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Annual regulation of adrenocortical function in migrant and resident subspecies of white-crowned sparrow

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Abstract

Corticosterone affects physiology and behavior both during normal daily processes but also in response to environmental challenges and is known to mediate life history trade-offs. Many studies have investigated patterns of corticosterone production at targeted times of year, while ignoring underlying annual profiles. We aimed to understand the annual regulation of hypothalamic-pituitary-adrenal (HPA) axis function of both migrant (*Zonotrichia leucophrys gambelii*; n=926) and resident (*Z. l. nutalli*; n=688) subspecies of white-crowned sparrow and how it is influenced by environmental conditions – wind, precipitation, and temperature. We predicted that more dramatic seasonal changes in baseline and stress-induced corticosterone would occur in migrants to precisely time the onset of breeding and cope with environmental extremes on their arctic breeding grounds, while changes in residents would be muted as they experience a more forgiving breeding schedule and comparatively benign environmental conditions in coastal California. During the course of a year, the harshest conditions were experienced the summer breeding grounds for migrants, at which point they had higher corticosterone levels compared to residents. For residents, the winter months coincided with harshest conditions at which point they had higher corticosterone levels than migrants. For both subspecies, corticosterone tended to rise as environmental conditions became colder and windier. We found that the annual maxima in stress-induced corticosterone occurred prior to egg lay for all birds except resident females. Migrants had much higher baseline and acute stress-induced corticosterone during breeding compared to residents; where in a harsher environment the timing

53 of the onset of reproduction is more critical because the breeding season is shorter. Interestingly,
54 molt was the only stage within the annual cycle in which subspecies differences were absent
55 suggesting that a requisite reduction in corticosterone may have to be met for feather growth.
56 These data suggest that modulation of the HPA axis is largely driven by environmental factors,
57 social cues, and their potential interactions with a genetic program.

Introduction

Appropriately timed expression of life history stages (e.g. breeding, molt, migration), allows for optimal temporal alignment of energetic demands with resource availability (Wingfield, Pérez et al. 2017). For instance, breeding coincides with the annual increase in food availability to ensure adequate provisioning of young and an inability to do so can result in reproductive failure (Wingfield, Pérez et al. 2017). The hypothalamic-pituitary-adrenal (HPA) axis, which produces the hormone corticosterone, is one of the key mediators controlling energetics and life history trade-offs (Wingfield and Sapolsky 2003, Bókony, Lendvai et al. 2009, Lattin, Breuner et al. 2016). HPA axis activity is often assessed by measuring circulating corticosterone at both baseline and stress-induced concentrations in plasma. Baseline corticosterone is thought to be positively correlated with energetic demands because it promotes energy mobilization and regulates immune function (Landys, Ramenofsky et al. 2006, Bonier, Martin et al. 2009). Stress-induced corticosterone represents a several fold increase over baseline levels, which on the acute time scale (minutes to hours) is often highly beneficial. Activation of the emergency life history stage by stress-induced corticosterone promotes survival by allocating resources away from non-essential physiological processes (such as breeding) towards those that are critical for self-preservation (Wingfield 2008). Generally in free-living birds, sex-based differences in baseline and peak corticosterone levels are observed during breeding (Romero 2002, Bókony, Lendvai et al. 2009, Romero and Wingfield 2015), but are often absent during non-breeding stages of the annual cycle in songbirds (Rogers, Ramenofsky et al. 1993, Romero 2006, Cornelius, Breuner et al. 2011, Krause, Dorsa et al. 2014). Therefore, disparities in corticosterone during breeding could be linked to sex-specific energetic demands associated with reproductive behaviors, investment in offspring (cost of laying an egg or feeding offspring).

and the body reserves maintained throughout breeding (Wingfield, O'Reilly et al. 1995, Bókonyi, Lendvai et al. 2009, Hau, Ricklefs et al. 2010).

Regardless of taxa, plasma corticosterone, at baseline and stress-induced levels, tend to be highest during breeding and lowest during molt (Romero 2002, Romero and Wingfield 2015), which may suggest an enhanced ability of the HPA axis to affect physiology and behavior during breeding relative to molt. Thus, transduction and integration of internal and external information by the HPA axis, as measured by plasma corticosterone, is likely under strong evolutionary selection. A continuum often exists from low ~~and to~~ high corticosterone responders, which each may offer individual fitness benefits based on a unique set of environmental and social pressures. Thus on evolutionary time-scales, a general shift along the stress phenotype continuum (low to high) at the species level may occur in response to selective pressures created by the environment (Cockrem 2013). We can hypothesize that unique and beneficial patterns of HPA axis function across species evolved in response to adaptation to different ecological niches and their associated challenges. As a consequence, the HPA axis function best matches the challenges associated with an environment - both in the absence and presence of a perturbation (Angelier and Wingfield 2013). For instance, some studies have found that circulating levels of corticosterone, and other hormones in general, are often positively correlated with latitude (L. Z. Garamszegi, K. Hirschenhauser et al. 2008, Hau, Ricklefs et al. 2010, Jessop, Woodford et al. 2013). The predicted enhanced endocrine signaling at high latitude, may suggest a selected phenotype that has resulted from exposure to a short breeding season, increased energetic demands associated with a more challenging environment, and/or greater cost of lifetime reproductive loss should a reproductive attempt fail (Eikenaar, Husak et al. 2012, Jessop, Woodford et al. 2013).

The assumptions about the functional significance of corticosterone can be myopic (Vera, Zenuto et al. 2017) if studies are focused only on key stages of interest (e.g. reproduction) within an animal's annual cycle. The full spectrum of actions of corticosteroids at baseline and stress-induced levels have been reviewed extensively in Sapolsky, Romero et al. (2000) and Wingfield and Romero (2016). As reviewed by Harris (2020), there are currently over 131 proposed hypotheses explaining the functional role of corticosterone for reproduction (n=43), stress coping mechanisms (n=18), and life history stage spanning models (n=37). Many of these hypotheses, focus on targeted relationships which may not consider the full scope of HPA axis function observed throughout an animal's annual cycle. For example, the parental care hypothesis (Wingfield, O'Reilly et al. 1995) states that corticosterone levels must decline from egg lay to feeding of young to promote parental care, while prediction 1 of the CORT-flexibility hypothesis (Lattin, Breuner et al. 2016) states that HPA axis function is elevated before breeding to fine-tune the onset of reproduction~~of the CORT-flexibility hypothesis, (Lattin, Breuner et al. 2016, Bonier, Martin et al. 2009).~~ Thus, data sets consisting of only breeding samples would be unable to distinguish if hormone concentrations were increasing prior to breeding to fine-tune reproductive onset or were decreasing from an annual high to promote parental care. Furthering the list of hypotheses, CORT-adaptation hypothesis predicts that baseline corticosterone can be positively correlated with reproductive investment, especially when factoring the challenges associated with the environment (Bonier, Martin et al. 2009). Thus, inferences that have been drawn from data collected for many of these hypotheses are likely limited without a full annual profile of corticosterone. A full annual profile provides a better understanding of how both current and preceding environmental conditions may affect corticosterone and thus influence these hypotheses.

