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Glucocorticoids and land cover: a largescale comparative approach to assess a physiological biomarker for avian conservation

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As humans alter landscapes worldwide, land and wildlife managers need reliable tools to assess and monitor responses of wildlife populations. Glucocorticoid (GC) hormone levels are one common physiological metric used to quantify how populations are coping in the context of their environments. Understanding whether GC levels can reflect broad landscape characteristics, using data that are free and commonplace to diverse stakeholders, is an important step towards physiological biomarkers having practical application in management and conservation. We conducted a phylogenetic comparative analysis using publicly available datasets to test the efficacy of GCs as a biomarker for large spatial-scale avian population monitoring. We used hormone data from HormoneBase (51 species), natural history information and US national land cover data to determine if baseline or stress-induced corticosterone varies with the amount of usable land cover types within each species' home range. We found that stress-induced levels, but not baseline, positively correlated with per cent usable land cover both within and across species. Our results indicate that GC concentrations may be a useful biomarker for characterizing populations across a range of habitat availability, and we advocate for more physiological studies on non-traditional species in less studied populations to build on this framework.

This article is part of the theme issue 'Endocrine responses to environmental variation: conceptual approaches and recent developments'.

1. Introduction

Habitat loss is one of the greatest threats to biodiversity, threatening 85% of all species on the IUCN's Red List [1]. Degradation of natural habitats has been caused by expansion of agricultural land, timber harvesting, overgrazing and other anthropogenic development in response to a growing human population, resulting in half of the world's non-glacial land being converted for human use [2,3]. Reduction in habitat quality or availability can cause rapid and lasting physiological responses in organisms. In particular, it is well documented that habitat alteration can cause physiological responses to stress [4–9], which can lead to decreased reproductive success, pathology and increased mortality rates in vertebrates [10,11]. As a result, a surge of research in the recent past decades has focused on refining the use of physiological metrics to assess animal responses to environmental change and inform conservation and management decisions [12–15].

Glucocorticoid hormones (GCs) have long been regarded as a candidate biomarker of physiological stress across vertebrates [16,17] and a valuable tool in conservation physiology [18]. This is due to their well-conserved role in mediating vertebrate behaviour and physiology in response to both long-term environmental changes and acute perturbations [19]. GCs are released from

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the adrenal gland via the hypothalamic–pituitary–adrenal (HPA) axis and regulate blood glucose levels and mobilize energy reserves. At baseline levels, GCs fluctuate daily and seasonally, reflecting the cumulative energetic demands required to maintain internal homeostasis across changing conditions [20]. For example, baseline GCs can vary during energetically demanding life-history stages such as breeding and migration [21,22], weather events [23,24] or periods of resource limitation [25]. In response to acute or unpredicted challenges, GCs rise quickly and promote immediate survival, e.g. by facilitating predator escape behaviour, territory defense behaviour or mounting of an immune response [26,27]. A fast and robust GC stress response has traditionally been considered critical to an individual's ability to maintain homeostasis through changing environmental conditions (i.e. allostasis) [19,28]. However, absolute levels of GCs and the extent to which they mediate responses to an individual's environment are highly context-dependent [17,29]. Both baseline and stress-induced GC levels can vary across life-history stage [30–33], age [34], sex [35] and species [36,37]. Whether an environmental challenge elicits a GC response, and whether the response is appropriate or harmful, also depends on the nature, frequency and context of the challenge [24,29]. Moreover, recent studies assert that proper interpretation of GC measures requires some knowledge of other components of the HPA axis (e.g. endocrine receptors and binding agents, or the efficiency of recovery from maximum levels) [29,38,39].

Despite this variability of GCs, large-scale comparative studies have been successful at detecting broad trends in GC profiles within and across species [17,40]. Indeed, while empirical studies are vital to our biological understanding of GCs, complementary meta-analyses provide support for using GCs as a physiological indicator of organismal response to environmental variation. For example, several reviews in the past decade have linked GC variation to latitude [41,42], ambient temperature [43,44], primary productivity [42] and food availability [45], suggesting that similar GC phenotypes may be selected for across species in similar environments. As a result, GCs have become one of the most common physiological biomarkers measured in the context of conservation [17,18]. However, there is a deficit in our understanding of how GCs relate to broad landscape characteristics, specifically those used by land and wildlife managers to remotely identify suitable habitat for species. Advancements in global satellite imaging, now able to accurately classify land cover types remotely [46], have become a widespread and common practice for assessing landscape and habitat characteristics [47–49]. Although they are still relatively crude estimates of habitat composition and overall quality compared to on-the-ground surveys [46,50], medium resolution satellite data offer a cost-effective way to assess land cover on very large scales and are accessible to organizations with limited time or resources [14,51,52]. As GCs have potential to be a valuable tool in conservation physiology across taxa [17,18,53], bridging the rich GC literature with broadscale, open-access data sources is of great value to ongoing management initiatives and to broadening global participation in conservation.

