

Corticosterone predicts double-brooding in female savannah sparrows (*Passerculus sandwichensis*)

Hayley A. Spina^{a,*}, D. Ryan Norris^a, Linda Nong^a, Sarah L. Dobney^b, Sarah D. Mueller^a,
Nikole E. Freeman^{a,1}, Stéphanie M. Doucet^b, Daniel J. Mennill^b, Amy E.M. Newman^a

^a Department of Integrative Biology, University of Guelph, 50 Stone Road, E, Guelph, Ontario N1G 2W1, Canada

^b Department of Integrative Biology, Windsor University, 401 Sunset Ave, Windsor, Ontario N9B 3P4, Canada

ARTICLE INFO

Keywords:

Double-brood
Cort-fitness hypothesis
Glucocorticoid
Migratory songbird
Quality

ABSTRACT

Given that double-brooding (rearing two broods within a season) can increase annual fecundity, it is unclear why some females in multi-brooded populations rear only one brood per season. The Quality Hypothesis proposes that double-brooded females are high quality and, thus, have sufficient energetic resources available to bear the costs of rearing two broods per season. Glucocorticoids — endocrine hormones that have a critical role in energy regulation — could reflect female quality, and, therefore, also have the potential to indicate whether a female will rear a second brood. Using 12 years of reproductive data on migratory Savannah sparrows (*Passerculus sandwichensis*) from a population in eastern Canada, we explored whether baseline corticosterone concentrations were correlated with measures of female quality (body condition and fat score) and whether a female's baseline corticosterone concentrations during her first brood would predict whether she attempted a second. We found weak evidence that baseline corticosterone was negatively correlated with female body condition and found strong evidence that baseline corticosterone was negatively correlated with fat score. There was weak evidence for a positive relationship between double-brooding and baseline corticosterone in females sampled during the first brood incubation stage. Additionally, there was moderate evidence to suggest that the probability of double-brooding was negatively related to baseline corticosterone in females sampled during the first brood nestling stage. Our results provide evidence that corticosterone can reflect female condition in the context of double-brooding and demonstrate the importance of considering breeding stage when assessing corticosterone concentrations in parents.

1. Introduction

Double-brooding (rearing two broods within one breeding season) can result in increased annual productivity (Bulluck et al., 2013; Den Boer-Hazewinkel, 1987; Evans Ogden and Stutchbury, 1996; Geupel and DeSante, 1990; Woodworth et al., 2017a) and positively impact lifetime reproductive success (Carro et al., 2014; Johns et al., 2018; Zabala et al., 2020). However, despite the reproductive benefits of double-brooding, producing a second brood after successfully rearing a first is typically only attempted by a subset of females in a given population (Geupel and DeSante, 1990; Kluijver, 1951; Middleton, 1979; Rodenhouse and Holmes, 1992). The question of what drives intra-population variation in double-brooding has interested ecologists for decades and multiple hypotheses have been developed to explain why some individuals

remain single-brooded, including hypotheses related to timing of breeding (Geupel and DeSante, 1990; Hoffmann et al., 2015; Jackson and Cresswell, 2017; Verboven and Verhulst, 1996; Woodworth et al., 2017a; Zabala et al., 2020), predation (Woodworth et al., 2017a), resource availability (Jackson and Cresswell, 2017; Zabala et al., 2020), environmental factors (Bukor et al., 2021; Den Boer-Hazewinkel, 1987), and female quality (Cornell and Williams, 2016; Geupel and DeSante, 1990; Hoffmann et al., 2015; Verboven and Verhulst, 1996).

The Quality Hypothesis proposes that “high quality” females — those with the energetic resources available to invest in reproduction (Patterson et al., 2011) — are more likely to rear two broods (van Noordwijk and de Jong, 1986; Verboven and Verhulst, 1996). The primary issue with testing predictions from this hypothesis is the challenge of reliably assessing variation in female ‘quality’. While some studies

* Corresponding author at: 50 Stone Road E, Guelph, Ontario N1G 2W1, Canada.

