009; Weingrill, Gray, Barrett, & Henzi, 2004) and inter-individual differences in stress responses and associated FCM levels (Rehnus & Palme, 2017) have also been shown in several species.

Despite this variety of drivers and associated sources of variance, most studies focus only on a small number of factors to assess their hypothesized effect on FCM levels. Inter- and intra-individual differences are often neglected (Goymann, 2012; Hadinger et al., 2015; Rehnus & Palme, 2017). Especially when non-invasive sampling methods are used, it is often unknown how many individual animals of a population have been sampled and possible pseudo-replication cannot be excluded (Rehnus & Palme, 2017). If and to which extent this may affect the results with regard to the effects of environmental stressors on FCM-levels has not yet been tested.

Declining and endangered in many Central European countries, grouse (Tetraoninae) have become a common model for conservation-related endocrinological studies. As these species are highly susceptible to human disturbance (Coppes, Ehrlacher, Thiel, Suchant, & Braunisch, 2017; Storch, 2013; Summers, McFarlane, & Pearce-Higgins, 2007; Thiel et al., 2011), their stress response has been elucidated particularly in relation to human recreation activities: Elevated FCM levels were found after repeated flushing in black grouse *Tetrao tetrix* (Arlettaz et al., 2015), with decreasing distance to recreational infrastructure in capercaillie *Tetrao urogallus* (Thiel et al., 2011) and in areas severely disturbed by winter sports in both species (Formenti et al., 2015; Thiel, Jenni-Eiermann, Braunisch, Palme, & Jenni, 2008). Furthermore, tree species composition and ambient temperature were found to affect FCM levels in capercaillie (Thiel et al., 2011). All studies were based on FCM extracted from faecal samples collected in

winter, when they are well-preserved in the cold environmental conditions. Although most studies distinguished between males and females, which are easily identified based on the size of their droppings, both inter- and intra-individual differences in FCM levels have not been considered so far.

In our study, we collected faecal samples across the entire geographical range of an endangered central European capercaillie population. We chose capercaillie as an ideal study model because FCM are calibrated (Thiel et al., 2005), they occur in various landscapes (Klaus et al., 1989) and they respond to various stressors such as predation (Kämmerle, Coppes, Ciuti, Suchant, & Storch, 2017), climate change (Braunisch et al., 2013), habitat degradation (Suchant & Braunisch, 2004) and human disturbance (Coppes et al., 2017) while being threatened throughout their central European range (Storch, 2007). By combining FCM measurements in three consecutive winters with genetic (i.e. to identify individual animals and determine their sex) and environmental analyses, we tested for individual variation in FCM levels and studied the effect of including or neglecting this information when investigating the effects of several potential environmental stressors on FCM levels: (1) habitat quality, (2) weather conditions, and (3) human recreational use. We expected a major effect of sex and individual animal on the FCM levels, which could considerably alter the results if not accounted for within the models. Furthermore we hypothesized that FCM would be higher in areas with low habitat quality (i.e. in dense forests) in cold weather conditions and close to recreational infrastructure.

2 | MATERIALS AND METHODS

2.1 | Study area and model species

The study was performed in the Black Forest, a lower mountain range in south-western Germany (Figure 1). The forest, dominated by spruce *Picea abies*, silver fir *Abies alba* and beech *Fagus sylvatica* (Kändler & Cullmann, 2014), is interspersed with pastures and small settlements, the latter predominantly located in the valleys. The Black Forest holds one of the largest capercaillie populations in central Europe, outside the Alps (Segelbacher, Höglund, & Storch, 2003; Storch, 2007), a species inhabiting well-structured, open mountain and boreal forests (Graf, Mathys, & Bollmann, 2009; Klaus et al., 1989; Storch, 2002). The Black Forest capercaillie population is isolated from other populations in central Europe (Segelbacher et al., 2003) and highly fragmented (Braunisch, Segelbacher, & Hirzel, 2010; Coppes et al., 2016). Population size and distribution have been continuously decreasing over the last 30 years (Coppes et al., 2016), with the cause considered to be multifactorial, including habitat deterioration (Suchant & Braunisch, 2004), habitat fragmentation (Braunisch et al., 2010), predator abundance (Kämmerle et al., 2017), disturbance through human recreation (Coppes et al., 2017) as well as climate change (Braunisch et al., 2013; Huntley, Green, Collingham, & Willis, 2007).

2.2 | Sampling method

Capercaillie faecal samples were collected in winter between November 2012 and May 2016, during periods with snow cover. Sampling areas were distributed over large parts of the capercaillie range in the Black Forest (Figure 1), and systematically searched between one and three times per winter. However, due to differences in weather, snow and topographical conditions, the surface searched within a single day varied greatly. Samples were collected 3–7 days after new snowfall. We only collected samples lying on snow, as Thiel et al. (2005) had shown in an experimental set-up that FCM levels in capercaillie droppings are stable for 7 days if samples were kept at temperatures below 9°C. When several samples were located within a radius of 25 m, only the freshest one (determined by visual assessment) was collected and its location taken using a handheld GPS (Garmin Etrex30). Samples were cooled during transport and stored at −32°C in the laboratory. Therefore, we assume our FCM measurements are not influenced by storage conditions after defecation.

2.3 | Model predictors

To study the importance of including information on individual heterogeneity when assessing the effect of environmental stressors,

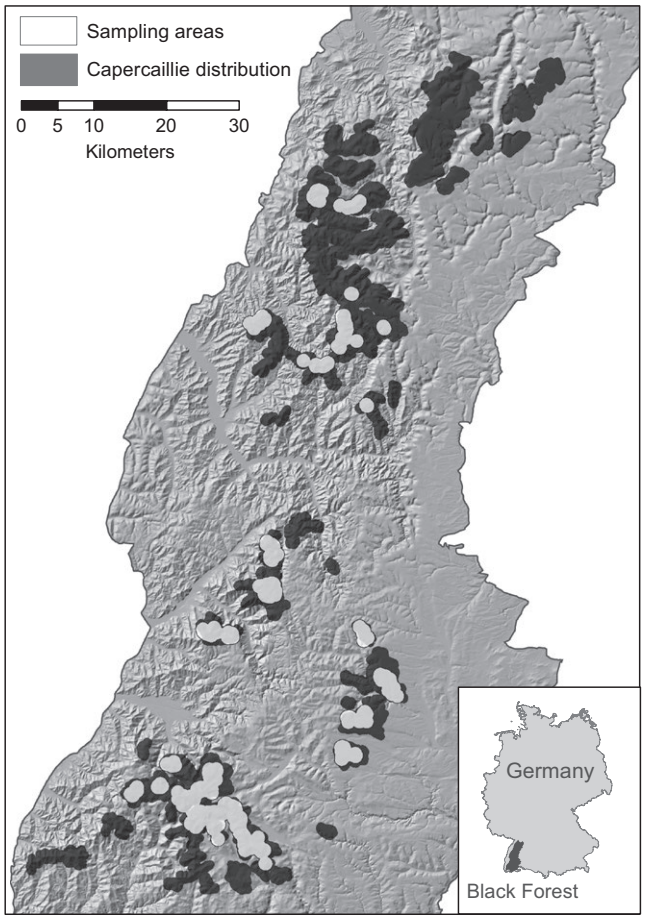

FIGURE 1 Capercaillie distribution in the Black Forest and the areas where samples were collected for faecal corticosteroid metabolites analysis. The inlay map shows the location of the Black Forest within Germany

