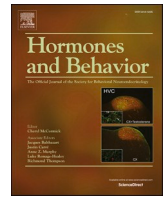




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Perspectives on environmental heterogeneity and seasonal modulation of stress response in neotropical birds

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ABSTRACT

Corticosterone (CORT), the main glucocorticoid in birds, regulates physiological and behavioral traits linked to predictable and unpredictable environmental fluctuations (i.e., stressors). Baseline and stress-induced CORT concentrations are known to fluctuate seasonally, linked to life history stages (LHS) such as breeding, molt, and wintering stage. These variations have been relatively well described in North American birds, but poorly addressed in neotropical species. To fill this gap, we explored how baseline and stress-induced CORT variation by LHS was affected by seasonality and environmental heterogeneity (i.e., frequency of unpredictable events such as droughts, flashfloods, etc) within the Neotropics using two approaches. First, we reviewed all currently available data about CORT concentrations for neotropical bird species. Second, we performed an in-depth analysis comparing the CORT responses of the two most common species of the *Zonotrichia* genus from North and South America (*Z. leucophrys* and *Z. capensis*, respectively) and their subspecies to seasonality and environmental heterogeneity. These species have been analyzed with the same methodology, allowing for an in-depth comparison of CORT variations. Despite scant data on neotropical bird species, we observed overlap between molt and breeding, and lower fluctuations of CORT among LHS. These patterns would be considered atypical compared to those described for North temperate species. Further, we found no significant associations between environmental heterogeneity and the stress-responses. In *Zonotrichia* we observed a positive association between baseline and stress-induced concentrations of CORT and latitude. We also observed differences by LHS. Both baseline and stress-induced CORT concentrations were higher during breeding and lower during molt. In addition, for both species, the overall pattern of seasonal modulation of stress response was heavily influenced by the migration strategy, with long-distance migrants showing significantly higher stress-induced CORT levels. Our results highlight the need for more data collection in the Neotropics. Comparative data would shed further light on the sensitivity of the adrenocortical response to stress under different scenarios of environmental seasonality and unpredictability.

1. Introduction

The adrenocortical response to stress allows for rapid response to unpredictable events by reallocating large amounts of energy and redirecting behavior to promote immediate survival (Wingfield et al., 2017;

Wingfield and Romero, 2011). Important physiological mediators of the stress response in vertebrates are glucocorticoids (Boonstra, 2004). In birds, the main glucocorticoid, corticosterone (CORT), not only regulates many physiological and behavioral traits linked to the stress response, but is also involved in everyday regulation of normal or

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predictable fluctuations in energy demand (Sapolsky et al., 2000). Thereby, baseline CORT levels regulate many functions such as carbohydrate, protein, and lipid metabolism on a daily and seasonal basis in accordance with predictable and cyclical variations of the environment (Landys et al., 2004). In contrast, when animals face unpredictable perturbations, individuals display an acute stress response (Landys et al., 2006).

If an unpredictable challenge triggers activation of the Hypothalamic-Pituitary-Adrenal (HPA) axis, then plasma CORT levels rise - first saturating the high affinity mineralocorticoid receptors (MR) and ultimately binding to low affinity glucocorticoid receptors (GR) that induce rapid physiological and behavioral changes (Sapolsky et al., 2000). These stress-induced CORT levels regulate physiological traits such as immune responses, gluconeogenesis and energy mobilization, and behavioral traits such as changes in reproductive behavior, foraging, and movement (Astheimer et al., 1995; Wingfield et al., 2017). All of these changes increase the immediate survival by suppressing current activities that may require extra energy expenditure - such as further investment in breeding or initiation of molt (Breuner et al., 1998b). Variations of the stress response across the annual life cycle may be related to the trade-off between survival, and current and future reproductive output (Zera and Harshman, 2001). For example, in North American species of song birds, stress-induced CORT levels tend to be lower during the parental care stage, reducing the probabilities of nest abandonment (Krause et al., 2021) - particularly in harsh environments and in migratory species (Cornelius et al., 2012; Wingfield et al., 1992). Migratory animals, and especially long-distance migrants which perform high energy investments for flight to reach their breeding grounds, have shorter and more inflexible time windows to display each LHS, adding to the value of the reproductive effort (Krause et al., 2021). Comparisons between subspecies of *Zonotrichia leucophrys* in North America, higher variations in CORT levels across LHS in migratory than in resident subspecies (Krause et al., 2021). Several studies also in North American species have reported a reduction in the stress response during molt, since acute elevations of corticosterone could impair production of quality feathers through the activation of catabolic pathways for flight which would negatively affect protein synthesis which necessary for feather replacement (Romero et al., 2005). Exceptions to this pattern have been found in species that exhibit a prolonged, low intensity molt that often fully overlaps with breeding (Cornelius et al., 2011; Echeverry-Galvis and Hau, 2012), without any impact on feather quality (Buttemer et al., 2015). Another example is the South American *Zonotrichia capensis*, which is year-round resident in the oases of the Atacama Desert and presents a completely atypical molt and overlap pattern (Echeverria et al., 2018; Gonzalez-Gomez et al., 2018).

Seasonal variations in circulating CORT levels and, to a lesser degree, other aspects of the HPA system (e.g., glucocorticoid receptors, carrier proteins (CBG) and bioactive enzymes, etc.) have been relatively well described for North American birds but poorly described in Neotropical birds. Consequently, almost all our knowledge and current theories about the HPA axis activity in birds are based on north temperate species studies (Breuner and Orchinik, 2001; Romero, 2002). Unlike North America which has been geographically connected to eastern Asia by Beringia from the Pleistocene (50–60 MYA), South America was isolated until the uplift of Central America (3 MYA), which explains the evolution of its highly endemic and biodiverse biota (Raven et al., 2020; Ricklefs, 2002; Simpson, 1980). Furthermore, this region has been shaped by a complex geological history which has created a high number of heterogenous environments (Antonelli, 2022; Raven et al., 2020). Accordingly, the degree to which Northern and Southern species respond to environmental conditions may vary as well. Especially considering that most birds rely on photoperiod to initiate their life history stages (LHSs; e.g.: breeding, molt, migration, etc.) but use local predictive cues such as rainfall, or ambient temperature to fine tune the progression and termination of LHSs.

The interaction between environmental cues and the expression of

LHSs could vary between bird species, depending on the seasonality associated with particular local habitats (Gonzalez et al., 2018; Lisovski et al., 2017; Wingfield, 2008a). For example, in temperate environments of North America, where the seasons have marked predictable differences along latitudinal gradients, birds mostly follow strict division of LHSs, aiming to reduce or avoid overlaps between LHSs and thus avoid high costs of expressing breeding and molt simultaneously (Zera and Harshman, 2001). Generally, in the Southern Hemisphere there is separation of LHSs, similarly to the Northern hemisphere. However, the number of species exhibiting life history overlap is much more common, such as in the Atacama Desert Oases, which exhibit benign environments that are less seasonal, or in some tropical environments where seasonal fluctuations in ecological parameters are often less severe. In this context, we hypothesize that environmental variability, which integrates both seasonality (i.e., predictable variation) and environmental heterogeneity (i.e., unpredictable variation), could have different effects on birds living in the Northern and Southern Hemisphere, and thus on the endocrine mechanisms associated with the expression of LHSs such as molt, breeding, wintering and/or migration.

In this study we aimed to address the broad gap in knowledge about variations in baseline and stress-induced CORT levels and their relationship with abiotic variables. Then, to assess the relationship between CORT variations (baseline and stress-induced) and environmental seasonality and heterogeneity in the Neotropics, we collected new data and performed a literature review of published data about the adrenal stress response of neotropical birds. We also carried out an in-depth data analyses using *Zonotrichia leucophrys* spp. and *Zonotrichia capensis* spp to compare these variables between species inhabiting Nearctic and Neotropical regions. These species were tested with the same protocol at different LHSs (Breuner et al., 1998b), and are excellent models for this comparison since they have a wide geographic distribution inhabiting different environments and latitudes (Chapman, 1940), allowing intra- and inter-hemisphere comparisons. Since the Neotropics offers a high diversity of environments at the same latitude, local predictive cues can be an important factor to initiate and terminate LHS, we expected positive effect of seasonality (i.e., latitude) on CORT levels in both *Zonotrichia* species. We also expect a positive effect of environmental heterogeneity (i.e., coefficient of variation of De Martone aridity Index, CV DMi) in *Z. capensis* but not in *Z. leucophrys*. The CV DMi is an index developed to assess the annual variation in aridity considering the monthly mean precipitation and temperature in a given locality (De Martonne, 1926), and thus it is a good proxy of heterogeneity, and has been used to study the effects of environmental heterogeneity on physiological traits (Cavieres and Sabat, 2008; Gonzalez-Gomez et al., 2018). With these two variables (i.e., latitude and CV DMi) we were able to separate the effects of predictable latitudinal effects from unpredictable variations in precipitation and temperature (CV DMi). Lastly, since the challenges that migration imposes in terms timing for the expression of LHSs are similar for different species in the same migration category (e.g., long-distance migrants), we expect a positive effect of this variable on both *Zonotrichia* species. Our overarching goal was to outline priorities and perspectives for the study of stress-responses in Neotropical birds.

2. Methods

2.1. Literature review and novel data on neotropical species

2.1.1. Literature review data

Literature searches were conducted in the database Web of Science with “corticosterone * season * neotropic * birds” as key words, or any combination and synonyms of these. We included all publications that measured circulating CORT levels in free-living birds. We considered only studies that had been done in the field (i.e., excluded captive experiments) in South and Central America. All CORT values were extracted from the publication text and estimated from the plots when

the data were not available in the text (see Table 1). We included the LHS data if provided in the article. In the few cases when LHS was not provided, we assigned the most likely LHS considering the month of the year and locality where the study was conducted. Assigned LHSs were: molting, breeding, wintering, overlap.

