

A meta-analysis of human disturbance effects on glucocorticoid hormones in free-ranging wild vertebrates

Betzi Pérez-Ortega^{1,2,*}  and Andrew P. Hendry¹ 

¹*McGill University, Redpath Museum and Department of Biology, 859 Sherbrooke Street West, Montreal, Quebec H3A 0C4, Canada*

²*Smithsonian Tropical Research Institute, PO Box 0843-03092, Panama, Republic of Panama*

ABSTRACT

Free-ranging wild vertebrates need to cope with natural and anthropogenic stressors that cause short and/or long-term behavioural and physiological responses. In areas of high human disturbance, the use of glucocorticoid (GC) hormones as biomarkers to measure stress responses is an increasingly common tool for understanding how animals cope with human disturbance. We conducted a meta-analysis to investigate how human disturbances such as habitat conversion, habitat degradation, and ecotourism influence baseline GC hormones of free-ranging wild vertebrates, and we further test the role of protected areas in reducing the impact of such disturbances on these hormones. A total of 58 studies met the inclusion criteria, providing 152 data points for comparing levels of GC hormones under disturbed and undisturbed conditions. The overall effect size suggests that human disturbance does not cause a consistent increase in levels of GC hormones (Hedges' $g = 0.307$, 95% CI = -0.062 to 0.677). However, when the data were analysed by disturbance type, living in unprotected areas or in areas with habitat conversion were found to increase GC hormone levels compared to living in protected or undisturbed areas. By contrast, we found no evidence that ecotourism or habitat degradation generates a consistent increase in baseline GC hormone levels. Among taxonomic groups, mammals appeared more sensitive to human disturbance than birds. We advocate the use of GC hormones for inferring major human-caused contributors to the stress levels of free-ranging wild vertebrates – although such information needs to be combined with other measures of stress and interpreted in the context of an organism's life history, behaviour, and history of interactions with human disturbance.

Key words: stress, glucocorticoid, cortisol, corticosterone, conservation physiology, human disturbance, systematic review, free-ranging wild vertebrates.

CONTENTS

I. Introduction	1460
II. Methods	1461
(1) Literature search and inclusion criteria	1461
(2) Effect size calculation and statistical analysis	1462
(3) Publication bias and heterogeneity	1463
III. Results	1463
IV. Discussion	1464
(1) Effects of human disturbance type	1464
(2) Variation among taxa in their responses to human disturbance	1466
(3) The role of protected areas	1467
V. Conclusions	1468
VI. Acknowledgements	1468

* Author for correspondence (Tel.: +507 6845 3482; E-mail: betziperez@yahoo.com).

VII. Author contributions	1468
VIII. References	1468
IX. Supporting information	1471

I. INTRODUCTION

Free-ranging wild vertebrates (i.e. not restrained, not feral, not raised or tested in laboratories or cages or enclosures) can be affected by a wide variety of ‘natural’ stressors, such as predation risk (Boonstra *et al.*, 1998), parasite infection (Seguel *et al.*, 2019; Defolie, Merklung & Fichtel, 2020), social interactions (Goymann & Wingfield, 2004; Sapolsky, 2005), and low food abundance (Bonier *et al.*, 2009). To the suite of natural stressors, modern times have added increasing human population sizes and their diverse environmental impacts (Hofer & East, 1998). Human-induced stressors can take many forms, including noise, light, deforestation, ecotourism, hunting, harvesting, and pollution (e.g. Thiel *et al.*, 2011; Balestri *et al.*, 2014; Blair *et al.*, 2016; Perez-Ortega *et al.*, 2021). These natural and anthropogenic changes (stressors) can produce stress responses that manifest as various physiological, behavioural, and psychological changes in animals (Romero & Butler, 2007).

The immediate physiological response to an external stressor is partly regulated by the hypothalamus–pituitary–adrenal (HPA) axis in mammals and birds, and the hypothalamus–pituitary–interrenal (HPI) axis in fish, reptiles, and amphibians. A common response of these axes to stress is the release of the glucocorticoid (GC) hormones cortisol and corticosterone (Romero, 2004). Both hormones are found in all vertebrate species; however, the stress response in fish and most mammals relies primarily on cortisol, whereas corticosterone plays this role in birds, reptiles, and amphibians (Romero & Butler, 2007; Sapolsky, 2002). Under normal conditions, GC hormones circulate in low to moderate levels and play an important role in energy regulation and homeostasis (Tsang, Barclay & Oster, 2014); that is, the maintenance of stability in parameters such as body temperature, blood glucose levels, blood pressure, and pH (Billman, 2020). During a stressful event, an acute stress response is activated that increases the level of circulating GC hormones, promoting gluconeogenesis, mobilisation of amino acids, and inhibition of the inflammatory response. These changes can serve to ensure a continued readiness to respond to recurring stresses (Sapolsky, 2002). However, these acute adaptive responses, when activated repeatedly, will direct energy away from growth, digestion, reproduction, and immune processes, thus generating a negative physiological impact typically referred to as chronic stress (Kirby *et al.*, 2009; Sapolsky, 2002; Boonstra *et al.*, 2001).

Given these expected associations between human disturbance, stress, and GC hormones, Wikelski & Cooke (2006) suggested that measuring GC hormone levels of wild organisms could be a valuable tool to understand the physiological responses of these organisms to their changed environment.

However, GC levels can differ depending on the species, age, and sex of individuals (Iglesias-Carrasco *et al.*, 2020) as well as the type, intensity, and duration of a given disturbance (Busch & Hayward, 2009) – making it hard to interpret what levels of hormones indicate chronic stress. Fortunately, the number of studies that examine relationships between GC hormones and human disturbance has increased dramatically in recent years, allowing scientists to identify patterns across species and disturbance types that can inform management decisions. For example, Dantzer *et al.* (2014) performed an early meta-analysis of the effects of human disturbance on GC hormone levels in free-ranging vertebrates. From there, Messina *et al.* (2018) conducted a meta-analysis to evaluate changes in GC hormones specifically in response to forest degradation in birds and mammals. More recently, Iglesias-Carrasco *et al.* (2020) conducted a meta-analysis on the effect of urbanisation on baseline and stress-induced GC hormone levels in wild vertebrates, and Kaisin *et al.* (2021) focused a meta-analysis on the effects of human disturbance in GC levels of wild primates. Our meta-analysis builds on these previous comparative analyses.

The overall conclusion of previous work is that, as long as interpretations are conditioned by the natural history of the organism under study, GC hormone assays can suggest stress levels that can inform appropriate management protocols, recovery strategies, and monitoring efforts (Coetzee & Chown, 2016; Busch & Hayward, 2009; Wikelski & Cooke, 2006). For example, some colonies of the African penguin (*Spheniscus demersus*) tolerate human exposure much better than others, highlighting the importance of context-specific measured stress responses (Pichegru *et al.*, 2016). Similarly, Magellan penguin (*Spheniscus magellanicus*) chicks do not habituate to human exposure as well as do adults (Walker, Boersma & Wingfield, 2005), suggesting that reducing human visitors during the reproductive season could mitigate negative impacts. Similar uses of stress hormones to infer – and then mitigate – human-induced stress on free-ranging invertebrates continue to expand across taxa and disturbance types (Adamo, 2012).

We conducted a meta-analysis to investigate how different types of human disturbance, such as habitat conversion, habitat degradation, and ecotourism, can affect baseline GC hormone levels of free-ranging wild vertebrates. Importantly, we also test for the role of protected areas in reducing the impact of such disturbances on these hormones. We specifically addressed the following questions: (i) what types of human disturbance cause the greatest increase in baseline GC hormones, with disturbance types classified into three categories: habitat conversion, habitat degradation, and ecotourism? (ii) To what extent do we see variation among taxa (e.g. mammals and birds) in their responses to human

disturbance – and can such differences be understood in light of their relative exposure to those disturbances or by their behaviour or life history? (iii) Do GC hormone levels differ between animals in protected areas *versus* those in unprotected areas? For instance, we might expect lower baseline GC hormone levels in protected areas as a result of reduced levels of disturbance. In answering these questions, we hope to improve our understanding of the utility of GC hormones in the conservation and management of animals and their habitats.

II. METHODS

(1) Literature search and inclusion criteria

A systematic literature search was conducted on July 15, 2019, with an update on October 1st, 2021. The search was conducted using the *Web of Science* database to identify English-language studies of the effect of human disturbance on the level of GC hormones in free-ranging wild vertebrates. The searches used all possible combinations of the following words, present either in the title, keywords, and/or abstract: (stress response OR cortisol OR glucocorticoid OR corticosterone) AND (free-ranging animals OR free-living animals OR wild

population) AND (anthropogenic impact OR human impact OR anthropogenic disturbance) AND (ecotourism OR habitat loss OR urbanisation). The search returned 1,787 studies, for which we then manually screened the titles and abstracts to remove duplicates and studies not relevant to our questions, resulting in 168 papers for detailed assessment. We also examined the cited literature to find additional papers not revealed by the online searches. A PRISMA flow diagram (Moher *et al.*, 2009) detailing the number of studies identified by the search, and the selection process is provided in Fig. 1.

