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

1 **Annual regulation of adrenocortical function in migrant and resident subspecies of white-**

2 **crowned sparrow**

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32   **Abstract**

33

34   Corticosterone affects physiology and behavior both during normal daily processes but

35   also in response to environmental challenges and is known to mediate life history trade-offs.

36   Many studies have investigated patterns of corticosterone production at targeted times of year,

37   while ignoring underlying annual profiles. We aimed to understand the annual regulation of

38   hypothalamic-pituitary-adrenal (HPA) axis function of both migrant (*Zonotrichia leucophrys*

39   *gambelii*; n=926) and resident (*Z. l. nutalli*; n=688) subspecies of white-crowned sparrow and

40   how it is influenced by environmental conditions – wind, precipitation, and temperature. We

41   predicted that more dramatic seasonal changes in baseline and stress-induced corticosterone

42   would occur in migrants to precisely time the onset of breeding and cope with environmental

43   extremes on their arctic breeding grounds, while changes in residents would be muted as they

44   experience a more forgiving breeding schedule and comparatively benign environmental

45   conditions in coastal California. During the course of a year, the harshest conditions were

46   experienced the summer breeding grounds for migrants, at which point they had higher

47   corticosterone levels compared to residents. For residents, the winter months coincided with

48   harshest conditions at which point they had higher corticosterone levels than migrants. For both

49   subspecies, corticosterone tended to rise as environmental conditions became colder and windier.

50   We found that the annual maxima in stress-induced corticosterone occurred prior to egg lay for

51   all birds except resident females. Migrants had much higher baseline and acute stress-induced

52   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),

82   and the body reserves maintained throughout breeding (Wingfield, O'Reilly et al. 1995, Bókony,

83   Lendvai et al. 2009, Hau, Ricklefs et al. 2010).

84   Regardless of taxa, plasma corticosterone, at baseline and stress-induced levels, tend to

85   be highest during breeding and lowest during molt (Romero 2002, Romero and Wingfield 2015),

86   which may suggest an enhanced ability of the HPA axis to affect physiology and behavior during

87   breeding relative to molt. Thus, transduction and integration of internal and external information

88   by the HPA axis, as measured by plasma corticosterone, is likely under strong evolutionary

89   selection. A continuum often exists from low and to high corticosterone responders, which each

90   may offer individual fitness benefits based on a unique set of environmental and social pressures.

91   Thus on evolutionary time-scales, a general shift along the stress phenotype continuum (low to

92   high) at the species level may occur in response to selective pressures created by the

93   environment (Cockrem 2013). We can hypothesize that unique and beneficial patterns of HPA

94   axis function across species evolved in response to adaptation to different ecological niches and

95   their associated challenges. As a consequence, the HPA axis function best matches the

96   challenges associated with an environment - both in the absence and presence of a perturbation

97   (Angelier and Wingfield 2013). For instance, some studies have found that circulating levels of

98   corticosterone, and other hormones in general, are often positively correlated with latitude

99   (L. Z. Garamszegi, K. Hirschenhauser et al. 2008, Hau, Ricklefs et al. 2010, Jessop, Woodford et

100   al. 2013). The predicted enhanced endocrine signaling at high latitude, may suggest a selected

101   phenotype that has resulted from exposure to a short breeding season, increased energetic

102   demands associated with a more challenging environment, and/or greater cost of lifetime

103   reproductive loss should a reproductive attempt fail (Eikenaar, Husak et al. 2012, Jessop,

104   Woodford et al. 2013).

105   The assumptions about the functional significance of corticosterone can be myopic (Vera,

106   Zenuto et al. 2017) if studies are focused only on key stages of interest (e.g. reproduction) within

107   an animal's annual cycle. The full spectrum of actions of corticosteroids at baseline and stress-

108   induced levels have been reviewed extensively in Sapolsky, Romero et al. (2000) and Wingfield

109   and Romero (2016). As reviewed by Harris (2020), there are currently over 131 proposed

110   hypotheses explaining the functional role of corticosterone for reproduction (n=43), stress coping

111   mechanisms (n=18), and life history stage spanning models (n=37). Many of these hypotheses,

112   focus on targeted relationships which may not consider the full scope of HPA axis function

113   observed throughout an animal's annual cycle. For example, the parental care hypothesis

114   (Wingfield, O'Reilly et al. 1995) states that corticosterone levels must decline from egg lay to

115   feeding of young to promote parental care, while prediction 1 of the CORT-flexibility hypothesis

116   (Lattin, Breuner et al. 2016) states that HPA axis function is elevated before breeding to fine-