In this cross-sectional study, we aimed to understand the annual regulation of HPA axis function in two subspecies of white-crowned sparrow (*Zonotrichia leucophrys*), with a focus on hormonal transitions during the sub-stages of breeding (Fig. 1). We were specifically interested in how the HPA axis has been influenced by evolutionary pressures associated with life history strategies and environmental niches in these two subspecies - which diverged from a single common ancestor around 50,000 years ago (Rand, 1948; Winger et al., 2014). We collected baseline and stress-induced corticosterone samples from free-living non-migratory (resident) Nuttall's white-crowned sparrows (*Z. L. nuttalli*) along the coast of Northern California, and the long distance migratory (migrant) Gambel's white-crowned sparrow (*Z. l. gambelii*) on their breeding grounds above the Arctic Circle in Alaska and on their wintering grounds in Northern California (Blanchard and Erickson 1949). We hypothesized that subspecies specific life history strategies and/or environmental conditions have promoted differences in circulating corticosterone levels, which in turn provide a mechanism for regulating energetic demands - assessed by baseline corticosterone, and the ability to respond to perturbations through acute stress-induced corticosterone (presumably to activate the emergency life history stage to modify behavior and physiology). The activation of the emergency life history stage is thought to occur more rapidly when corticosterone is produced at a faster rate and at higher concentrations (Boonstra 2004, Angelier and Wingfield 2013). ~~In~~For animals with few life history stages, who experience benign conditions and a long breeding season, the energetic demands and precise timing of a life history stage and its potential suspension may be less critical and thus would have lower plasma corticosterone compared to species constrained by a greater number of life history stages and a short breeding season (Jacobs and Wingfield, 2000). We predicted that more dramatic seasonal changes in baseline and stress-induced corticosterone would occur in migrants

during onset of breeding to cope with environmental extremes on their arctic breeding grounds, while changes in residents would be muted as they experience a more forgiving breeding schedule and comparatively benign environmental conditions in alignment with the CORT-adaptation Hypothesis (See Fig. 1 for subspecies life history stage alignment).

Materials and Methods

Study species

Gambel's white-crowned sparrows (*Zonotrichia leucophrys gambelii*; hereafter migrants) breed in Alaska and Canada and migrate to their wintering grounds in the Southwest United States and Northern Mexico (Blanchard and Erickson 1949, Lisovski, Németh et al. 2019). Nuttall's white-crowned sparrows (*Zonotrichia leucophrys nuttalli*; hereafter residents) are a non-migratory subspecies that remain year-round on the California coast (Mewaldt and King 1977). Migrants exhibit territorial behaviors soon after arrival on the breeding grounds until the end of egg lay. Residents have been described as being territorial year-round, though large mixed flocks, primarily of juveniles and unpaired females, also form during the winter months (DeWolfe, Baptista et al. 1989, Krause, Németh et al. 2016). There is partial temporal overlap of breeding between the two subspecies with residents raising 2-3 broods between March and July and migrants raising a single brood between May and June (Mewaldt and King 1977, Baker, Mewaldt et al. 1981, Krause, Németh et al. 2016, Boelman, Krause et al. 2017). Both subspecies are socially monogamous, have female only intermittent incubation, and display biparental care both during the nestling and post fledging period (Blanchard 1936, Blanchard 1941, Pérez, Krause et al. 2016). The pre-basic molt is initiated at the termination of breeding and occurs between June and September for residents and between July and August for migrants and no

differences in the timing of pre-basic molt have been observed between the sexes (Morton, King et al. 1969, Mewaldt and King 1977). Figure 1 shows the subspecies alignment of life history stages (molt, migration, breeding) and breeding sub-stages (territoriality, egg lay, incubation, feeding young) based on Krause, Németh et al. (2016) and sample sizes are indicated. It is important to note that not all life history stages are expressed by each subspecies (e.g. residents do not migrate, nor do they have a clear pre-alternate molt, Fig. 1).

Capture and Blood Sampling

Birds were opportunistically captured between 2010 and 2016 using seed-baited Potter traps and Japanese mist nets. Distinct migrant populations were captured at Toolik Research Station, Alaska, USA (N 68°38', W 149°36') during breeding and pre-basic molt, and in Davis, California, USA (N 38° 33', W 121°44') during fall arrival/winter. Residents were captured year-round at Bodega Bay (N 38° 31', W 123°06') and Point Reyes, CA, USA (N 37°59', W 122°58'). Birds were banded with aluminum United States Fish & Wildlife Service bands. Wing chord, tarsus length, beak and skull length were measured with calipers to the nearest tenth of a millimeter. Mass was measured to the nearest tenth of a gram using a Pesola spring scale. During the breeding season, sex was determined by secondary sexual characteristics such as presence of a brood patch in females or an enlarged cloacal protuberance in males (Mewaldt and King 1977, Wingfield and Farner 1978). For both subspecies, brood patch development, behavioral observations, and nest information were used to classify birds into the sub-stages of breeding (see Boelman et al. 2017, Chmura et al. 2018, and Krause et al. 2016 for timing of migrant spring events, nesting success of migrants, and characterization of annual event of both migrants and residents, respectively). During the non-breeding season sex was determined by using a combination of wing chord and body mass (Fugle and Rothstein 1985).

Blood samples were collected between 07:30 and 17:00 for both subspecies. Immediately following capture, a 26-gauge needle was used to pierce the alar vein and blood was collected into heparinized microcapillary tubes (VWR: 15401-560). Baseline blood samples were collected under 3 minutes of entering the net or trap and time was recorded to the nearest second (Romero and Reed 2005). To assess stress-induced corticosterone, birds were placed in an opaque cloth bag and sampled again 30 minutes post-capture (a standardized stress of capture, handling and restraint). Blood samples were stored on ice until they could be processed in the laboratory later in the day. Plasma was separated from red blood cells by centrifugation for 5 minutes at 13,000 g, aspirated using a Hamilton syringe, and placed into a microcentrifuge tube. The samples were stored at -30°C until corticosterone assay quantification. All procedures were approved by UC Davis Institutional Animal Care and Use Committee (IACUC) under protocol #17812.

Meteorological data

Daily mean wind speed (m/s), temperature (°C) and total daily precipitation (mm/day) data from Toolik Research Station, AK, Point Reyes National Seashore, Bodega Bay Marine Laboratory, and Davis were retrieved from Toolik Field Station's Environmental Data Center, Western Regional Climate Center, Bodega Marine Laboratory Observation Node, and University of California, Davis - Atmospheric Sciences, respectively.