The following criteria were used to select studies for inclusion in the analysis: (i) the study had to investigate free-ranging wild vertebrates rather than domestic, laboratory, or wild animals in captivity (e.g. cages or zoos). (ii) Stress was not induced by the investigator due to manipulations such as the administration of exogenous hormones, translocation, restraint challenge, playbacks, controlled laboratory conditions. (iii) The study had to report baseline GC hormone measurements for both disturbed (experimental group) and undisturbed (control group) conditions (details on these categories are provided in Section II.2). In the original studies, baseline GC was defined as the concentration of the hormones prior to its elevation in response to a stressor; therefore, it was expected that animals under disturbed conditions showing elevated baseline GC levels were

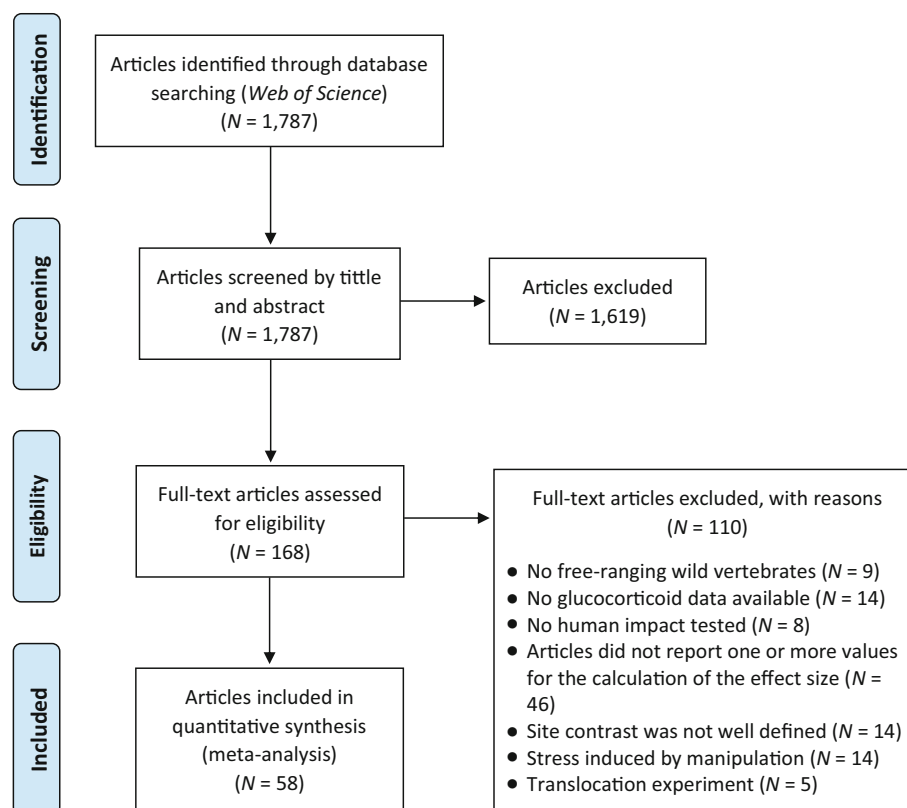


Fig. 1. PRISMA flow diagram detailing the number of studies identified by the search, and the selection process for inclusion of studies in our meta-analysis.

experiencing chronic stress. (iv) For studies using blood samples to measure baseline GC hormone levels, capture and manipulation time of the animals had to be less than 3 min – as recommended by Romero & Reed (2005). For hair, faeces, saliva, or feathers, no such time limit was imposed because GC hormone levels in these matrices are more stable (see online supporting information, Appendix S1). (v) The study had to report mean values and either standard deviation (SD) or standard errors (SE) of GC hormone levels, as well as sample sizes (N) for both disturbed and undisturbed conditions. Finally, our study employed a paired design, with disturbed and undisturbed areas (or populations) paired by study. This is a particularly powerful design for inferring the effects of treatment while controlling for (some) variation among studies.

Of the 168 studies, 58 met the above criteria for inclusion (Fig. S1, Table S1), including studies on birds (32 species), mammals (31 species), amphibians (one species), and reptiles (four species); and 110 studies were excluded (see online supporting information, Appendix S2). As many studies reported more than one sampling location (often with different disturbance types) or more than one species, we obtained 152 data points from these 58 studies with which to compare baseline levels of GC hormones in free-ranging wild vertebrates under disturbed *versus* undisturbed conditions. For each study selected, we recorded the type of disturbance, taxonomic group, location of the study (inside and/or outside of a protected area), the mean baseline GC hormone value and either the SD or SE of the mean value, and sample size from both disturbed and undisturbed conditions. When the data were reported for a gradient (e.g. urban-to-rural gradient), we selected the most extreme values of the gradient for comparison. Additional data extracted included sex (male or female), age group (adult or non-adult), and matrix type (i.e. blood, faeces, feathers, hair). When the original studies controlled for sex and/or age group and reported separate values, we considered each of those values as a different data point (note: our analysis controls for ‘study’ as a random effect – see Section II.2). However, for some studies it was not possible to determine the sex or age group of the animals sampled because the samples were opportunistically collected in the field or because the data were reported as an average. In these studies, if differences between sex and age groups were not specified by the authors, we assumed no significant differences and that the reported values included all sex and/or age groups. Additional analysis to estimate the effect size for each of these categorical moderator variables is provided in Fig. S1. All data were extracted from published tables and text, or when necessary, from figures using Plot Digitizer v1.9. All searches and assessments were conducted by B. P.-O.

(2) Effect size calculation and statistical analysis

The data were grouped within three moderators: disturbance type, taxonomic group, and land protection. Disturbance type was divided into three categories: (i) ‘Habitat

conversion’ included urbanisation, agriculture, wind farms, and cattle ranches. (ii) ‘Habitat degradation’ included studies that classified the disturbance as logging, forest fragmentation, and edge effects. (iii) ‘Ecotourism’ included activities that promote access to wildlife in their natural habitat or the use of their habitat for recreation (e.g. hiking, snowshoeing, skiing). The presence of humans in these ‘Ecotourism’ environments is higher during daylight hours and during some periods of the year than others (e.g. summer *versus* winter). Taxonomic group included four taxa: birds, mammals, amphibians, and reptiles. Land protection was divided into three habitat contrasts depending on where the study was performed: (i) ‘Outside *versus* inside protected area’ was used when the experimental group (disturbed condition) was sampled in unprotected areas whereas the control group (undisturbed condition) was sampled inside protected areas. (ii) ‘High *versus* low disturbance inside protected area’ was used when both samples (experimental and control) were collected within protected areas but in sub-areas characterised by different levels of human disturbance. (iii) ‘High *versus* low disturbance outside protected area’ was used when both samples (experimental and control) were collected in unprotected areas, but in sub-areas characterised by different levels of human disturbance. Protected areas were natural areas legally protected by the government under any category (e.g. national parks, natural monuments, reserves) or were private nature reserves.

In the studies included in our meta-analysis, GC hormones were extracted from a variety of different sample matrices (blood, feathers, hair, or faeces), with the most common measurement methods being enzyme-immunoassay (EIA), radio-immunoassay (RIA), and liquid chromatography with tandem mass spectrometry (LC-MS/MS). Our explicitly paired design could account for this among-study variation. Standardised mean difference Hedges’ g , multiplied by a small sample bias correction factor (Hedges & Olkin, 1985), was used to calculate the effect size for each pair according to the equation:

$$\text{Hedges}'g = \frac{M_1 - M_2}{\text{SD}_{\text{pooled}}} \times \left(1 - \frac{3}{4N - 9}\right)$$

where $M_1 - M_2$ = difference in means, $\text{SD}_{\text{pooled}}$ = pooled and weighted standard deviation, and N = total sample size of the study. For our study, M_1 was the mean GC value for the experimental (disturbed condition) group and M_2 was the mean GC value for the control (undisturbed condition) group. A positive effect size (experiment > control) suggests that anthropogenic disturbance increases GC levels, whereas negative values (experiment < control) suggest the opposite.

All four taxa (including all sexes and age groups) were included for the estimation of the meta-analytic mean and for the estimation of the effect size according to each level of the disturbance category and land protection moderators. Amphibians and reptiles were excluded from the taxonomic group analyses because the number of case studies involved four species or fewer. Calculation of the effect size and subsequent

analyses were conducted in R v.4.0.3 (R Core Team, 2020) and RStudio v.1.2.5042 (R Studio Team, 2020) using the *rma.mv* function in the *metafor* package v.2.4.0 (Viechtbauer, 2010). See online supporting information Appendix S3 for extracted data and Appendix S4 for R code.

Because multiple treatments sometimes were compared to the same control group (some studies contributed more than one effect size to the analysis) and due to the phylogenetic relatedness of species, the sampling variances were non-independent (i.e. the correlation between sampling variances >0) (Gleser & Olkin, 2009; Nakagawa & Santos, 2012). To account for this non-independence, we conducted a sensitivity analysis that assumes a correlation between sampling variances of repeatedly measured individuals, using a within-study covariance matrix, assuming a correlation coefficient of $r = 0.5$ (Wei & Higgins, 2013).

We started with an intercept-only meta-regression model using maximum likelihood (ML) to select the structure of the random effects. Three random effects were chosen to construct the models: study ID, species, and observation ID. After model selection using the Akaike Information Criterion (AIC) and variance components (σ^2), study ID and observation ID were retained as random effects (Table S2). We then estimated the meta-analytic mean (i.e. all data points) using an intercept-only meta-regression model with the two random effects retained in the previous model, and using restricted maximum likelihood (REML). Finally, we ran univariate meta-regression models (i.e. one moderator at a time) to estimate how much of the variation in GC hormone levels could be explained by each categorical moderator variable (disturbance type, taxonomic group, and land protection category). Finally, we used multivariate models to test the effect of disturbance type and land protection within each taxonomic group (mammals and birds only). We did not use a phylogenetic correction for three reasons. First, 'taxa' was a moderator that we were interested in exploring, and that implicitly includes (some) phylogenetic information. Second, we are interested in the effects on the subset of species and populations that are commonly studied using these methods. Thus, if particular groups (e.g. primates) are over-represented, we do not necessarily want to correct for that over-representation, beyond considering 'taxa' as a moderator. Third, recent assessments suggest that correction for phylogeny has little or no influence on conclusions (Foo *et al.*, 2017; Defolie *et al.*, 2020; Dougherty, 2020; Iglesias-Carrasco *et al.*, 2020).

(3) Publication bias and heterogeneity

A contour-enhanced funnel plot (Peters *et al.*, 2008) plotting the effect size against the inverse standard error (Sterne & Egger, 2001), and an Egger's regression test (Egger *et al.*, 1997), were used to identify and assess publication bias in the meta-analysis (Rothstein, Sutton & Borenstein, 2005; Nakagawa *et al.*, 2017). A funnel plot will appear asymmetrical when publication bias is present. To improve the interpretation of the funnel plot, Peters *et al.*

(2008) suggested adding a contour of statistical significance (i.e. $P < 0.05$ or 0.01). If studies appear to be missing in the area of low statistical significance, then it is possible that the asymmetry is due to publication bias, whereas if studies appear to be missing in the area of high statistical significance, then the asymmetry is assumed to be due to other factors such as heterogeneity, study size, and study quality (Peters *et al.*, 2008). The Egger's test systematically examines asymmetry of the funnel plot (Egger *et al.*, 1997), which is commonly visually inferred (e.g. Villar *et al.*, 1997). As an indicator of heterogeneity, we used the Q statistic (Hedges & Olkin, 1985), which follows a chi-squared distribution with $(k - 1)$ degrees of freedom (where $k =$ number of studies), and a $P < 0.05$. To assess heterogeneity, we calculated the I^2 index, which measures the percentage of variation not attributable to sampling error variance (Higgins & Thompson, 2002).