117   tune the onset of reproduction~~of the CORT-flexibility hypothesis ,(Lattin, Breuner et al.~~

118   ~~2016,Bonier, Martin et al. 2009)~~. Thus, data sets consisting of only breeding samples would be

119   unable to distinguish if hormone concentrations were increasing prior to breeding to fine-tune

120   reproductive onset or were decreasing from an annual high to promote parental care. Furthering

121   the list of hypotheses, CORT-adaptation hypothesis predicts that baseline corticosterone can be

122   positively correlated with reproductive investment, especially when factoring the challenges

123   associated with the environment (Bonier, Martin et al. 2009). Thus, inferences that have been

124   drawn from data collected for many of these hypotheses are likely limited without a full annual

125   profile of corticosterone. A full annual profile provides a better understanding of how both

126   current and preceding environmental conditions may affect corticosterone and thus influence

127   these hypotheses.

128   In this cross--sectional study, we aimed to understand the annual regulation of HPA axis

129   function in two subspecies of white-crowned sparrow (*Zonotrichia leucophrys*), with a focus on

130   hormonal transitions during the sub-stages of breeding (Fig. 1). We were specifically interested

131   in how the HPA axis has been influenced by evolutionary pressures associated with life history

132   strategies and environmental niches in these two subspecies - which diverged from a single

133   common ancestor around 50,000 years ago (Rand, 1948; Winger et al., 2014). We collected

134   baseline and stress-induced corticosterone samples from free-living non-migratory (resident)

135   Nuttall's white-crowned sparrows (*Z. L. nuttalli*) along the coast of Northern California, and the

136   long distance migratory (migrant) Gambel's white-crowned sparrow (*Z. l. gambelii*) on their

137   breeding grounds above the Arctic Circle in Alaska and on their wintering grounds in Northern

138   California (Blanchard and Erickson 1949). We hypothesized that subspecies specific life history

139   strategies and/or environmental conditions have promoted differences in circulating

140   corticosterone levels, which in turn provide a mechanism for regulating energetic demands -

141   assessed by baseline corticosterone, and the ability to respond to perturbations through acute

142   stress-induced corticosterone (presumably to activate the emergency life history stage to modify

143   behavior and physiology). The activation of the emergency life history stage is thought to occur

144   more rapidly when corticosterone is produced at a faster rate and at higher concentrations

145   (Boonstra 2004, Angelier and Wingfield 2013). In For animals with few life history stages, who

146   experience benign conditions and a long breeding season, the energetic demands and precise

147   timing of a life history stage and its potential suspension may be less critical and thus would

148   have lower plasma corticosterone compared to species constrained by a greater number of life

149   history stages and a short breeding season (Jacobs and Wingfield, 2000). We predicted that more

150   dramatic seasonal changes in baseline and stress-induced corticosterone would occur in migrants

151   during onset of breeding to cope with environmental extremes on their arctic breeding grounds,

152   while changes in residents would be muted as they experience a more forgiving breeding

153   schedule and comparatively benign environmental conditions in alignment with the CORT-

154   adaptation Hypothesis (See Fig. 1 for subspecies life history stage alignment).

155

156   **Materials and Methods**

157   *Study species*

158   Gambel's white-crowned sparrows (*Zonotrichia leucophrys gambelii*; hereafter migrants)

159   breed in Alaska and Canada and migrate to their wintering grounds in the Southwest United

160   States and Northern Mexico (Blanchard and Erickson 1949, Lisovski, Németh et al. 2019).

161   Nuttall's white-crowned sparrows (*Zonotrichia leucophrys nuttalli*; hereafter residents) are a

162   non-migratory subspecies that remain year-round on the California coast (Mewaldt and King

163   1977). Migrants exhibit territorial behaviors soon after arrival on the breeding grounds until the

164   end of egg lay. Residents have been described as being territorial year-round, though large mixed

165   flocks, primarily of juveniles and unpaired females, also form during the winter months

166   (DeWolfe, Baptista et al. 1989, Krause, Németh et al. 2016). There is partial temporal overlap of

167   breeding between the two subspecies with residents raising 2-3 broods between March and July

168   and migrants raising a single brood between May and June (Mewaldt and King 1977, Baker,

169   Mewaldt et al. 1981, Krause, Németh et al. 2016, Boelman, Krause et al. 2017). Both subspecies

170   are socially monogamous, have female only intermittent incubation, and display biparental care

171   both during the nestling and post fledging period (Blanchard 1936, Blanchard 1941, Pérez,

172   Krause et al. 2016). The pre-basic molt is initiated at the termination of breeding and occurs

173   between June and September for residents and between July and August for migrants and no

174 differences in the timing of pre-basic molt have been observed between the sexes (Morton, King

175 et al. 1969, Mewaldt and King 1977). Figure 1 shows the subspecies alignment of life history

176 stages (molt, migration, breeding) and breeding sub-stages (territoriality, egg lay, incubation,

177 feeding young) based on Krause, Németh et al. (2016) and sample sizes are indicated. It is

178 important to note that not all life history stages are expressed by each subspecies (e.g. residents

179 do not migrate, nor do they have a clear pre-alternate molt, Fig. 1).