To relate CORT values to seasonality and environmental heterogeneity, we used latitude, as a proxy of seasonality, and the Coefficient of Variation of the De Martone Index (CV DMi) as a proxy of heterogeneity. Latitude represents a measure of predictability since it is directly related to variations in photoperiod and seasonality across the year, triggering the beginning and duration of LHSs via endocrine mechanisms (Farner, 1964; Ramenofsky and Németh, 2014). The De Martone index (DMi), was proposed to describe the degree of climate dryness, based on mean monthly precipitation and temperature (De Martonne, 1926):

$$(\text{DMi} = \Sigma P / T^{\circ} + 10)$$

where P is the monthly precipitation (mm) and T° is the average monthly temperature ($^{\circ}\text{C}$) of a particular locality. Thus, the low DMi defines the driest and hottest the region, and higher DMi represents colder regions with higher precipitation through the year (Pellicone et al., 2019). The Coefficient of variation (CV), on the other hand, is a measure of data dispersion around the mean, and it is calculated (Standard Deviation / Mean) * 100. Thus, the coefficient of variation of the DMi (CV DMi) can be used as a measure of environmental heterogeneity where higher CV DMi values indicate higher environmental heterogeneity. For migratory birds, we calculated CV DMi for the months in which birds were present in the locality (e.g., breeding grounds) where the samples were obtained. We searched for the nearest city from localities and/or geographical references given in each publication to assess the climatic data. All climate data were obtained from climate-data.org through the site climatic charts.

2.1.2. New data for the review analysis

For the review analysis we also include data from nine free-living bird species collected by J.M.C. at the Organization for Tropical Studies biological station ($8^{\circ} 47' 7'' \text{N}$, $82^{\circ} 57' 32'' \text{W}$, 1200 m elevation), Las Cruces, Costa Rica, from June until August of 2011. Most captures occurred after dawn and before 12 pm, though some individuals were captured in the early afternoon. Birds were captured using mist nets positioned at forest edges/pathways or using fruit-baited walk-in traps. In both cases, birds were removed immediately, and baseline blood samples collected within three minutes of capture. Approximately 80 μL of whole blood was collected in heparinized microcapillary tubes following venipuncture of the alar vein. Birds were then placed in a soft cloth bag and held for 30 min, after which a second 80 μL blood sample was collected. Samples were stored on ice for up to four hours until centrifugation (8 min at 10,000 RPM in an IEC Clinical Centrifuge) and the plasma component was stored at -80°C until assay. Birds were then weighed to the nearest 0.5 g using a Pesola spring scale, measured for wing chord, tarsus and keel lengths to the nearest 0.1 mm using calipers. All birds were banded with uniquely numbered metal bands. Reproductive status was assigned based on the presence or absence of a brood patch (BP) or swollen cloacal protuberance (CP). Birds were classified as actively molting if $>20\%$ of the feathers in the body were growing or more than one flight feather. Then, each individual was assigned to one of the following LHS: neither molting nor breeding (0), molting only (1), breeding only (2), molting and breeding overlap (3).

Samples collected at Las Cruces were assayed for plasma CORT concentrations at the Max Planck Institute for Ornithology by enzyme immunoassay (Enzo Life Sciences, Cat. No. ADI 900–097). Samples were extracted prior to assay using diethyl-ether, were dried under nitrogen stream and reconstituted at 4°C overnight in Tris-buffered saline at a 1:30 dilution. Average percent recovery during extraction was determined previously (mean \pm SD; 85 % \pm 2.7; Baugh et al., 2014). All samples, standards and controls were assayed in duplicate. Assay

sensitivity was 27 pg/ml. Intra-assay variation was 9.3 %. Inter-assay variation across 4 plates was 8.5 % and was determined using stripped chicken plasma spiked to 20 ng/ml CORT.

2.2. Data analysis

The studies included for the review analysis (Table 1) were performed with variable assay methodologies (i.e., radioimmunoassay or enzyme immunoassay), which can produce differences in the absolute values of CORT (Fanson et al., 2017). Therefore, we worked with the relative amount of change in CORT levels between LHSs (i.e., CORT suppression, Δ CORT) (Cornelius et al., 2011). To assess these changes, and link them to environmental heterogeneity, for each species we calculated:

$$\Delta \text{CORT} = (1 - (\text{CORT LHS1} / \text{CORT LHS2}))^{\ast} 100$$

Thus, we were able to assess differences between breeding and non-breeding (i.e., molt and wintering lumped together), as well as molt and non-molt (i.e., breeding and wintering lumped together). If molt and breeding overlapped, then we included those samples in both the breeding and the molt categories to maintain adequate sample sizes for analysis (N overlap = 11 species). In addition, we also analyzed the overlap category as a separate group.

To test the role of phylogenetic relations in the association between corticosterone levels and latitude or CV DMi, we performed a Phylogenetic Generalized Least Squares (PGLS) with Brownian motion as evolutionary model (Grafen, 1989). Phylogeny was generated using BirdTree.com (Jetz et al., 2012) (Figs. 1S, 2S). Analyses were performed with packages Ape, Geiger and Phyltools in software R (version 3.5.0). Because we did not detect a significant effect of phylogenetic relations in our statistical models (i.e., the results were similar with and without PGLS, see Supplemental Materials, and Figs. 1S, 2S), we used the raw data. We tested for associations between the change in baseline and stress-induced CORT, and environmental seasonality and heterogeneity (i.e., Latitude and CV DMi) using Linear Models (LM) in R (Version 3.2.2, lme4 package; Bates et al., 2014). Considering our reduced sample size, we ran separate simple regression models to test the effect of the independent variables, latitude and CV DMi on the variations in the baseline and stress-induced CORT values. To test the effect size for each variable, we calculated the squared Pearson's r .

2.2.1. In-depth *Zonotrichia* genus analysis

Our study models were the Nearctic *Zonotrichia leucophrys* (25 g) and the neotropical *Zonotrichia capensis* (22 g). Although both species inhabit a variety of environments, *Z. capensis* show a greater diversification with 29 subspecies versus 5 subspecies in *Z. leucophrys* (Handford, 1985; Loughheed et al., 2013; Weckstein et al., 2001). Both species are granivorous-herbivorous -insectivorous and present resident and long-distance migratory subspecies (Clark et al., 2018; Krause et al., 2021; Landys et al., 2004; Novoa et al., 1996). For this analysis we evaluated the hypothesis that baseline and stress-induced CORT levels in species from the Northern and Southern hemispheres are differentially affected by seasonality and environmental heterogeneity (i.e., Latitude and CV DMi). We compared two related species, the northern *Zonotrichia leucophrys* and the southern *Zonotrichia capensis*, taking advantage of the fact that both species include residents, altitudinal and long-distance migrants. In the northern species, we used data from *Z. l. oriantha* (altitudinal migrant) (JC Wingfield, unpublished data), *Z. l. gambelii* (Long-distance migrant), and *Z. l. nuttalli* (resident), previously published (Krause et al., 2021). In the southern *Z. capensis*, we used data on *Z. c. peruviansis* (resident), *Z. c. chilensis* (resident), *Z. c. sanborni* (altitudinal migrant), *Z. c. australis* (long-distance migrant). For this analysis, and in contrast to the new data obtained in Costa Rica for the neotropical review analysis, we only include data from northern and southern *Zonotrichia* that were processed in the J.C.W. laboratory, following the

Table 1

Studies on corticosterone levels in neotropical birds. Life history stage LHS), 0 = wintering, 1 = molt, 2 = breeding, 3 = overlap between molt and breeding. ~ CORT values were obtained from the manuscript plot.

Reference	Species	Locality	Georeference	Latitude	CV DMi	LHS	Baseline CORT (ng/ml)	Stress-induced CORT (ng/ml)	Sample size
Martin et al., 2005. General and Comparative Endocrinology 140: 126–135	House sparrows (<i>Passer domesticus</i>)	Colon, Panama	9°1'N, 80°, 1°W	9.3593	2.676	2	~2.9 ± 0.1	~13 ± 3.1	11
						0	~4 ± 1.4	~13.3	5
Mark and Rubenstein, 2013. Hormones and Behavior 63:717–722	Rufous-and-white wren (<i>Thryophilus rufalbus</i>)	Pacific slope of Nicaragua	6°55'N, 43°24'W		4.302	2	~5 ± 1	~38 ± 5	13
						2	~9 ± 2	~37 ± 2.5	23
						2	~12.5 ± 2.5	~38 ± 3.5	18
Mata et al., 2009. Annals of the New York Academy of Sciences 1163: 460–463	Semipalmated sandpiper (<i>Calidris pusilla</i>)	La Restinga, Margarita Island, Venezuela	10°84'N, 064°09'W		0.247	0		~160 ± 15	12
Marra and Holberton, 1998. Oecologia 116:284–292	American redstart (<i>Setophaga ruticilla</i>)	Font Hill Nature Preserve, Jamaica	18°03'N, 77°56'W		0.656	0	15.125 ± 1.75		12
						0	24.975 ± 3.2		78
						0	~4.8 ± 1.4		78
Tarlow E.M., et al. 2003, Hormones and Behavior 43: 402–407	Nazca boobies (<i>Sula granti</i>)	Isla Española, Galapagos Island, Ecuador	1°23'S, 89°37'W		1.056	0			8
Quispe et al., 2018. Frontiers in Zoology volume 15:39	Silver-beaked tanager (<i>Ramphocelus carbo</i>)	Belem, Brazil	1°12'07"S, 48°18'07"W	1.4558	1.624	1	4.6 ± 0.9		23
						0	8.3 ± 1.7		34
Quirici et al., 2014. General and Comparative Endocrinology 198: 39–46	Thorn-tailed Rayadito (<i>Aphrastura spinicauda</i>)	Fray Jorge, Chile	30°38'S, 71°40'W	30.652	0.764	2	7.24 ± 1.21	22.72 ± 1.20	25
						2	15.57 ± 1.17	41.22 ± 1.14	55
						2	7.38 ± 1.22	30.02 ± 1.26	47
Schwabl et al., 2016. Frontiers in Zoology 13:19	Orange-bellied euphonia (<i>Euphonia xanthogaster</i>)	Choco, Ecuador	0°9' N, 78° 51'W	0.15	2.333	0	6.034	39.96	1
						1	12.09	124.88	1
						2	2.85 ± 0.16	43.26741513	2
	Wedge-billed woodcreeper (<i>Glyphorhynchus spirurus</i>)	Choco, Ecuador	0°9' N, 78° 51'W	0.15	2.333	0	9.45 ± 3.62	63.80 ± 14.93	3
						1	12.09 ± 2.67	42.88 ± 9.77	7
						2	3.94 ± 2.56	46.96 ± 10.55	6
						3	3.31 ± 3.62	37.88 ± 14.93	3
	Slaty-capped flycatcher (<i>Leptopogon superciliaris</i>)	Choco, Ecuador	0°9' N, 78° 51'W	0.15	2.333	0	10.79 ± 3.62	81.55 ± 14.93	3
						1	10.33 ± 4.4	85.58 ± 18.29	2
	Club-winged manakin (<i>Machaeropterus deliciosus</i>)	Choco, Ecuador	0°9' N, 78° 51'W	0.15	2.333	0	5.08	121.85 ± 18.28	2
1						1.79	42.03	1	
2						10.63	57.83	1	
Tawny-faced gnatwren (<i>Microbates cinereiventris</i>)	Choco, Ecuador	0°9' N, 78° 51'W	0.15	2.333	1	3.4	67.42	1	