III. RESULTS

More than half (62.1%) of the 58 studies we analysed reported an increase in the baseline level of GC hormones due to some form of human disturbance. Figure 2 shows selected examples for three species of birds (Fig. 2A) and three species of mammal (Fig. 2B) to represent the variation of results from the original studies. Our meta-analysis revealed that, relative to undisturbed habitats, GC hormone levels were higher in habitats disturbed by habitat conversion, were higher outside than inside protected areas, and were higher for mammals in disturbed relative to undisturbed habitats (Fig. 3; Table 1). No other significant effects were found, although moderate effect sizes combined with limited study size suggest that additional significant effects may emerge after more studies are conducted. Below, we draw attention to several trends that warrant attention in future work.

The meta-analytic mean effect size (i.e. all data points) (0.307, 95% CI = -0.062 to 0.677), suggests that human disturbance tends to increase the levels of GC hormones, although this did not reach statistical significance, potentially due to large heterogeneity among studies (Table 1) examining different species and types of disturbance. The moderator disturbance categories ranked in order of estimated effect sizes were habitat conversion (0.653, 95% CI = 0.092 to 1.215), followed by ecotourism, then habitat degradation, although the effect size was significant only for habitat conversion (Fig. 3; Table 1). The positive effect size estimate for habitat conversion and perhaps for ecotourism suggests that human disturbances result in increased baseline levels of GC hormones. Mammals (0.562, 95% CI = 0.049 to 1.075) showed a significantly positive effect size, unlike birds (Fig. 3; Table 1). Finally, across all land protection categories, GC hormone levels were significantly higher outside protected areas than within them (1.200, 95% CI = 0.400 to 1.999). For the other land protection categories, effect sizes were not significant (Fig. 3; Table 1).

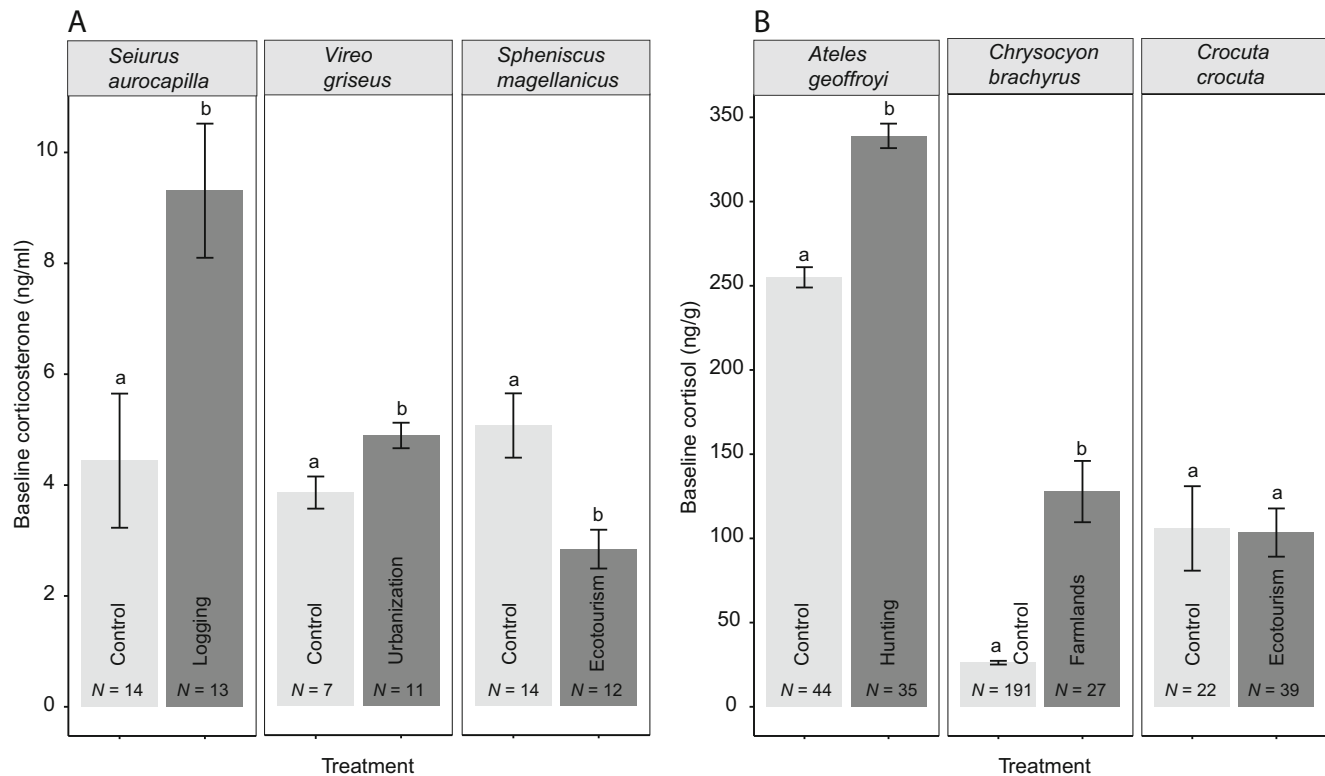


Fig. 2. Selected examples from the studies included in our meta-analysis showing the variation of baseline GC hormone levels for different species of birds (A) [*S. aurocapilla* (Leshyk *et al.*, 2012), *V. griseus* (Butler *et al.*, 2013), and *S. magellanicus* (Fowler, 1999)], and mammals (B) [*A. geoffroyi* (Ordóñez-Gómez *et al.*, 2016), *C. brachyurus* (Spercoski *et al.*, 2012), and *C. crocuta* (Van Meter *et al.*, 2009)] under different types of human disturbance. Values are means \pm SEM from the original studies. *N* is given within each column. Different letters above the bars indicate a statistically significant difference at $P = 0.05$.

We also tested for different effects of the disturbance and land protection categories within mammals and birds; too few species were available for amphibians ($N = 1$) and reptiles ($N = 4$). For mammals, disturbance categories ranked in order of effect size were habitat conversion (0.990, 95% CI = 0.297, 1.682), followed by ecotourism and habitat degradation, although effect sizes in the latter two were not significant (Fig. 4A; Table 2). In the case of birds, none of the effects were significant, although effect sizes were greatest for habitat conversion and ecotourism and were in the opposite direction for habitat degradation (Fig. 4B; Table 2). Mammals outside protected areas showed increased GC hormone levels compared to those inside protected areas (1.350, 95% CI = 0.489 to 2.210) (Fig. 4A; Table 2); with a similar, borderline non-significant, trend for birds (0.970, 95% CI = -0.058 to 1.997). There were no significant effects for birds or mammals inhabiting areas of high human disturbance compared with areas of low human disturbance, either inside or outside protected areas (Fig. 4; Table 2).

Overall heterogeneity (I^2) was 96.9% (Table 1). High heterogeneity values are commonly found in ecology and evolutionary studies (Senior *et al.*, 2016). Visual inspection of the contour-enhanced funnel plot (Fig. S2) indicated minimal, or no publication bias based on the statistical significance of

the study. An Egger's regression test provided no evidence of funnel plot asymmetry ($z = 1.338$, $P = 0.183$).

IV. DISCUSSION

In their meta-analysis, Dantzer *et al.* (2014) found that human disturbances were associated with increased levels of GC hormones in free-ranging vertebrates (0.32, 95% CI = 0.26 to 0.38). We identified the same trend, but our mean effect size did not reach statistical significance. Our study differs from that of Dantzer *et al.* (2014) in our inclusion of non-adults (i.e. juveniles), our inclusion of 40 more recent studies, and our analysis of different types of protected areas. These differences in our data set added heterogeneity that can be used to explore factors that modify the effects of disturbance on GC hormones.

(1) Effects of human disturbance type

Among the three disturbance categories in our meta-analysis, habitat conversion was the most important, showing a positive and significant effect size. Baseline GC hormone levels