180 *Capture and Blood Sampling*

181 Birds were opportunistically captured between 2010 and 2016 using seed-baited Potter

182 traps and Japanese mist nets. Distinct migrant populations were captured at Toolik Research

183 Station, Alaska, USA (N 68°38', W 149°36') during breeding and pre-basic molt, and in Davis,

184 California, USA (N 38° 33', W 121°44') during fall arrival/winter. Residents were captured

185 year-round at Bodega Bay (N 38° 31', W 123°06') and Point Reyes, CA, USA (N 37°59', W

186 122°58'). Birds were banded with aluminum United States Fish & Wildlife Service bands. Wing

187 chord, tarsus length, beak and skull length were measured with calipers to the nearest tenth of a

188 millimeter. Mass was measured to the nearest tenth of a gram using a Pesola spring scale. During

189 the breeding season, sex was determined by secondary sexual characteristics such as presence of

190 a brood patch in females or an enlarged cloacal protuberance in males (Mewaldt and King 1977,

191 Wingfield and Farner 1978). For both subspecies, brood patch development, behavioral

192 observations, and nest information were used to classify birds into the sub-stages of breeding

193 (see Boelman et al. 2017, Chmura et al. 2018, and Krause et al. 2016 for timing of migrant

194 spring events, nesting success of migrants, and characterization of annual event of both migrants

195 and residents, respectively). During the non-breeding season sex was determined by using a

196 combination of wing chord and body mass (Fugle and Rothstein 1985).

197   Blood samples were collected between 07:30 and 17:00 for both subspecies. Immediately

198   following capture, a 26-gauge needle was used to pierce the alar vein and blood was collected

199   into heparinized microcapillary tubes (VWR: 15401-560). Baseline blood samples were

200   collected under 3 minutes of entering the net or trap and time was recorded to the nearest second

201   (Romero and Reed 2005). To assess stress-induced corticosterone, birds were placed in an

202   opaque cloth bag and sampled again 30 minutes post-capture (a standardized stress of capture,

203   handling and restraint). Blood samples were stored on ice until they could be processed in the

204   laboratory later in the day. Plasma was separated from red blood cells by centrifugation for 5

205   minutes at 13,000 g, aspirated using a Hamilton syringe, and placed into a microcentrifuge tube.

206   The samples were stored at -30°C until corticosterone assay quantification. All procedures were

207   approved by UC Davis Institutional Animal Care and Use Committee (IACUC) under protocol

208   #17812.

209

210   *Meteorological data*

211   Daily mean wind speed (m/s), temperature (°C) and total daily precipitation (mm/day)

212   data from Toolik Research Station, AK, Point Reyes National Seashore, Bodega Bay Marine

213   Laboratory, and Davis were retrieved from Toolik Field Station's Environmental Data Center,

214   Western Regional Climate Center, Bodega Marine Laboratory Observation Node, and University

215   of California, Davis - Atmospheric Sciences, respectively.

216

217   *Corticosterone Assay*

218   Corticosterone concentrations were determined using a radioimmunoassay as previously

219   described by Wingfield, Vleck et al. (1992). In brief, 15 µL from baseline plasma samples and

220   10 µL from the 30 minute samples were combined with 2000 CPM of tritiated corticosterone

221   (Perkin Elmer NET399250UC). 4 mL of freshly distilled dichloromethane was used to extract

222   corticosterone from the plasma samples. Aspirated dichloromethane was placed into a water bath

223   at 35°C and was dried using nitrogen gas. Dried extracts were reconstituted in 550 µL of

224   phosphate buffer saline with gelatin (PBSG). 100 µL of reconstituted extract was added to a

225   scintillation vial and combined with 3 mL of scintillation fluid (Perkin Elmer Ultima Gold:

226   6013329) to determine extraction recovery percentages. Next, 200 µL of reconstituted extracts

227   were added to duplicate RIA assay tubes with 100 µL of tritiated corticosterone (Perkin Elmer

228   NET399250UC) and 100 µL of antiserum (MP Biomedical 07–120016, lot 3R3-PB-20E

229   antibody). Unbound steroid was separated from bound steroid using 500 µL of dextran coated

230   charcoal solution. Samples were then placed in a centrifuge for 10 minutes at 4°C at 3000 rpm.