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Table 1 (continued)

Reference	Species	Locality	Georeference	Latitude	CV DMI	LHS	Baseline CORT (ng/ml)	Stress-induced CORT (ng/ml)	Sample size
This study	Olive-striped flycatcher (<i>Mionectes olivaceus</i>)	Choco, Ecuador	0°9' N, 78° 51'W	0.15	2.333	2	3.86	36.98	1
						3	1.89 ± 0.60	42.85 ± 5.86	2
						0	5.76 ± 0.62	80.17 ± 13.35	6
						1	12.41 ± 4.43	137.77 ± 46.02	2
						2	9.18 ± 5.57	132.47 ± 46.82	4
	Black-Striped Sparrow (<i>Arremonops conirostris</i>)	Las Cruces OTS Biol. Station, Costa Rica	8° 47' 3.826" N, 82° 57' 32.302" W	8.785278	4.125	1	1.27	21.3	1
						2	7.02 ± 3.22	42.8 ± 7.12	2
						1	0.82	23.9 ± 7.07	1–2
	Buff Throated Saltator (<i>Saltator maximus</i>)	Las Cruces OTS Biol. Station, Costa Rica	8° 47' 3.826" N, 82° 57' 32.302" W	8.785278		0	1.72 ± 0.54	29.16 ± 1.33	3
						1	2.13 ± 0.67	23.1 ± 1.33	2
	Cherrie's Tanager (<i>Ramphocelus costaricensis</i>)	Las Cruces OTS Biol. Station, Costa Rica	8° 47' 3.826" N, 82° 57' 32.302" W	8.785278		3	1.49	9.98	1
						0	21.6	51.6	1
	Clay Colored Robin (<i>Turdus grayi</i>)	Las Cruces OTS Biol. Station, Costa Rica	8° 47' 3.826" N, 82° 57' 32.302" W	8.785278		1	7.7	44.6	1
						0	0.64	24.3	1
	Common Bush Tanager (<i>Chlorospingus flavopectus</i>)	Las Cruces OTS Biol. Station, Costa Rica	8° 47' 3.826" N, 82° 57' 32.302" W	8.785278		2	0.95	34.4	1
3						2.51 ± 0.15	57.57 ± 18.31	2	
Golden Hooded Tanager (<i>Tangara larvata</i>)	Las Cruces OTS Biol. Station, Costa Rica	8° 47' 3.826" N, 82° 57' 32.302" W	8.785278		3	2.58	116	1	
					0	2.41 ± 0.67	29.87 ± 10.97	4	
Orange Billed Nightingale Thrush (<i>Catharus aurantiirostris</i>)	Las Cruces OTS Biol. Station, Costa Rica	8° 47' 3.826" N, 82° 57' 32.302" W	8.785278		2	8.48 ± 6.71	48.27 ± 19.19	7	
					3	13.85 ± 7.99	80.95 ± 5.16	2	
Orange Billed Sparrow (<i>Arremon aurantiirostris</i>)	Las Cruces OTS Biol. Station, Costa Rica	8° 47' 3.826" N, 82° 57' 32.302" W	8.785278		0	1.62 ± 0.27	16.4 ± 9.89	2	
					0	6.11 ± 1.61	51.05 ± 18.22	3	
Palm Tanager (<i>Thraupis palmarum</i>)	Las Cruces OTS Biol. Station, Costa Rica	8° 47' 3.826" N, 82° 57' 32.302" W	8.785278		2	3.86 ± 1.14	31.06 ± 13.17	2	
					0	3.63 ± 0.64	17.41 ± 5.61	4	
Wingfield et al., 2018. General and Comparative Endocrinology 259:20–33	Galapagos Mockingbird (<i>Mimus parvulus</i>)	Isla Isabella, Galapagos, Ecuador	0° 49' 45.12"S, 91° 8' 7.08" W	0.8292	0.558	0	6.11 ± 1.61	51.05 ± 18.22	3
						2	3.86 ± 1.14	31.06 ± 13.17	2
	Galapagos Penguin (<i>Spheniscus mendiculus</i>)	Isla Isabella, Galapagos, Ecuador	0° 49' 45.12"S, 91° 8' 7.08" W	0.8292		0	3.63 ± 0.64	17.41 ± 5.61	4
						1	3.22 ± 1.93	11.59 ± 8.41	2
	Large Cactus Finch (<i>Geospiza conirostris</i>)	Isla Isabella, Galapagos, Ecuador	0° 49' 45.12"S, 91° 8' 7.08" W	0.8292		0	5.14	32.27	1
						2	8.46 ± 2.05	43.19 ± 18.38	3
	Large Ground Finch (<i>Geospiza magirostris</i>)	Isla Isabella, Galapagos, Ecuador	0° 49' 45.12"S, 91° 8' 7.08" W	0.8292		0	6.97 ± 1.48	66.69 ± 18.77	2
						2	6.01	53.74	1
	Medium Ground Finch (<i>Geospiza fortis</i>)	Isla Isabella, Galapagos, Ecuador	0° 49' 45.12"S, 91° 8' 7.08" W	0.8292		2	6.01	53.74	1

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Table 1 (continued)

Reference	Species	Locality	Georeference	Latitude	CV DMI	LHS	Baseline CORT (ng/ml)	Stress-induced CORT (ng/ml)	Sample size
	Sharp-beaked Ground Finch (<i>Geospiza difficilis</i>)	Isla Isabella, Galapagos, Ecuador	0° 49' 45.12"S, 91° 8' 7.08" W	0.8292		0	9.63 ± 1.74	62.29 ± 5.45	9
						2	4.57 ± 2.21	60.42 ± 13.85	3
	Small Ground Finch (<i>Geospiza fuliginosa</i>)	Isla Isabella, Galapagos, Ecuador	0° 49' 45.12"S, 91° 8' 7.08" W	0.8292		1	2.3	9.2	1
	Galapagos Mockingbird (<i>Mimus parvulus</i>)	Isla Genovesa, Galapagos, Ecuador	0° 19' 23.16"N, 89° 53' 24"W	0.3231	1.314	0	3.93	24.17	1
						1	5 ± 1.15	21.13 ± 2.75	6
						2	5.87	27.95	1
	Large Cactus Finch (<i>Geospiza conirostris</i>)	Isla Genovesa, Galapagos, Ecuador	0° 19' 23.16"N, 89° 53' 24"W	0.3231		3	1	16.7	1
						1	5.63 ± 1.24	44.23 ± 8.08	3
						3	10.2	47.013	1
	Large Ground Finch (<i>Geospiza magnirostris</i>)	Isla Genovesa, Galapagos, Ecuador	0° 19' 23.16"N, 89° 53' 24"W	0.3231		1	4.99 ± 2.54	46.17 ± 15.64	2
	Sharp-beaked Ground Finch (<i>Geospiza difficilis</i>)	Isla Genovesa, Galapagos, Ecuador	0° 19' 23.16"N, 89° 53' 24"W	0.3231		0	6.42 ± 3.03	53.83 ± 14.82	4
						1	2.57 ± 0.45	25.73 ± 5.56	7
	Medium Ground Finch (<i>Geospiza fortis</i>)	Isla Santa Cruz, Galapagos, Ecuador	0° 38' 21.84"S 90° 20' 13.919"W	0.6394	0.013	1	2.5	24.6	1
	Small Ground Finch (<i>Geospiza fuliginosa</i>)	Isla Santa Cruz, Galapagos, Ecuador	0° 38' 21.84"S 90° 20' 13.919"W	0.6394		0	1.65 ± 0.21	23.35 ± 16.89	2
						1	3.03 ± 0.46	42.3 ± 16.55	4
						3	13.4 ± 8.90	48.53 ± 26.98	3
Busch et al., 2011. Journal of Animal Ecology 80(3): 640–9	Song wren (<i>Cyphorhinus phaeocephalus</i>)	Panama	9° 4' 53.148"N 79° 42' 2.663"W	9.08143	2.09800	0	4.18 ± 0.79	28.09 ± 3.47	12
	Rufous-collared sparrows (<i>Zonotrichia capensis</i>)	Papallacta, Ecuador	0° 22' 30.356"S 78° 8' 42.622"W	0.3772	0.198	1	0.1 ± 0.01	0.78 ± 0.5	37
						2	0.39 ± 0.5	1.15 ± 0.9	37
Busch D.S., et al., 2010. Physiological and Biochemical Zoology: Ecological and Evolutionary Approaches 83: 87–93	Costa rican rufous-collared sparrows (<i>Zonotrichia capensis costaricensis</i>)	Cuerici Biological Station and Finca dos Lados, Costa Rica	09°33'13.9"N, 83°40'04.1"W and 10°10'8.4"N, 84°17'2.0"W	9.55386	4.594 and 3.795	0	~3.84 ± 0.23		39
						1	~5.63 ± 0.39		11
Gonzalez-Gomez et al., 2018. Journal of Animal Ecology 87 (5): 1364–1382	Rufous-collared sparrows (<i>Zonotrichia capensis</i>)	Fray Jorge, Chile	30°30'S, 71°35'W	30.652	0.764	0	2.26 ± 2.59	18.76 ± 3.11	36
						1	1.25 ± 3.05	20.65 ± 3.64	26
						2	10.62 ± 3.78	39.73 ± 4.89	15
	Rufous-collared sparrows (<i>Zonotrichia capensis</i>)	Arica, Chile	18°20'S, 70°20'W	18.4783	0.039	0	7.8 ± 1.82	17.01 ± 2.23	72
						1	3.5 ± 1.44	12.62 ± 1.77	114
						2	7.9 ± 1.81	19.28 ± 2.13	74
						3	4.25 ± 2.12	21.78 ± 2.6	51

(continued on next page)

Table 1 (continued)

Reference	Species	Locality	Georeference	Latitude	CV DMi	LHS	Baseline CORT (ng/ml)	Stress-induced CORT (ng/ml)	Sample size
Clark et al., 2019. Journal of Ornithology 160 (1), 61–70	Rufous-collared sparrows (<i>Zonotrichia capensis</i>)	Quebrada de la Plata, Chile	33.5°S, 71°W	33.4939	2.056	2	8.64 ± 1.99	56.96 ± 6.74	19
	Rufous-collared sparrows (<i>Zonotrichia capensis</i>)	Farellones, Chile	33°21'S, 70°18'W	33.3	3.507	2	4.78 ± 0.65	70.25 ± 6.08	10
	Rufous-collared sparrows (<i>Zonotrichia capensis</i>)	Isla Navarino, Chile	55°S, 68°W	55.0567	0.141	2	14.04 ± 3.51	84.43 ± 4.13	12
Poblete et al., 2021. Journal of Ornithology 162:487–496	Rufous-collared sparrows (<i>Zonotrichia capensis</i>)	Picarquin, Chile	33°57'S, 70°37'W	33.95972	2.7437	2	3.4 ± 0.61	38.05 ± 14.03	15
	Rufous-collared sparrows (<i>Zonotrichia capensis</i>)	Cantalao, Chile	33°27'S, 70°30'W	33.4719	4.869	0	5.27 ± 1.19	33.23 ± 6.73	9
	Rufous-collared sparrows (<i>Zonotrichia capensis</i>)	Farellones, Chile	33°21'S, 70°18'W	33.3	3.507	2	1.32 ± 0.6	3.69 ± 0.60	22
							7.03 ± 3.1	16.27 ± 3.74	12

same radioimmunoassay protocol (Breuner et al., 1998a) and antibodies to avoid biases linked with differences in hormone analyses (Fanson et al., 2017), and to perform a quantitative comparison.