Corticosterone Assay

Corticosterone concentrations were determined using a radioimmunoassay as previously described by Wingfield, Vleck et al. (1992). In brief, 15 µL from baseline plasma samples and

10 μ L from the 30 minute samples were combined with 2000 CPM of tritiated corticosterone (Perkin Elmer NET399250UC). 4 mL of freshly distilled dichloromethane was used to extract corticosterone from the plasma samples. Aspirated dichloromethane was placed into a water bath at 35°C and was dried using nitrogen gas. Dried extracts were reconstituted in 550 μ L of phosphate buffer saline with gelatin (PBSG). 100 μ L of reconstituted extract was added to a scintillation vial and combined with 3 mL of scintillation fluid (Perkin Elmer Ultima Gold: 6013329) to determine extraction recovery percentages. Next, 200 μ L of reconstituted extracts were added to duplicate RIA assay tubes with 100 μ L of tritiated corticosterone (Perkin Elmer NET399250UC) and 100 μ L of antiserum (MP Biomedical 07–120016, lot 3R3-PB-20E antibody). Unbound steroid was separated from bound steroid using 500 μ L of dextran coated charcoal solution. Samples were then placed in a centrifuge for 10 minutes at 4°C at 3000 rpm. The supernatant containing bound steroids was decanted into scintillation vials and 3 mL of scintillation fluid were added. Each sample was counted on a Beckman 6500 liquid scintillation counter for 6 minutes or within 2% accuracy. Mean recoveries were 83.72 % and intra- (calculated using C.V. between duplicates) and inter-assay variations were 7.62 and 11.12%, respectively. The assay has been previously validated in white-crowned sparrows (Krause, Pérez et al. 2017).

Statistical Analyses

Statistical analyses were conducted in the R statistical environment version 3.6.1 (R Core Development Team 2018). All meteorological data were combined with individual captures by date. Corticosterone concentrations were analyzed using the following packages: lme4 (Bates, Maechler et al. 2014), lmerTest (Kuznetsova, Brockhoff et al. 2014), multcomp (Hothorn, Bretz

et al. 2016), stringi (Gagolewski and Tartanus 2017), and emmeans (Lenth and Lenth 2018). Weather data including temperature, wind speed, and daily precipitation were analyzed using the AOV function in the Base R package to compare differences between environments experienced by subspecies and post hoc tests were conducted using emmeans with a Tukey's correction. Cohen's d effects size estimates were performed using the emmeans package. Weather data corresponded to the day of capture for each bird in the study which were then averaged by month. We performed model selection on a series of candidate models that included all possible interactions of subspecies, sex, life history stage or month, and time point (baseline vs stress-induced sample) as well as weather variables as either main effects or additional interaction terms on plasma corticosterone levels. Model performance was compared using Akaike's Information Criterion (AIC) (Supp. Tables 1 & 3). We considered any model that had a delta AIC less than 2 to be a viable candidate model. Models compared corticosterone based on life history or month in order to 1) directly compare temporal variation and 2) absolute differences within a life history stage regardless of calendar date. The coefficient of determination (R^2) was used to estimate the proportion of the variance explained by the statistical model based on Nakagawa, Johnson et al. (2017) using the package MuMIn. All post hoc tests were performed using the contrast function in emmeans package with a Tukey's correction for the P-value (Supp. Table 2 & 4).

Results

Comparison of Wind Speed, Temperature, and Precipitation experienced by migrants and residents

A significant interaction between subspecies and month was found for average daily temperature (Fig. 2a, $F_{11,5570}=61.38$, $P<0.001$), average daily wind speed (Fig. 2b, $F_{11,5344}=10.84$, $P<0.001$), and total daily precipitation (Fig 2c, $F_{11,5586}=31.58$, $P<0.0001$).

For migrant habitats, average monthly temperatures ranged from a low of 4.11 and 6.38°C in May and June during breeding and a high of 18.45°C in October during fall arrival (Fig. 2c). The temperature experienced by residents across the annual cycle did not fluctuate as much as it did for migrants with the annual low temperature of 9.88°C observed in February during dispersal and the high in August of 14.38°C during pre-basic molt. Thus, migrants experienced temperatures that ranged by 14.3°C vs 4.5 °C by residents. Average environmental temperature experienced by each subspecies was different for each month except for July.

The coastal habitat residents occupied was characterized as having higher average daily wind speeds compared to the Arctic and central California sites of migrants for all months except September. Within migrant's annual cycle, wind speed was highest from March to August, averaging above 2 m/s while the rest of the year windspeeds were below 2m/s. For residents, wind speed was fairly constant across months, near 4 m/s, while in January, March, and September wind speed was reduced by half on average.

Precipitation was higher at the resident's habitat in August and December but lower during February, and April compared to the migrant's habitat (Fig. 2 c, d, & e).

Effects of month on corticosterone

The best fit model using AIC included all two-way interactions of month, subspecies, and time point plus the interaction of wind, temperature, and precipitation (Supp. Table 1). The main effect of month ($F_{11,2184}= 19.35$, $P<0.001$), subspecies ($F_{1,1549}=11.14$, $P<0.001$) and time point

($F_{1,2004}=519.49$, $P<0.001$) and the interactions of month and sex ($F_{11,2167}=7.46$, $P<0.001$), month and time point ($F_{11,2002}=12.21$, $P<0.001$), and month and species ($F_{11,2223}=17.41$, $P<0.001$), time point and temperature ($F_{1,2004}=4.21$, $P=0.04$), and time point and wind ($F_{1,2002}=9.26$, $P=0.002$) and the interaction of time point, temp and wind ($F_{1,2001}=11.71$, $P=0.003$) while no other factors were significant (Fig. 2a&b). The coefficient of determination (R^2) estimated that the model explained 84.2% of the variance.

The following cross sectional comparisons address changes across month at the population and not the individual level. Baseline corticosterone in female migrants was elevated during June, July, and September compared to all other months (Fig. 2a; see Supp Table 2 for all post hoc tests involving month), while stress-induced levels were elevated during June and July (Fig. 2b). In female residents, baseline corticosterone was lower in March compared to December, while stress-induced levels were highest in April, declined through August, and then rose through December. In female migrants, baseline and stress-induced corticosterone levels were lower than female residents in December, January and April, while this pattern was reversed in June, and July.

Male migrants had higher baseline (Fig. 2a) and stress-induced corticosterone (Fig. 2b) in May and June compared to all other months. Male resident baseline corticosterone was higher in April compared to February, June, July, and August while stress-induced levels were at an annual low during July and August and at an annual high during April and May. Male migrants had lower baseline and stress-induced corticosterone levels compared to male residents from October through April but this pattern was reversed in June and July. No differences were detected between migrants and residents during the months of July, August, and September.