TABLE 1 Predictor variables tested for their effect on faecal corticosteroid metabolites levels in capercaillie. Predictors retained in the models are indicated, otherwise the reason for discarding them is provided ("Decision"). Spatial predictors (i.e. Recr_dist and PropOpen) were calculated at two scales: local scale (average values in a 20 m radius) and home-range scale (average values in a 400 m radius)

Group	Name	Description (unit)	Type	Decision
Human recreation	Recr_dist	Average distance to recreational infrastructure within a 20 and 400 m radius (m)	Continuous	Retained
	Recr_dens	Density (sum of line feature lengths) within a 20 and 400 m radius (1,257 m^2 and 502 655 m^2)	Continuous	corr. with Recr_dist
	Prop_recr	Proportion of 20 or 400 m buffer covered by a 50 m buffer around recreational infrastructure	Continuous	corr. with recr_dist
Habitat	PropOpen	Proportion of open forest (<70% canopy cover) within a 20 and 400 m radius (%)	Continuous	Retained
	Altitude	Elevation of sample above sea level (m)	Continuous	corr. with Tmin3d
Weather conditions	Tmin3d	Minimum temperature in the 3 days before sample was collected (°C)	Continuous	Retained
	Tmean3d	Mean temperature over 3 day window before sample was collected (°C)	Continuous	corr. with Tmin3d
	Tmin7d	Minimum temperature in the 7 days before sample was collected (°C)	Continuous	corr. with Tmin3d
	Tmean7d	Mean temperature over 7 day window before sample was collected (°C)	Continuous	corr. with Tmin3d
	PrecDays	Number of days without precipitation before the day of sampling (range 3–7 days)	Continuous	Retained
Season	Day	Day of the winter season with 1 as start of winter and 212 as the end of winter	Continuous	Retained
Individual	Sex	The sex of the animal (male/female)	Categorical	Retained
	Indiv	The ID of the individual animal	Categorical	Retained

we tested several environmental predictors. These included spatial information on habitat quality and human recreation, temporal information on weather and season as well as information on sex and identity of the individual (Table 1). To account for the mobility of the species and the time-lag between blood corticosteroid levels and the excretion of their metabolites in the droppings (Thiel et al., 2005), we extracted the spatial environmental covariates using circular buffers at two spatial scales; at the "local scale" the predictors were considered within a 20 m radius (to account for GPS inaccuracies) around the faecal sample location. In addition, we considered the environmental conditions within a 400 m radius, which is equivalent to an area of 50 ha (i.e. the size of a small winter home range of capercaillie in the Black Forest; Coppes et al., 2017). The predictors were prepared using ArcGIS 10.4 (ESRI 2014).

2.3.1 | Habitat quality

We calculated the proportion of open forest (<70% canopy cover) as a proxy for habitat quality, as it has been identified as a key structural habitat characteristic in various European capercaillie populations (Graf et al., 2009; Storch, 2002; Suchant & Braunisch, 2004). We used a digital vegetation surface model (1 × 1 m resolution), which was derived from stereo aerial images of the years 2015 and 2016 as described in Zielewska-Büttner, Adler, Ehmann, and Braunisch (2016). In a first step, canopy cover was calculated as the proportion of pixels with vegetation of at least 2 m height within a 25 m radius around every raster cell (Zielewska-Büttner et al., 2016). We then calculated the proportion of pixels classified as "open forest" (canopy cover <70%) in a 20 and 400 m radius around our samples.

2.3.2 | Human recreation

To test for an influence of human recreational activities on FCM levels, we calculated the mean distance of each sample to the nearest winter recreation infrastructure (i.e. winter hiking paths, cross-country skiing trails, skiing pistes, snowshoe trails) as well as the density (as length per aerial unit) of infrastructure within a 20 and 400 m radius respectively. In addition, we applied a 50 m buffer around all recreational infrastructure and estimated the proportion of buffer area within the two radii. The data on recreational infrastructure were adopted from the official Tourism and Recreation Information System of Baden-Württemberg (TFIS) and complemented with data of snowshoe trails, back-country skiing tours or winter hiking trails provided by specific user groups on the Internet (www.outdooractive.de, www.gpsies.com, www.bergfex.de). Since capercaillie are most likely not affected by recreation activities at distances over 400 m (Coppes et al., 2017; Thiel et al., 2011), we truncated the distance to recreation at 400 m based on the frequency distribution of the data that is excluding extreme outliers (Figure S1).

2.3.3 | Weather

To test for weather effects on FCM levels, weather data were obtained from the German meteorological service (Deutscher Wetterdienst, www.dwd.de). Precipitation and temperature data of the nearest meteorological station were used for each sample. We corrected for differences in elevation between sample and station by adjusting temperature with −0.6°C per 100 m of elevation increase (Liston & Elder, 2006). We prepared a number of weather predictors: the number of days without precipitation before the date of sampling (PrecDays) and the minimum temperature as well as the mean temperature over three as well as 7 days before the date of sampling (Tmin3d, Tmean3d, Tmin7d, Tmean7d).

2.3.4 | Season

Since photoperiod and season can affect FCM levels (Corlatti et al., 2011), all samples were numbered based on the collection date, starting with 1 for the start of winter (1 November) and ending with 212 at the end of Winter (31 May) for every year. This resulted in a continuous variable depicting the time of the year.

2.4 | Endogenous predictors: Genetic analysis

Genomic DNA was extracted from capercaillie droppings using spin columns (QIAamp DNA Stool Mini Kit, Qiagen, Hilden, Germany) according to the manufacturer's protocols. To minimize contamination risks, amplification and post-PCR procedures were conducted separately from DNA extraction. All samples were genotyped using 12 microsatellite loci (Jacob, Debrunner, Gugerli, Schmid, & Bollmann, 2010) and one sex marker (Kahn, St. John, & Quin, 1998) arranged in four multiplex-PCR reactions based on the protocol by Jacob et al. (2010). To avoid genotyping errors, a multiple tube approach with three replicates was implemented. Additionally, negative controls were included in the PCR amplification procedure to exclude contaminations. PCR products were sized on an ABI 3130 DNA Analyzer (Applied Biosystems, Darmstadt, Germany). Fragment length was scored using the program GeneMapper v.4.0 (Applied Biosystems). Individuals were identified using GenAlEx 6.503 (Peakall & Smouse, 2006) by searching for multilocus genotype matches. Samples that shared all alleles at all loci, excluding loci with missing values, were considered as identical.

2.5 | FCM analysis

To avoid effects of the sample humidity on the FCM measurements, all samples were dried at 80°C. After careful homogenization, glucocorticoid metabolites were extracted with 60% methanol (0.5 g droppings plus 5 ml) as described by Palme, Touma, Arias, Dominchin, and Lepschy (2013). FCM metabolites were measured using a cortisone enzyme immunoassay (EIA; Rettenbacher, Möstl, Hackl, Ghareeb, & Palme, 2004), which has been successfully validated for capercaillie (Thiel et al., 2005). To exclude any bias due to storage, analysis or other conditions, all faecal samples were stored and analysed under the same conditions in the same laboratory.