In this study, we asked whether broad trends in GC profiles exist across avian species in relation to landscape characteristics, using free, publicly available land cover data and natural history databases. We gathered GC data from HormoneBase, a comprehensive database containing records of GCs collected from adult, free-living vertebrates over the past six decades [54]. Since its publication, HormoneBase has facilitated the investigation of many similar comparative physiological questions at broad spatial and temporal scales, such as relating GCs to life-history traits [55], metabolic rate [56], energetic costs of thermoregulation [57] and conservation status [58], but no studies have assessed the utility of GCs in predicting organismal responses to available land cover types. We focused our study on birds due to their broad distributions, utilization of diverse habitats, extensively studied natural history and well-documented variation in GC (corticosterone in birds) concentrations across life-history stages. We hypothesized that if GC levels reflect the status of an organism's interaction with its surrounding environment, then populations will differ in their GC characteristics along a gradient of appropriate landscape type availability. Specifically, we predicted that populations inhabiting areas characterized by more usable land cover types may have lower baseline GC levels and a robust stress-induced response. Alternatively, baseline levels could be higher if populations inhabiting more suitable habitat exist at greater densities and experience more intraspecific competition, as predicted by ideal free distribution [59].

2. Material and methods

(a) Data extraction

We gathered data on plasma GC concentration, species natural history and land cover from three databases for our analysis. These databases include (1) HormoneBase, a database containing extensive published and unpublished records of circulating androgens and GCs collected from free-living adult vertebrates [54]; (2) Birds of North America (BNA), a Cornell Lab of Ornithology database providing detailed species accounts of North American bird biology written by recognized experts [60], and (3) the National Land Cover Database (NLCD) [61–64], a US Geological Survey database characterizing the US land surface at a medium resolution of 30 m^2.

We used HormoneBase to obtain records of plasma corticosterone (cort) sampled from free-living adult bird populations in the USA from 1969 through 2015. The HormoneBase dataset was compiled via searches through online academic databases for published (and some unpublished) data of free-living, unmanipulated, adult populations where plasma hormone levels were measured (see [46] for a detailed description of HormoneBase inclusion criteria). We limited our study to the USA due to the availability of high-resolution land cover data for the entire country (i.e. Landsat products). We initially selected all records that included baseline circulating GC measurements. Because the goal of this study was to test for habitat effects, we limited our dataset to records with accurate reported coordinates and excluded records with estimated study locations. To account for sampling variance across studies, we also limited our dataset to studies with reported standard errors. These criteria resulted in the inclusion of 281 records of 51 species collected between 1982 and 2012. A subset of these records included measurements of both baseline and stress-induced cort concentrations, but the presence of a stress-induced cort measurement was not required for inclusion in our dataset. Stress-induced cort values were available in 216 records, representing 44 species. From HormoneBase, we also noted whether records were obtained during 'breeding' or 'non-breeding' periods to account for variance in life-history stage. Although more specific information about the timing within the annual cycle (e.g. moulting, migration) was not consistently available, we assumed breeding periods to be separate from migration.

We obtained species' diet and average adult mass data from BNA because these variables have been shown to play a role in