231   The supernatant containing bound steroids was decanted into scintillation vials and 3 mL of

232   scintillation fluid were added. Each sample was counted on a Beckman 6500 liquid scintillation

233   counter for 6 minutes or within 2% accuracy. Mean recoveries were 83.72 % and intra-

234   (calculated using C.V. between duplicates) and inter-assay variations were 7.62 and 11.12%,

235   respectively. The assay has been previously validated in white-crowned sparrows (Krause, Pérez

236   et al. 2017).

237

238   *Statistical Analyses*

239   Statistical analyses were conducted in the R statistical environment version 3.6.1 (R Core

240   Development Team 2018). All meteorological data were combined with individual captures by

241   date. Corticosterone concentrations were analyzed using the following packages: lme4 (Bates,

242   Maechler et al. 2014), lmerTest (Kunznetsova, Brockhoff et al. 2014), multcomp (Hothorn, Bretz

243   et al. 2016), stringi (Gagolewski and Tartanus 2017), and emmeans (Lenth and Lenth 2018).

244   Weather data including temperature, wind speed, and daily precipitation were analyzed using the

245   AOV function in the Base R package to compare differences between environments experienced

246   by subspecies and post hoc tests were conducted using emmeans with a Tukey's correction.

247   Cohen's d effects size estimates were performed using the emmeans package. Weather data

248   corresponded to the day of capture for each bird in the study which were then averaged by

249   month. We performed model selection on a series of candidate models that included all possible

250   interactions of subspecies, sex, life history stage or month, and time point (baseline vs stress-

251   induced sample) as well as weather variables as either main effects or additional interaction

252   terms on plasma corticosterone levels. Model performance was compared using Akiake's

253   Information Criterion (AIC) (Supp. Tables 1 & 3). We considered any model that had a delta

254   AIC less than 2 to be a viable candidate model. Models compared corticosterone based on life

255   history or month in order to 1) directly compare temporal variation and 2) absolute differences

256   within a life history stage regardless of calendar date. The coefficient of determination (R^2) was

257   used to estimate the proportion of the variance explained by the statistical model based on

258   Nakagawa, Johnson et al. (2017) using the package MuMIn. All post hoc tests were performed

259   using the contrast function in emmeans package with a Tukey's correction for the P-value (Supp.

260   Table 2 & 4).

261

262   **Results**

263   *Comparison of Wind Speed, Temperature, and Precipitation experienced by migrants and*

264   *residents*

265   A significant interaction between subspecies and month was found for average daily

266   temperature (Fig. 2a, $F_{11,5570}$=61.38, P<0.001), average daily wind speed (Fig. 2b, $F_{11,5344}$=10.84,

267   P<0.001), and total daily precipitation (Fig 2c, $F_{11,5586}$=31.58, P<0.0001).

268   For migrant habitats, average monthly temperatures ranged from a low of 4.11 and

269   6.38°C in May and June during breeding and a high of 18.45°C in October during fall arrival

270   (Fig. 2c). The temperature experienced by residents across the annual cycle did not fluctuate as

271   much as it did for migrants with the annual low temperature of 9.88°C observed in February

272   during dispersal and the high in August of 14.38°C during pre-basic molt. Thus, migrants

273   experienced temperatures that ranged by 14.3°C vs 4.5 °C by residents. Average environmental

274   temperature experienced by each subspecies was different for each month except for July.

275   The coastal habitat residents occupied was characterized as having higher average daily

276   wind speeds compared to the Arctic and central California sites of migrants for all months except

277   September. Within migrant's annual cycle, wind speed was highest from March to August,

278   averaging above 2 m/s while the rest of the year windspeeds were below 2m/s. For residents,

279   wind speed was fairly constant across months, near 4 m/s, while in January, March, and

280   September wind speed was reduced by half on average.

281   Precipitation was higher at the resident's habitat in August and December but lower

282   during February, and April compared to the migrant's habitat (Fig. 2 c, d, & e).