For both subspecies, the 3D plots (Fig 3) and model estimates showed that both baseline and stress-induced levels increased as the temperatures become colder and windspeed increased. In considering the relationship between the environment and corticosterone levels, residents had higher baseline and stress-induced corticosterone levels compared to migrants during the months in which they experienced the coldest conditions of their annual cycle. This pattern was reversed for migrants during the months of May and June when they were experiencing the coldest temperatures of their annual cycle.

Life history stage and sub-stage and corticosterone

Model selection using AIC indicated that the best fit model contained all possible two-way interactions between life history stage, subspecies, and time point plus the interaction of time point with the covariates of wind, precipitation, and temperature (Supp. Table 2). Corticosterone concentrations were affected by the interactions of stage and subspecies ($F_{7,1608}=39.0$, $P<0.001$), stage and time point ($F_{7,1533}=14.04$, $P<0.001$), stage and sex ($F_{7,1565}=8.285$, $P<0.001$), sex and subspecies ($F_{1,1195}=4.50$, $P=0.03$), time point and wind ($F_{1,1537}=4.78$, $P=0.02$), time point, temp, and wind ($F_{1,1627}=4.78$, $P=0.02$) and the main effects of wind ($F_{1,1747}=13.52$, $P<0.001$), temperature ($F_{1,1537}=8.12$, $P=0.004$), rain ($F_{1,1408}=10.85$, $P=0.001$), but not by sex and time point ($F_{1,1266}=0.02$, $P=0.61$, Fig. 4). The coefficient of determination (R^2) estimated that the model explained 84.4% of the variance.

In female migrants, baseline corticosterone levels were elevated throughout breeding, from arrival on the breeding grounds through incubation, and then declined during feeding of young and pre-basic molt before reaching their annual nadir during the autumn arrival and winter stages (Fig. 4a; all post hoc tests involving life history stage are found in Supp. Table 4). In

migrant males, baseline corticosterone increased from winter through egg lay and then declined through pre-basic molt and autumn arrival (Fig. 4a). Stress-induced corticosterone concentrations in female migrants were at their annual maximum from arrival through incubation while levels were lowest during molt, although not significantly different from autumn arrival (Fig. 4b). In male migrants, stress-induced corticosterone levels increased from winter and reached an annual maximum during territorial and egg lay sub-stages and declined to the annual low at pre-basic molt (Fig. 4b). Migrant males had higher baseline and stress-induced corticosterone from spring arrival through incubation compared to migrant females.

Baseline corticosterone levels were lowest in male residents during feeding of young and pre-basic molt, while in female residents corticosterone was lowest during incubation (Fig. 4a). Stress-induced corticosterone levels in female residents were lowest during pre-basic molt, although not significantly different from feeding young or autumn flocking stages (Fig. 4b). In male residents, stress-induced corticosterone increased from winter through egg lay and declined to their lowest points during pre-basic molt and autumn flocking stages (Fig. 4b).

For both sexes, subspecies differences in baseline and stress-induced corticosterone were present in all life history stages except for pre-basic molt. In both sexes, migrants had lower corticosterone levels during winter and autumn arrival stages than residents, but higher levels from spring arrival through feeding of young (Fig. 4a&b). Males for both subspecies had higher baseline and stress-induced corticosterone than females during territorial and egg lay stages, only.

Discussion

Corticosterone is a pleiotropic hormone that has been implicated in numerous physiological and behavioral processes. As a consequence, hundreds of hypotheses have been put forward describing the functional role of corticosterone (Harris 2020). However, many of these studies utilize small data sets to draw their inferences. Our data set, collected from 1614 individuals, highlights that circulating corticosterone levels reflect a nuanced interplay between the shifting demands of individual life history stages and environmental conditions that are unique to each subspecies. Temporal patterns and absolute amounts of corticosterone differed between the subspecies. Yet, regardless of subspecies, baseline and stress-induced corticosterone levels increased as wind speed increased, and temperatures declined (Fig 3).

Breeding and Corticosterone

As the breeding season progressed, both baseline and stress-induced corticosterone reached an annual maximum during the territorial and egg lay sub-stages of breeding but was higher in migrants compared to residents. Our data provide support for prediction 1 of the CORT-Flexibility Hypothesis which states that the annual maximum in corticosterone occurs prior to egg lay (Lattin, Breuner et al. 2016). The CORT-Flexibility hypothesis proposes that modulation of various HPA axis regulatory elements for circulating levels of hormones, CBG, enzymes and receptors allow for rapid and robust suppression of the HPG axis which can delay the onset of egg lay. Thus, further studies are required to fully support the CORT-Flexibility hypothesis by determining if tissue sensitivity is changing in addition to plasma glucocorticoids. Our data then give pause to the Parental Care Hypothesis (Wingfield, O'Reilly et al. 1995) which states that HPA axis function declines at the transition from the pre-parental to parental stage, but in many ways ignores the initial cause of the annual increase in peak corticosterone (Wingfield, O'Reilly et al. 1995, Chastel, Lacroix et al. 2005). The seasonal peak in HPA axis function may

be critical for fine-tuning reproduction onset, while the decline observed starting at incubation serves to prevent nest abandonment and foster parental care. Thus, the Parental Care Hypothesis is valid in that high corticosterone levels can directly interfere with parental care, but corticosterone is really declining towards levels that are observed through most of the calendar year. Lastly, our data provide support for the CORT- adaptation hypothesis because higher baseline and stress-induced corticosterone levels were observed in the migrant during breeding who have just a single opportunity to breed during the year and are faced with harsher environmental conditions. The CORT-adaptation hypothesis predicts that brood value or environmental harshness can be mutually exclusive or additive which was recently confirmed in a meta analysis by Vitousek, Johnson et al. (2019)