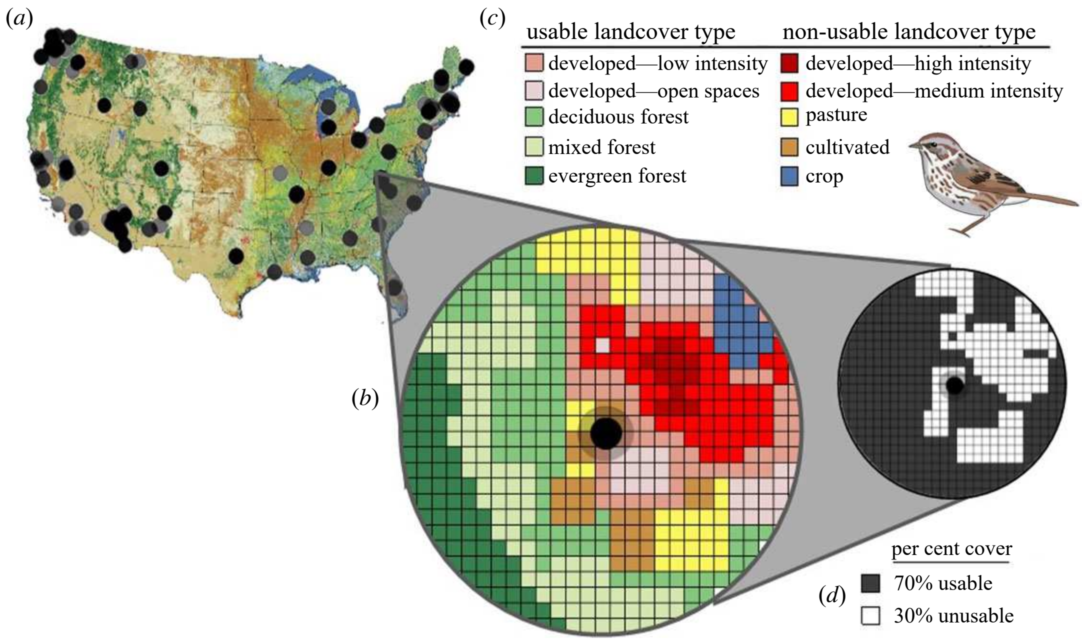

Figure 1. Methods for extracting per cent availability of appropriate landcover cover using the National Land Cover Database (NLCD) and Birds of North America (BNA) natural history information, demonstrated using a song sparrow (*Melospiza melodia*). For each HormoneBase record, we assigned a binned home range area (less than 1, 1–10, 10–100, greater than 100 ha). Centring each home range area on the coordinates of the HormoneBase cort sample (*a*), we layered each home range area on the NLCD raster (*b*). Using natural history information from the Birds of North America database (*c*), we extracted per cent cover of usable habitat for each HormoneBase record in our study based on each record's binned home range area (*d*). Map courtesy of NLCD. (Online version in colour.)

the variation of GC levels, as well as in responses to anthropogenic change [65,66]. We selected the species' diet (carnivore, herbivore or omnivore) and average mass from BNA that most closely matched the sex, life-history stage (i.e. breeding or non-breeding), geographical location and time of sample collection for each HormoneBase record. For example, for widespread species with different specific diets across its range, we selected the diet representative of populations in the same region or same ecosystem type of the HormoneBase record. Similarly, when diet was reported to vary seasonally or across life-history stages, we selected a relevant time of sample collection as the same month, season, or life-history stage. See electronic supplementary material, table S1 for all designations.

We used BNA to identify each species' approximate home range and the types of land cover that each species is reported to use (figure 1). Again, we extracted the most relevant information corresponding to the sex, life-history stage, geographical location and season of each HormoneBase record. For example, if a HormoneBase record was obtained during breeding, we extracted the home range and usable land types as specified on BNA for breeding populations specifically. In the absence of a reported home range, we used reported distances travelled from the nest, defining this distance as the home range radius. We categorized appropriate land cover types for each species using the 18 land surface classes defined in the NLCD [62]. Habitat classes include forested (coniferous, deciduous, mixed), wetland (woody wetlands, herbaceous wetlands), short vegetated (shrub/scrub, grassland/herbaceous), northern latitude-associated (dwarf scrub, sedge/herbaceous), unvegetated (open water, perennial snow and ice, barren land) and developed habitats (developed open spaces; low-, medium- and high-intensity development; cultivated crops; hay/pasture; figure 1). For species that use edge habitats, we included land cover categories for both types of land cover forming the edge.

We then used the 'raster' package [67] in R v.3.4.3 [68] to extract the percentage of cover of each NLCD land cover class within a species' home range, centred on the geographical coordinate of each HormoneBase record (figure 1). We pooled home ranges into four bins (1 ha, 10 ha, 100 ha and 1000 ha), resulting in similar numbers of species in each bin (1 ha = 10 species, 10 ha = 26 species, 100 ha = 8 species, 1000 ha = 9 species). Because NLCD produced land cover datasets in 1992, 2001, 2006 and 2011 and land cover can change over time, we extracted land cover data from the NLCD dataset closest in year to each HormoneBase record. We then calculated the percentage of usable land cover available by summing the per cent cover of land classes that species have been reported to use (figure 1).

(b) Analyses

All analyses were conducted in R v.3.5.2 [68]. We used phylogenetic generalized linear mixed models (PGLMM) to assess the impact of life history and per cent usable land cover on cort concentrations ([55,69]. We used the package 'MCMCglmm' and incorporated phylogeny using the 'pedigree' argument within the 'MCMCglmm' function [69]. For our phylogeny, we constructed a consensus tree using 1000 Hackett *et al.* [70] backbone trees downloaded from BirdTree.org [71]. Specifically, we built a 50% majority-rule consensus tree [72] using the Python program 'SumTrees' in 'DendroPy' [73] following the methods of Rubolini *et al.* [74]. We trimmed trees to include species in the baseline and stress-induced data subsets using the R package 'APE' [75].