283

284   *Effects of month on corticosterone*

285   The best fit model using AIC included all two-way interactions of month, subspecies, and

286   time point plus the interaction of wind, temperature, and precipitation (Supp. Table 1). The main

287   effect of month ($F_{11,2184}$= 19.35, P<0.001), subspecies ($F_{1,1549}$=11.14, P<0.001) and time point

288   ($F_{1,2004}$=519.49, P<0.001) and the interactions of month and sex ($F_{11,2167}$= 7.46, P<0.001), month

289   and time point ($F_{11,2002}$=12.21, P<0.001), and month and species ($F_{11, 2223}$=17.41, P<0.001), time

290   point and temperature ($F_{1,2004}$=4.21, P=0.04), and time point and wind ($F_{1,2002}$=9.26, P=0.002)

291   and the interaction of time point, temp and wind ($F_{1,2001}$=11.71, P=0.003) while no other factors

292   were significant (Fig. 2a&b). The coefficient of determination (R^2) estimated that the model

293   explained 84.2% of the variance.

294   The following cross sectional comparisons address changes across month at the

295   population and not the individual level. Baseline corticosterone in female migrants was elevated

296   during June, July, and September compared to all other months (Fig. 2a; see Supp Table 2 for all

297   post hoc tests involving month), while stress-induced levels were elevated during June and July

298   (Fig. 2b). In female residents, baseline corticosterone was lower in March compared to

299   December, while stress-induced levels were highest in April, declined through August, and then

300   rose through December. In female migrants, baseline and stress-induced corticosterone levels

301   were lower than female residents in December, January and April, while this pattern was

302   reversed in June, and July.

303   Male migrants had higher baseline (Fig. 2a) and stress-induced corticosterone (Fig. 2b) in

304   May and June compared to all other months. Male resident baseline corticosterone was higher in

305   April compared to February, June, July, and August while stress-induced levels were at an

306   annual low during July and August and at an annual high during April and May. Male migrants

307   had lower baseline and stress-induced corticosterone levels compared to male residents from

308   October through April but this pattern was reversed in June and July. No differences were

309   detected between migrants and residents during the months of July, August, and September.

310   For both subspecies, the 3D plots (Fig 3) and model estimates showed that both baseline

311 and stress-induced levels increased as the temperatures become colder and windspeed increased.

312 In considering the relationship between the environment and corticosterone levels, residents had

313 higher baseline and stress-induced corticosterone levels compared to migrants during the months

314 in which they experienced the coldest conditions of their annual cycle. This pattern was reversed

315 for migrants during the months of May and June when they were experiencing the coldest

316 temperatures of their annual cycle.

317

318 *Life history stage and sub-stage and corticosterone*

319   Model selection using AIC indicated that the best fit model contained all possible two-

320 way interactions between life history stage, subspecies, and time point plus the interaction of

321 time point with the covariates of wind, precipitation, and temperature (Supp. Table 2).

322 Corticosterone concentrations were affected by the interactions of stage and subspecies

323 ($F_{7,1608}$=39.0, P<0.001), stage and time point ($F_{7,1533}$=14.04, P<0.001), stage and sex

324 ($F_{7,1565}$=8.285, P<0.001), sex and subspecies ($F_{1.1195}$=4.50, P=0.03), time point and wind

325 ($F_{1.1537}$=4.78. P=0.02), time point, temp, and wind ($F_{1,1627}$=4.78. P=0.02) and the main effects of

326 wind ($F_{1,1747}$=13.52, P<0.001), temperature ($F_{1,1537}$=8.12. P=0.004), rain ($F_{1,1408}$=10.85.

327 P=0.001), but not by sex and time point ($F_{1,1266}$=0.02. P=0.61, Fig. 4). The coefficient of

328 determination (R^2) estimated that the model explained 84.4% of the variance.

329   In female migrants, baseline corticosterone levels were elevated throughout breeding,

330 from arrival on the breeding grounds through incubation, and then declined during feeding of

331 young and pre-basic molt before reaching their annual nadir during the autumn arrival and winter

332 stages (Fig. 4a; all post hoc tests involving life history stage are found in Supp. Table 4). In

333   migrant males, baseline corticosterone increased from winter through egg lay and then declined

334   through pre-basic molt and autumn arrival (Fig. 4a). Stress-induced corticosterone concentrations

335   in female migrants were at their annual maximum from arrival through incubation while levels

336   were lowest during molt, although not significantly different from autumn arrival (Fig. 4b). In

337   male migrants, stress-induced corticosterone levels increased from winter and reached an annual

338   maximum during territorial and egg lay sub-stages and declined to the annual low at pre-basic

339   molt (Fig. 4b). Migrant males had higher baseline and stress-induced corticosterone from spring

340   arrival through incubation compared to migrant females.