Within breeding, sex-based differences persisted from spring arrival through feeding of young in migrants, while in residents differences were only detected during territorial and egg lay sub-stages. It has often been reported that the sex providing the bulk of the parental care has lower corticosterone levels, which is made evident in examples where males incubate and provision offspring (Wingfield, O'Reilly et al. 1995, Bókony, Lendvai et al. 2009). The division in parental care and HPA axis function are likely intricately tied to social information, daily activity budgets and its effects on energy balance. Peak hormone levels for testosterone and corticosterone occur prior to- or around egg lay suggesting that social information is having a direct effect on hormone patterns during the period of greatest social instability (Goymann, Moore et al. 2019). For male white-crowned sparrows, territorial and egg lay sub-stages occur during the annual nadir in fat reserves, which is likely a consequence of shifts in time budgeting away from foraging to mate guarding, singing and copulation (King and Farner 1966). Females maintain much higher fat reserves throughout the breeding season and a greater proportion of

their time is allocated to foraging - especially during egg lay (Wingfield and Farner 1978). Clear relationships have been demonstrated between energetic reserves and peak corticosterone which suggests a threshold effect for triggering an increase in HPA axis function (Lynn, Breuner et al. 2003, Krause, Pérez et al. 2017). Males are much closer to the threshold for enhancing HPA axis activity during breeding because of their low fat reserves (perturbation resistance potential, PRP, Wingfield et al., 2017) and could be a contributing factor to their higher overall HPA axis function. Greater fat reserves in females after the transition to parental phase might allow the female to be more attentive to incubation, especially during inclement weather (Wingfield, O'Reilly et al. 1995, Krause, Chmura et al. 2015, Walker, Meddle et al. 2015)

Corticosterone and Molt

During pre-basic molt following clutch completion, baseline and peak corticosterone levels reached an annual low and there were no significant differences between either sex or subspecies. It is not clear whether HPA axis function is reduced to an absolute minimum in all groups, or it has been reduced to a requisite level for optimum feather growth. Increased corticosterone during molt results in poor feather quality, which likely affects flight performance, but more importantly insulation (Cornelius, Perfito et al. 2011, Lattin, Reed et al. 2011). It should also be noted that molt was the only stage in which we did not detect a significant difference between subspecies throughout the annual cycle. Interestingly, molt also coincides with the warmest months and highest food availability for migrants - as measured by arthropod biomass, vegetation greenness (NDVI), seed and berry production, on the breeding grounds (Sweet, Asmus et al. 2014, Asmus, Chmura et al. 2018) but is also most likely true for residents as well. From pre-basic molt through the end of the wintering stage, no sex differences in corticosterone were detected for either subspecies which also coincides with the increased total

body lipid reserves and a period in which the reproductive axis is inactive (King, Barker et al. 1963).

Lessons from migrants and residents

In alignment with our prediction, greater annual modulation of stress-induced corticosterone occurred in the migrants with males showing 4.25x and females a 2.5x fold change, compared to the residents in which males showed a 2.5x and females a 1.5x fold change from the annual low to maximum. Other studies have observed higher corticosterone peak concentrations in populations facing harsher or more unpredictable environmental conditions (Rogers, Ramenofsky et al. 1993, Dunlap and Wingfield 1995, Wingfield, Kubokawa et al. 1995, Holberton and Able 2000, Krause, Chmura et al. 2015, Walker, Meddle et al. 2015, Krause, Pérez et al. 2016). Of particular note, a similar pattern in the magnitude of change in baseline and peak corticosterone levels across stages of the annual cycle was found in rufous collared sparrows, *Zonotrichia capensis*, in South America comparing subspecies in a highly stable environment versus a highly unpredictable environment (González-Gómez, Echeverria et al. 2018).

Work by Barbara Blanchard and Donald Farner asserted over 70 years ago that distinct time schedules existed between migrants and residents, based on gonad morphology and function. Photoinduced residents initiate recrudescence as early as December - while photoperiod is still declining, as opposed to mid-March for migrants, when photoperiod is increasing (Blanchard 1941, Blanchard and Erickson 1949, Farner and Follett 1966). Genetic differences between subspecies in critical day length may ultimately determine the initiation of seasonal changes in the HPG and HPA axes. However, the seasonal dynamics of HPA axis function in

terms of circulating levels of baseline and stress-induced corticosterone do not appear from genetic differences alone, as a common garden experiment with migrants and residents did not detect any HPA differences during winter or spring months (Ramenofsky, Campion et al. 2017). Similarly, Romero and Wingfield (1999), showed that captive Gambel's white-crowned sparrows did not show baseline and stress-induced changes across their annual cycle.

It is evident that environmental conditions and social cues were a major driver of corticosterone levels (see Fig. 4). Migrants faced the most thermally challenging conditions within their annual cycle during breeding - with annual lows in temperature and highs in wind speed, and at this time had higher corticosterone levels than residents. Conversely, for residents the winter was the most thermally challenging period – with annual highs in rain and wind, and lows in temperature (but the latter only slightly), and at these points residents had higher corticosterone levels compared to migrants. Thus, the subspecies experiencing the most thermally challenging period of their annual cycle had the highest measured corticosterone levels. A recent review by de Bruijn and Romero (2018) found that challenging conditions associated with weather often resulted in elevated glucocorticoids. Environmental conditions may necessitate rapid switches in substrate utilization (fats, protein and carbohydrates) for metabolism during the transition points across the three stages of fasting – these transitions are thought to be mediated by increasing corticosterone concentrations as body mass declines (Cherel, Robin et al. 1988, Cherel, Robin et al. 1992, Landys, Ramenofsky et al. 2006). Migrants may have a lower margin for error during breeding based on their environmental conditions, which has selected for a more robust and fast acting physiological response to environmental challenges (Angelier and Wingfield 2013). These data suggest that modulation of the HPA axis is largely driven by environmental factors, social cues, and their potential interactions with a

genetic program. A common garden experiment in which migrants and residents were exposed to each others' environment would be critical for separating the environmental and genetic determinants of HPA axis function.

Conclusion

There are temporal differences in seasonal HPA axis activity between resident and migrant white-crowned sparrows. The results clearly support the first prediction of the CORT-Flexibility hypothesis that states that the highest corticosterone levels are observed at the onset of the breeding season and may be critical for fine-tuning the onset of reproduction. The magnitude of the change in baseline and peak corticosterone was better predicted when weather variables were included in the model. This study builds on a growing literature that shows populations exposed to harsher environmental conditions often have higher circulating levels of corticosterone. Thus, the challenges associated with an animal's environment may be critical in regulating transitions across life history stages. We propose that further studies should elucidate seasonal changes in tissue sensitivity through measurements of receptors and metabolizing enzymes as well as negative feedback control mechanisms that govern the observed seasonal glucocorticoid patterns.