2.6 | Statistical analysis

2.6.1 | Individual variation in FCM levels

In order to evaluate and visualize inter-individual differences in mean FCM levels, we calculated individual means and associated standard errors (*SE*) and confidence intervals (CI = 1.96 × *SE*) for each animal with ≥3 samples. Prior to that, we tested whether the mean was correlated with the sample size (i.e. resampling rate) of each individual using Pearson's product-moment correlations. We conducted a repeatability analysis to assess the consistency of FCM among individuals (i.e. the intraclass correlation coefficient, ICC), calculating (1) ANOVA-based and linear mixed-effect model (LMM)-based agreement repeatability with confidence intervals and (2) adjusted repeatability after accounting for environmental covariates (i.e. human recreation, habitat and weather conditions, Table 1; Nakagawa & Schielzeth, 2010; Wolak, Fairbairn, & Paulsen, 2012). Using the capercaillie individual as a grouping factor, we calculated agreement repeatability as implemented in the R-packages ICC (ANOVA-based Wolak et al., 2012) and RPTR (LMM-based; Stoffel, Nakagawa, & Schielzeth, 2017). Adjusted repeatability in package RPTR was estimated based on the final LMM structure specified below (see next section). All ICCs were estimated assuming Gaussian error distributions.

2.6.2 | Model generation

In a first step, the initial set of predictors (Table 1) was tested for collinearity by calculating pairwise Pearson correlations (Dormann et al., 2012; Zuur, Ieno, Walker, Saveliev, & Smith, 2009). Of variables with a pairwise correlation coefficient of $|r| > .5$, we retained the one we considered to be of higher ecological relevance. Pre-selection of variables resulted in seven predictors that we hypothesized to be related to the FCM levels: the proportion of open forest, the distance to winter tourism infrastructure, the number of days since the last precipitation event and the minimum temperature within a 3-day window. Furthermore, the day of season, the sex of the animal and an interaction term was included, as we expected FCM patterns to differ between sexes as a function of the advancement of the mating season. All data were standardized by subtracting the mean and dividing by the *SD* to aid model convergence and to allow for a comparison of effect sizes.

We modelled FCM levels using generalized additive mixed models (GAMM, e.g. Wood, 2006) from the R-package GAMM4 (Wood & Scheipl, 2017) with a Gaussian error distribution and a log-transformed response variable to meet parametric assumptions and to achieve model convergence. We accounted for variation in the mean FCM levels between individuals and study years by including a random intercept for individual and the year of study. One full GAMM containing all pre-selected predictors was calibrated for each scale (i.e. 20 and 400 m radius) using cubic regression splines

with shrinkage (Wood, 2006) to penalize non-relevant predictors to zero.

In a first validation step, we compared the results of the GAMMs to LMMs (package LME4, Bates, Mächler, Bolker, & Walker, 2015) of equal structure as our GAMMs, including higher order terms for each predictor as indicated by the degrees of freedom estimated for each predictor in our GAMMs (LMM model results are provided in Table S1).

We then evaluated the performance of GAMMs as compared to LMMs using fivefold cross-validation (CV; with the five random partitions containing equal proportions of our data to detect overfitting) by comparing the root mean square error (RMSE) of our final models to the mean RMSE of the CV iterations. Finally, we obtained effect plots with 95% confidence intervals conditional on the estimated smoothing parameters of the model, while holding all other covariates at the mean (package MGCV, Wood, 2004, 2011). All statistical analyses were performed using the program R 2.15.0 (R Development Core Team, 2017).

2.6.3 | Assessing the importance of individual effects

In order to quantify the effect of inter-individual variation in FCM levels, we partitioned the reduction in model deviance that could be ascribed to the fixed effect (i.e. environmental predictors) and random effect part (i.e. inter-individual differences) of our GAMMs respectively. Fitting both models using maximum likelihood estimation allowed for comparison across different fixed effect structures.

The model deviance was quantified as the squared sum of residuals (RSS) and we related this to the deviance of a null model to obtain a measure of variance explained (i.e. a pseudo-R^2). We constructed the null model as a GAMM containing a single intercept only, but adding the random effect structure of our models as:

$$\text{Nullmod} = \text{GAMM}(y \sim 1 + r_{j,i} + \varepsilon)$$

with random intercepts $r_{j,i}$ of equal structure to our full models. To obtain null deviance, the RSS was calculated based on population level predictions of the null model (i.e. discarding the random effects for predicting).

The variance explained by the models was quantified as the reduction in model deviance attributed to fixed effects, random effects or both combined. We calculated the variance explained by (*A*) our full model (using RSS of predicting with the full model on the data), (*B*) the fixed effects for unknown random effects (using RSS of population level predictions, i.e. disregarding the random term) and (*C*) the fixed effects given our known random effects (as the difference between the RSS of *A* and the RSS of the full model prediction of our null model that is including the random term for predicting). Finally, we dropped the random intercept for the year of the study from the model to estimate the amount of variance explained by individuals alone. For this comparison of the two random effect terms, we used models fitted using restricted maximum likelihood. Finally, we validated our calculations by estimating the variance explained by individual differences in the LMMs using the marginal and conditional R^2 (Nakagawa & Schielzeth, 2013).

To test how the results of our analysis were influenced by including the information on inter-individual differences, we refitted our GAMMs keeping all settings the same except that individual was not included as a random effect. We compared these "naïve" models with the corresponding full models (i.e. including individual as a random effect) in terms of significance of predictors and the shape of the effect plots.

3 | RESULTS

3.1 | Individual variation in FCM levels

A total of 894 samples were collected and genetically analysed in the three winter seasons. Across all seasons, 232 individual capercaillies could be genetically identified, of which 139 were males and 93 females (Table S2). The probability of two unrelated individuals sharing the same genotype (PI) was 1.7×10^{-10} while the probability of siblings sharing the same genotype (PISib) was 1.0×10^{-4}. Individual birds were resampled between 1 and 32 times ($M = 3.8$, median = 2 times). There was large inter-individual variation in FCM levels (Figure 2), which did not correlate with sample size ($r = -.05$, $t = -0.56$, $p = .57$). ICCs were accordingly low with confidence intervals excluding zero (ANOVA-based $R = .21$ [0.14–0.28]; LMM-based $R = .235$ [0.151–0.314]).

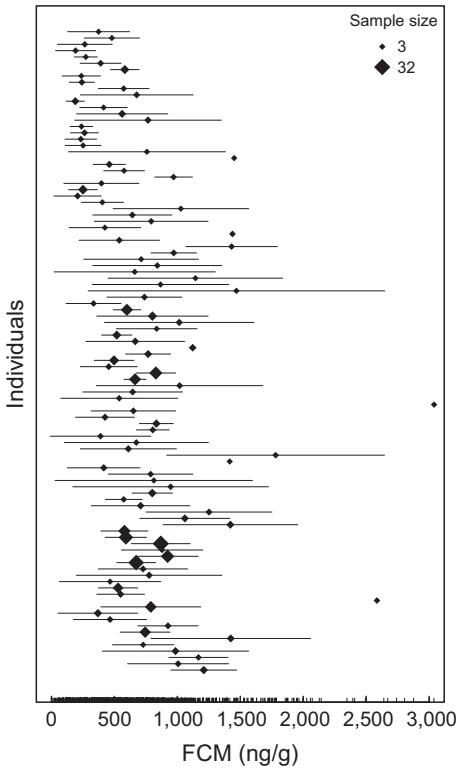

FIGURE 2 Mean (±95%CI) faecal corticosteroid metabolites levels of individual capercaillie which were sampled at least three times. Samples size is illustrated by the size of the diamonds, with larger diamonds indicating larger sample sizes. For individuals for which no error bars are shown, error bars exceed beyond the extent of the box

Adjusted repeatability was slightly lower than agreement repeatability (LMM-based 20 m: R = .21 [0.14–0.29] and 400 m: R = .21 [0.14–0.29]).