2.2.2. Data analysis of *Zonotrichia* genus

For the *Zonotrichia* in-depth analysis, we analyzed data on baseline and stress-induced CORT values using Linear Models (LMs). Baseline CORT and stress-induced CORT data were first normalized using log-transformation. The migration strategy was included as “subspecies”, considering that both species present resident, altitudinal, and long-distance migrants. We used the *Dredge* function in R to compare all possible models including “species”, “subspecies”, “LHS”, “CV DMi”, “Latitude”, and the interaction between LHS and Subspecies (R version 4.2.1 packages MuMIn and Rethinking). For model selection, each model set was assessed using values of Akaike Information Criterion Corrected for small sample sizes (AICc), and model weights for model comparisons. We then estimated the parameter and 95 % Confidence Intervals (CI) using the *Summary* function in R (version 3.5.0). We assessed the importance of effect sizes based on whether the 95 % CI overlapped zero. In addition, to assess the effect size we standardized the parameters for each top model and calculated the 95 % Confidence Intervals (CI) using the *Effectsize* function in R. Post-hoc comparisons were assessed using Tukey tests.

3. Results

3.1. Literature review and novel data on neotropical species

We found baseline and/or stress-induced CORT levels values for 38 neotropical bird species, 29 from literature and 9 previously unpublished data collected in Costa Rica (Table 1). From all these species, approximately ~90 % of them were passerines. We found that most of the studies ($N = 15$), except for four (Gonzalez-Gomez et al., 2018; Gonzalez-Gomez et al., 2013; Merrill et al., 2015; Quispe et al., 2018) reported sampling periods shorter than a year. Long-term monitoring allows a comprehensive view of the seasonal modulation of the adrenocortical stress response. The fact that most of the studies we found for our analysis are shorter, reiterate the gap in knowledge in the study of Neotropical birds, and although it is a limitation in our study, our data showed important comparative changes in CORT levels between LHS.

3.1.1. Neotropical molt: corticosterone levels and their relationship with seasonality and environmental variability

Out of 38 neotropical bird species, 18 had data to compare modulation of the stress response during the molt versus non-molt periods. Ten species showed lower levels of baseline CORT during molt (range ~ -180 % to -4 %) compared to other LHSs, regardless of whether the non-molt season was compared to breeding or wintering (Table 1). The remaining 8 species increased their baseline CORT levels during molt (range ~ 11 % to ~80 %). We found no impact of Latitude (Table 2a) or CV DMi (Table 2b) on the change of baseline or stress-induced CORT levels. In addition, we observed overlap of molt and breeding in eight species. In species overlapping breeding and molt, we did not observe lower levels of baseline CORT during molt in comparison with other LHSs in the same species. In contrast, as it has commonly described for North American species, in Neotropical species which do not overlap molt and breeding, we observed lower baseline CORT levels during molt (non-overlap: -79.88 % ± 34.55, overlap: 16.83 % ± 24.79, mean ± s.e.), but this difference was not significant, $t = 1.90$, $df = 17$, $p = 0.07$. Birds overlapping did not show significant differences in stress-induced CORT levels in comparison with birds not overlapping (non-overlap: -48.35 % ± 21.85, overlap: 7.38 % ± 18.71, mean ± s.e., $t = 1.77$, $df = 16$, $p = 0.09$). Interestingly, in those localities where we had data from more than one species (i.e., Costa Rica, Choco Ecuador, and Ecuador Galapagos), we observed different strategies in terms of CORT change during molt (Fig. 1). For example, in the three localities we found species increasing both, baseline and stress-induced CORT levels during molt (e.g., Orange-bellied euphonia and Olive-striped flycatcher in Choco-Ecuador) to increase. We also observed that in all localities some species decreased both levels (e.g., Club-winged manakin in Choco-Ecuador), and other species decreased the stress-induced CORT levels and slightly increased baseline levels (e.g., Tawny-faced gnatwren and Wedge-billed woodcreeper in Choco-Ecuador). We also detected species which showed almost no change in CORT levels during molt (e.g., Slaty-capped flycatcher in Choco-Ecuador).

3.1.2. Neotropical breeding: corticosterone levels and their relationship with seasonality and environmental variability

We have data from 21 neotropical bird species or sub-species from which to compare hormone levels at breeding and non-breeding LHSs. We found that 80 % of these species increased their baseline CORT levels when breeding (range ~ 1 % to 87 %); whereas the remaining species decreased baseline CORT levels during breeding (range ~ -15 to -140 %). We found no association between the values of change of baseline

Table 2

Relation between variations of CORT levels during molt in relation to non-molt season (Δ CORT molting = $(1 - (\text{CORT molting} / \text{CORT non-molting})) * 100$) and latitude (2a) and CV DMi (2b) for the neotropical species included in Table 1.

Table 2a.

Predictors	Baseline CORT levels			Stress-induced CORT levels		
	Estimates	CI	p	Estimates	CI	P
(Intercept)	79.91	31.31–128.52	0.003	63.47	36.40–90.53	<0.001
Latitude	1.74	–3.55–7.04	0.498	–1.53	–4.41–1.35	0.276
Observations	20			18		
R ² / R ² adjusted	0.026 / -0.028			0.074 / 0.016		
Effect size	0.02			0.07		

Table 2b.

Predictors	Baseline CORT levels			Stress-induced CORT levels		
	Estimates	CI	p	Estimates	CI	P
(Intercept)	87.53	16.31–158.75	0.019	44.28	4.81–83.75	0.030
CV DMi	0.50	–27.95–28.95	0.971	6.03	–10.30–22.36	0.445
Observations	20			18		
R ² / R ² adjusted	0.000 / -0.055			0.037 / -0.023		
Effect size	0.01			0.004		

Bold font indicates statistical significance, $p < 0.05$.

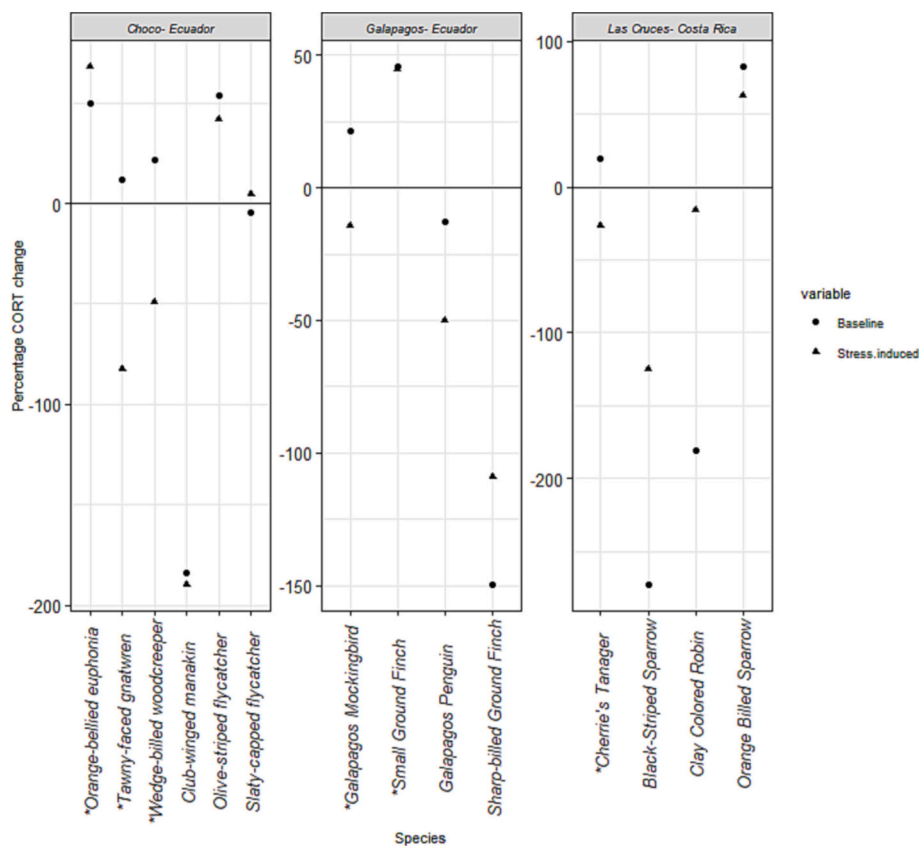


Fig. 1. Baseline CORT and stress-induced CORT change in birds molting and not molting in three study sites. (*) Species in which we observed overlap between molt and breeding.

CORT values and latitude or CV DMi (Table 3 a, b). The effect size values were close to 0, supporting the idea of a negligible effect of these variables even when the sample size was small.

In addition, we found that 70 % of species increased their stress-induced CORT levels during breeding (~2.0 to ~54 %), whereas 30 % decreased their stress-induced CORT levels during breeding (~ -15 % - ~ -190 %). We found no significant association between change in

stress-induced CORT levels and latitude or CV DMi (Table 3 a, b). The values of effect size for these variables were close to 0, supporting the results despite the small sample size.

Table 3

Relation between variations of CORT levels during breeding season in relation to other (Δ CORT = (1-(CORT breeding / CORT non- breeding)) *100) and latitude (3a) and CV DMi (3b) for the neotropical species included in Table 1.