We created separate models for baseline and stress-induced cort. We applied a $\log_{10}$ transformation to both baseline and stress-induced cort population means to improve fit to a normal distribution. Our fixed effects included latitude, sex, mean mass ($\log_{10}$-transformed), home range size, diet, life-history stage (breeding or non-breeding), per cent usable land cover type, and the times at which baseline and stress-induced cort were measured post-capture [76]. Most (92%) of baseline levels were collected < 3 min, and all were collected within 7.5 min, post-capture. Similarly, most (80%) of stress-induced levels were collected at 30 ± 5 min, with only eight records collected in less than 25 min and all collected within 60 min post-capture. We included species as a random effect, as well as laboratory ID to control for inter-laboratory variation [77]. We included standard error reported with each record as a random effect, with parameter-expanded priors to control for potential measurement biases. We defined non-informative priors for both R and G as inverse-Wishart distributions

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Figure 2. Phylogeny of species included in our analyses. We selected records from HormoneBase that reported mean population baseline cort, standard error and study site geographical coordinates for bird populations in North America (281 records of 51 species). Records that lacked stress-induced cort values are noted with asterisks. Within-species analyses were conducted for five species with more than 2 population records (denoted by +).

(expected variance, $V = 1$; degree of belief, $\nu = 0.002$). For all models, we ran three chains of 200 000 iterations, with a burn-in of 50 000 and thinning interval of 100. We diagnosed convergence visually and with Gelman-Rubin statistics [78].

For species with multiple (more than 2) populations represented in HormoneBase, we also ran individual within-species linear models. These species were the house sparrow (*Passer domesticus*), semi-palmated sandpiper (*Calidris pusilla*), dark-eyed junco (*Junco hyemalis*), song sparrow (*Melospiza melodia*) and white-crowned sparrow (*Zonotrichia leucophrys*). For each species, we ran separate models for baseline and stress-induced cort, including sex, life-history stage (breeding or non-breeding) and per cent usable land cover type as fixed effects. All models met assumptions of a normal distribution. These models are redundant with the main model (which included species as a random effect) but were included to demonstrate the variability in trends across species, and to provide an example of how our methods can be applied to a single focal species (figure 2).

3. Results

(a) Baseline cort

Baseline cort correlated with sex and species mean mass, but not with per cent usable land cover type. Males had higher baseline cort compared to females, with the 95% credible interval not overlapping zero (table 1). Baseline cort decreased with increasing mean mass, with the 95% credible interval not overlapping zero (table 1). There was no support for an effect of latitude, diet type, home range size, life-history stage, late sample time

Table 1. Results from the PGLMMs for baseline and stress-induced corticosterone models. In the p_{MCMC} columns, values in bold are significant for the 95% credible interval (CI); those in parentheses are significant for the 90% credible interval.

	baseline				stress-induced			
	post. mean	lower 95% CI	upper 95% CI	p_{MCMC}	post. mean	lower 95% CI	upper 95% CI	p_{MCMC}
usable landcover (%)	0.102	−0.034	0.245	0.171	0.144	0.048	0.253	**0.004**
latitude	0.003	−0.003	0.008	0.345	<0.001	−0.004	0.004	0.845
late cort (sample time)	0.028	−0.002	0.006	0.073	0.002	−0.003	0.007	0.351
diet (carnivore)	0.089	−0.211	0.392	0.524	0.145	−0.039	0.347	0.143
diet (herbivore)	0.015	−0.231	0.303	0.913	−0.075	−0.304	0.137	0.467
breeding cycle (non-breeding)	−0.056	−0.127	0.023	0.156	−0.112	−0.173	−0.035	**<0.001**
mass	−0.434	−0.872	−0.011	**0.044**	−0.204	−0.458	0.040	**(0.103)**
sex (male)	0.056	−0.003	0.110	**0.045**	0.067	0.023	0.110	**0.005**
home range size	< 0.001	< −0.001	< 0.001	0.544	< 0.001	< −0.001	< 0.001	0.966

or per cent usable land cover type (table 1). The random effect of species, which incorporates the underlying phylogeny, was important (posterior mean = 0.437, lower credible interval (LCI) = 0.149, upper credible interval (UCI) = 0.790), whereas laboratory identity (posterior mean = 0.062, LCI = 0.006, UCI = 0.140) explained a much smaller proportion of the variance and the effect of study standard error was negligible (posterior mean = 0.0005, LCI = 0.0001, UCI = 0.001).