3.2 | Relative importance of individual effects

Our full models (i.e. fixed and random effects combined) explained approximately 44.0% (20 m) and 45.1% (400 m) of the variance in the data. The random term of our model explained the majority of variance, while fixed effects only accounted for 4.0% (20 m) and 5.1% (400 m) of the explained variance for known random effects (i.e. if individuals and years were known) and only 0.5% (20 m) and 0.8% (400 m) of the variance in population level predictions (i.e. for unknown individuals and years). Removing the year of study caused a drop in overall variance explained to 3.0% (20 m) and 3.2% (400 m), respectively, thus attributing the bulk of variance explained to the inter-individual differences (20 m: 37.0%; 400 m: 36.8%). The same pattern was found for the LMMs, with a marginal R^2 of.081 (20 m) and .082 (400 m) and conditional R^2 of .428 (20 m) and .438 (400 m), respectively.

3.3 | Effect of including individual variation on model outcomes

The GAMMs performed well in CV, with only a slight increase in RMSE in CV as compared to the full model at the 20 m scale (full model RMSE = 0.90; mean CV RMSE = 0.92; Δ = 0.02) and 400 m scale (full model RMSE = 0.89 mean CV RMSE = 0.91; Δ = 0.02). In addition, GAMMs performed better in CV than the respective LMMs of similar structure (20 m: Δ = 0.22; 400 m: Δ = 0.12). FCM levels were not related to weather conditions (PrecDay or Tmin3D) at the home-range scale (i.e. 400 m radius), but affected by the minimum temperature 3 days before sampling (Tmin3D) at the local scale (Table 2). We found a significant, albeit small decrease in FCM levels with increasing proportions of open forest (ProbOpen) at the local, but not at the home-range scale (Table 2, Figure S2). Distance to human winter recreation infrastructure was significantly related to an increase in FCM levels at the home-range scale, but not at the local scale (Table 2). FCM levels were, however, only elevated if the average distance to recreation infrastructure within the home range was less than approximately 180 m (Figure S2). In both models, we found a significant interaction between the sex and day of season. Female capercaillie had higher FCM levels than males in November, which continuously decreased during the course of winter (Figure S3). Male capercaillie, in contrast, showed a more complex, bimodal pattern: Low FCM levels in early winter were followed by a first peak in mid-winter (January). Thereafter, FCM levels decreased, before peaking again in April–May (Figure S3).

The naïve models, not including individual as a random effect, differed considerably from the full models (Table 2, and Figures 3

TABLE 2 Generalized additive mixed models explaining the faecal corticosteroid metabolites levels on both scales for both the full model (including individual as a random effect, panel a and c) as well as the naïve model (without individuals as random effect, panel b and d). Codes and descriptions of the predictors are given in Table 1. Predictors highlighted in bold become significant when not including the individual as a random effect

	(a) Full model local scale (20 m radius)				(b) Naïve model local scale (20 m radius)			
	Estimate	***SE***	***T* value**	**Pr(>|*t*|)**	**Estimate**	***SE***	***T* value**	**Pr(>|*t*|)**
	6.176	0.266	23.22	<0.001	6.141	0.296	20.74	<0.001
Predictors	E*df*			*p*	E*df*			*p*
Recr_dist	1.017			.071	0.861			.087
PrecDay	**1.887**			**.108**	**8.468**			**.004**
Tmin3D	2.022			.044	5.540			.035
ProbOpen	1.084			.007	5.540			.020
Day:SexF	2.375			<.001	6.046			<.001
Day:SexM	4.485			<.001	2.926			<.001
	(c) Full model home-range scale (400 m radius)				**(c) Naïve model home-range scale (400 m radius)**			
	Estimate	***SE***	***T* value**	**Pr(>|*t*|)**	**Estimate**	***SE***	***T* value**	**Pr(>|*t*|)**
	6.195	0.258	24.01	<0.001	6.148	0.275	22.37	<0.001
Predictors	E*df*			*p*	E*df*			*p*
Recr_dist	2.917			.001	6.007			.006
PrecDay	**1.947**			**.077**	**8.407**			**.003**
Tmin3D	**1.947**			**.061**	**5.831**			**.019**
ProbOpen	0.678			.193	8.415			.124
Day:SexF	2.398			.001	6.641			<.001
Day:SexM	4.403			<.001	2.911			<.001

and 4). In the local scale model, one predictor (PrecDay) additionally appeared significant which were not significant in the full model. Similarly, at the home-range scale two additional predictors were found significant in the naïve model (PrecDay, Tmin3D; Table 2, and Figures 3 and 4). The extreme increase in Edf (Table 2) indicates an overfitting of the naïve models, and effect plots revealed ecologically meaningless patterns, regardless of significance in the model (Figures 3 and 4).

4 | DISCUSSION

Our study is one of the first to investigate FCM levels combined with genetic analysis to identify the individuals in the sample within a free-ranging population over several years. Our results highlight the importance of considering individual heterogeneity when analysing FCM. While our models explained approximately 44.0%–45.1% of the variance in capercaillie FCM levels, only 4.0% and 5.1% thereof could be ascribed to environmental conditions, 36.8% and 37.0% being associated with inter-individual variation (Figure 2). This pattern was independent of the scale at which environmental conditions were measured and was supported by the low repeatability values and the fact that the adjusted repeatability was not larger than the agreement repeatability.

Differences in FCM levels between individual animals could be explained by differences in how individuals metabolize corticosterone (Goymann, 2012). However, individual animals can also respond differently to environmental stressors (Dickens & Romero, 2013; Ganswindt, Tordiffe, Stam, Howitt, & Jori, 2012). Our results suggest that neglecting these differences may lead to erroneous results, notably an overestimation of environmental effects on FCM levels: Several predictors which had no significant effect in the full models (accounting for inter-individual variance) were found to be significant in the naïve models (Table 2). Moreover, the latter models showed strange, ecologically meaningless effect patterns, partly due to the fact that they were more prone to overfitting (Figures 3 and 4). These findings indicate that one should be cautious when interpreting results without information on the number of individuals sampled (Rehnus & Palme, 2017) and their respective resampling rates. If a genetic assignment is not possible, due to financial or other constraints, the sampling design should be adapted so as to maximize the number of sampled individuals while simultaneously minimizing repeated sampling of the same individual. While extending the sampling area will increase the chance of sampling many individuals, the latter bias may be reduced by applying an adequate minimum distance between samples (e.g. corresponding to the territory size in territorial species). Another method would be to attribute samples found within close distance to the same individual (Thiel et al., 2008, 2011). This could, however, further blur the results if the samples of two or more individuals are erroneously pooled. Using genetic analysis to obtain information on sex and individual is therefore a major advantage, especially for non-territorial, elusive and disturbance-sensitive species, where samples have to be collected non-invasively and without observing the individual (Rehnus & Palme, 2017).