341   Baseline corticosterone levels were lowest in male residents during feeding of young and

342   pre-basic molt, while in female residents corticosterone was lowest during incubation (Fig. 4a).

343   Stress-induced corticosterone levels in female residents were lowest during pre-basic molt,

344   although not significantly different from feeding young or autumn flocking stages (Fig. 4b). In

345   male residents, stress-induced corticosterone increased from winter through egg lay and declined

346   to their lowest points during pre-basic molt and autumn flocking stages (Fig. 4b).

347   For both sexes, subspecies differences in baseline and stress-induced corticosterone were

348   present in all life history stages except for pre-basic molt. In both sexes, migrants had lower

349   corticosterone levels during winter and autumn arrival stages than residents, but higher levels

350   from spring arrival through feeding of young (Fig. 4a&b). Males for both subspecies had higher

351   baseline and stress-induced corticosterone than females during territorial and egg lay stages,

352   only.

353

354   **Discussion**

355   Corticosterone is a pleiotropic hormone that has been implicated in numerous

356   physiological and behavioral processes. As a consequence, hundreds of hypotheses have been

357   put forward describing the functional role of corticosterone (Harris 2020). However, many of

358   these studies utilize small data sets to draw their inferences. Our data set, collected from 1614

359   individuals, highlights that circulating corticosterone levels reflect a nuanced interplay between

360   the shifting demands of individual life history stages and environmental conditions that are

361   unique to each subspecies. Temporal patterns and absolute amounts of corticosterone differed

362   between the subspecies. Yet, regardless of subspecies, baseline and stress-induced corticosterone

363   levels increased as wind speed increased, and temperatures declined (Fig 3).

364   *Breeding and Corticosterone*

365   As the breeding season progressed, both baseline and stress-induced corticosterone

366   reached an annual maximum during the territorial and egg lay sub-stages of breeding but was

367   higher in migrants compared to residents. Our data provide support for prediction 1 of the

368   CORT-Flexibility Hypothesis which states that the annual maximum in corticosterone occurs

369   prior to egg lay (Lattin, Breuner et al. 2016). The CORT-Flexibility hypothesis proposes that

370   modulation of various HPA axis regulatory elements for circulating levels of hormones, CBG,

371   enzymes and receptors allow for rapid and robust suppression of the HPG axis which can delay

372   the onset of egg lay. Thus, further studies are required to fully support the CORT-Flexibility

373   hypothesis by determining if tissue sensitivity is changing in addition to plasma glucocorticoids.

374   Our data then give pause to the Parental Care Hypothesis (Wingfield, O'Reilly et al. 1995) which

375   states that HPA axis function declines at the transition from the pre-parental to parental stage, but

376   in many ways ignores the initial cause of the annual increase in peak corticosterone (Wingfield,

377   O'Reilly et al. 1995, Chastel, Lacroix et al. 2005). The seasonal peak in HPA axis function may

378   be critical for fine-tuning reproduction onset, while the decline observed starting at incubation

379   serves to prevent nest abandonment and foster parental care. Thus, the Parental Care Hypothesis

380   is valid in that high corticosterone levels can directly interfere with parental care, but

381   corticosterone is really declining towards levels that are observed through most of the calendar

382   year. Lastly, our data provide support for the CORT- adaptation hypothesis because higher

383   baseline and stress-induced corticosterone levels were observed in the migrant during breeding

384   who have just a single opportunity to breed during the year and are faced with harsher

385   environmental conditions. The CORT-adaptation hypothesis predicts that brood value or

386   environmental harshness can be mutually exclusive or additive which was recently confirmed in

387   a meta analysis by Vitousek, Johnson et al. (2019)

388   Within breeding, sex-based differences persisted from spring arrival through feeding of

389   young in migrants, while in residents differences were only detected during territorial and egg

390   lay sub-stages. It has often been reported that the sex providing the bulk of the parental care has

391   lower corticosterone levels, which is made evident in examples where males incubate and

392   provision offspring (Wingfield, O'Reilly et al. 1995, Bókony, Lendvai et al. 2009). The division

393   in parental care and HPA axis function are likely intricately tied to social information, daily

394   activity budgets and its effects on energy balance. Peak hormone levels for testosterone and

395   corticosterone occur prior to- or around egg lay suggesting that social information is having a

396   direct effect on hormone patterns during the period of greatest social instability (Goymann,

397   Moore et al. 2019). For male white-crowned sparrows, territorial and egg lay sub-stages occur

398   during the annual nadir in fat reserves, which is likely a consequence of shifts in time budgeting

399   away from foraging to mate guarding, singing and copulation (King and Farner 1966). Females

400   maintain much higher fat reserves throughout the breeding season and a greater proportion of

401 their time is allocated to foraging - especially during egg lay (Wingfield and Farner 1978). Clear

402 relationships have been demonstrated between energetic reserves and peak corticosterone which

403 suggests a threshold effect for triggering an increase in HPA axis function (Lynn, Breuner et al.