(b) Stress-induced cort

Stress-induced cort significantly correlated with per cent usable land cover type, sex, life-history stage and species mean mass. Stress-induced cort levels increased with increasing percentage of usable habitat, with the 95% credible interval not overlapping zero (table 1). Males had higher average stress-induced cort than females, with the 95% credible interval not overlapping zero (table 1). Breeding populations had higher stress-induced cort levels than non-breeding, with the 95% credible intervals not overlapping zero (table 1). There was also support for a negative effect of mean mass, with the 90% credible intervals not overlapping zero (table 1). There was no support for an effect of latitude, late sample time, diet type or home range size. The random effect of species was important (posterior mean = 0.104, 95% LCI = 0.036, UCI = 0.183), though less so than in the baseline cort model. Again, laboratory identity explained much less of the variance (posterior mean = 0.017, 95% LCI = 0.0003, UCI = 0.048) and the effect of study standard error was negligible (posterior mean = 0.0001, 95% LCI = 0.00008, UCI = 0.0002).

(c) Within-species relationships between cort and per cent usable land cover type

Within-species analysis of five taxa all showed similar trending relationships between cort and per cent usable land cover type as the PGLMM. There was no relationship between baseline cort and land cover availability for any species, and the direction of the slopes differed among species (table 2). For stress-induced cort, all species showed positive trending relationships with per cent usable land cover type (figure 3), although significance level and effect sizes varied (table 2). House sparrow (n = 25), semi-palmated sandpiper (n = 6) and dark-eyed junco (n = 5) models showed significant effects of per cent usable land cover on stress-induced cort, while song sparrow (n = 11) and white-crowned sparrow (n = 22) models did not (table 2).

4. Discussion

As landscape alteration and habitat loss increase globally, land managers and conservation practitioners need reliable tools to assess and monitor wildlife populations. Within-individual physiological responses can scale up to population-level responses and have great potential to increase the effectiveness of conservation decisions [40,79,80], yet identifying practical physiological biomarkers is challenging. We sought to evaluate the efficacy of GCs as a physiological biomarker that may help assess how populations are coping with their surrounding environment. Specifically, we tested whether the availability of appropriate landcover types within a population's generalized home range correlates with GC profiles, across 51 species of birds. We intentionally used free, accessible environmental data from the National Land Cover Database, and life-history information from the Birds of North America dataset, to generate insights that are relevant and accessible to any land and wildlife manager. We found that stress-induced cort, but not baseline cort, correlated with differences in usable habitat, where populations surrounded by higher percentages of usable habitat had higher stress-induced cort levels. We discuss our results in light of what is known about avian GC responses and recommend how this framework may be applied to conservation in the future.

As in other comparative studies, sex and mass explained variance in both baseline and stress-induced cort levels across species. Males had higher baseline and stress-induced cort than females [55,81], and larger species (by average mass) had lower baseline and stress-induced levels of cort, consistent with other avian comparative analyses [28,66,82]. Here, mass represents a mean per sex per species because

Table 2. Within-species linear models testing the effect of per cent useable landcover type on baseline and stress-induced corticosterone levels. Corticosterone values were obtained from HormoneBase and filtered for records that with accurate GPS coordinates and standard errors recorded. Species were selected for individual analysis if records from more than 2 locations existed in HormoneBase. Models include life-history stage, sex and per cent usable landcover type, except for the semi-palmated sandpiper and song sparrow. All stress-induced corticosterone records of the song sparrow came from males during breeding, so sex and life-history stage were excluded from this model. All records of the semi-palmated sandpiper came from males during breeding, so sex and life-history stage were excluded from both models. Bold values indicate $p < 0.05$.

	baseline				stress-induced			
	estimate	s.e.	*t*	*p*-value	estimate	s.e.	*t*	*p*-value
dark-eyed junco								
usable landcover (%)	−0.35	0.47	−0.74	0.487	−0.25	0.002	−122.94	**0.005**
breeding cycle (non-breeding)	−0.54	0.23	−2.30	0.061	−0.60	0.001	−535.77	**0.001**
sex	−0.15	0.24	−0.61	0.562	−0.08	0.001	−82.96	**0.007**
house sparrow								
usable landcover (%)	−1.77	1.01	−1.76	0.122	0.26	0.089	2.91	**0.008**
breeding cycle (non-breeding)	0.24	0.21	1.18	0.278	−0.05	0.061	−0.83	0.414
sex	0.23	0.21	1.18	0.122	0.03	0.064	0.53	0.603
song sparrow								
usable landcover (%)	0.21	0.16	1.4	0.202	0.09	0.118	0.81	0.440
breeding cycle (non-breeding)	−0.30	0.13	−2.3	**0.040**	—	—	—	—
sex	0.18	0.12	1.48	0.165	—	—	—	—
white-crowned sparrow								
usable landcover (%)	−0.15	0.21	−0.71	0.486	0.16	0.194	0.82	0.425
breeding cycle (non-breeding)	−0.34	0.26	−1.28	0.212	−0.19	0.220	−0.86	0.403
sex	0.17	0.13	1.40	0.176	0.20	0.098	2.05	0.056
semi-palmated sandpiper								
usable landcover (%)	−0.14	0.24	−0.57	0.600	3.97	0.524	7.58	**0.002**