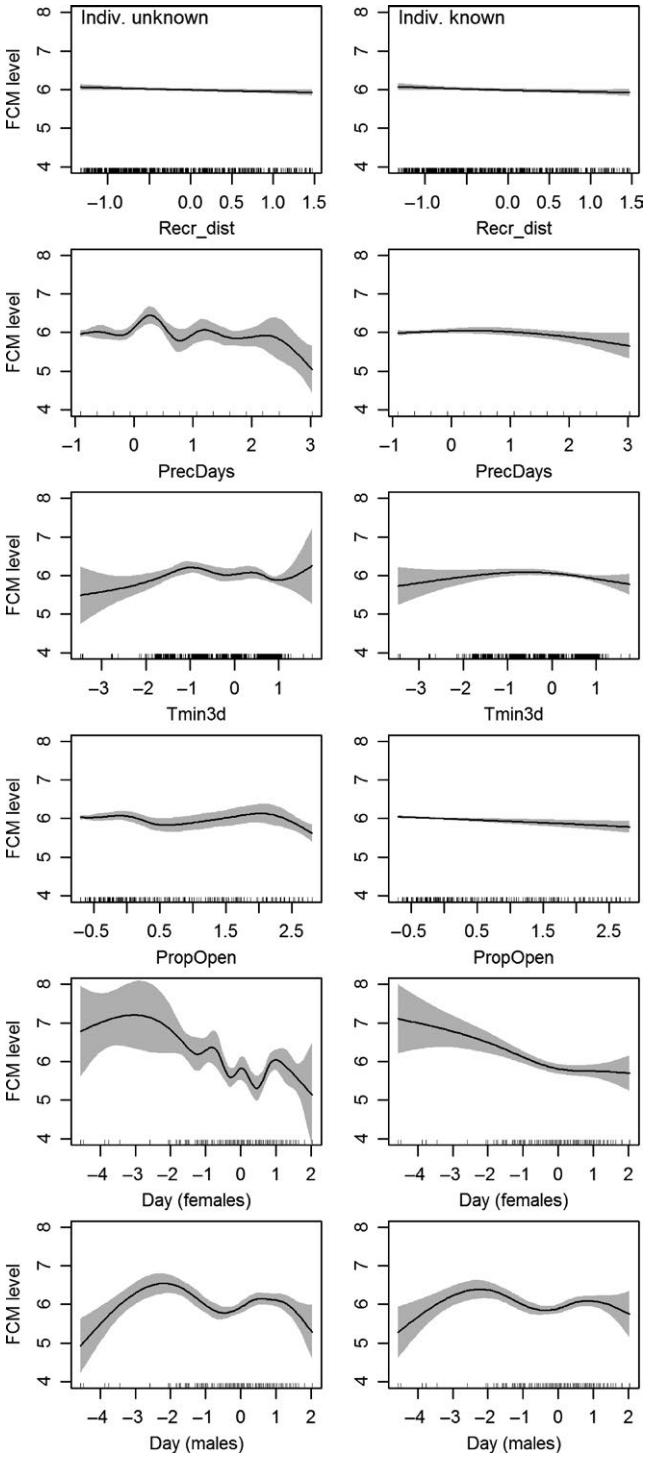

FIGURE 3 Effect plots showing faecal corticosteroid metabolites levels as a function of the environmental predictor variables, measured at the local scale (i.e. within a 20 m radius) for the models excluding (left) and including (right) information on individual heterogeneity. Grey areas indicate the 95% confidence intervals conditional on the estimated smoothing parameters of the model, while holding all other covariates at the mean. Variable codes and descriptions are provided in Table 1

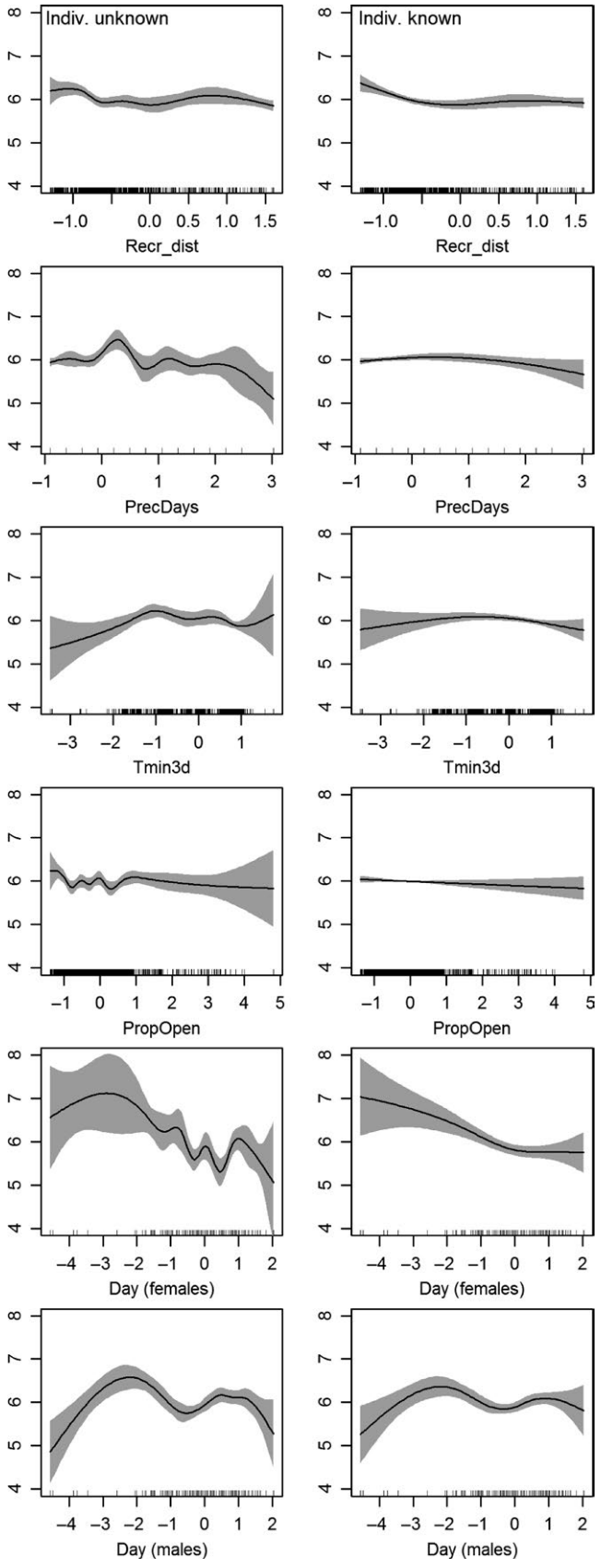

FIGURE 4 Effect plots showing faecal corticosteroid metabolites levels as a function of the environmental predictor variables, measured at the home-range scale (i.e. within a 400 m radius) for models excluding (left) and including (right) information on individual heterogeneity. Grey areas indicate the 95% confidence intervals conditional on the estimated smoothing parameters of the model, while holding all other covariates at the mean. Variable codes and descriptions are provided in Table 1

Despite the large proportion of variance explained by the individual animal, we could still confirm significant environmental effects on FCM levels in capercaillie. The strongest effect was found for human recreation at the home-range scale (Table 2), even though we did not account for the number and distribution of recreationists within the area and between years, but only focused on recreation infrastructure. Birds exposed to high densities of recreation infrastructure within their winter-home range showed elevated FCM levels, this effect levelled off, however, when the average distance of recreation infrastructure within the 400 m radius exceeded 180 m. A similar pattern, with an effect up to 500 m was found by Thiel et al. (2011). The difference between their and our threshold can most likely be attributed to averaging within 400 m in our study, the fact, however, that effects of recreational infrastructure on capercaillie FCM levels are only measurable up to a certain distance, is also supported by behavioural studies (e.g. Coppes et al., 2017).

Habitat quality is likely to affect FCM levels (Davies et al., 2013; Rangel-Negrın et al., 2009; Suorsa et al., 2003); therefore, we expected to find lower FCM levels in samples from areas with a high proportion of open forest representing the habitat favoured by capercaillie, (Rolstad & Wegge, 1987; Storch, 1995, 2002) compared to dense forests, which represent less suitable habitats. This hypothesis was supported at the local scale, where FCM levels were significantly lower at locations with a high proportion of open forest in the immediate vicinity (Table 2, Figure S2). We did not find this effect at the home-range scale, though (i.e. within 400 m radius), possibly due to the fact that there are only few, scattered and small areas with open forest in the Black Forest, which only marginally affect values when averaging the canopy cover within a 400 m radius (home-range scale).