404 2003, Krause, Pérez et al. 2017). Males are much closer to the threshold for enhancing HPA axis

405 activity during breeding because of their low fat reserves (perturbation resistance potential, PRP,

406 Wingfield et al., 2017) and could be a contributing factor to their higher overall HPA axis

407 function. Greater fat reserves in females after the transition to parental phase might allow the

408 female to be more attentive to incubation, especially during inclement weather (Wingfield,

409 O'Reilly et al. 1995, Krause, Chmura et al. 2015, Walker, Meddle et al. 2015)

410 *Corticosterone and Molt*

411   During pre-basic molt following clutch completion, baseline and peak corticosterone

412 levels reached an annual low and there were no significant differences between either sex or

413 subspecies. It is not clear whether HPA axis function is reduced to an absolute minimum in all

414 groups, or it has been reduced to a requisite level for optimum feather growth. Increased

415 corticosterone during molt results in poor feather quality, which likely affects flight performance,

416 but more importantly insulation (Cornelius, Perfito et al. 2011, Lattin, Reed et al. 2011). It

417 should also be noted that molt was the only stage in which we did not detect a significant

418 difference between subspecies throughout the annual cycle. Interestingly, molt also coincides

419 with the warmest months and highest food availability for migrants - as measured by arthropod

420 biomass, vegetation greenness (NDVI), seed and berry production, on the breeding grounds

421 (Sweet, Asmus et al. 2014, Asmus, Chmura et al. 2018) but is also most likely true for residents

422 as well. From pre-basic molt through the end of the wintering stage, no sex differences in

423 corticosterone were detected for either subspecies which also coincides with the increased total

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

425 1963).

426

427 *Lessons from migrants and residents*

428   In alignment with our prediction, greater annual modulation of stress-induced

429 corticosterone occurred in the migrants with males showing 4.25x and females a 2.5x fold

430 change, compared to the residents in which males showed a 2.5x and females a 1.5x fold change

431 from the annual low to maximum. Other studies have observed higher corticosterone peak

432 concentrations in populations facing harsher or more unpredictable environmental conditions

433 (Rogers, Ramenofsky et al. 1993, Dunlap and Wingfield 1995, Wingfield, Kubokawa et al. 1995,

434 Holberton and Able 2000, Krause, Chmura et al. 2015, Walker, Meddle et al. 2015, Krause,

435 Pérez et al. 2016). Of particular note, a similar pattern in the magnitude of change in baseline and

436 peak corticosterone levels across stages of the annual cycle was found in rufous collared

437 sparrows, *Zonotrichia capensis,* in South America comparing subspecies in a highly stable

438 environment versus a highly unpredictable environment (González-Gómez, Echeverria et al.

439 2018).

440   Work by Barbara Blanchard and Donald Farner asserted over 70 years ago that distinct

441 time schedules existed between migrants and residents, based on gonad morphology and

442 function. Photoinduced residents initiate recrudescence as early as December - while photoperiod

443 is still declining, as opposed to mid-March for migrants, when photoperiod is increasing

444 (Blanchard 1941, Blanchard and Erickson 1949, Farner and Follett 1966). Genetic differences

445 between subspecies in critical day length may ultimately determine the initiation of seasonal

446 changes in the HPG and HPA axes. However, the seasonal dynamics of HPA axis function in

447   terms of circulating levels of baseline and stress-induced corticosterone do not appear from

448   genetic differences alone, as a common garden experiment with migrants and residents did not

449   detect any HPA differences during winter or spring months (Ramenofsky, Campion et al. 2017).

450   Similarly, Romero and Wingfield (1999), showed that captive Gambel's white-crowned

451   sparrows did not show baseline and stress-induced changes across their annual cycle.