Table 3a.						
Predictors	Baseline CORT levels			Stress-induced CORT levels		
	Estimates	CI	p	Estimates	CI	P
(Intercept)	7.36	-27.77-42.48	0.666	-10.75	-43.37-21.87	0.499
Latitude	1.54	-0.80-3.89	0.184	1.27	-0.91-3.45	0.238
Observations	21			21		
R ² / R ² adjusted	0.091 / 0.043			0.073 / 0.024		
Effect size	0.18			0.01		

Table 3b.						
Predictors	Baseline CORT levels			Stress-induced CORT levels		
	Estimates	CI	p	Estimates	CI	P
(Intercept)	23.39	-26.50-73.28	0.339	21.78	-21.81-65.36	0.309
CV DMi	-2.77	-25.11-19.57	0.798	-13.58	-33.10-5.93	0.161
Observations	21			21		
R ² / R ² adjusted	0.004 / -0.049			0.101 / 0.053		
Effect size	0.10			0.01		

3.2. In-depth comparison with North and South American species of the *Zonotrichia* genus

We compared two related species, the northern *Zonotrichia leucophrys* including data from three subspecies: *Z. l. gambelii*, *Z. l. oriantha*, and *Z. l. nuttalli* previously published (Krause et al., 2021); and the

southern *Zonotrichia capensis* with four subspecies: *Z. c. peruviansis*, *Z. c. chilensis*, *Z. c. sanborni*, *Z. c. australis* at different LHS (Table 4).

3.2.1. Baseline CORT levels

Our analysis showed two top ranked statistical models for baseline CORT levels. Model 1 included “Latitude”, “LHS”, “Subspecies” and the

Table 4

Baseline and stress-induced levels of CORT in *Zonotrichia capensis* (Neotropical) and *Zonotrichia leucophrys* (Nearctic) with different migration strategies at different localities and life history stages (0 = wintering, 1 = molting, 2 = breeding, 3 = overlapping molt and breeding, 4 = migration). Subspecies GWCS = *Z. l. gambelii* (long-distance migrant), MWCS = *Z. l. oriantha* (altitudinal migrant), NWCS = *Z. l. nuttalli* (resident), ZCAU = *Z. c. australis* (long-distance migrant), ZCCHI = *Z. c. chilensis* (resident), ZCPE = *Z. c. peruviansis* (resident), ZCSB = *Z. c. sanborni* (altitudinal migrant).

Species	SubSpecies	Field Site	Latitude	CV DMi	LHSs	cort0			cort30					
						Mean	Std Err	N	Mean	Std Err	N			
<i>Z. leucophrys</i>	GWCS	Davis	38.333	2.207	0	2.68	0.25	125	24.45	0.91	125			
						1	2.64	0.17	148	20.528	0.85	148		
						4	2.97	0.297	54	22.45	1.37	54		
		Fairbanks	64.8378	0.208	1	3.02	0.17	44	17.95	2.14	44			
					2	6.97	0.49	131	55.73	3.26	131			
					4	6.26	0.81	50	57.79	5.73	50			
	Franklin Bluffs	70.241954	0.185	1	2.44	0.23	11	23.43	3.60	11				
				2	6.83	1.36	32	47.51	5.895	32				
				4	2.87	.	1	39.82	.	1				
	Happy Valley	69.1507	0.185	1	4.35	.	1	37.07	.	1				
				2	8.27	1.52	30	74.78	9.64	30				
				0	2.83	0.38	12	23.37	2.17	12				
	Point Reyes/Bodega Bay	38.30745	0.814	1	3.2	0.95	3	23.397	7.52	3				
				4	2.67	0.17	11	21.45	2.64	11				
				4	3.62	0.49	18	40.41	4.69	18				
	Tioga Pass	37.90852802	0.289	4	3.62	0.49	18	40.41	4.69	18				
				Toolik Lake Field Station (TLFS)	68.62581667	0.043	1	3.99	0.47	85	22.92	1.55	85	
							2	6.31	0.37	379	51.87	1.96	379	
NWCS	Point Reyes/Bodega Bay	38.30745	0.814	0	4.48	0.24	165	35.05	0.96	165				
				1	3.08	0.24	64	18.42	0.99	64				
				2	4.06	0.23	195	34.695	1.46	195				
MWCS	Tioga Pass	37.90852802	0.289	1	4.55	1.38	9	35.55	3.38	10				
				2	5.52	1.58	12	45.33	3.78	12				
<i>Z. capensis</i>	ZCAU	Navarino	54.93333	1.141	0	4.86	0.96	7	66.32	12.44	6			
					2	20.43	6.18	6	91.89	4.69	6			
	ZCCHI	Fray Jorge	30.652	0.764	0	2.27	0.26	36	18.79	1.93	36			
					1	1.29	0.15	25	17.08	1.52	24			
					2	5.13	1.02	15	39.74	4.71	15			
	ZCPE	Quebrada de la Plata	33.49045	2.056	2	8.64	1.91	12	64.57	6.37	11			
					Arica	18.4783	0.039	0	2.45	0.297	69	12.28	1.65	63
								1	2.60	0.24	114	10.20	0.76	107
	ZCSB	Farellones	33.36025	3.507	2	4.05	0.65	69	19.05	2.061	65			
					2	4.63	.	1	69.48	.	1			
					2	4.8	0.76	9	74.28	8.53	8			

interaction between “Subspecies” and “LHS” (Tables 5.1, Table 6.1). Model 2 included the same variables adding “Species”. In both models we found that “Latitude” had a positive association with baseline CORT levels (Table 6.1, 6.2, Fig. 2). In both models the effect sizes (Table 6.1, 6.2) reported as the standardized coefficients, supported the significance of variables. In terms of “Subspecies”, we observed that one resident of *Z. capensis* subspecies (*Z.c. chilensis*, 0.79 ± 0.09) showed significantly lower levels of baseline CORT than the resident subspecies in *Z. leucophrys* (i.e., *Z.l. nuttalli*, 1.19 ± 0.03 , mean \pm s.e., Tukey’s HSD Test for multiple comparisons $p = 0.03$, 95 % C.I. [−0.5, −0.01]). The altitudinal subspecies of both species (*Z.c. sanborni*, 1.45 ± 1.16 , *Z. l. oriantha* 2.00 ± 0.17 , $p = 0.15$) did not show significant differences in baseline CORT levels, however in the long-distance migrants we observed higher levels of baseline CORT in *Z.c australis* (2.09 ± 0.28) than in *Z.c. gambelli* (1.30 ± 0.02 , mean \pm s.e., Tukey’s HSD Test for multiple comparisons $p < 0.001$, 95 % C.I. [0.24, 1.42]). These findings are also supported by the effect sizes (measured as standardized coefficients) where the relations are not significant despite an important effect size (e.g., model 1, *Z. capensis australis*). These effects could be explained by limited sample sizes. In both models we found higher CORT baseline levels during the breeding and migration LHSs in comparison with wintering and molt seasons (Table 6.1, 6.2). During wintering CORT baseline levels in the resident *Z. c. peruviansis* were significantly lower than in the North American species (Table 7, Fig. 3). During molt, all species showed lower levels of baseline CORT than during breeding (Fig. 3, Table 7), and during breeding, we observed *Z.c. australis* showing the highest levels of baseline CORT than any other species in our analysis (Fig. 3, Table 7).

3.2.2. Stress-induced CORT levels

We found that the top two models for stress-induced CORT levels, included “Species”, “LHS”, “Subspecies” and the interaction between “Subspecies” and “LHS” (Table 5.1). Model 2 included “Species” (Table 5.2). In both models we found a positive effect of “Latitude”

Table 5

Candidate models for effects on a) baseline and b) stress-induced CORT levels for *Z. leucophrys* and *Z. capensis*, with number of parameters (k), AICc values (AICc), the difference between each model and the best fit model (Δ AICc), and AIC model weights (Wi). Baseline CORT levels.

5.1. Baseline candidate models				
Model	K	AICc	Δ AICc	Wi
Null	5	10,359.9	77.4	0
Latitude + LHS + Subspecies + Subspecies * LHS	21	4383.2	0	0.37
Latitude + LHS + Species + Subspecies + Subspecies * LHS	21	4383.2	0	0.37
Latitude + LHS + CV DMi + Subspecies + Subspecies * LHS	22	4386.3	1.98	0.13
Latitude + LHS + CV DMi + Species + Subspecies + LHS * Subspecies	22	4386.3	1.98	0.13
5.2. Stress-induced candidate models.				
Model	K	AICc	delta AICc	Weight
Null	5	12,253.2	200.9	0
Latitude + LHS + Subspecies + Subspecies * LHS	21	4437.5	0	0.30
Latitude + LHS + Species + Subspecies + Subspecies * LHS	21	4437.5	0	0.30
Latitude + LHS + CV DMi + Species + Subspecies + Subspecies * LHS	21	4438.3	0.72	0.20
Latitude + LHS + CV DMi + Subspecies + Subspecies * LHS	21	4438.3	0.72	0.20

Bold font indicates statistical significance, $p < 0.05$.

(Fig. 2, Table 6.3, 6.4) and “Subspecies” (Table 6.3, 6.4), supported by the effect sizes reported (Table 6.3, 6.4). In long-distance migrant subspecies, we found significantly higher stress-induced CORT levels in the South American species (*Z.c. australis* 4.3 ± 0.12) than in the North American one (*Z.c. gambelli* 3.5 ± 0.02 , mean \pm s.e., Tukey’s HSD Test for multiple comparisons $p < 0.001$, 95 % C.I. [0.62, 1.82]), and the same pattern in altitudinal migrants (*Z.c. sanborni*, 4.3 ± 0.13 ; *Z. l. oriantha* 3.6 ± 0.06 , mean \pm s.e., Tukey’s HSD Test for multiple comparisons $p = 0.01$, 95 % C.I. [0.08, 1.65]). We found significant differences in stress-induced CORT levels among all the resident South American species (*Z.c.peruviansis* = 2.12 ± 0.06 , *Z.c. chilensis*, 3.05 ± 0.08 mean \pm s.e., Tukey’s HSD Test for multiple comparisons $p < 0.001$, 95 % C.I. [−0.93, −0.41]), and between these species and *Z.l. nuttalli* (3.3 ± 0.02 , mean \pm s.e., Tukey’s HSD Test for multiple comparisons $p = 0.003$, 95 % C.I. [0.06, 0.55] for *Z.c. chilensis*-*Z.l. nuttalli*, and $p < 0.001$ 95 % C.I. [−0.53,] *Z.c. peruviansis* - *Z.l. nuttalli*). Although we did not find a significant effect associated with *Z. l. oriantha* in the two top ranked models, the effect size indicates this is a relevant factor. Most likely this result is associated with the small sample size for this subspecies. When LHS was considered in the analysis, we observed the highest stress-induced CORT levels during breeding in the migrants *Z. l. gambelli*, *Z.c australis*, and *Zc. sanborni* in comparison with the resident *Z. c. peruviansis* (Table 7, Fig. 3). We observed lower levels of stress-induced CORT during molt in all species in comparison with breeding LHS (Fig. 3, Table 6.3, 6.4). Overall, we observed higher levels of stress-induced CORT in *Z. leucophrys* (3.4 ± 0.01) than in *Z. capensis* (2.5 ± 0.06 , mean \pm s.e., Tukey’s HSD Test for multiple comparisons $p < 0.001$, 95 % C.I. [0.14, 0.30]).