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References

- Angelier, F. and J. C. Wingfield (2013). "Importance of the glucocorticoid stress response in a changing world: Theory, hypotheses and perspectives." General and Comparative Endocrinology **190**(0): 118-128.
- Asmus, A. L., H. E. Chmura, T. T. Høye, J. S. Krause, S. K. Sweet, J. H. Perez, N. T. Boelman, J. C. Wingfield and L. Gough (2018). "Shrub shading moderates the effects of weather on arthropod activity in arctic tundra." Ecological Entomology **43**(5): 647-655.
- Baker, M. C., L. R. Mewaldt and R. M. Stewart (1981). "Demography of White-Crowned Sparrows (*Zonotrichia Leucophrys Nuttalli*)." Ecology **62**(3): 636-644.
- Bates, D., M. Maechler, B. Bolker and S. Walker (2014). lme4: Linear mixed-effects models using Eigen and S4. R package version 1.1-7.
- Blanchard, B. D. (1936). "Continuity of behavior in the Nuttall white-crowned sparrow." The Condor **38**(4): 145-150.
- Blanchard, B. D. (1941). "The white-crowned sparrows (*Zonotrichia leucophrys*) of the Pacific Seaboard: Environment and annual cycle." University of California Publication Zoology **46**: 1-176.
- Blanchard, B. D. and M. M. Erickson (1949). "The cycle of the Gambel sparrow." University of California Publication Zoology **47**(11): 255-318.
- Boelman, N. T., J. S. Krause, S. K. Sweet, H. E. Chmura, J. H. Perez, L. Gough and J. C. Wingfield (2017). "Extreme spring conditions in the Arctic delay spring phenology of long-distance migratory songbirds." Oecologia.
- Bókony, V., Ádám Z. Lendvai, A. Liker, F. Angelier, John C. Wingfield and O. Chastel (2009). "Stress response and the value of reproduction: Are birds prudent parents?" The American Naturalist **173**(5): 589-598.
- Bonier, F., P. R. Martin, I. T. Moore and J. C. Wingfield (2009). "Do baseline glucocorticoids predict fitness?" Trends Ecol Evol **24**.
- Bonier, F., P. R. Martin, I. T. Moore and J. C. Wingfield (2009). "Do baseline glucocorticoids predict fitness?" Trends in Ecology & Evolution **24**(11): 634-642.
- Boonstra, R. (2004). "Coping with changing northern environments: The role of the stress axis in birds and mammals." Integrative and Comparative Biology **44**(2): 95-108.

Chastel, O., A. Lacroix, H. Weimerskirch and G. W. Gabrielsen (2005). "Modulation of prolactin but not corticosterone responses to stress in relation to parental effort in a long-lived bird." Hormones and Behavior **47**(4): 459-466.

Cherel, Y., J.-P. Robin, A. Heitz, C. Calgari and Y. le Maho (1992). "Relationships between lipid availability and protein utilization during prolonged fasting." Journal of Comparative Physiology B **162**(4): 305-313.

Cherel, Y., J. P. Robin, O. Walch, H. Karmann, P. Netchitailo and Y. L. Maho (1988). "Fasting in king penguin. I. Hormonal and metabolic changes during breeding." American Journal of Physiology-Regulatory, Integrative and Comparative Physiology **254**(2): R170-R177.

Chmura, H. E., J. S. Krause, J. H. Pérez, A. Asmus, S. K. Sweet, K. E. Hunt, S. L. Meddle, R. McElreath, N. T. Boelman, L. Gough and J. C. Wingfield (2018). "Late-season snowfall is associated with decreased offspring survival in two migratory arctic-breeding songbird species." Journal of Avian Biology **49**(9): e01712.

Cockrem, J. F. (2013). "Corticosterone responses and personality in birds: individual variation and the ability to cope with environmental changes due to climate change." General and Comparative Endocrinology **190**: 156-163.

Cornelius, J. M., C. W. Breuner and T. P. Hahn (2011). "Coping with the extremes: stress physiology varies between winter and summer in breeding opportunists." Biology Letters.

Cornelius, J. M., N. Perfito, R. Zann, C. W. Breuner and T. P. Hahn (2011). "Physiological trade-offs in self-maintenance: plumage molt and stress physiology in birds." The Journal of Experimental Biology **214**(16): 2768-2777.

de Bruijn, R. and L. M. Romero (2018). "The role of glucocorticoids in the vertebrate response to weather." General and Comparative Endocrinology.

DeWolfe, B. B., L. F. Baptista and L. Petrinovich (1989). "Song development and territory establishment in Nuttall's white-crowned sparrows." The Condor **91**(2): 397-407.

Dunlap, K. D. and J. C. Wingfield (1995). "External and internal influences on indices of physiological stress. I. Seasonal and population variation in adrenocortical secretion of free-living lizards, *Sceloporus occidentalis*." Journal of Experimental Zoology **271**(1): 36-46.

Eikenaar, C., J. Husak, C. Escallón and I. T. Moore (2012). "Variation in testosterone and corticosterone in amphibians and reptiles: Relationships with latitude, elevation, and breeding season length." The American Naturalist **180**(5): 642-654.

Environmental Data Center. 2018. Meteorological monitoring program at Toolik, Alaska. Toolik Field Station, Institute of Arctic Biology, University of Alaska Fairbanks, Fairbanks, AK 99775. http://toolik.alaska.edu/edc/abiotic_monitoring/data_query.php.

Farner, D. S. and B. K. Follett (1966). "Light and other environmental factors affecting avian reproduction." Journal of Animal Science **25**(Supplement): 90-115.

Fugle, G. N. and S. I. Rothstein (1985). "Age- and sex-related variation in size and crown plumage brightness in wintering white-crowned sparrows." Journal of Field Ornithology **56**(4): 356-368.

Gagolewski, M. and B. Tartanus (2017). "R package stringi: Character string processing facilities." Computer software manual]. Retrieved from <http://www.gagolewski.com/software/stringi>.

González-Gómez, P. L., V. Echeverria, C. F. Estades, J. H. Perez, J. S. Krause, P. Sabat, J. Li, D. Kültz and J. C. Wingfield (2018). "Contrasting seasonal and aseasonal environments across stages of the annual cycle in the rufous-collared sparrow, *Zonotrichia capensis*: Differences

in endocrine function, proteome and body condition." Journal of Animal Ecology **87**(5): 1364-1382.

Goymann, W., I. T. Moore and R. F. Oliveira (2019). "Challenge Hypothesis 2.0: A Fresh Look at an Established Idea." BioScience **69**(6): 432-442.

Harris, B. N. (2020). "Stress hypothesis overload: 131 hypotheses exploring the role of stress in tradeoffs, transitions, and health." General and Comparative Endocrinology **288**: 113355.

Hau, M., R. E. Ricklefs, M. Wikelski, K. A. Lee and J. D. Brawn (2010). "Corticosterone, testosterone and life-history strategies of birds." Proc R Soc B Biol Sci **277**.

Holberton, R. L. and K. P. Able (2000). "Differential migration and an endocrine response to stress in wintering dark-eyed juncos (*Junco hyemalis*)." Proceedings of the Royal Society of London. Series B: Biological Sciences **267**(1455): 1889-1896.

Hothorn, T., F. Bretz, P. Westfall, R. M. Heiberger, A. Schuetzenmeister, S. Scheibe and M. T. Hothorn (2016). "Package 'multcomp'." Simultaneous inference in general parametric models. Project for Statistical Computing, Vienna, Austria.