Interestingly, we found strong seasonal patterns in FCM levels, which differed markedly between the sexes (Figure S3). For females, the highest FCM levels were detected during early winter when the first snow appeared, their level dropped later in winter. This pattern may be linked to food constraints: During winter, capercaillie feed almost exclusively on conifer needles, a low-caloric food which is hard to digest (Klaus et al., 1989). Towards the end of winter additional new food sources, especially buds of trees and dwarf shrubs are available. This may explain a decrease in FCM levels in females, which strongly depend on sufficient energy supplies to be in good conditions for reproduction (Schoech et al., 2007). For males, we found two distinctive FCM-peaks. Although the first peak during mid-winter (January) might be due to the start of winter conditions, and associated change to a low-caloric diet, the second peak at the end of winter (April) is very likely linked to the start of the mating season. Capercaillie are polygynous birds, at the end of winter males display and defend territories at a lekking site to attract females

(Klaus et al., 1989). This competitive mating behaviour is likely to contribute elevated stress levels in male capercaillie (Figures 3 and 4; Thiel et al., 2011).

In addition, we expected that weather conditions affect animal physiology and Thiel et al. (2011) found increased FCM levels in capercaillie in cold conditions. Our model confirmed these results for the local scale: With colder temperatures in the 3 days before the collection of the samples, significantly higher FCM levels were recorded (Table 2 and Figure 3).

Finally, due to a lack of reliable data across the large extent of the study area, we were not able to test for potential predator effects. The presence of predators can be an important driver for increased glucocorticoid levels in prey species (Sheriff et al., 2009) and high predator densities were the main factor affecting FCM levels in rabbits *Oryctolagus cuniculus* (Monclús et al., 2009). Collecting sound data and including this potential stressor in the models would therefore be an important subject to be addressed in further studies.

5 | CONCLUSIONS

We demonstrate the importance of including inter-individual differences when studying FCM-levels in wildlife. Individual effects may account for the vast majority of variance in FCM levels and may lead to erroneous results, in our case an overestimation of environmental effects, when disregarded. Adding to the benefits of using FCM instead of invasive blood samples, we see it as a major advantage to combine genetic analysis with FCM measurements to gain more knowledge on the endogenous and exogenous factors influencing FCM levels in wildlife. If genetic individual assessment is not possible, we recommend avoiding pseudo-replication by adopting a sampling strategy that reduces multiple sampling of the same individual. Furthermore, as we found strong sex-specific and seasonal FCM patterns, distinguishing between sexes and ensuring that samples are collected at the same time of season when comparing different areas are of crucial importance for correctly appraising the effects of environmental and human-induced "stressors" affecting FCM levels in wildlife.

ACKNOWLEDGEMENTS

We are grateful to all field-assistants, foresters, ornithologists and hunters who helped collecting samples according to the field protocols. We thank Petra Adler and Selina Ganz for the help with preparing the aerial imagery to assess the proportion of open forest. Thanks also to Leonie Culmann for preparing the samples, Edith Klobetz-Rassam for running the EIAs and Sandra Würstlin for performing the DNA extraction and PCR. Rudi Suchant, Ursula Nopp-Mayr and Veronika Grünschachner-Berger supported the study and helped improve the manuscript. We thank James David Hale for language proofreading.

AUTHORS' CONTRIBUTIONS

V.B., J.-L.K., M.W. and J.C. conceived the ideas and designed methodology; J.C., M.W. collected and prepared the field data; R.P. supervised and analysed the corticosteroids; A.K. supervised the genetic analysis; J.-L.K. performed the statistical analysis; J.C., V.B. and J.-L.K. led the writing of the original manuscript. All authors contributed critically to the drafts and gave final approval for publication.

DATA ACCESSIBILITY

Data available from the Dryad Digital Repository https://doi.org/10.5061/dryad.mr62h68 (Coppes et al., 2018).

ORCID

Joy Coppes http://orcid.org/0000-0002-5295-8638

REFERENCES

Arlettaz, R., Nusslé, S., Baltic, M., Vogel, P., Palme, R., Jenni-Eiermann, S., … Genoud, M. (2015). Disturbance of wildlife by outdoor winter recreation: Allostatic stress response and altered activity-energy budgets. *Ecological Applications*, *25*, 1197–1212. https://doi.org/10.1890/14-1141.1

Arlettaz, R., Patthey, P., Baltic, M., Leu, T., Schaub, M., Palme, R., & Jenni-Eiermann, S. (2007). Spreading free-riding snow sports represent a novel serious threat for wildlife. *Proceedings of the Royal Society B. Series Biological Sciences*, *274*, 1219–1224. https://doi.org/10.1098/rspb.2006.0434

Bates, D., Mächler, M., Bolker, B., & Walker, S. (2015). Fitting linear mixed-effects models using lme4. *Journal of Statistical Software*, *67*, 1–48.

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

Braunisch, V., Coppes, J., Schmid, H., Suchant, R., Arlettaz, R., & Bollmann, K. (2013). Selecting from correlated climate variables: A major source of uncertainty for predicting species distributions under climate change. *Ecography*, *36*, 1–13.

Braunisch, V., Segelbacher, G., & Hirzel, A. H. (2010). Modelling functional landscape connectivity from genetic population structure: A new spatially explicit approach. *Molecular Ecology*, *19*, 3664–3678. https://doi.org/10.1111/j.1365-294X.2010.04703.x

Broom, D. M., & Johnson, K. G. (1993). *Stress and animal welfare*. London, UK: Chapman & Hall. https://doi.org/10.1007/978-94-024-0980-2

Buehler, D. M., Bhola, N., Barjaktarov, D., Goymann, W., Schwabl, I., Tieleman, B. I., & Piersma, T. (2008). Constitutive immune function responds more slowly to handling stress than corticosterone in a shorebird. *Physiological and Biochemical Zoology*, *81*, 673–681. https://doi.org/10.1086/588591

Cockrem, J. (2007). Stress, corticosterone responses and avian personalities. *Journal of Ornithology*, *148*, 169–178. https://doi.org/10.1007/s10336-007-0175-8

Cockrem, J., & Silverin, B. (2002). Variation within and between birds in corticosterone responses of great tits (*Parus major*). *General and Comparative Endocrinology*, *125*, 197–206. https://doi.org/10.1006/gcen.2001.7750

Coppes, J., Ehrlacher, J., Müller, G., Roth, K., Schroth, K.-E., Braunisch, V., & Suchant, R. (2016). Decline in capercaillie *Tetrao urogallus* numbers and distribution area in the Black Forest. *Der Ornithologische Beobachter*, *113*, 235–248.