E-mail address: hayleyannspina@gmail.com (H.A. Spina).

¹ Present address: School of Environmental and Natural Sciences, Bangor University, Bangor, Gwynedd, United Kingdom, LL57 2DG.

have found that double-brooding was positively related to female body mass and body condition (Jacobs et al., 2013; Laet and Dhont, 1989; Pilastro et al., 2003; Smith et al., 1987 in 1 of 4 years), others have found no relationship between these same body metrics and double-brooding (Cornell and Williams, 2016; Evans Ogden and Stutchbury, 1996; Lindén, 1988; Nomi et al., 2018a, 2018b; Tinbergen and Verhulst, 2000). Body mass could reflect structural size, rather than fats or other energy reserves that would be expected to reflect energy availability (Green, 2001). Moreover, daily mass can fluctuate up to 15 % in small songbirds (Clark, 1979), which questions any method using mass to estimate body condition. Thus, expanding the exploration of the Quality Hypothesis to other condition-related metrics, such as physiological ones, may offer additional insight as to whether and how individual condition can help explain intra-population variation in double-brooding.

Because the concept of “quality” invokes the availability of energy that can be devoted to reproduction, glucocorticoids — steroid hormones that play a critical role in mobilizing internal energy reserves — are good candidates as physiological measures of quality and may, therefore, be reliable predictors of double-brooding. In wild vertebrates, glucocorticoid hormones play roles in both daily energy regulation and in the mobilization of energy in response to stressors (Wingfield and Romero, 2015). Glucocorticoids help stimulate increases in blood glucose levels through sustaining catecholamine-induced glycogenolysis, upregulating glucose transport, stimulating gluconeogenesis, and influencing appetite (Dallman et al., 1993; Dallman et al., 2004; Horner et al., 1987; Munck and Koritz, 1962; Taborsky and Porte, 1981; Wingfield and Romero, 2015). During resting, non-stressful conditions, glucocorticoids regulate diurnal fluctuations in activity through circadian rhythms, aligning biological processes such as metabolism and hormone secretion with daily activities (Breuner et al., 1999; Schwabl et al., 2016). Importantly, glucocorticoids can act as mediators contributing to allostasis: the maintenance of physiological stability (homeostasis) through changing internal or external conditions (Sterling and Eyer, 1988). In the reactive scope model of homeostasis, glucocorticoids act as homeostatic mediators and will rise and fall within a range of values to achieve internal stability over time (Romero et al., 2009). Such baseline glucocorticoids mediate energetic changes across different life-history stages, such as during reproduction and migration, by reallocating resources in ways that support these behaviours (Bonier et al., 2009a; Crespi et al., 2013; Holberton, 1999; Landys-Ciannelli et al., 2002; Landys et al., 2006; Romero, 2002).

The role of glucocorticoids as mediators connecting an organism with its environment has led to numerous studies exploring a relationship between glucocorticoids and fitness in free-living animals (reviewed in Bonier et al., 2009a; Breuner et al., 2008). The Cort-Fitness Hypothesis (Bonier et al., 2009a, 2009b) proposes that higher levels of baseline corticosterone, the primary circulating glucocorticoid in birds (Holmes and Phillips, 1976), can reflect poor condition and reduced fitness. When environmental challenges increase glucocorticoids, energy is directed toward behaviours that support an individual’s ability to cope with the challenges (Bonier et al., 2009a). Thus, less energy may be available for reproduction. It is important to recognise that elevated glucocorticoid levels are not equivalent to “stress” (MacDougall-Shackleton et al., 2019), but rather that increases in glucocorticoids may be one of many physiological responses to challenging conditions that contribute to allostasis. The Cort-Adaptation Hypothesis (Bonier et al., 2009b) predicts a positive relationship between baseline corticosterone and fitness during times of high reproductive effort (i.e., when corticosterone elevations direct energy toward reproduction). In birds, baseline total glucocorticoids (combined measure of corticosterone bound to the transport protein corticosteroid binding globulin, CBG, and free corticosterone) have been negatively correlated with other metrics of energy storage, such as fat scores (Jenni et al., 2000; Long and Holberton, 2004; Müller et al., 2006), body mass (Hau et al., 2010; Marra and Holberton, 1998), and body condition (meta-analysis by Bókony et al., 2009).