we did not have information about variation in mass across specific HormoneBase records. Thus, the relationship between GCs and mass that we detected likely indicates an evolutionary life-history phenomenon, rather than a reflection of individual populations being smaller or of poor body condition. It is also important to note that one other study assessing avian data (separate from HormoneBase) found that mass was not an important predictor of GCs when accounting for latitude [42], which also covaries with body size among species, but we did not find the same result here.

We found that stress-induced cort was higher for breeding populations. This is contrary to some previous avian empirical studies and reviews that show stress-induced cort is lowered during breeding [66,83]. Higher levels of stress-induced cort are sometimes associated with reduced breeding success [84,85]. However, cort phenotypes also vary temporally within the breeding season [22,55] and depend on brood value [66], which can reflect a combination of many variables such as relative clutch size, age, mate choice etc. It could be that that the coarse dichotomy of 'breeding' and 'non-breeding' available on HormoneBase does not capture the complexity of circannual cort variation. There is not a consistent correlation between breeding and stress-induced cort in birds according to the literature, to date [55,82,86].

No correlation was found between baseline cort levels and per cent usable land cover type, in agreement with several other meta-analyses that suggest baseline cort may not be a reliable biomarker on broad comparative scales [22,30,45]. This is likely because individual variation in baseline cort is high, due to its role in metabolism, energy expenditure and reflection of daily and seasonally fluctuating conditions (e.g. weather or breeding/migration status, respectively [23,87,88]. Our results support other recent literature advocating for caution when interpreting single measurements of baseline GCs [38,89]. Measurements of baseline circulating cort may be a valuable metric for individual health on a case-by-case basis if investigators take repeated measurements and incorporate species biology into sampling design [45,90–92].

We did find a relationship between population-level stress-induced cort profiles and per cent usable land cover type. Populations living in areas with more usable land cover had higher stress-induced plasma cort levels. In contrast to baseline levels, stress-induced cort levels represent an acute physiological response that is relatively more repeatable over an individual's lifetime [93–96] and is heritable [97–99]. The positive relationship between stress-induced cort and land cover availability is consistent with the traditional view that a robust GC stress response is an adaptive mechanism critical to the maintenance of homeostasis and may be indicative of population health [11,100–102]. However, it is becoming increasingly evident that the benefits of a robust response may depend on the efficacy of recovery