Jessop, T. S., R. Woodford and M. R. E. Symonds (2013). "Macrostress: do large-scale ecological patterns exist in the glucocorticoid stress response of vertebrates?" Functional Ecology **27**(1): 120-130.

King, J. R., S. Barker and D. S. Farner (1963). "A Comparison of energy reserves during autumnal and vernal migratory periods in the White-crowned sparrow, *Zonotrichia leucophrys gambelii*." Ecology **44**(3): 513-521.

King, J. R. and D. S. Farner (1966). "The adaptive role of winter fattening in the white-crowned sparrow with comments on its regulation." The American Naturalist **100**(914): 403-418.

Krause, J. S., H. E. Chmura, J. H. Pérez, L. N. Quach, A. Asmus, K. R. Word, M. A. McGuigan, S. K. Sweet, S. L. Meddle, L. Gough, N. Boelman and J. C. Wingfield (2015). "Breeding on the leading edge of a northward range expansion: differences in morphology and the stress response in the arctic Gambel's white-crowned sparrow." Oecologia **180**(1): 33-44.

Krause, J. S., D. Dorsa and J. C. Wingfield (2014). "Changes in plasma concentrations of progesterone, dehydroepiandrosterone and corticosterone in response to acute stress of capture, handling and restraint in two subspecies of white-crowned sparrows." Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology **177**(0): 35-40.

Krause, J. S., Z. Németh, J. H. Pérez, H. E. Chmura, M. Ramenofsky and J. C. Wingfield (2016). "Annual hematocrit profiles in two subspecies of white-crowned sparrow: A migrant and a resident comparison." Physiological and Biochemical Zoology **89**(1): 51-60.

Krause, J. S., J. H. Pérez, H. E. Chmura, S. K. Sweet, S. L. Meddle, K. E. Hunt, L. Gough, N. Boelman and J. C. Wingfield (2016). "The effect of extreme spring weather on body condition and stress physiology in Lapland longspurs and white-crowned sparrows breeding in the Arctic." General and Comparative Endocrinology **237**: 10-18.

Krause, J. S., J. H. Pérez, S. L. Meddle and J. C. Wingfield (2017). "Effects of short-term fasting on stress physiology, body condition, and locomotor activity in wintering male white-crowned sparrows." Physiology & Behavior **177**: 282-290.

Kunznetsova, A., P. B. Brockhoff and R. H. B. Christensen (2014). lmerTest: Tests for random and fixed effects for linear mixed effects models (lmer objects of lme4 package). R package version 2.0-11.

L. Z. Garamszegi, K. Hirschenhauser, V. Bókony, M. Eens, S. Hurtrez-Boussès, A. P. Møller, R. F. Oliveira and J. C. Wingfield (2008). "Latitudinal Distribution, Migration, and Testosterone Levels in Birds." The American Naturalist **172**(4): 533-546.

Landys, M. M., M. Ramenofsky and J. C. Wingfield (2006). "Actions of glucocorticoids at a seasonal baseline as compared to stress-related levels in the regulation of periodic life processes." General and Comparative Endocrinology **148**(2): 132-149.

Lattin, C. R., C. W. Breuner and L. M. Romero (2016). "Does corticosterone regulate the onset of breeding in free-living birds?: The CORT-Flexibility Hypothesis and six potential mechanisms for priming corticosteroid function." Hormones and behavior **78**: 107-120.

Lattin, C. R., J. M. Reed, D. W. DesRochers and L. M. Romero (2011). "Elevated corticosterone in feathers correlates with corticosterone-induced decreased feather quality: a validation study." Journal of Avian Biology **42**(3): 247-252.

Lenth, R. and M. R. Lenth (2018). "Package 'lsmeans'." The American Statistician **34**(4): 216-221.

Lisovski, S., Z. Németh, J. C. Wingfield, J. S. Krause, K. A. Hobson, N. E. Seavy, J. Gee and M. Ramenofsky (2019). "Distribution and migration pattern of Gambel's White-crowned sparrow along the Pacific flyway." J Ornithol In Press.

Lynn, S. E., C. W. Breuner and J. C. Wingfield (2003). "Short-term fasting affects locomotor activity, corticosterone, and corticosterone binding globulin in a migratory songbird." Hormones and Behavior **43**(1): 150-157.

Mewaldt, L. R. and J. R. King (1977). "The annual cycle of white-crowned sparrows (*Zonotrichia leucophrys nuttalli*) in Coastal California." The Condor **79**(4): 445-455.

Morton, M. L., J. R. King and D. S. Farner (1969). "Postnuptial and postjuvenile molt in White-crowned sparrows in Central Alaska." The Condor **71**(4): 376-385.

Nakagawa, S., P. C. D. Johnson and H. Schielzeth (2017). "The coefficient of determination R^2 and intra-class correlation coefficient from generalized linear mixed-effects models revisited and expanded." Journal of The Royal Society Interface **14**(134): 20170213.

Pérez, J. H., J. S. Krause, H. E. Chmura, S. Bowman, M. McGuigan, A. L. Asmus, S. L. Meddle, K. E. Hunt, L. Gough and N. T. Boelman (2016). "Nestling growth rates in relation to food abundance and weather in the Arctic." The Auk.

Quirici, V., C. I. Venegas, P. L. González-Gómez, G. J. Castaño-Villa, J. C. Wingfield and R. A. Vásquez (2014). "Baseline corticosterone and stress response in the Thorn-tailed Rayadito (*Aphrastura spinicauda*) along a latitudinal gradient." General and Comparative Endocrinology **198**: 39-46.

R Core Development, T. (2018). R: a language and environment for statistical computing. R Foundation for Statistical Computing. Vienna, Austria.

Ramenofsky, M., A. W. Campion, J. H. Pérez, J. S. Krause and Z. Németh (2017). "Behavioral and physiological traits of migrant and resident white-crowned sparrows: a common garden approach." The Journal of Experimental Biology **220**(7): 1330-1340.

Rogers, C. M., M. Ramenofsky, E. D. Ketterson, V. Nolan, Jr. and J. C. Wingfield (1993). "Plasma corticosterone, adrenal mass, winter weather, and season in nonbreeding populations of dark-eyed juncos (*Junco hyemalis hyemalis*)." The Auk **110**(2): 279-285.

Romero, L. M. (2006). "Seasonal changes in hypothalamic-pituitary-adrenal axis sensitivity in free-living house sparrows (*Passer domesticus*)." General and Comparative Endocrinology **149**(1): 66-71.

Romero, L. M. and J. M. Reed (2005). "Collecting baseline corticosterone samples in the field: is under 3 min good enough?" Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology **140**(1): 73-79.

Romero, L. M. and J. C. Wingfield (2015). Tempests, poxes, predators, and people: stress in wild animals and how they cope, Oxford University Press.