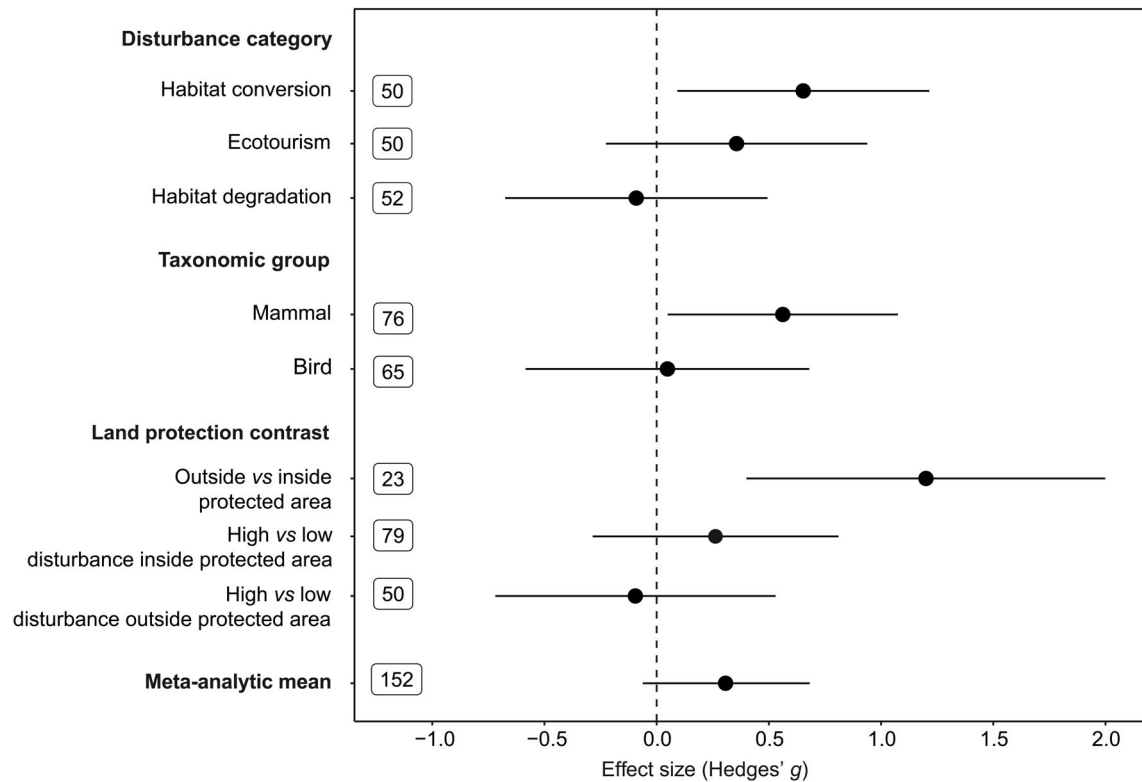


Fig. 3. Forest plot reporting the mean effect sizes (Hedges' g) and their 95% confidence intervals (CI) for changes in baseline glucocorticoid (GC) hormone levels of free-ranging wild vertebrates in disturbed *versus* undisturbed conditions. Estimates were obtained using random effect meta-regression models. The mean effect sizes are given for each level of the three categorical moderator variables. For taxonomic group, we excluded amphibians and reptiles due to the low number of species ($N = 1$ and 4, respectively) representing each group. The meta-analytic mean (i.e. all data points) is also shown. The dotted vertical line represents no effect. Positive values indicate higher GC levels in disturbed relative to undisturbed conditions whereas negative values indicate the reverse. The effect size is considered significant when CI do not overlap 0. Sample size (N) is given inside the box beside each category.

were higher for animals in environments with intensive human land use than they were for animals in undisturbed environments (Fig. 3). Human land use is responsible for transformation of the Earth's surface through activities such as urbanisation, agriculture, and cattle ranching (Hooke & Martín-Duque, 2012). Such activities are often associated with reduced-quality habitat (Kija *et al.*, 2020; Wang *et al.*, 1997), changes in home ranges, and decreases in food availability and shelter (Foley, 2005). The increase in baseline levels of GC hormones that our meta-analysis revealed suggests that there are substantial energy costs for organisms coping with such environments (McEwen & Wingfield, 2003), which could negatively impact important physiological conditions such as foraging, resting, and mating. For example, during hunting weekends, the little bustard (*Tetrax tetrax*) spent more time being vigilant and flying and showed higher GC hormone levels, whereas during the afternoon before hunting weekends and the morning after those weekends (i.e. in the absence of hunting activity), the level of GC hormones was lower and the animals spent more time foraging (Tarjuelo *et al.*, 2015), illustrating that a shift in

energy budget can occur under stress as a response to human activity.

Human land use is a main driver of habitat degradation, which has been associated with major population declines (Bender, Contreras & Fahrig, 1998). Surprisingly, then, our meta-analysis did not find a significant effect for baseline GC levels in animals exposed to habitat degradation (Fig. 3). There may be several reasons for this lack of a significant effect. First, different species respond to habitat degradation differently as a result of variation in habitat specialisation, trophic level, and vagility (Ewers & Didham, 2006). Further, some species can adjust to living in fragmented habitats by increasing their home range to compensate (e.g. Wegge & Rolstad, 1986) or because they can tolerate modest losses of home range (e.g. Forsman, Meslow & Wight, 1984), especially if the remaining fragments still have good resource availability (Doherty, Fist & Driscoll, 2019). Additional work will be necessary to disentangle these and other possibilities.

We were also interested in whether ecotourism affected baseline levels of GC hormones in free-ranging vertebrates.

Most protected areas include ecotourism as a conservation strategy (Stronza, Hunt & Fitzgerald, 2019), yet such activities might be problematic if resident animals perceive humans as a threat (Frid & Dill, 2002). Our meta-analysis suggests, however, that ecotourism does not generally increase stress levels. One possible explanation is that

Table 1. Summary of the mean effect sizes (ES), with the lower (lb) and upper (ub) bounds of the 95% confidence interval (CI). Estimates were obtained using univariate random effect meta-regression models for each categorical moderator variable, and for the meta-analytic mean including all studies.

	ES	CI.lb	CI.ub
Disturbance category			
Habitat conversion	0.653	0.092	1.215
Ecotourism	0.356	-0.226	0.938
Habitat degradation	-0.091	-0.675	0.494
Heterogeneity: $I^2 = 97\%$			
Taxonomic group			
Mammal	0.562	0.049	1.075
Bird	0.048	-0.584	0.680
Heterogeneity: $I^2 = 97.1\%$			
Land protection contrast			
Outside versus inside PA	1.200	0.400	1.999
High versus low dist. inside PA	0.262	-0.285	0.810
High versus low dist. outside PA	-0.095	-0.719	0.530
Heterogeneity: $I^2 = 96.9\%$			
Meta-analytic mean	0.307	-0.062	0.677
Heterogeneity: $I^2 = 96.9\%$			

dist., disturbance; PA, protected area.

animals can retreat to refuges (Foltz *et al.*, 2015). For example, four species of birds studied in urban parks with different levels of human disturbance and vegetation cover showed increased tolerance to humans in parks with greater vegetation cover and tree height (Fernández-Juricic, Jimenez & Lucas, 2001). Thus, even when animals are stressed by the presence of humans, this effect might be ameliorated by a safe place that offers good opportunities to escape from the disturbance. Further, the restricted period of ecotourism activities (i.e. certain times of day or days of the week) in many cases probably allows animals to recover during periods when humans are not present. Finally, ecotourism often focuses on habituated animals, which no longer experience human-exposure related stress, as a result of extended periods of conditioning (Shutt *et al.*, 2014). Thus, ecotourism might only be stressful in situations where habituation is incomplete or absent.

(2) Variation among taxa in their responses to human disturbance

Our meta-analysis suggests that mammals might be more sensitive than birds in terms of stress responses associated with human disturbance (Fig. 3). One explanation for this difference could be associated with the social nature of the species included in the meta-analysis. In particular, 47% of the species in our study were mammals that live in groups. Group-living animals – as is the case for most mammals (Prox & Farine, 2020) – tend to have higher baseline levels of glucocorticoids due to the constant social interactions resulting from competition and reproduction (Goymann &

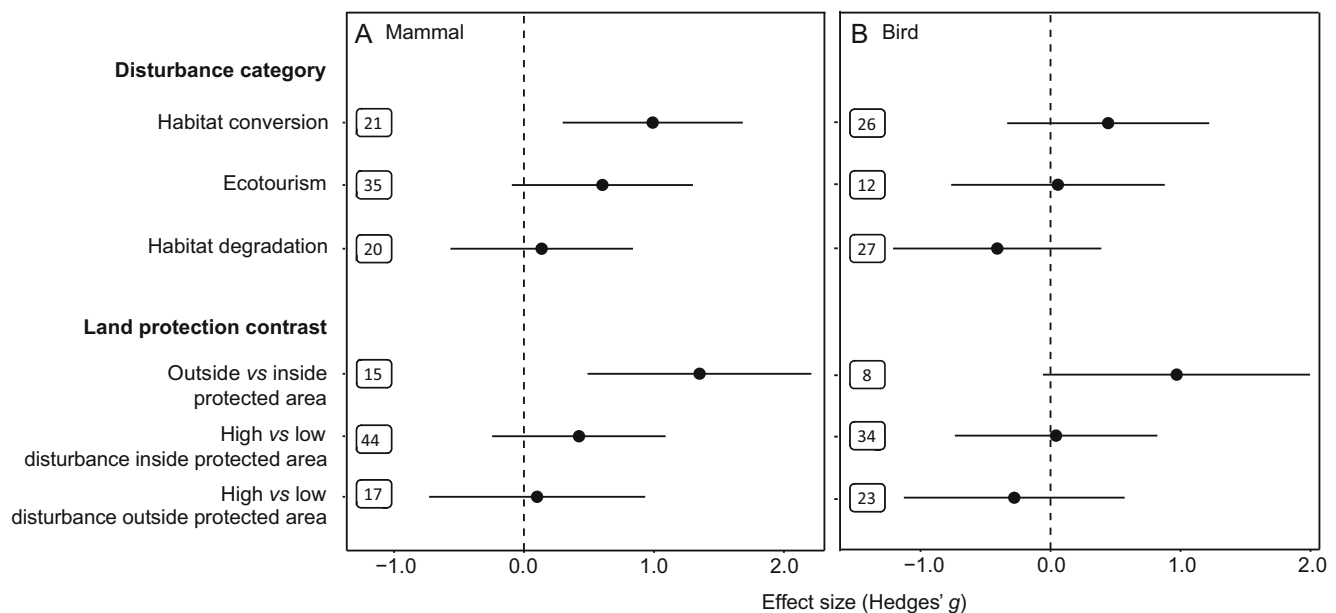


Fig. 4. Forest plot reporting the mean effect sizes (Hedges' g) and their 95% confidence intervals (CI) for changes in glucocorticoid (GC) levels in response to disturbance category and land protection contrast in (A) mammals, and (B) birds. Estimates were obtained using multivariate random effect meta-regression models. We excluded amphibians and reptiles due to the low number of species for which data were available ($N = 1$ and 4, respectively). Other details are as in Fig. 3.

Table 2. Summary of the mean effect size (ES), with the lower (lb) and upper (ub) bounds of the 95% confidence interval (CI). Estimates were obtained using a multivariate random effect meta-regression model with two categorical moderator variables: disturbance category and land protection, to investigate the effects of the disturbance and land protection categories within mammals and birds separately.