452   It is evident that environmental conditions and social cues were a major driver of

453   corticosterone levels (see Fig. 4). Migrants faced the most thermally challenging conditions

454   within their annual cycle during breeding - with annual lows in temperature and highs in wind

455   speed, and at this time had higher corticosterone levels than residents. Conversely, for residents

456   the winter was the most thermally challenging period – with annual highs in rain and wind, and

457   lows in temperature (but the latter only slightly), and at these points residents had higher

458   corticosterone levels compared to migrants. Thus, the subspecies experiencing the most

459   thermally challenging period of their annual cycle had the highest measured corticosterone

460   levels. A recent review by de Bruijn and Romero (2018) found that challenging conditions

461   associated with weather often resulted in elevated glucocorticoids. Environmental conditions

462   may necessitate rapid switches in substrate utilization (fats, protein and carbohydrates) for

463   metabolism during the transition points across the three stages of fasting – these transitions are

464   thought to be mediated by increasing corticosterone concentrations as body mass declines

465   (Cherel, Robin et al. 1988, Cherel, Robin et al. 1992, Landys, Ramenofsky et al. 2006). Migrants

466   may have a lower margin for error during breeding based on their environmental conditions,

467   which has selected for a more robust and fast acting physiological response to environmental

468   challenges (Angelier and Wingfield 2013). These data suggest that modulation of the HPA axis

469   is largely driven by environmental factors, social cues, and their potential interactions with a

470   genetic program. A common garden experiment in which migrants and residents were exposed to

471   each others' environment would be critical for separating the environmental and genetic

472   determinants of HPA axis function.

473

474   **Conclusion**

475   There are temporal differences in seasonal HPA axis activity between resident and

476   migrant white-crowned sparrows. The results clearly support the first prediction of the CORT-

477   Flexibility hypothesis that states that the highest corticosterone levels are observed at the onset of

478   the breeding season and may be critical for fine-tuning the onset of reproduction. The magnitude

479   of the change in baseline and peak corticosterone was better predicted when weather variables

480   were included in the model. This study builds on a growing literature that shows populations

481   exposed to harsher environmental conditions often have higher circulating levels of

482   corticosterone. Thus, the challenges associated with an animal's environment may be critical in

483   regulating transitions across life history stages. We propose that further studies should elucidate

484   seasonal changes in tissue sensitivity through measurements of receptors and metabolizing

485   enzymes as well as negative feedback control mechanisms that govern the observed seasonal

486   glucocorticoid patterns.

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716
717
718 **Figure and Table Legends**

719
720 Figure 1.
721 Summary of life-history stages, sub-stages, and the months in which they occur for both migrant
722 Gambel's (grey trapezoids) and resident Nuttall's (white trapezoids) white-crowned sparrows.
723 Samples sizes are included for each species <u>by life history stage and calendar month</u>.
724
725 Figure 2.
726 The effect of month on a) temperature (^{o}C), b) wind speed (m/s) and c) precipitation, (mm/day)
727 d) baseline corticosterone and e) stress-induced circulating concentrations of plasma
728 corticosterone (ng/ml) migrant (symbol: *circles,* line: *solid*) and resident (symbol: *squares,* line:
729 *dashed*) white-crowned sparrows. Males are denoted by filled symbols and females by unfilled
730 symbols. The study uses a cross sectional design which reflects changes at the population and not
731 the individual level. Subspecies differences in corticosterone within each month is indicated by
732 an asterisks (*) for females and by the cross (†) for males. Subspecies differences in weather
733 parameters for each month are indicated by section sign (§).
734
735 Figure 3.
736 3D plots showing the interaction of average wind speed (m/s) and temperature (oC) on baseline
737 (a-d) and stress-induced plasma corticosterone (CORT; ng/ml) (e-h) in (a,e) female migrants,
738 (b,f) male migrants, (c, g) female residents, and (d, h) male residents, respectively. The color
739 gradient from blue to red indicates increasing concentrations of corticosterone.
740
741 Figure 4.
742 The effects of life history stage and sub-stages of the annual cycle on a) baseline and b) stress-
743 induced circulating concentrations of plasma corticosterone (ng/ml). Letters that are different
744 from one another denote significance across stages within a sex and subspecies using post hoc
745 contrasts with Tukey's correction. (UPPERCASE = Migrant females, lowercase = migrant
746 males, *UPPERCASE ITALICIZED* = resident females, *lower case italicized* = resident males).
747 Asterisks (*) indicate differences between sexes within a sub-stage and subspecies. The cross (†)
748 indicates subspecies differences within a sex and life history stage.
749
750
751
752 Supplemental Table 1.
753 Linear mixed effects models were tested for the best fit using Akiake's information criterion
754 (AIC) and compared against a null model. All four, three, and two-way interactions were run for
755 subspecies, stress, sex and sub-stage of the annual cycle. Weather variables were added as a
756 covariate or the interaction with the categorical factor.
757
758 <u>Supplemental Table 2.</u>

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.
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