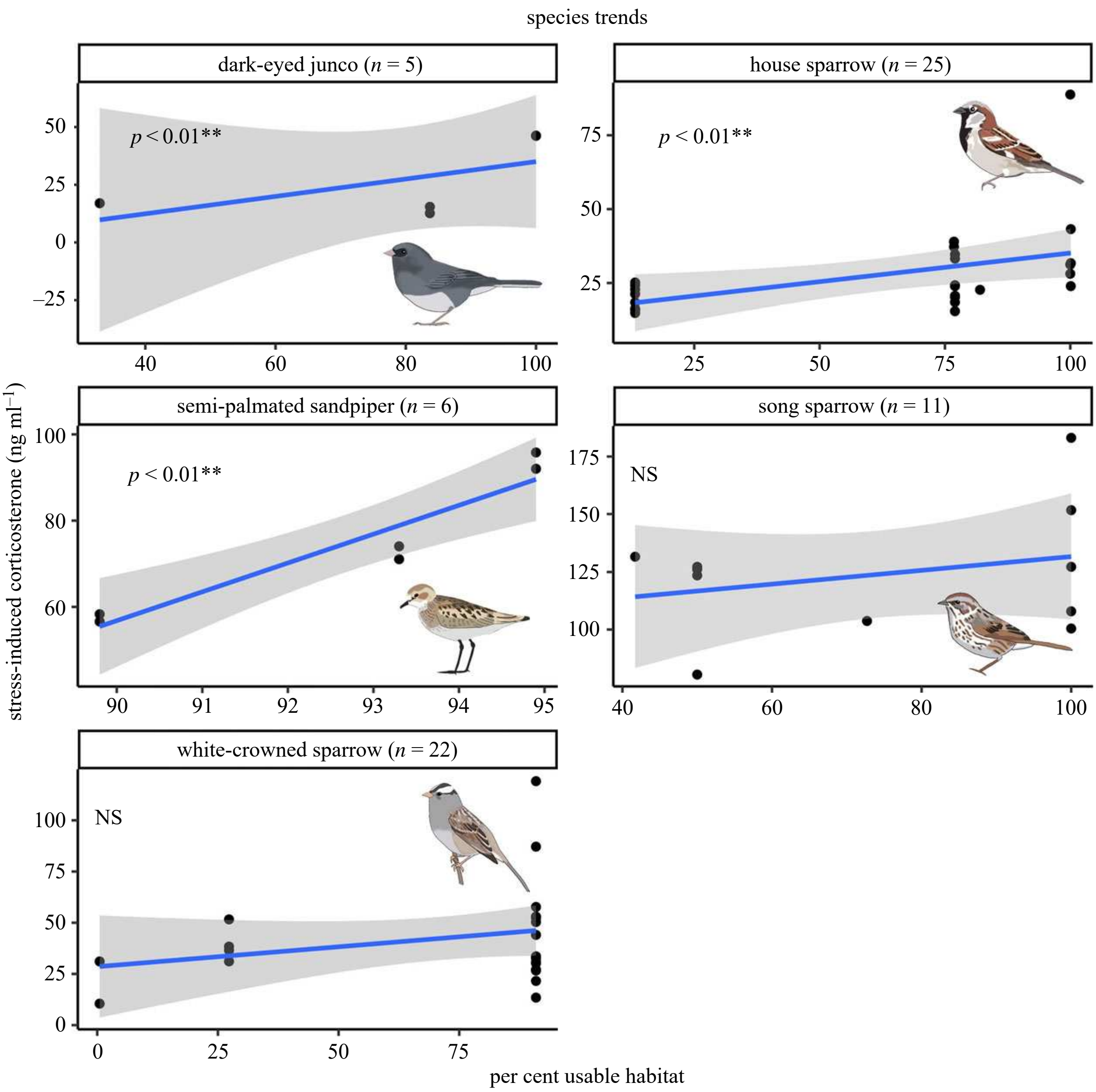

Figure 3. Individual species trends for the relationship between stress-induced corticosterone and per cent availability of appropriate landcover; p-values indicate significance based on linear models. Positive trends within species models support findings from interspecific models. (Online version in colour.)

to baseline levels, i.e. the rate at which individual GC levels return to baseline after a disturbance [39,44].

While our results show that land use can affect physiology on a population level, the magnitude and direction of the effect should be carefully interpreted on a case-by-case basis. For example, the positive relationship between stress-induced cort levels and per cent usable land cover could reflect a dampened stress response in populations inhabiting sub-optimal, habitat-limited areas. There are several possible mechanisms that explain a reduced stress response when habitat availability is reduced. First, dampening of the stress response may be adaptive for populations in poor-quality environments that invest more in reproduction than immediate survival, because their likelihood of surviving to the next breeding season is relatively low [66]. Second, populations surrounded by lower amounts of usable land cover types could exhibit lower stress-induced cort as a result of habituation or maternal effects, as might be expected in a chronically stressful environment [103]. For example, nutritionally restricted maternal environments can lead to decreased responsiveness of the hypothalamic–pituitary–adrenal axis in fledglings [104], leading to long-term changes in populations. Decreased GC responsiveness may not be maladaptive, as some populations in urban environments exhibit dampened stress responses while survivorship remains high [105–107], which is important to consider when interpreting GC variation at the population level. Finally, areas with less usable habitat might be disproportionately occupied by subordinate or younger/inexperienced individuals [108], with more dominant/older individuals controlling more optimal habitats. Results may reflect differences in population structure that can vary across temporal and spatial scales and that we are unable to deduce from the HormoneBase database (although all samples here represent adult populations).

It is also important to acknowledge that the trend we detected might be driven by species' land-use specificity. We classified surrounding usable land cover types based on information about the habitat types used by each species. So, species that are more generalist in regard to habitat have, by default, more 'options' of usable land cover types. As a result, more generalist species should show a higher probability of being surrounded by a high percentage of suitable land-cover types, regardless of the specific mosaic of land-cover classifications.