	Mammal			Bird		
	ES	CI.lb	CI.ub	ES	CI.lb	CI.ub
Disturbance category						
Habitat conversion	0.990	0.297	1.682	0.443	-0.334	1.221
Ecotourism	0.603	-0.094	1.300	0.056	-0.765	0.878
Habitat degradation	0.136	-0.566	0.838	-0.410	-1.211	0.390
Heterogeneity: $I^2 = 97.3\%$						
Land protection contrast						
Outside <i>versus</i> inside PA	1.350	0.498	2.210	0.970	-0.058	1.997
High <i>versus</i> low dist. inside PA	0.423	-0.244	1.090	0.042	-0.736	0.821
High <i>versus</i> low dist. outside PA	0.101	-0.730	0.932	-0.279	-1.128	0.570
Heterogeneity: $I^2 = 97.2\%$						

dist., disturbance; PA, protected area.

Wingfield, 2004). Hence, these animals might show more scope for those hormones to change in response to human disturbances to their habitats and perhaps to their social interactions (Shutt *et al.*, 2014). For example, in a capture and restraint experiment of female golden-mantled ground squirrels (*Spermophilus saturatus*), individuals showed higher baseline GC hormone levels during the breeding season than before hibernation; but, in both cases, GC levels were higher 30 min after capture (Romero *et al.*, 2008). These results suggest that having a higher baseline concentration of GC hormones does not always limit the capacity of an animal to respond to stress when additional disturbances are present. Among mammals, primates represented 50% of the species in our meta-analysis. Primates, as well as many other large mammals, might be subject to higher disturbance because of poaching (even in protected areas) or through other interactions outside protected areas (e.g. crop raiding; Carlitz *et al.*, 2016). More data are needed for both primates and non-primates if we are to parse these various influences more precisely.

Animals that are exposed to human disturbance on a daily basis are thought to habituate by increasing their tolerance to environmental changes and human presence. This topic has been widely discussed, especially for birds inhabiting urban *versus* rural areas. In particular, birds living in urban areas have a higher degree of tolerance to human disturbance than birds living in rural areas (Bonier, Martin & Wingfield, 2007a; Samia *et al.*, 2015; Tryjanowski *et al.*, 2020; Gotanda, 2020). Interestingly, some species in urban areas have higher levels of GC hormones (Bonier *et al.*, 2007a; Strasser & Heath, 2013; Zhang *et al.*, 2011), suggesting that some species or populations may be better adapted physiologically to tolerating higher levels of GC hormones, or that some individuals in these environments are not habituated and therefore have higher GC hormone levels.

Habituation does not necessarily mean that an animal is no longer stressed. For example, the behaviour of animals

can appear unaltered despite having an elevated heart rate (Coetzee & Chown, 2016; Ellenberg *et al.*, 2006). Such cryptic effects show why it is important to measure levels of GC hormones and other indicators in combination to infer stress levels in free-ranging wild vertebrates. Yet GC hormones are not a panacea because they can be influenced by capture, handling, and sampling, because sampling times might not match peak hormone levels, and because animals might experience other types of costs (e.g. avoiding areas with human activity) that are not reflected by their GC hormone levels. In such cases, GC hormones might not suggest chronic stress, but the animal might still be affected by human disturbance.

Another factor potentially causing variation among animals in how GC hormones are influenced by human disturbance is the precise nature of the interaction with humans. For example, Carlitz *et al.* (2016) found that chimpanzees (*Pan troglodytes*) living in a fragmented forest had higher GC hormone levels than those living in continuous forest. The same study, however, also reported lower GC hormone levels in a group living in an area with illegal logging, which is one of the main causes of forest fragmentation. At both sites humans were present; however, the nature of the interaction with the animals was different. In the fragmented forest, the chimpanzees were harassed by local villagers, whereas such harassment was not reported in the area with logging (Carlitz *et al.*, 2016). This example raises some hope that regulation of aspects of human behaviour could reduce the impacts of environmental disturbance.

(3) The role of protected areas

Protected areas play an important role in the conservation of biodiversity by providing habitat and protection from human disturbance (Dosso *et al.*, 2012; Naughton-Treves, Holland & Brandon, 2005). Animals living in such areas would be expected to have lower baseline levels of GC hormones, as

was confirmed by our analyses (Fig. 3). Moreover, a significant effect was found for the inside *versus* outside protected areas comparison, unlike some types of disturbance (e.g. habitat degradation). This highlights the important role of protected areas *per se* (as opposed to just a reduction in disturbance) in reducing stress for animals. However, even within protected areas, differences in GC hormone levels can occur between areas with different levels of human disturbance (low *versus* high). For example, the Magellan penguin (Fowler, 1999) and the European pine marten (*Martes martes*) (Barja *et al.*, 2007) show increased GC hormone levels in areas with high tourism compared with low tourism. Thus, not only are protected areas important for reducing stress but so is the limitation of disturbance within those protected areas.

V. CONCLUSIONS

(1) A wide range of species from different taxonomic groups respond to human disturbance by increasing their levels of GC hormones, yet the magnitude and direction of the effect depend not only on the type of disturbance but also on its duration and intensity. More studies – particularly of non-primates – are necessary before we can obtain a clear picture of their effects, although we found a significant effect for habitat conversion.

(2) Human disturbances will be perceived as a threat by many animals; yet the way that different animals respond will depend on various species-level (e.g. sociality) or population-level (e.g. past exposure) differences, as well as the individual-level perception of threat or harm. Our study revealed high heterogeneity in GC responses to human disturbance, even within a given combination of moderators.

(3) Our meta-analysis supports the expectation that animals in protected areas show lower levels of GC hormones, which we can translate into a reduction in the stress they experience. However, it also underlines the importance of limiting human disturbances even within protected areas.

(4) The high levels of heterogeneity identified by our meta-analysis imply that management decisions that regulate land use, human activity, or human behaviour could be used to mitigate stress in free-ranging vertebrates.

VI. ACKNOWLEDGEMENTS

We would like to thank Rose E. O’Dea for her advice with the analysis, William Wcislo and Gregory Gilbert for helpful comments on an early version of this review, and Danielle Brown for her comments. Thanks to the three anonymous reviewers and editor whose comments/suggestions helped improve and clarify this manuscript. This work was supported by the Organization of American States (OAS) and Secretaría Nacional de Ciencia y Tecnología of Panama

(SENACYT) scholarships to B. P.-O. The Neotropical Environment Option (NEO) and the NSERC-BESS Biodiversity, Ecosystem Services and Sustainability program of the McGill University, and the Smithsonian Tropical Research Institute (STRI) are acknowledged for additional funding.

VII. AUTHOR CONTRIBUTIONS

B. P.-O. and A. P. H. conceived the study and designed the project goals. B. P.-O. collected the data. B. P.-O. analysed the data with the input of Rose E. O’Dea. Both authors drafted and revised the manuscript.

VIII. REFERENCES

- References marked with an asterisk (*) were used in the meta-analysis; those identified with a dagger symbol (†) are used only in the supporting information.
- ADAMO, S. A. (2012). The effects of the stress response on immune function in invertebrates: an evolutionary perspective on an ancient connection. *Hormones and Behavior* **62**(3), 324–330.
- *AGNEW, R. C. N., SMITH, V. J. & FOWKES, R. C. (2016). Wind turbines cause chronic stress in badgers (*Meles meles*) in Great Britain. *Journal of Wildlife Diseases* **52**, 459–467.
- *AGUILAR-MELO, A. R., ANDRESEN, E., CRISTÓBAL-AZKARATE, J., ARROYO-RODRÍGUEZ, V., CHAVIRA, R., SCHONDUPE, J., SERIO-SILVA, J. C. & CUARÓN, A. D. (2013). Behavioral and physiological responses to subgroup size and number of people in howler monkeys inhabiting a forest fragment used for nature-based tourism. *American Journal of Primatology* **75**, 1108–1116.
- *ALLEN, L. C., TURMELLE, A. S., WIDMAIER, E. P., HRISTOV, N. I., MCCracken, G. F. & KUNZ, T. H. (2011). Variation in physiological stress between bridge- and cave-roosting brazilian free-tailed bats: stress in cave- and bridge-roosting bats. *Conservation Biology* **25**, 374–381.
- *ANGELIER, F., MEILLÈRE, A., GRACE, J. K., TROUVÉ, C. & BRISCHOUX, F. (2016). No evidence for an effect of traffic noise on the development of the corticosterone stress response in an urban exploiter. *General and Comparative Endocrinology* **232**, 43–50.
- *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: Biological Sciences* **274**, 1219–1224.
- *BALESTRI, M., BARRESI, M., CAMPERA, M., SERRA, V., RAMANAMANJATO, J. B., HEISTERMANN, M. & DONATI, G. (2014). Habitat degradation and seasonality affect physiological stress levels of *Eulemur collaris* in littoral forest fragments. *PLoS One* **9**, e107698.
- *BARBOSA, A., DE MAS, E., BENZAL, J., DIAZ, J. I., MOTAS, M., JEREZ, S., PERTIERRA, L., BENAYAS, J., JUSTEL, A., LAZURICA, P., GARCIA-PEÑA, F. J. & SERRANO, T. (2013). Pollution and physiological variability in gentoo penguins at two rookeries with different levels of human visitation. *Antarctic Science* **25**, 329–338.
- BARJA, I., SILVÁN, G., ROSELLINI, S., PIÑEIRO, A., GONZÁLEZ-GIL, A., CAMACHO, L. & ILLERA, J. C. (2007). Stress physiological responses to tourist pressure in a wild population of European pine marten. *The Journal of Steroid Biochemistry and Molecular Biology* **104**, 136–142.
- *BEHIE, A. M., PAVELKA, M. S. M. & CHAPMAN, C. A. (2010). Sources of variation in fecal cortisol levels in howler monkeys in Belize. *American Journal of Primatology* **72**, 600–606.
- BENDER, D. J., CONTRERAS, T. A. & FAHRIG, L. (1998). Habitat loss and population decline: a meta-analysis of the patch size effect. *Ecology* **79**, 517–533.
- *BICUDO, T., ANCIAES, M., ARREGUI, L. & GIL, D. (2020). Effects of forest fragmentation on feather corticosterone levels in an Amazonian avian community. *Ardeola* **67**, 229–245.
- BILLMAN, G. E. (2020). Homeostasis: the underappreciated and far too often ignored central organizing principle of physiology. *Frontiers in Physiology* **11**, 200.
- BLAIR, H. B., MERCHANT, N. D., FRIEDLAENDER, A. S., WILEY, D. N. & PARKS, S. E. (2016). Evidence for ship noise impacts on humpback whale foraging behaviour. *Biology Letters* **12**, 20160005.
- BONIER, F., MARTIN, P. R., MOORE, I. T. & WINGFIELD, J. C. (2009). Do baseline glucocorticoids predict fitness? *Trends in Ecology & Evolution* **24**, 634–642.
- *BONIER, F., MARTIN, P. R., SHELDON, K. S., JENSEN, J. P., FOLTZ, S. L. & WINGFIELD, J. C. (2007b). Sex-specific consequences of life in the city. *Behavioral Ecology* **18**, 121–129.