Coppes, J., Ehrlacher, J., Thiel, D., Suchant, R., & Braunisch, V. (2017). Outdoor recreation causes effective habitat reduction in capercaillie *Tetrao urogallus*: A major threat for geographically restricted populations. *Journal of Avian Biology*, *48*, 1583–1594. https://doi.org/10.1111/jav.01239

Coppes, J., Kämmerle, J.-L., Willert, W., Kohnen, A., Palme, R., & Braunisch, V. (2018). Data from: The importance of individual heterogeneity for interpreting faecal glucocorticoid metabolite levels in wildlife studies. *Dryad Digital Repository*, https://doi.org/10.5061/dryad.mr62h68

Corlatti, L., Palme, R., Frey-Roos, F., & Hackländer, K. (2011). Climatic cues and glucocorticoids in a free-ranging riparian population of red deer (*Cervus elaphus*). *Folia Zoologica*, *60*, 176–180. https://doi.org/10.25225/fozo.v60.i2.a1.2011

Cyr, N., Earle, K., Tam, C., & Romero, L. M. (2007). The effect of chronic psychological stress on corticosterone, plasma metabolites, and immune responsiveness in European starlings. *General and Comparative Endocrinology*, *154*, 59–66. https://doi.org/10.1016/j.ygcen.2007.06.016

Davies, N. A., Gramotnev, G., McAlpine, C., Seabrook, L., Baxter, G., Lunney, D, … Bradley, A. (2013). Physiological stress in koala populations near the arid edge of their distribution. *PLoS ONE*, *8*, e79136. https://doi.org/79110.71371/journal.pone.0079136 https://doi.org/10.1371/journal.pone.0079136

Dickens, M. J., & Romero, L. M. (2013). A consensus endocrine profile for chronically stressed wild animals does not exist. *General and Comparative Endocrinology*, *191*, 177–189. https://doi.org/10.1016/j.ygcen.2013.06.014

Dormann, C. F., Elith, J., Bacher, S., Buchmann, C., Carl, G., Carré, G., … Lautenbach, S. (2012). Collinearity: A review of methods to deal with it and a simulation study evaluating their performance. *Ecography*, *36*, 27–46.

Formenti, N., Vigano, R., Bionda, R., Ferrari, N., Trogu, T., Lanfranchi, P., & Palme, R. (2015). Increased hormonal stress reactions induced in an Alpine Black Grouse (*Tetrao tetrix*) population by winter sports. *Journal of Ornithology*, *156*, 317–321. https://doi.org/10.1007/s10336-014-1103-3

Frigerio, D., Dittami, J., Möstl, E., & Kotrschal, K. (2004). Excreted corticosterone metabolites co-vary with air temperature and air pressure in male Greylag geese (*Anser anser*). *General and Comparative Endocrinology*, *137*, 29–36. https://doi.org/10.1016/j.ygcen.2004.02.013

Ganswindt, A., Tordiffe, A., Stam, E., Howitt, M., & Jori, F. (2012). Determining adrenocortical endocrine activity as a measure of stress in African buffalo (*Syncerus caffer*) based on faecal analysis. *African Zoology*, *47*, 261–269. https://doi.org/10.1080/15627020.2012.11407558

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

Graf, R. F., Mathys, L., & Bollmann, K. (2009). Habitat assessment for forest dwelling species using LiDAR remote sensing: Capercaillie in the Alps. *Forest Ecology and Management*, *257*, 160–167. https://doi.org/10.1016/j.foreco.2008.08.021

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

Huntley, B., Green, R. E., Collingham, Y. C., & Willis, S. G. (2007). *A climatic atlas of European breeding birds*. Barcelona, ES: Lynx Edicions.

Jacob, G., Debrunner, R., Gugerli, F., Schmid, B., & Bollmann, K. (2010). Field surveys of capercaillie (*Tetrao urogallus*) in the Swiss Alps underestimated local abundance of the species as revealed by genetic analyses of non-invasive samples. *Conservation Genetics*, *11*, 33–44. https://doi.org/10.1007/s10592-008-9794-8

Jenni-Eiermann, S., Glaus, E., Grübler, M., Schabl, H., & Jenni, L. (2008). Glucocorticoid response to food availability in breeding barn swallows (*Hirundo rustica*). *General and Comparative Endocrinology*, *155*, 558–565. https://doi.org/10.1016/j.ygcen.2007.08.011

Kahn, N. W., St. John, J., & Quin, T. W. (1998). Chromosome-specific intron size differences in the Avian CHD gene provide an efficient method for sex identification in birds. *The Auk*, *115*, 1074–1078.

Kämmerle, J.-L., Coppes, J., Ciuti, S., Suchant, R., & Storch, I. (2017). Range loss of a threatened grouse species is related to the relative abundance of a mesopredator. *Ecosphere*, *8*, e01934. 01910.01002/ecs01932.01934. https://doi.org/10.1002/ecs2.1934

Kändler, G., & Cullmann, D. (2014). *Der Wald in Baden-Württemberg. Ausgewählte Ergebnisse der dritten Bundeswaldinventur*. Freiburg, DE: Forstliche Versuchs- und Forschungsanstalt Baden-Württemberg.

Klaus, S., Andreev, V., Bergmann, H. H., Müller, F., Porkert, J., & Wiesner, J. (1989). *Die Auerhühner*. Magdeburg, DE: Westarp Wissenschaften.

Liston, G., & Elder, K. (2006). A meteorological distribution system for high-resolution terrestrial modeling (MicroMet). *Journal of Hydrometeorology*, *7*, 217–234. https://doi.org/10.1175/JHM486.1

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

Moberg, G. P., & Mench, J. A. (2000). *The biology of animal stress: Basic principles and implications for animal welfare*. Wallingford, UK: CABI Publishing.

Monclús, R., Palomares, F., Tablado, Z., Martínez-Fontúrbel, A., & Palme, R. (2009). Testing the threat-sensitive predator avoidance hypothesis: Physiological responses and predator pressure in wild rabbits. *Oecologia*, *158*, 615–623. https://doi.org/10.1007/s00442-008-1201-0

Möstl, E., Maggs, J. L., Schrötter, G., Besenfelder, U., & Palme, R. (2002). Measurement of cortisol metabolites in faeces of ruminants. *Veterinary Research Communications*, *26*, 127–139. https://doi.org/10.1023/A:1014095618125

Möstl, E., & Palme, R. (2002). Hormones as indicators of stress. *Domestic Animal Endocrinology*, *23*, 67–74. https://doi.org/10.1016/S0739-7240(02)00146-7

Nakagawa, S., & Schielzeth, H. (2010). Repeatability for Gaussian and non-Gaussian data: A practical guide for biologists. *Biological Reviews*, *85*, 935–956.

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

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

Palme, R., Touma, C., Arias, N., Dominchin, M. F., & Lepschy, M. (2013). Steroid extraction: Get the best out of faecal samples. *Wiener Tierärztl Monatsschrift – Veterinary Medicine Austria*, *100*, 238–246.

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

Preisser, E. L., Bolnick, D. I., & Benard, M. F. (2005). Scared to death? The effects of intimidation and consumption in predator-prey interactions. *Ecology and Society*, *86*, 501–509. https://doi.org/10.1890/04-0719

R Development Core Team. (2017). *R: A language and environment for statistical computing*. Vienna, Austria: R Foundation for Statistical Computing. ISBN 3-900051-07-0. Retrieved from: http://www.R-project.org

Rangel-Negrın, A., Alfaro, J. L., Valdez, R. A., Roman, M. C., & Serio-Silva, J. C. (2009). Stress in Yucatan spider monkeys: Effects of environmental conditions on fecal cortisol levels in wild and

captive populations. *Animal Conservation*, *12*, 496–502. https://doi.org/10.1111/j.1469-1795.2009.00280.x

Rehnus, M., & Palme, R. (2017). How genetic data improve the interpretation of results of faecal glucocorticoid metabolite measurements in a free-living population. *PLoS ONE*, *12*, e0183718. https://doi.org/0183710.0181371/journal.pone.0183718 https://doi.org/10.1371/journal.pone.0183718

Rettenbacher, S., Möstl, E., Hackl, R., Ghareeb, K., & Palme, R. (2004). Measurement of corticosterone metabolites in chicken droppings. *British Poultry Science*, *45*, 704–711. https://doi.org/10.1080/00071660400006156

Rolstad, J., & Wegge, P. (1987). Habitat characteristics of capercaillie *Tetrao urogallus* display grounds in southeastern Norway. *Holarctic Ecology*, *10*, 219–229.