4. Discussion

In this analysis we reviewed the data on circulating levels of CORT in birds of the Neotropics in relation to LHSs as well as with environmental seasonality and heterogeneity (i.e., Latitude and CV DMi) in southern and northern hemisphere populations. Our results revealed general patterns of seasonal variation in CORT levels in southern hemisphere populations similar to what has been observed in North American species, although with there were some notable differences (see below). We found that critical information in terms of geographic locations, temporal sampling, and number of species is generally lacking for southern hemisphere populations.

4.1. Perspectives in avian neotropical endocrinology

The neotropical region is characterized by a high diversity of species, yet there is a paucity of information about its fauna, including birds, which needs to be addressed (Theuerkauf et al., 2022). According to Bird Life International (<http://datazone.birdlife.org>) there are 3562 bird species in South America alone and in Central America there are 1188 species, making 4750 neotropical bird species the highest biodiversity richness of the biosphere (Jenkins et al., 2013). Contrast this with the 1321 species found in North America, or the 552 species found in Europe, where almost all published studies are focused. The 29 species for which there are CORT data available from multiple seasons included in this review comprise only ~0.6 % of all the species found in the neotropics. In addition, we found only four longitudinal studies of more than one year that have measured CORT levels throughout the annual cycle. Thus, we clearly cannot broadly describe inter-annual variation of circulating CORT levels in neotropical bird species. The Neotropics is further divided into 10 bioregions, each of them with a high diversity of ecoregions. The studies that we found do not represent all of them, and there is only one study in the Amazon and one in central Andes (Quispe et al., 2018; Wada et al., 2006), respectively). The heterogeneity of environments across the neotropics imposes diverse conditions, which may determine the seasonal dynamics of CORT production in different bird species. There may be diverse ways in which CORT control

Table 6

Observed relationships (β estimates $\pm 95\%$ CIs, calculated using $\pm (1.96 * SE)$) and standardized coefficients between response variables and parameters for the two top-ranked models on Baseline CORT and Stress-induced CORT levels for *Z. capensis* (*Zc*) and *Z. leucophys*. (*Zl*). Comparisons against LHS Wintering and Subspecies *Z.l. gambelii*. Subspecies: MWCS = *Z. l. oriantha*, NWCS = *Z. l. nuttalli*, ZCAU = *Z. c. australis*, ZCCHI = *Z. c. chilensis*, ZCPE = *Z. c. peruviansis*.

6.1. Baseline CORT levels Model 1					
Parameter	β estimate*	95 % CI	P-value	Std. Coef.	95 % CI
Intercept	0.13	-0.06-0.33	0.168	-0.34	-0.50 - -0.18
Latitude	0.02	0.01-0.02	<0.001	0.38	0.29-0.47
LHSs [Molt]	-0.05	-0.20-0.10	0.511	-0.06	-0.26 - 0.13
LHSs [Breeding]	0.27	0.10-0.45	0.002	0.35	0.13-0.57
LHSs [Migration]	0.39	0.22-0.55	<0.001	0.49	0.28-0.70
Sub [MWCS]	0.68	0.27-1.10	0.001	0.87	0.35-1.40
Sub [NWCS]	0.52	0.38-0.75	<0.001	0.66	0.46-0.86
Sub [ZCAU]	0.44	-0.12-1.01	0.125	0.57	-0.16 - 1.29
Sub [ZCCHI]	0.03	-0.23-0.28	0.831	0.04	-0.29 - 0.36
Sub [ZCPE]	0.14	-0.08-0.36	0.222	0.17	-0.11 - 0.46
Sub [ZCSB]	0.49	0.04-0.95	0.033	0.63	0.05-1.20
LHSs [Molt] * Sub [MWCS]	0.94	0.35-1.53	0.002	1.20	0.45-1.95
LHSs [Molt] * Sub [NWCS]	-0.23	-0.55 to -0.02	0.038	-0.30	-0.62 - 0.02
LHSs [Breeding] * Sub [NWCS]	-0.38	-0.68 to -0.19	0.001	-0.49	-0.78 - -0.20
LHSs [Breeding] * Sub [ZCAU]	0.93	0.13-1.73	0.023	1.18	0.16-2.20
LHSs [Molt] * Sub [ZCCHI]	-0.5	-0.89 to -0.12	0.011	-0.64	-1.13 - -0.15
LHSs [Breeding] * Sub [ZCCHI]	0.63	0.24-1.01	0.002	0.80	0.30-1.29
LHSs [Molt] * Sub [ZCPE]	0.13	-0.13-0.39	0.334	0.16	-0.17 - 0.50
LHSs [Breeding] * Sub [ZCPE]	0.08	-0.22-0.38	0.599	0.10	-0.28 - 0.48

6.2 Baseline CORT levels Model 2					
Parameter	β estimate*	95 % CI	p-value	Std. Coef.	95 % CI
Intercept	0.63	0.18-1.07	0.006	0.29	-0.33 - 0.90
Latitude	0.02	0.01-0.02	<0.001	0.38	0.29-0.47
LHSs [Molt]	-0.05	-0.20-0.10	0.511	-0.06	-0.26 - 0.13
LHSs [Breeding]	0.27	0.10-0.45	0.002	0.35	0.13-0.57
LHSs [Migration]	0.39	0.22-0.55	<0.001	0.49	0.28-0.70
Species [Zl]	-0.49	-0.95 to -0.04	0.033	-0.63	-1.20 - -0.05
Sub [MWCS]	0.68	0.27-1.10	0.001	0.87	0.35-1.40
Sub [NWCS]	0.57	0.38-0.75	<0.001	0.66	0.46-0.86
Sub [ZCAU]	-0.05	-0.79-0.69	0.898	-0.06	-1.00 - 0.88
Sub [ZCCHI]	-0.46	-0.98-0.05	0.075	-0.59	-1.24 - 0.06
Sub [ZCPE]	-0.36	-0.84-0.13	0.149	-0.45	-1.07 - 0.16
LHSs [Molt] * Sub [MWCS]	0.94	0.35-1.53	0.002	1.20	0.45-1.95
LHSs [Molt] * Sub [NWCS]	-0.28	-0.55 to -0.02	0.038	-0.30	-0.62 - 0.02
LHSs [Breeding] * Sub [NWCS]	-0.43	-0.68 to -0.19	0.001	-0.49	-0.78 - -0.20
LHSs [Breeding] * Sub [ZCAU]	0.93	0.13-1.73	0.023	1.18	0.16-2.20
LHSs [Molt] * Sub [ZCCHI]	-0.5	-0.89 to -0.12	0.011	-0.64	-1.13 - -0.15
LHSs [Breeding] * Sub [ZCCHI]	0.63	0.24-1.01	0.002	0.80	0.30-1.29
LHSs [Molt] * Sub [ZCPE]	0.13	-0.13-0.39	0.334	0.16	-0.17 - 0.50
LHSs [Breeding] * Sub [ZCPE]	0.08	-0.22-0.38	0.599	0.10	-0.28 - 0.48

6.3. Stress-induced top ranked model 1					
Parameter	β estimate*	95 % CI	p-value	Std. Coef.	95 % CI
Intercept	3.68	3.23-4.14	<0.001	1.15	0.60-1.70
Lat	0.01	0.01-0.02	<0.001	0.25	0.17-0.33
LHSs [Molt]	-0.4	-0.55 to -0.25	<0.001	-0.45	-0.63 to -0.28
LHSs [Breeding]	0.22	0.05-0.40	0.013	0.25	0.05-0.45
LHSs [Migration]	0.35	0.18-0.51	<0.001	0.39	0.21-0.58
Species [Zl]	-1.05	-1.51 to -0.59	<0.001	-1.18	-1.70 to -0.66
Sub [MWCS]	0.35	-0.07-0.77	0.102	0.39	-0.08-0.87
Sub [NWCS]	0.38	0.19-0.57	<0.001	0.31	0.13-0.49
Sub [ZCAU]	-0.27	-1.02-0.48	0.484	-0.30	-1.15-0.54
Sub [ZCCHI]	-1.35	-1.87 to -0.83	<0.001	-1.52	-2.11 to -0.94
Sub [ZCPE]	-1.83	-2.32 to -1.34	<0.001	-2.07	-2.62 to -1.52
LHSs [Molt] * Sub [MWCS]	0.43	-0.16-1.03	0.155	0.49	-0.19-1.17
LHSs [Molt] * Sub [NWCS]	-0.27	-0.54-0.00	0.054	-0.18	-0.47-0.10
LHSs [Breeding] * Sub [NWCS]	-0.34	-0.58 to -0.09	0.008	-0.26	-0.52-0.00
LHSs [Breeding] * Sub [ZCAU]	0.2	-0.62-1.01	0.637	0.22	-0.70-1.14
LHSs [Molt] * Sub [ZCCHI]	0.43	0.03-0.82	0.034	0.48	0.04-0.92
LHSs [Breeding] * Sub [ZCCHI]	0.84	0.44-1.23	<0.001	0.95	0.50-1.39
LHSs [Molt] * Sub [ZCPE]	0.24	-0.03-0.50	0.081	0.27	-0.03-0.57
LHSs [Breeding] * Sub [ZCPE]	0.2	-0.10-0.50	0.184	0.23	-0.11-0.57