- BONIER, F., MARTIN, P. R. & WINGFIELD, J. C. (2007a). Urban birds have broader environmental tolerance. *Biology Letters* **3**, 670–673.
- 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.
- BOONSTRA, R., HUBBS, A. H., LACEY, E. A. & MCCOLL, C. J. (2001). Seasonal changes in glucocorticoid and testosterone concentrations in free-living arctic ground squirrels from the boreal forest of the Yukon. *Canadian Journal of Zoology* **79**, 49–58.
- BUSCH, D. S. & HAYWARD, L. S. (2009). Stress in a conservation context: a discussion of glucocorticoid actions and how levels change with conservation-relevant variables. *Biological Conservation* **142**, 2844–2853.
- *BUTLER, L. K., RIES, L., BISSON, I.-A., HAYDEN, T. J., WIKELSKI, M. M. & ROMERO, L. M. (2013). Opposite but analogous effects of road density on songbirds with contrasting habitat preferences: effects of roads on breeding songbirds. *Animal Conservation* **16**, 77–85.
- *CANTARELLI, V. I., PEREZ-RUEDA, M. A., KOWALEWSKI, M. M., MASTROMONACO, G. F. & PONZIO, M. F. (2017). Validation of an enzyme immunoassay and comparison of fecal cortisol metabolite levels in black and gold howler monkeys (*Alouatta caraya*) inhabiting fragmented and continuous areas of the humid Chaco region, Argentina. *American Journal of Primatology* **79**, e22625.
- *CARLITZ, E. H. D., MILLER, R., KIRSCHBAUM, C., GAO, W., HÄNNI, D. C. & VAN SCHAİK, C. P. (2016). Measuring hair cortisol concentrations to assess the effect of anthropogenic impacts on wild chimpanzees (*Pan troglodytes*). *PLoS One* **11**, e0151870.
- *CASAS, F., BENÍTEZ-LÓPEZ, A., TARJUELO, R., BARJA, I., VIÑUELA, J., GARCÍA, J. T., MORALES, M. B. & MOUGEOT, F. (2016). Changes in behaviour and faecal glucocorticoid levels in response to increased human activities during weekends in the pin-tailed sandgrouse. *The Science of Nature* **103**, 91.
- COETZEE, B. W. T. & CHOWN, S. L. (2016). A meta-analysis of human disturbance impacts on Antarctic wildlife. *Biological Reviews* **91**, 578–596.
- *CREEL, S., FOX, J. E., HARDY, A., SANDS, J., GARROTT, B. & PETERSON, R. O. (2002). Snowmobile activity and glucocorticoid stress responses in wolves and elk. *Conservation Biology* **16**, 809–814.
- DANTZER, B., FLETCHER, Q. E., BOONSTRA, R. & SHERIFF, M. J. (2014). Measures of physiological stress: a transparent or opaque window into the status, management and conservation of species?. *Conservation Physiology* **2**, cou023.
- DEFOLIE, C., MERKLING, T. & FICHTEL, C. (2020). Patterns and variation in the mammal parasite–glucocorticoid relationship. *Biological Reviews* **95**, 74–93.
- DOHERTY, T., FIST, C. & DRISCOLL, D. (2019). Animal movement varies with resource availability, landscape configuration and body size: a conceptual model and empirical example. *Landscape Ecology* **34**, 603–614.
- DOSSO, K., YÉO, K., KONATÉ, S. & LINSÉNMAIR, K. E. (2012). Importance of protected areas for biodiversity conservation in Central Côte d'Ivoire: comparison of termite assemblages between two neighboring areas under differing levels of disturbance. *Journal of Insect Science* **12**(131), 1–18.
- DOUGHERTY, L. R. (2020). Meta-analysis reveals that animal sexual signalling behaviour is honest and resource based. *Nature Ecology & Evolution* **5**, 688–699.
- EGGER, M., SMITH, G. D., SCHNEIDER, M. & MINDER, C. (1997). Bias in meta-analysis detected by a simple, graphical test. *BMJ* **315**, 629–634.
- *EL HASSANI, M. S., EL HASSAN, E. M., SLIMANI, T. & BONNET, X. (2019). Morphological and physiological assessments reveal that freshwater turtle (*Mauremys leprosa*) can flourish under extremely degraded-polluted conditions. *Chemosphere* **220**, 432–441.
- ELLENBERG, U., MATTERN, T., SEDDON, P. J. & JORQUERA, G. L. (2006). Physiological and reproductive consequences of human disturbance in Humboldt penguins: the need for species-specific visitor management. *Biological Conservation* **133**, 95–106.
- EWERS, R. M. & DIDHAM, R. K. (2006). Confounding factors in the detection of species responses to habitat fragmentation. *Biological Reviews* **81**, 117–142.
- FERNÁNDEZ-JURICIC, E., JIMENEZ, M. D. & LUCAS, E. (2001). Bird tolerance to human disturbance in urban parks of Madrid (Spain): management implications. In *Avian Ecology and Conservation in an Urbanizing World* (eds J. M. MARZLUFF, R. BOWMAN and R. DONNELLY), pp. 259–273. Springer, US.
- FOLEY, J. A. (2005). Global consequences of land use. *Science* **309**, 570–574.
- *FOLTZ, S. L., DAVIS, J. E., BATTLE, K. E., GREENE, V. W., LAING, B. T., ROCK, R. P., ROSS, A. E., TALLANT, J. A., VEGA, R. C. & MOORE, I. T. (2015). Across time and space: effects of urbanization on corticosterone and body condition vary over multiple years in song sparrows (*Melospiza melodia*): year-dependent effects of urbanization. *Journal of Experimental Zoology Part A: Ecological Genetics and Physiology* **323**, 109–120.
- FOO, Y. Z., NAKAGAWA, S., RHODES, G. & SIMMONS, L. W. (2017). The effects of sex hormones on immune function: a meta-analysis: sex hormones and immune function. *Biological Reviews* **92**, 551–571.
- *FORMENTI, N., VIGANÓ, R., FRAQUELLI, C., TROGU, T., BONFANTI, M., LANFRANCHI, P., PALME, R. & FERRARI, N. (2018). Increased hormonal stress response of Apennine chamois induced by interspecific interactions and anthropogenic disturbance. *European Journal of Wildlife Research* **64**, 68.
- FORSMAN, E. D., MESLOW, E. C. & WIGHT, H. M. (1984). Distribution and biology of the spotted owl in Oregon. *Wildlife Monographs* **87**, 3–64.
- *FOURIE, N. H., TURNER, T. R., BROWN, J. L., PAMPUSH, J. D., LORENZ, J. G. & BERNSTEIN, R. M. (2015). Variation in vervet (*Chlorocebus aethiops*) hair cortisol concentrations reflects ecological disturbance by humans. *Primates* **56**, 365–373.
- *FOWLER, G. S. (1999). Behavioral and hormonal responses of Magellanic penguins (*Spheniscus magellanicus*) to tourism and nest site visitation. *Biological Conservation* **90**, 143–149.
- FRID, A. & DILL, L. M. (2002). Human-caused disturbance stimuli as a form of predation risk. *Conservation Ecology* **6**(1), art11.
- *GARCÍA-PEREIRA, R. J., BARBANTI DUARTE, J. M. & NEGRAO, J. A. (2006). Effects of environmental conditions, human activity, reproduction, antler cycle and grouping on fecal glucocorticoids of free-ranging pampas deer stags (*Ozotoceros bezoarticus bezoarticus*). *Hormones and Behavior* **49**, 114–122.
- GLESER, L. J. & OLKIN, I. (2009). Stochastically dependent effect sizes. In *The Handbook of Research Synthesis and Meta-Analysis* (eds H. COOPER, L. V. HEDGES and J. C. VALENTINE), pp. 357–376. New York: Russell Sage Foundation.
- †GORMALLY, B. M. G. & ROMERO, L. M. (2020). What are you actually measuring? A review of techniques that integrate the stress response on distinct time-scales. *Functional Ecology* **34**, 2030–2044.
- GOTANDA, K. M. (2020). Human influences on antipredator behaviour in Darwin's finches. *Journal of Animal Ecology* **89**, 614–622.
- GOYMANN, W. & WINGFIELD, J. C. (2004). Allostatic load, social status, and stress hormones: the costs of social status matter. *Animal Behaviour* **67**, 591–602.
- *GRUNST, M. L., ROTENBERRY, J. T. & GRUNST, A. S. (2014). Variation in adrenocortical stress physiology and condition metrics within a heterogeneous urban environment in the song sparrow *Melospiza melodia*. *Journal of Avian Biology* **45**, 574–583.
- HEDGES, L. V. & OLKIN, I. (1985). *Statistical Methods for Meta-Analysis*. Academic Press, Orlando.
- HIGGINS, J. P. T. & THOMPSON, S. G. (2002). Quantifying heterogeneity in a meta-analysis. *Statistics in Medicine* **12**, 1539–1558.
- HOFER, H. & EAST, M. L. (1998). Biological conservation and stress. In *Advances in the Study of Behavior* (Volume 27, eds A. P. MÖLLER, M. MILINSKI and P. J. B. SLATER), pp. 405–525. San Diego: Academic Press.
- HOOKE, R. L. B. & MARTÍN-DUQUE, J. F. (2012). Land transformation by humans: a review. *GSA Today* **12**, 4–10.
- *HUNT, K. E., INNIS, C. J., MERIGO, C. & ROLLAND, R. M. (2016). Endocrine responses to diverse stressors of capture, entanglement and stranding in leatherback turtles (*Derموchelys coriacea*). *Conservation Physiology* **4**, cow022.
- IGLESIAS-CARRASCO, M., AICH, U., JENNIONS, M. D. & HEAD, M. L. (2020). Stress in the city: meta-analysis indicates no overall evidence for stress in urban vertebrates. *Proceedings of the Royal Society B: Biological Sciences* **287**, 20201754.
- *IGLESIAS-MERCHAN, C., HORCAJADA-SÁNCHEZ, F., DIAZ-BALTEIRO, L., ESCRIBANO-ÁVILA, G., LARA-ROMERO, C., VIRGÓS, E., PLANILLO, A. & BARJA, I. (2018). A new large-scale index (AcED) for assessing traffic noise disturbance on wildlife: stress response in a roe deer (*Capreolus capreolus*) population. *Environmental Monitoring and Assessment* **190**(4), 185.
- KAISIN, O., FUZESSY, L., PONCIN, P., BROTCORNE, F. & CULOT, L. (2021). A meta-analysis of anthropogenic impacts on physiological stress in wild primates. *Conservation Biology* **35**, 101–114.
- KIJA, H. K., OGUTU, J. O., MANGEWA, L. J., BUKOMBE, J., VERONES, F., GRAAE, B. J., KIDEGHESHO, J. R., SAID, M. Y. & NZUNDA, E. F. (2020). Spatio-temporal changes in wildlife habitat quality in the greater Serengeti ecosystem. *Sustainability* **12**(6), 2440.
- KIRBY, E. D., GERAGHTY, A. C., UBUKA, T., BENTLEY, G. E. & KAUFER, D. (2009). Stress increases putative gonadotropin inhibitory hormone and decreases luteinizing hormone in male rats. *Proceedings of the National Academy of Sciences* **106**, 11324–11329.
- *KNAPP, C. R., HINES, K. N., ZACHARIAH, T. T., PEREZ-HEYDRICH, C., IVERSON, J. B., BUCKNER, S. D., HALACH, S. C., LATTIN, C. R. & ROMERO, L. M. (2013). Physiological effects of tourism and associated food provisioning in an endangered iguana. *Conservation Physiology* **1**, cot032.
- *LESHYK, R., NOL, E., BURKE, D. M. & BURNES, G. (2012). Logging affects fledgling sex ratios and baseline corticosterone in a forest songbird. *PLoS One* **7**, e33124.
- *LESHYK, R., NOL, E., CHIN, E. H. & BURNES, G. (2013). Adult ovenbirds (*Seiurus aurocapilla*) show increased stress-responsiveness in logged forests. *General and Comparative Endocrinology* **194**, 295–299.
- *ŁOPUCKI, R., KLICH, D., ŚCIBIOR, A. & GOŁĘBIOWSKA, D. (2019). Hormonal adjustments to urban conditions: stress hormone levels in urban and rural populations of *Apodemus agrarius*. *Urban Ecosystem* **22**, 435–442.
- *LYNCH, M. A., YOUNGFLESH, C., AGHA, N. H., OTTINGER, M. A. & LYNCH, H. J. (2019). Tourism and stress hormone measures in Gentoo Penguins on the Antarctic Peninsula. *Polar Biology* **42**, 1299–1306.