5. Future directions for research

Overall, we interpret the significant positive relationship between appropriate land cover availability and stress-induced cort levels as notable, especially given the resolution of our land cover data and inclusivity of our criteria for identifying usable habitat for a population. Using BNA life-history information, we identified land cover types as 'usable' for a population if the species had any record of inhabiting the type of land cover at the appropriate time of year/life-history stage, without attempting to estimate differences in the quality or preference, likely overestimating the area that a population could realistically exploit. Still, examining the limitations of our study provides an opportunity to recommend some ways in which researchers can increase the utility of GC measures in conservation and management in the future. First, while HormoneBase offers the most comprehensive hormone database in existence to date, data were strongly biased toward passerine bird species and some species lack records from multiple populations or are limited to data from only one sex or life-history stage. Even for the five species highlighted here with records for more than 3 populations, there were sometimes only one or two representative populations per sex, per life-history stage (see results for song sparrow and semi-palmated sandpiper), limiting the power of these analyses. More empirical data are needed to test whether our findings are biologically meaningful for all taxa and to examine interactive effects (e.g. whether the effect of surrounding land cover varies between age classes or between breeding and non-breeding individuals of a population). Assessing interactions could be important for managers to interpret variation within and across populations and identify particularly vulnerable groups. Additionally, many of the GPS points recorded for populations in HormoneBase are approximate, (e.g. representing the research station rather than exact capture points). Remote sensing technology and satellite data are currently advancing at an exciting rate. As high-resolution land cover data becomes more accurate and available, efforts to record precise GPS coordinates will be especially worthwhile, allowing future studies to consider the existence of microrefugia within an otherwise unusable landscape, or degree of connectivity with other usable habitat areas.

6. Conservation application

What application do GCs have in management and conservation? We show that, despite the known individual variability and complexity of the GC response, access to suitable land cover types can affect GC phenotypes at the population level. This is an important contribution, demonstrating a utility for GCs at the scales that managers are most concerned with (populations) and a framework for predicting how changes in land-use will affect populations. For example, it may be possible to calculate a threshold of per cent usable land cover at which populations will have a physiological response when making decisions about converting landscapes (e.g. for agriculture, urbanization, or restoration etc.). Contrary to occupancy and demographic surveys, GC measurements may offer a way to monitor and assess populations before declines occur. Below we offer several suggestions for conservation practitioners and wildlife managers applying this framework to monitor species in their jurisdictions.

First, when using GC data in an applied context, we strongly suggest incorporating more specific natural history information into investigations than we were able to here. Our method of quantifying the per cent of surrounding habitat variability could be much improved by wildlife managers who have more accurate information about their species of interest. For example, a more comprehensive definition of usable 'habitat' might be more closely related to food abundance, foraging efficiency, competitor densities, or other abiotic and biotic factors unrelated to land cover [109]. Even within a species, we found that the per cent of usable land cover sometimes varies depending on the life-history stage or sex of individuals in the same population. When possible, it would be informative to incorporate the extent to which species depend on specific resources in their environment, and how this dependency may vary across life history or ontogeny.

Second, it is important to consider the context in which GC measures were collected before making judgements about populations. The conditions under which GC responsiveness is adaptive depend on the nature of the stressor [29]. For example, factors such as the abruptness of a landscape change, history of a landscape and concomitant responses of competitors or predators will determine the extent to which a change in land cover imposes 'stress' on a population [89].

Additionally, whether a GC response is considered adaptive or harmful in a particular context may vary between species or life-history stages within species. For example, studies have shown that the relationship between GC levels and fitness may be highly variable across breeding stages [22,31,33,110], but also can depend on the lifetime reproductive opportunity of a species [82,111]. Pairing GC measures with information about survivorship and recruitment will help better understand the relationship between GC levels and fitness for different species or populations.

Finally, conservation practitioners should be cautious in using one-time plasma samples of GCs to make long-term judgements about populations. While it is possible to detect broad trends using metrics as crude as population means of one-time measures, it is becoming increasingly evident that other aspects of the HPA axis are equally (or more) important for wholistically interpreting the GC response. As new data emerge, we hope that it becomes possible to start incorporating more integrative measures into wildlife assessments. Our results represent a meaningful starting point, but more repeated measures of various endocrine traits within species and across geographically distinct populations will strengthening the utility of GC as a conservation tool.

Ethics. This work did not require ethical approval from a human subject or animal welfare committee.

Data accessibility. No primary data were generated in the production of this manuscript. Datasets curated and used in analyses are available in electronic supplementary material [112], table S1 and from the GitHub digital repository: https://github.com/valaasam/GC_and_landcover_2023 [113].

Declaration of AI use. We have not used AI-assisted technologies in creating this article.

Authors' contributions. V.J.A.: conceptualization, data curation, formal analysis, methodology, visualization, writing—original draft, writing—review and editing; T.L.B.: conceptualization, data curation, formal analysis, methodology, writing—original draft, writing—review and editing; A.R.G.: conceptualization, data curation,

writing—review and editing; J.Q.O.: conceptualization, data curation, project administration, supervision, writing—review and editing.

All authors gave final approval for publication and agreed to be held accountable for the work performed therein.

Conflict of interest declaration. The authors declare that we have no competing interests.

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