Romero, M. L. (2002). "Seasonal changes in plasma glucocorticoid concentrations in free-living vertebrates." General and Comparative Endocrinology **128**(1): 1-24.

Sapolsky, R. M., L. M. Romero and A. U. Munck (2000). "How do glucocorticoids influence stress responses? Integrating permissive, suppressive, stimulatory, and preparative actions." Endocrine Reviews **21**(1): 55-89.

Sweet, S. K., A. Asmus, M. E. Rich, J. Wingfield, L. Gough and N. T. Boelman (2014). "NDVI as a predictor of canopy arthropod biomass in the Alaskan arctic tundra." Ecological Applications.

Vera, F., R. Zenuto and C. D. Antenucci (2017). "Expanding the actions of cortisol and corticosterone in wild vertebrates: A necessary step to overcome the emerging challenges." General and Comparative Endocrinology **246**: 337-353.

Vitousek, M. N., M. A. Johnson, C. J. Downs, E. T. Miller, L. B. Martin, C. D. Francis, J. W. Donald, M. J. Fuxjager, W. Goymann, M. Hau, J. F. Husak, B. K. Kircher, R. Knapp, L. A. Schoenle and T. D. Williams (2019). "Macroevolutionary patterning in glucocorticoids suggests different selective pressures shape baseline and stress-induced levels." The American Naturalist **193**(6): 866-880.

Walker, B. G., S. L. Meddle, L. M. Romero, M. M. Landys, J. Reneerkens and J. C. Wingfield (2015). "Breeding on the extreme edge: Modulation of the adrenocortical response to acute stress in two high arctic passerines." Journal of Experimental Zoology Part A: Ecological Genetics and Physiology **323**(4): 266-275.

Wingfield, J. C. (2008). "Comparative endocrinology, environment and global change." General and Comparative Endocrinology **157**(3): 207-216.

Wingfield, J. C. and D. S. Farner (1978). "The annual cycle of plasma irLH and steroid hormones in feral populations of the white-crowned sparrow, *Zonotrichia leucophrys gambelii*." Biology of Reproduction **19**(5): 1046-1056.

Wingfield, J. C., K. Kubokawa, K. Ishida, S. Ishii and M. Wada (1995). "The adrenocortical response to stress in male bush warblers, *Cettia diphone*: a comparison of breeding populations in Honshu and Hokkaido, Japan." Zoological Science **12**(5): 615-621.

Wingfield, J. C., K. M. O'Reilly and L. B. Astheimer (1995). "Modulation of the adrenocortical responses to acute stress in Arctic birds: A possible ecological basis." American Zoologist **35**(3): 285-294.

Wingfield, J. C., J. H. Pérez, J. S. Krause, K. R. Word, P. L. González-Gómez, S. Lisovski and H. E. Chmura (2017). "How birds cope physiologically and behaviourally with extreme climatic events." Philosophical Transactions of the Royal Society B: Biological Sciences **372**(1723).

Wingfield, J. C. and L. Romero (2016). Tempests, Predators, Poxes, and People: Stress in Wild Animals and how They Cope, Oxford University Press.

Wingfield, J. C. and R. M. Sapolsky (2003). "Reproduction and resistance to stress: When and how." Journal of Neuroendocrinology **15**(8): 711-724.

Wingfield, J. C., C. M. Vleck and M. C. Moore (1992). "Seasonal changes of the adrenocortical response to stress in birds of the Sonoran desert." Journal of Experimental Zoology **264**(4): 419-428.

Figure and Table Legends

Figure 1.

Summary of life-history stages, sub-stages, and the months in which they occur for both migrant Gambel's (grey trapezoids) and resident Nuttall's (white trapezoids) white-crowned sparrows. Samples sizes are included for each species by life history stage and calendar month.

Figure 2.

The effect of month on a) temperature ($^{\circ}\text{C}$), b) wind speed (m/s) and c) precipitation, (mm/day) d) baseline corticosterone and e) stress-induced circulating concentrations of plasma corticosterone (ng/ml) migrant (symbol: *circles*, line: *solid*) and resident (symbol: *squares*, line: *dashed*) white-crowned sparrows. Males are denoted by filled symbols and females by unfilled symbols. The study uses a cross sectional design which reflects changes at the population and not the individual level. Subspecies differences in corticosterone within each month is indicated by an asterisks (*) for females and by the cross (†) for males. Subspecies differences in weather parameters for each month are indicated by section sign (§).

Figure 3.

3D plots showing the interaction of average wind speed (m/s) and temperature ($^{\circ}\text{C}$) on baseline (a-d) and stress-induced plasma corticosterone (CORT; ng/ml) (e-h) in (a,e) female migrants, (b,f) male migrants, (c, g) female residents, and (d, h) male residents, respectively. The color gradient from blue to red indicates increasing concentrations of corticosterone.

Figure 4.

The effects of life history stage and sub-stages of the annual cycle on a) baseline and b) stress-induced circulating concentrations of plasma corticosterone (ng/ml). Letters that are different from one another denote significance across stages within a sex and subspecies using post hoc contrasts with Tukey's correction. (UPPERCASE = Migrant females, lowercase = migrant males, *UPPERCASE ITALICIZED* = resident females, *lower case italicized* = resident males). Asterisks (*) indicate differences between sexes within a sub-stage and subspecies. The cross (†) indicates subspecies differences within a sex and life history stage.

Supplemental Table 1.

Linear mixed effects models were tested for the best fit using Akiake's information criterion (AIC) and compared against a null model. All four, three, and two-way interactions were run for subspecies, stress, sex and sub-stage of the annual cycle. Weather variables were added as a covariate or the interaction with the categorical factor.

Supplemental Table 2.

759 Post hoc tests investigating the effects of month, subspecies, and sex on plasma levels of
760 corticosterone at both baseline and stress-induced levels (30 min). Tests statistics were calculated
761 using Tukey's Honestly Significant Difference Test with the emmeans package in R statistical
762 analysis software. Effects size was estimated using Cohen's D.

763
764 Supplemental Table 3.

765 Linear mixed effects models were tested for the best fit using Akiake's information criterion
766 (AIC) and compared against a null model. All four, three, and two-way interactions were run for
767 subspecies, stress, sex and sub-stage of the annual cycle. Weather variables were added as a
768 covariate or the interaction with the categorical factor.

769
770 Supplemental Table 4

771 Post hoc tests investigating the effects of life history stage, subspecies, and sex on plasma levels
772 of corticosterone at both baseline and stress-induced levels (30 min). Tests statistics were
773 calculated using Tukey's Honestly Significant Difference Test with the emmeans package in R
774 statistical analysis software. Effects size was estimated using Cohen's D.
775