- *MAJELANTLE, T. L., MCINTYRE, T. & GANSWINDT, A. (2020). Monitoring the effects of land transformation on African clawless otters (*Aonyx capensis*) using fecal glucocorticoid metabolite concentrations as a measure of stress. *Integrative Zoology* **15**, 293–306.
- *MARTÍNEZ-MOTA, R., VALDESPINO, C., SÁNCHEZ-RAMOS, M. A. & SERIO-SILVA, J. C. (2007). Effects of forest fragmentation on the physiological stress response of black howler monkeys. *Animal Conservation* **10**, 374–379.
- MCÉWEN, B. S. & WINGFIELD, J. C. (2003). The concept of allostasis in biology and biomedicine. *Hormones and Behavior* **43**, 2–15.
- *MCLENNAN, M. R., HOWELL, C. P., BARDI, M. & HEISTERMANN, M. (2019). Are human-dominated landscapes stressful for wild chimpanzees (*Pan troglodytes*)? *Biological Conservation* **233**, 73–82.
- MESSINA, S., EDWARDS, D. P., EENS, M. & COSTANTINI, D. (2018). Physiological and immunological responses of birds and mammals to forest degradation: a meta-analysis. *Biological Conservation* **224**, 223–229.
- *MESSINA, S., EDWARDS, D. P., MARASCO, V., CANOINE, V., COSSET, C. C. P., TOMASSI, S., BENEDICK, S., EENS, M. & COSTANTINI, D. (2020). Glucocorticoids link forest type to local abundance in tropical birds. *Functional Ecology* **34**, 1814–1825.
- MOHER, D., LIBERATI, A., TETZLAFF, J., ALTMAN, D. G. & THE PRISMA GROUP (2009). Preferred reporting items for systematic reviews and meta-analyses: the PRISMA statement. *PLoS Medicine* **6**, e1000097.
- *MORGAN, G. M., WILCOXEN, T. E., RENDEL, M. A. & SCHOECH, S. J. (2012). Are roads and traffic sources of physiological stress for the Florida scrub-jay? *Wildlife Research* **39**, 301.
- *MUEHLENBEIN, M. P., ANCRENAZ, M., SAKONG, R., AMBU, L., PRALL, S., FULLER, G. & RAGHANTI, M. A. (2012). Ape conservation physiology: fecal glucocorticoid responses in wild *Pongo pygmaeus morio* following human visitation. *PLoS One* **7**, e33357.
- *MÜLLNER, A., LINSENMAIR, K. E. & WIKELSKI, M. (2004). Exposure to ecotourism reduces survival and affects stress response in hoatzin chicks (*Opisthocomus hoazin*). *Biological Conservation* **118**, 549–558.
- *NABI, G., HAO, Y., MCLAUGHLIN, R. W. & WANG, D. (2018). The possible effects of high vessel traffic on the physiological parameters of the critically endangered Yangtze finless porpoise (*Neophocaena asiaterialis* ssp. *asiaterialis*). *Frontiers in Physiology* **9**, 1665.
- NAKAGAWA, S., NOBLE, D. W. A., SENIOR, A. M. & LAGISZ, M. (2017). Meta-evaluation of meta-analysis: ten appraisal questions for biologists. *BMC Biology* **15**(1), 18.
- NAKAGAWA, S. & SANTOS, E. S. A. (2012). Methodological issues and advances in biological meta-analysis. *Evolutionary Ecology* **26**, 1253–1274.
- NAUGHTON-TREVES, L., HOLLAND, M. & BRANDON, K. (2005). The role of protected areas in conserving biodiversity and sustaining local livelihoods. *Annual Review of Environmental and Resources* **17**, 219–252.
- *NAVARRO-CASTILLA, Á., BARJA, I., OLEA, P. P., PIÑEIRO, A., MATEO-TOMÁS, P., SILVÁN, G. & ILLERA, J. C. (2014). Are degraded habitats from agricultural crops associated with elevated faecal glucocorticoids in a wild population of common vole (*Microtus arvalis*)? *Mammalian Biology* **79**, 36–43.
- *NELSON, J. L., CREEL, S. & CYPHER, B. L. (2015). Fecal glucocorticoid levels of endangered San Joaquin kit foxes (*Vulpes macrotis mutica*) in natural and urban habitats. *Western North American Naturalist* **75**, 52–57.
- *NEWCOMB HOMAN, R., REGOSIN, J. V., RODRIGUES, D. M., REED, J. M., WINDMILLER, B. S. & ROMERO, L. M. (2003). Impacts of varying habitat quality on the physiological stress of spotted salamanders (*Ambystoma maculatum*). *Animal Conservation* **6**, 11–18.
- *ORDÓÑEZ-GÓMEZ, J. D., CRISTÓBAL-AZKARATE, J., ARROYO-RODRÍGUEZ, V., SANTILLÁN-DOHERTY, A. M., VALDEZ, R. A. & ROMANO, M. C. (2016). Proximal and distal predictors of the Spider Monkey's stress levels in fragmented landscapes. *PLoS One* **11**, e0149671.
- *PALMA, A., BLAS, J., TELLA, J. L., CABEZAS, S., MARCHANT, T. A. & CARRETE, M. (2020). Differences in adrenocortical responses between urban and rural burrowing owls: poorly-known underlying mechanisms and their implications for conservation. *Conservation Physiology* **8**(1), coaa054.
- PEREZ-ORTEGA, B., DAW, R., PARADEE, B., GIMBRERE, E. & MAY-COLLADO, L. J. (2021). Dolphin-watching boats affect whistle frequency modulation in bottlenose dolphins. *Frontiers in Marine Science* **8**, 618420.
- PETERS, J. L., SUTTON, A. J., JONES, D. R., ABRAMS, K. R. & RUSHTON, L. (2008). Contour-enhanced meta-analysis funnel plots help distinguish publication bias from other causes of asymmetry. *Journal of Clinical Epidemiology* **61**, 991–996.
- PICHEGRU, L., EDWARDS, T. B., DILLEY, B. J., FLOWER, T. P. & RYAN, P. G. (2016). African penguin tolerance to humans depends on historical exposure at colony level. *Bird Conservation International* **26**, 307–322.
- PROX, L. & FARINE, D. (2020). A framework for conceptualizing dimensions of social organization in mammals. *Ecology and Evolution* **10**, 791–807.
- R CORE TEAM (2020). *R: A Language and Environment for Statistical Computing*. R Foundation for Statistical Computing, Vienna.
- R STUDIO TEAM (2020). *RStudio: Integrated Development for R*. RStudio, Inc., Boston. Electronic file available at <http://www.rstudio.com/>.
- *RANGEL-NEGRÍN, A., ALFARO, J. L., VALDEZ, R. A., ROMANO, 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.
- *RANGEL-NEGRÍN, A., COYOHUA-FUENTES, A., CHAVIRA, R., CANALES-ESPINOSA, D. & DIAS, P. A. D. (2014). Primates living outside protected habitats are more stressed: the case of Black howler monkeys in the Yucatán Peninsula. *PLoS One* **9**, e112329.
- *REHNUS, M., WEHRLE, M. & PALME, R. (2014). Mountain hares *Lepus timidus* and tourism: stress events and reactions. *Journal of Applied Ecology* **51**, 6–12.
- *RIMBACH, R., LINK, A., HEISTERMANN, M., GOMEZ-POSADA, C., GALVIS, N. & HEYMANN, E. W. (2013). Effects of logging, hunting, and forest fragment size on physiological stress levels of two sympatric ateline primates in Colombia. *Conservation Physiology* **1**, cot031.
- *RIVERS, J. W., LIEBL, A. L., MARTIN, L. B. & BETTS, M. G. (2014). Corticosterone in territorial male Swainson's Thrushes varies in relation to forest age but not vegetation cover. *Journal of Ornithology* **155**, 539–548.
- *ROLLAND, R., MCLELLAN, W., MOORE, M., HARMS, C., BURGESS, E. & HUNT, K. (2017). Fecal glucocorticoids and anthropogenic injury and mortality in North Atlantic right whales *Eubalaena glacialis*. *Endangered Species Research* **34**, 417–429.
- ROMERO, L. M. (2004). Physiological stress in ecology: lessons from biomedical research. *Trends in Ecology & Evolution* **19**, 249–255.
- ROMERO, L. M., MEISTER, C. J., CYR, N. E., KENAGY, G. J. & WINGFIELD, J. C. (2008). Seasonal glucocorticoid responses to capture in wild free-living mammals. *American Journal of Physiology-Regulatory, Integrative and Comparative Physiology* **294**, R614–R622.
- ROMERO, L. M. & REED, J. M. (2005). Collecting baseline corticosterone samples in the field: is under 3 min good enough? *Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology* **140**, 73–79.
- *ROMERO, L. M. & WIKELSKI, M. (2002). Exposure to tourism reduces stress-induced corticosterone levels in Galápagos marine iguanas. *Biological Conservation* **108**, 371–374.
- ROMERO, M. L. & BUTLER, L. K. (2007). Endocrinology of stress. *International Journal of Comparative Psychology* **20**, 89–95.
- ROTHSTEIN, H. R., SUTTON, A. J. & BORENSTEIN, M. J. (eds) (2005). *Publication Bias in Meta-Analysis: Prevention, Assessment, and Adjustments*. Wiley, Chichester.
- SAMIA, D. S. M., NAKAGAWA, S., NOMURA, F., RANGEL, T. F. & BLUMSTEIN, D. T. (2015). Increased tolerance to humans among disturbed wildlife. *Nature Communications* **6**, 8877.
- SAPOLSKY, R. M. (2002). Endocrinology of the stress-response. In *Behavioral endocrinology*, Second Edition, pp. 409–450. Cambridge: MIT Press.
- SAPOLSKY, R. M. (2005). The influence of social hierarchy on primate health. *Science* **308**, 648–652.
- SEGUEL, M., PEREZ-VELEGAS, D., GUTIÉRREZ, J., CROCKER, D. E. & DERANGO, E. J. (2019). Parasitism elicits a stress response that allocates resources for immune function in south American fur seals (*Arctocephalus australis*). *Physiological and Biochemical Zoology* **92**, 326–338.
- SENIOR, A. M., GRUEBER, C. E., KAMIYA, T., LAGISZ, M., O'DWYER, K., SANTOS, E. S. A. & NAKAGAWA, S. (2016). Heterogeneity in ecological and evolutionary meta-analyses: its magnitude and implications. *Ecology* **97**, 3293–3299.
- *SHIMAMOTO, T., UCHIDA, K., KOIZUMI, I., MATSUI, M. & YANAGAWA, H. (2020). No evidence of physiological stress in an urban animal: comparison of fecal cortisol metabolites between urban and rural Eurasian red squirrels. *Ecological Research* **35**, 243–251.
- *SHUTT, K., HEISTERMANN, M., KASIM, A., TODD, A., KALOUSOVA, B., PROFOSOUVA, I., PETRZELKOVA, K., FUH, T., DICKY, J.-F., BOPALANZOGNAKO, J.-B. & SETCHELL, J. M. (2014). Effects of habituation, research, and ecotourism on faecal glucocorticoid metabolites in wild western lowland gorillas: implications for conservation management. *Biological Conservation* **172**, 72–79.
- *SPERCOSKI, K. M., MORAIS, R. N., MORATO, R. G., DE PAULA, R. C., AZEVEDO, F. C., MAY-JÚNIOR, J. A., SANTOS, J. P., REGHELIN, A. L., WILDT, D. E. & SONGSASEN, N. (2012). Adrenal activity in maned wolves is higher on farmlands and park boundaries than within protected areas. *General and Comparative Endocrinology* **179**, 232–240.
- STERNE, J. A. C. & EGGER, M. (2001). Funnel plots for detecting bias in meta-analysis: guidelines on choice of axis. *Journal of Clinical Epidemiology* **10**, 1046–1055.
- STRASSER, E. H. & HEATH, J. A. (2013). Reproductive failure of a human-tolerant species, the American kestrel, is associated with stress and human disturbance. *Journal of Applied Ecology* **50**, 912–919.
- STRONZA, A. L., HUNT, C. A. & FITZGERALD, L. A. (2019). Ecotourism for conservation? *Annual Review of Environment and Resources* **44**, 229–253.
- TARJUELO, R., BARJA, I., MORALES, M. B., TRABA, J., BENÍTEZ-LÓPEZ, A., CASAS, F., ARROYO, B., DELGADO, M. P. & MOUGEOT, F. (2015). Effects of