6.4. Stress-induced top ranked model 2

Parameter	β estimate*	CI	p-value	Std. Coef.	95 % CI
Intercept	2.64	2.44–2.83	<0.001	−0.03	−0.17–0.11
Lat	0.01	0.01–0.02	<0.001	0.25	0.17–0.33
LHSs [Molt]	−0.4	−0.55 to −0.25	<0.001	−0.45	−0.63 to −0.28
LHSs [Breeding]	0.22	0.05–0.40	0.013	0.25	0.05–0.45
LHSs [Migration]	0.35	0.18–0.51	<0.001	0.39	0.21–0.58
Sub [MWCS]	0.35	−0.07–0.77	0.102	0.39	−0.08–0.87
Sub [NWCS]	0.38	0.19–0.57	<0.001	0.31	0.13–0.49
Sub [ZCAU]	0.78	0.20–1.36	0.008	0.88	0.23–1.53
Sub [ZCCHI]	−0.3	−0.56 to −0.04	0.023	−0.34	−0.63 to −0.05
Sub [ZCPE]	−0.78	−1.01 to −0.56	<0.001	−0.89	−1.14 to −0.63
Sub [ZCSB]	1.05	0.59–1.51	<0.001	1.18	0.66–1.70
LHSs [Molt] * Sub [MWCS]	0.43	−0.16–1.03	0.155	0.49	−0.19–1.17
LHSs [Molt] * Sub [NWCS]	−0.27	−0.54–0.00	0.054	−0.18	−0.47–0.10
LHSs [Breeding] * Sub [NWCS]	−0.34	−0.58 to −0.09	0.008	−0.26	−0.52–0.00
LHSs [Breeding] * Sub [ZCAU]	0.2	−0.62–1.01	0.637	0.22	−0.70–1.14
LHSs [Molt] * Sub [ZCCHI]	0.43	0.03–0.82	0.034	0.48	0.04–0.92
LHSs [Breeding] * Sub [ZCCHI]	0.84	0.44–1.23	<0.001	0.95	0.50–1.39
LHSs [Molt] * Sub [ZCPE]	0.24	−0.03–0.50	0.081	0.27	−0.03–0.57
LHSs [Breeding] * Sub [ZCPE]	0.2	−0.10–0.50	0.184	0.23	−0.11–0.57

Bold font indicates statistical significance, $p < 0.05$.

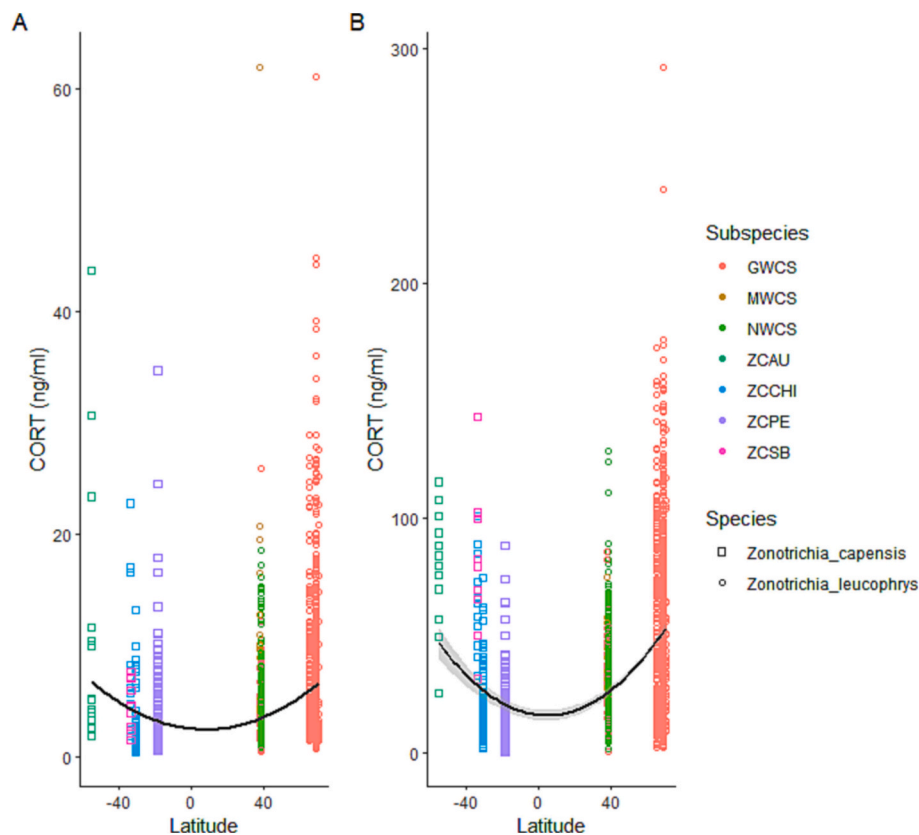


Fig. 2. Relation between baseline (A) and stress-induced CORT levels (B) in relation with seasonality (latitude) in *Zonotrichia capensis* and *Zonotrichia leucophrys*.

mechanisms regulate customized responses of individuals and populations to diverse environmental conditions found throughout the Neotropics. On the other hand, individual bird species can inhabit contrasting regions that require flexible adjustments in physiology and behavior. Thus, more comparative studies about CORT production in birds throughout the neotropical regions are urgently needed to know how they perceive the changing environment and transduce the signals to generate an appropriate response. Understanding how birds regulate such responses both within and across species in diverse environments is important for understanding fitness and, ultimately, species distributions and how they may respond to climate and other anthropogenic

perturbations of the environment (Wingfield et al., 2015). Overall, given the apparent lack of information, the significant differences highlighted here are informative and provide a focus for future work interpreting impact of environmental conditions on stress physiology.

4.2. Seasonal modulation of the adrenocortical response to stress

In general, animals exhibit seasonal changes in sensitivity and regulation of the adrenocortical stress response (Romero, 2002). In temperate zone birds, it is common to find patterns such as the increase of CORT levels in the early stages of the breeding season, and a down-

Table 7

Post-hoc analysis between subspecies of *Z. capensis* (Zc) and *Z. leucophrys* (Zl). Subspecies GWCS = *Z. l. gambelii* (long-distance migrant), MWCS = *Z. l. oriantha* (altitudinal migrant), NWCS = *Z. l. nuttalli* (resident), ZCAU = *Z. c. australis* (long-distance migrant), ZCCHI = *Z. c. chilensis* (resident), ZCPE = *Z. c. peruviansis* (resident), ZCSB = *Z. c. sanborni* (altitudinal migrant).

CORT	Contrast	LHSs	Estimate	SE	t ratio	p-value	
Baseline	GWCS -	Wintering	-0.520	0.080	-6.518	0.000	
	NWCS						
	NWCS -						
	ZCCHI		0.492	0.128	3.848	0.002	
	NWCS -						
	ZCPE		0.383	0.110	3.484	0.009	
	MWCS -						
	ZCCHI		2.104	0.260	8.082	0.000	
	GWCS -						
	MWCS		-1.628	0.224	-7.268	0.000	
	MWCS -						
	ZCPE		1.361	0.232	5.875	0.000	
	MWCS -						
	NWCS	Molt	1.342	0.235	5.724	0.000	
	ZCCHI -						
	ZCPE		-0.743	0.158	-4.704	0.000	
	NWCS -						
	ZCCHI		0.761	0.166	4.596	0.000	
	GWCS -						
	ZCCHI		0.476	0.153	3.108	0.031	
	GWCS -						
	ZCAU		-1.372	0.285	-4.821	0.000	
	NWCS -						
	ZCAU		-1.236	0.288	-4.290	0.000	
	GWCS -		Breeding	ZCCHI	-0.654	0.156	-4.178
	ZCAU -						
	ZCPE	1.155		0.304	3.803	0.003	
	NWCS -						
	ZCCHI	-0.518		0.145	-3.578	0.007	
	GWCS -						
	MWCS	-0.684		0.210	-3.254	0.020	
	NWCS -						
ZCPE	1.059	0.111		9.508	0.000		
GWCS -							
ZCPE	0.785	0.114		6.882	0.000		
ZCAU -							
ZCPE	1.564	0.308	5.074	0.000			
NWCS -							
ZCCHI	0.575	0.130	4.433	0.000			
ZCAU -							
ZCCHI	1.080	0.313	3.455	0.010			
GWCS -							
NWCS	-0.274	0.081	-3.384	0.013			
ZCCHI -							
ZCPE	Wintering	0.484	0.148	3.261	0.019		
MWCS -							
ZCPE		1.331	0.235	5.669	0.000		
NWCS -							
ZCPE		0.661	0.117	5.637	0.000		
GWCS -		Molt	0.548	0.105	5.203	0.000	
ZCPE							
ZCCHI -							
ZCPE			0.672	0.160	4.201	0.001	
GWCS -							
MWCS			-0.783	0.227	-3.448	0.010	
ZCPE -							
ZCSB	-1.628	0.240	-6.793	0.000			
ZCCHI -							
ZCPE	Breeding	1.118	0.165	6.787	0.000		
NWCS -							
ZCPE		0.626	0.108	5.785	0.000		
ZCAU -							
ZCPE		1.556	0.308	5.055	0.000		
GWCS -							
ZCSB	-1.047	0.234	-4.468	0.000			
Stress-induced	NWCS -						
	ZCSB	-1.003	0.227	-4.414	0.000		

Table 7 (continued)

CORT	Contrast	LHSs	Estimate	SE	t ratio	p-value
	GWCS -		0.582	0.137	4.260	0.000
	ZCPE					
	MWCS -		0.930	0.223	4.163	0.001
	ZCPE					
	GWCS -		-0.536	0.159	-3.380	0.013
	ZCCHI					
	GWCS -		-0.974	0.288	-3.378	0.013
	ZCAU					
	NWCS -		-0.492	0.147	-3.355	0.014
	ZCCHI					
	NWCS -		-0.931	0.292	-3.186	0.025
	ZCAU					

regulation of the stress response later during the breeding season (parental phase) and during molt (Cornelius et al., 2011), possibly to reduce behavioral distractions during rearing and avoid the detrimental effects of protein catabolism on feather quality (Jenni-Eiermann et al., 2015; Romero et al., 2005; Wingfield, 2005). There is an evident scarcity of studies from the neotropics, and we observed patterns that are atypical from those described in the north temperate zone, where CORT levels increased during molt in some species and decreased during the breeding period in others. For example, *Euphonia xanthogaster* showed overlap between molt and breeding, higher levels of both baseline and stress-induced CORT levels during molt, and lower levels during breeding. On the other hand, *Glyphorhynchus spirurus*, also showed overlap between molt and breeding, and increase in the baseline but decrease in the stress-induced levels of CORT during molt - although our data should be taken with caution given the low sample sizes and we could not monitor CORT levels in the same individual across LHSs. We did not observe a significant association between latitude or CV DMi and stress-response in neotropical birds. Despite that the effect size analysis suggested that these results are valid beyond the reduced sample sizes, our findings could be the result of a small number of species or habitats sampled. This translated in a poor representation of the environmental variability in the region and reinforces the need for long-term field studies in the neotropics. Furthermore, since many species are widely distributed within the region, we expect to find a synergistic effect between unpredictable events (i.e., Environmental heterogeneity, CV DMi) and latitude on baseline and stress-induced CORT levels if we increase sampling efforts. For example, this phenomenon could be found in resident high-altitude populations in Mediterranean localities. In these environments the complex interaction between natural seasonal variation and global change has resulted in shifts in temperature and rainfall patterns increasing droughts and heatwaves with detrimental consequences over food resources (Ortiz et al., 2023). Thus, the role of the HPA axis in different LHS could be crucial to adapt to those changes. Furthermore, sampling in these localities will help to elucidate the role of unpredictable events and the degree of seasonality on the reactivity of the HPA axis in birds.