Sapolsky, R. M. (2002). Endocrinology of the stress-response. In J. Becker, S. Breedlove, S. Crews & M. McCarthy(Eds.), *Behavioral endocrinology* (2nd ed., pp. 409–450). Cambridge, MA: MIT Press.

Sapolsky, R. M., Romero, L. M., & Minck, A. U. (2000). How do glucocorticoids influence stress responses? Integrating permissive, suppressive, stimulatory and preparative actions. *Endocrine Reviews*, *21*, 55–89.

Schmitz, O. J., Beckerman, A. P., & O'Brien, K. M. (1997). Behaviorally mediated throphic cascades: Effects of predation risk on food web interactions. *Ecology*, *78*, 1388–1399. https://doi.org/10.1890/0012-9658(1997)078[1388:BMTCEO]2.0.CO;2

Schoech, S. J., Bowman, R., Bridge, E. S., & Boughton, R. K. (2007). Baseline and acute levels of corticosterone in Florida Scrub-Jays (*Aphelocoma coerulescens*): Effects of food supplementation, suburban habitat, and year. *General and Comparative Endocrinology*, *154*, 150–160. https://doi.org/10.1016/j.ygcen.2007.05.027

Segelbacher, G., Höglund, J., & Storch, I. (2003). From connectivity to isolation: Genetic consequences of population fragmentation in capercaillie across Europe. *Molecular Ecology*, *12*, 1773–1780. https://doi.org/10.1046/j.1365-294X.2003.01873.x

Sheriff, M. J., Dantzer, B., Delehanty, B., Palme, R., & Boonstra, R. (2011). Measuring stress in wildlife: Techniques for quantifying glucocorticoids. *Oecologia*, *166*, 869–887. https://doi.org/10.1007/s00442-011-1943-y

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

Stier, K. S., Almasi, B., Gasparini, J., Piault, R., Roulin, A., & Jenni, L. (2009). Effects of corticosterone on innate and humoral immune functions and oxidative stress in barn owl nestlings. *Journal of Experimental Biology*, *212*, 2085–2091. https://doi.org/10.1242/jeb.024406

Stoffel, M. A., Nakagawa, S., & Schielzeth, H. (2017). rptR: Repeatability estimation and variance decomposition by generalized linear mixed-effects models. *Methods in Ecology and Evolution*, *8*, 1639–1644. https://doi.org/10.1111/2041-210X.12797

Storch, I. (1995). Habitat requirements of capercaillie. S. 151–154. Proceedings of the 6th International Grouse Symposium, Udine, IT.

Storch, I. (2002). On spatial resolution in habitat models: Can small-scale forest structure explain capercaillie numbers? *Conservation Ecology*, *6*, 25.

Storch, I. (2007). *Grouse: Status survey and conservation action plan 2006-2010*. Gland, Switzerland/Fordingbridge, UK: IUCN/World Pheasant Association.

Storch, I. (2013). Human disturbance of grouse – Why and when? *Wildlife Biology*, *19*, 390–403. https://doi.org/10.2981/13-006

Suchant, R., & Braunisch, V. (2004). Multidimensional habitat modelling in forest management – A case study using capercaillie in the Black Forest, Germany. *Ecological Bulletins*, *51*, 455–649.

Summers, R. W., McFarlane, J., & Pearce-Higgins, J. (2007). Measuring avoidance by capercaillie *Tetrao urogallus* of woodlands close to tracks. *Wildlife Biology*, *13*, 19–27. https://doi.org/10.2981/0909-6396(2007)13[19:MABCTU]2.0.CO;2

Suorsa, P., Huhta, E., Nikula, A., Nikinmaa, M., Jäntii, A., Helle, H., & Hakkarainen, H. (2003). Forest management is associated with physiological stress in an old-growth forest passerine. *Proceedings of the Royal Society B: Biological Sciences*, *270*, 963–969. https://doi.org/10.1098/rspb.2002.2326

Thiel, D., Jenni-Eiermann, S., Braunisch, V., Palme, R., & Jenni, L. (2008). Ski tourism affects habitat use and evokes a physiological stress response in capercaillie *Tetrao urogallus*: A new methodological approach. *Journal of Applied Ecology*, *45*, 845–853.

Thiel, D., Jenni-Eiermann, S., & Palme, R. (2005). Measuring corticosterone metabolites in droppings of capercaillies (*Tetrao urogallus*). *Annals of the New York Academy of Sciences*, *1046*, 96–108. https://doi.org/10.1196/annals.1343.009

Thiel, D., Jenni-Eiermann, S., Palme, R., & Jenni, L. (2011). Winter tourism increases stress hormone levels in the capercaillie *Tetrao urogallus*. *IBIS*, *153*, 122–133. https://doi.org/10.1111/j.1474-919X.2010.01083.x

Thierry, A.-M., Ropert-Coudert, Y., & Raclot, T. (2013). Elevated corticosterone levels decrease reproductive output of chick-rearing Adélie penguins but do not affect chick mass at fledging. *Conservation Physiology*, *1*, 1–12.

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

Weingrill, T., David, A., Gray, D. A., Louise Barrett, L., & Henzi, S. P. (2004). Fecal cortisol levels in free-ranging female chacma baboons: Relationship to dominance, reproductive state and environmental factors. *Hormones and Behavior*, *45*, 259–269. https://doi.org/10.1016/j.yhbeh.2003.12.004

Wolak, M. E., Fairbairn, D. J., & Paulsen, Y. R. (2012). Guidelines for estimating repeatability. *Methods in Ecology and Evolution*, *3*, 129–137. https://doi.org/10.1111/j.2041-210X.2011.00125.x

Wood, S. N. (2004). Stable and efficient multiple smoothing parameter estimation for generalized additive models. *Journal of the American Statistical Association*, *99*, 673–686. https://doi.org/10.1198/016214504000000980

Wood, S. N. (2006). *Generalized additive models: An introduction with R*. Boca Raton, FL: Chapman and Hall/CRC.

Wood, S. N. (2011). Fast stable restricted maximum likelihood and marginal likelihood estimation of semiparametric generalized linear models. *Journal of the Royal Statistical Society*, *73*, 3–36. https://doi.org/10.1111/j.1467-9868.2010.00749.x

Wood, S., & Scheipl, F. (2017). gamm4: Generalized additive mixed models using 'mgcv' and 'lme4'. R package version 0.2-5. https://CRAN.R-project.org/package=gamm4.

Zielewska-Büttner, K., Adler, P., Ehmann, M., & Braunisch, V. (2016). Automated detection of forest gaps in spruce dominated stands using canopy height models derived from stereo aerial imagery. *Remote Sensing*, *8*, 175. https://doi.org/10.3390/rs8030175

Zuur, A. F., Ieno, E. N., Walker, N., Saveliev, A. A., & Smith, G. M. (2009). *Mixed effects models and extensions in ecology with R*. Newburgh, UK: Highland Statistics. https://doi.org/10.1007/978-0-387-87458-6

SUPPORTING INFORMATION

Additional Supporting Information may be found online in the supporting information tab for this article.

How to cite this article: Coppes J, Kämmerle J-L, Willert M, Kohnen A, Palme R, Braunisch V. The importance of individual heterogeneity for interpreting faecal glucocorticoid metabolite levels in wildlife studies. *J Appl Ecol*. 2018;55:2043–2054. https://doi.org/10.1111/1365-2664.13140