- human activity on physiological and behavioral responses of an endangered steppe bird. *Behavioral Ecology* **26**, 828–838.
- *TECOT, S. R., IRWIN, M. T. & RAHARISON, J.-L. (2019). Faecal glucocorticoid metabolite profiles in diademed sifakas increase during seasonal fruit scarcity with interactive effects of age/sex class and habitat degradation. *Conservation Physiology* **7**(1), coz001.
- THIEL, D., JENNI-EIERMANN, S., PALME, R. & JENNI, L. (2011). Winter tourism increases stress hormone levels in the Capercaillie *Tetrao urogallus*: tourism and stress in Capercaillies. *Ibis* **153**, 122–133.
- †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.
- TRYJANOWSKI, P., KOSICKI, J. Z., HROMADA, M. & MIKULA, P. (2020). The emergence of tolerance of human disturbance in Neotropical birds. *Journal of Tropical Ecology* **36**, 1–5.
- TSANG, A. H., BARCLAY, J. L. & OSTER, H. (2014). Interactions between endocrine and circadian systems. *Journal of Molecular Endocrinology* **52**, R1–R16.
- *TYAGI, A., KUMAR, V., KITTUR, S., REDDY, M., NAIDENKO, S., GANSWINDT, A. & UMAPATHY, G. (2020). Physiological stress responses of tigers due to anthropogenic disturbance especially tourism in two central Indian tiger reserves. *Conservation Physiology* **7**(1), coz045.
- *VAN METER, P. E., FRENCH, J. A., DLONIAK, S. M., WATTS, H. E., KOLOWSKI, J. M. & HOLEKAMP, K. E. (2009). Fecal glucocorticoids reflect socio-ecological and anthropogenic stressors in the lives of wild spotted hyenas. *Hormones and Behavior* **55**, 329–337.
- VIECHTBAUER, W. (2010). Conducting meta-analyses in R with the metafor package. *Journal of Statistical Software* **36**, 1–48.
- VILLAR, J., PIAGGIO, G., CARROLI, G. & DONNER, A. (1997). Factors affecting the comparability of meta-analyses and largest trials results in perinatology. *Journal of Clinical Epidemiology* **50**, 997–1002.
- *WALKER, B. G., BOERSMA, P. D. & WINGFIELD, J. C. (2005). Physiological and behavioral differences in Magellanic penguin chicks in undisturbed and tourist-visited locations of a colony. *Conservation Biology* **19**, 1571–1577.
- WANG, L., LYONS, J., KANEHL, P. & GATTI, R. (1997). Influences of watershed land use on habitat quality and biotic integrity in Wisconsin streams. *Fisheries* **22**, 6–12.
- WEGGE, P. & ROLSTAD, J. (1986). Size and spacing of capercaillie leks in relation to social behavior and habitat. *Behavioral Ecology and Sociobiology* **19**, 401–408.
- WEI, Y. & HIGGINS, J. P. (2013). Estimating within-study covariances in multivariate meta-analysis with multiple outcomes. *Statistics in Medicine* **32**, 1191–1205.
- WIKELSKI, M. & COOKE, S. J. (2006). Conservation physiology. *Trends in Ecology & Evolution* **21**, 38–46.
- *ZHANG, S., LEI, F., LIU, S., LI, D., CHEN, C. & WANG, P. (2011). Variation in baseline corticosterone levels of tree sparrow (*Passer montanus*) populations along an urban gradient in Beijing, China. *Journal of Ornithology* **152**, 801–806.
- *ZWIJACZ-KOZICA, T., SELVA, N., BARJA, I., SILVÁN, G., MARTÍNEZ-FERNÁNDEZ, L., ILLERA, J. C. & JODŁOWSKI, M. (2013). Concentration of fecal cortisol metabolites in chamois in relation to tourist pressure in Tatra National Park (South Poland). *Acta Theriologica* **58**, 215–222.

IX. SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Appendix S1. Assessing for other factors affecting glucocorticoid hormone levels.

Table S1. Details of the 58 studies included in the meta-analysis.

Appendix S2. Excluded studies with reasons for their exclusion.

Fig. S1. Forest plot reporting effect sizes (Hedges' g) and their 95% confidence intervals (CIs) for changes in glucocorticoid (GC) levels of free-ranging wild vertebrates according to sex, age group and matrix.

Appendix S3. Data extracted from the studies selected for the meta-analysis.

Appendix S4. R code for all analyses.

Table S2. Model selection for random effect factors using the Akaike Information Criterion (AIC) values and variance component (σ^2).

Fig. S2. Visual representation of publication bias.

(Received 11 July 2021; revised 31 March 2023; accepted 3 April 2023; published online 24 April 2023)