4.3. Comparison between North and South American species: *Zonotrichia* genus

In this study we assessed the effect of seasonality and environmental heterogeneity (i.e., Latitude and CV DMi) on baseline and stress-induced CORT levels in Nearctic and Neotropical species of the *Zonotrichia* genus across LHSs. We found similarities in the role of seasonality, LHSs, migratory strategy, and environmental heterogeneity on CORT levels in both species. Latitude, our proxy for seasonality, positively affected baseline and stress-induced levels of CORT in both species. Seasonality is linked to cyclic predictable environmental cues such as variation in precipitation, temperature, and photoperiod which are the major source of information to initiate and terminate LHSs (Lisovski et al., 2017).

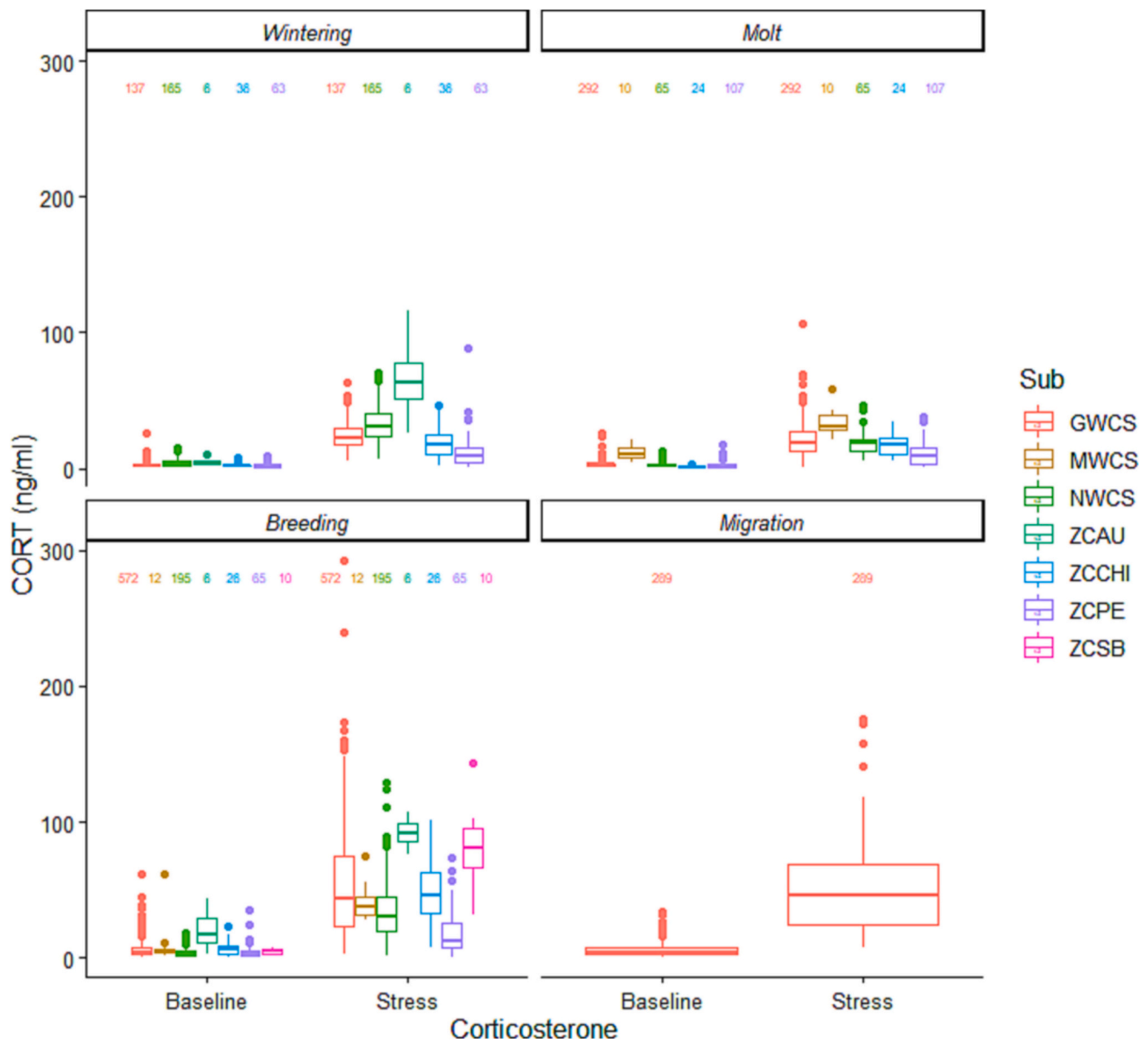


Fig. 3. Baseline and stress-induced (stress) CORT levels during different life history stages and number of individuals in four subspecies of *Zonotrichia capensis* (ZCPE = *Z.c. peruviansis*, ZCCH = *Z.c. chilensis*, ZCSB = *Z.c. sanborni*, ZCAU = *Z.c. australis*), and three subspecies of *Zonotrichia leucophrys* (GWCS = *Z. l. gambellii*, NWCS = *Z. l. nuttalli*, and MWCS = *Z.l. oriantha*).

Higher latitudes are in general linked to well defined seasons with narrower windows for the display of LHSs such as breeding (Gonzalez-Gomez et al., 2018; Wingfield, 2005; Wingfield, 2008b). Accordingly, in higher latitudes animals exhibit more strict division of LHSs (Stutchbury and Morton, 2001; Stutchbury and Morton, 2008; Wingfield, 2008b). Our results are consistent with the idea that species with shorter breeding seasons have higher modulation of the adrenocortical stress response (Krause et al., 2015; Krause et al., 2021); thus, preventing the high fitness cost associated with giving up the only opportunity for producing young during the year (Astheimer et al., 1995; Breuner et al., 2003; Krause et al., 2016b; Meddle et al., 2002).

Our *Z. leucophrys* data corresponds to a narrower range of values of environmental heterogeneity (CV DMi range 0.043–2.207) than in *Z. capensis* (CV DMi range 0.039–3.507), and we did not detect an effect of this variable on CORT levels in any of these species. Moreover, although microclimatic data shows that local variables such as wind speed and temperature are correlated with an increased baseline and stress-induced CORT levels in *Z.l. gambellii* and *Z.l. nuttalli* (Krause et al.,

2021; Krause et al., 2016c), we did not find differences in the role of environmental heterogeneity (CV DMi) on the baseline and stress-induced CORT levels between *Z. leucophrys* and *Z. capensis*. This could be the result of the much stronger effect of seasonality (i.e., predictable events), since it is a more stable and reliable cue, which most likely has shaped the variations in CORT levels across the year during the evolutionary history of these species. However, considering that in this study we are comparing 60 % and just 14 % of the total number of subspecies in *Z. leucophrys* and *Z. capensis* respectively, this statement should be tested increasing the number of subspecies in both species. This would be important since several studies have shown heterogeneity in the timing and duration of LHSs in subspecies of the southern *Z. capensis* across their geographic range (Class and Moore, 2011; Class et al., 2011) in comparison with *Zonotrichia leucophrys* which shows higher seasonality and synchronicity in their breeding and molt schedules (Class et al., 2011; Krause et al., 2016a; Wingfield, 2005). Therefore, it is possible that the effect of unpredictable events (i.e., Environmental heterogeneity, CV DMi) is more dramatic in species that show inflexible and

narrower reproductive windows such as *Z. leucophrys* than in more flexible species such as *Z. capensis*.

We found differences in CORT levels related to subspecies – and therefore migration strategy, and the LHS in both *Z. capensis* and *Z. leucophrys*. We observed that northern and southern hemisphere long-distance migrants which have breeding grounds at high latitudes (*Z. c. australis* and *Z. l. gambellii*), show higher baseline and stress-induced CORT levels than resident species during reproduction. Although our sample size is extremely limited for *Z. c. australis*, baseline CORT levels were high in the southern sub-species, which is coincident with the elevation of CORT levels at the beginning of the reproductive season in long distance migrants in the northern hemisphere (Krause et al., 2021; Krause et al., 2016b; Krause et al., 2016c). In altitudinal migrants (i.e., *Z. c. sanborni* and *Z. l. oriantha*) we also observed higher stress-induced levels of CORT in comparison with resident species; however, we do not have data on other LHSs. In resident sub-species (*Z. c. peruviansis* and *Z. l. nuttalli*), we observed a similar overall pattern of seasonal modulation of stress response. However, the northern sub-species showed stronger increases in CORT levels during breeding. This difference could be related to the fact that even in the resident *Z. l. nuttalli* which occurs in coastal habitats, the breeding seasons are significantly shorter than in the resident sub-species of *Z. capensis* which can breed year-round, and even overlap breeding and molt extensively in an aseasonal environment (Class et al., 2011; Gonzalez-Gomez et al., 2018). Overall, the migration strategy played an important role in the modulation of CORT across LHS. In our opinion, migrants face high energetic challenges as well as limited timing for displaying LHS. Both factors could explain why within the same migratory strategy in both species, we found similar patterns of CORT levels across the year.

Interestingly, we found less CORT changes in *Z. capensis* sub-species than in *Z. leucophrys* during molt, which is similar to what we observed for other neotropical species in this study. This raises the question of whether the relationship between CORT levels and feather quality is different in neotropical environments than in north (Echeverria et al., 2018). However, we lack data for most of the 29 known subspecies of *Z. capensis*, including basic natural history, informing for example if the duration of molt is similar in northern and southern species. Our results highlight the need to achieve a better balance between northern and southern hemisphere research and to focus greater effort on data collection in the neotropics. In addition to baseline and stress-induced CORT levels, information on glucocorticoid receptors and circulating binding proteins across different avian species and geographic ranges would make valuable contributions to the field of avian endocrinology. We encourage researchers to categorize their data in LHSs for more salient comparisons across species, and especially for longer than a single year. Such data would shed light on the sensitivity of the adrenocortical response to stress under different scenarios of environmental seasonality and unpredictability, which could be different in the northern and southern hemispheres. We envision this work as a call to action for more complete studies in this field.

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