Baseline glucocorticoids have also been negatively associated with reproductive success (Angelier et al., 2010), providing support for the Cort-Fitness Hypothesis. While double-brooding can substantially increase annual fecundity, whether baseline corticosterone relates to double-brooding has only been tested in European starlings (*Sturnus vulgaris*), with no evidence for a relationship between baseline corticosterone measured during the first brood and the probability of initiating a second brood (Fowler et al., 2018).

Despite the critical role of glucocorticoids for energy regulation and mobilization, it is not always clear how to interpret high or low levels of corticosterone, which poses challenges for creating a priori predictions following hypotheses that relate corticosterone to fitness. Multiple reviews have found that there is no consistent corticosterone-fitness relationship: individual studies demonstrated either positive or negative relationships between baseline or post-stress corticosterone and fitness-related traits (Bonier et al., 2009a; Breuner et al., 2008; Crespi et al., 2013). One reason for discrepancies could be due to variation in sampling individuals across different life-history stages (Bonier et al., 2009b; Hau et al., 2010; MacDougall-Shackleton et al., 2013). Given that individual corticosterone concentrations are temporally variable and are thought to change in association with energetic resource needs across different life-history stages, a relationship between corticosterone and double-brooding could depend on breeding stage (Bonier et al., 2009b; Duckworth and Jawor, 2018; Romero, 2002). In a study on tree swallows (*Tachycineta bicolor*), for example, the direction of the corticosterone-fitness relationship depended on breeding stage, with baseline corticosterone negatively correlating with clutch mass during early incubation but positively correlating with clutch mass during the nestling stage (Bonier et al., 2009b). As a consequence, it is important to control for breeding stage when detecting and interpreting relationships between corticosterone and components of fitness.

In this study, we aimed to determine whether corticosterone varied with double-brooding in a population of Savannah sparrows (*Passerculus sandwichensis*) on Kent Island, New Brunswick. Previous work with migratory Savannah sparrows in this population has revealed that double-brooding was negatively related to predation, first lay date, and local density, and positively related to age (Woodworth et al., 2017a). In this population, double-brooded females have higher return rates compared to single-brooded females (*unpublished data*), suggesting that double-brooded individuals are high quality (i.e., capable of rearing two broods in one breeding season without incurring a survival cost). However, it is unknown whether metrics related to female physiological condition predict double-brooding in this population. First, we explored whether corticosterone related to other measures of female condition. We predicted that individuals with a higher body condition index and fat score would have lower total baseline corticosterone. Second, we explored factors predicting double-brooding, including factors previously demonstrated to influence double-brooding in this population (timing of breeding, predation, and age; Woodworth et al., 2017a), and corticosterone concentrations measured during the first brood period in the focal season. Following the Cort-Fitness Hypothesis (Bonier et al., 2009a, 2009b), we predicted that females with lower baseline corticosterone concentrations during the first brood would be more likely to double-brood rather than remain single-brooded in a given breeding season. Because the nestling stage is nearer to when females initiate a second brood, we expected that corticosterone concentrations during this time might show a stronger relationship with the probability of double-brooding.

2. Methods

2.1. Study species and field site

Savannah sparrows are migratory, grassland songbirds that are abundant throughout North America (Wheelwright and Rising, 2020). They overwinter in the southern United States or Mexico and return to

their temperate breeding areas to reproduce in the spring and summer (Wheelwright and Mauck, 1998; Wheelwright and Rising, 2020). Savannah sparrows breed in open areas such as meadows, pastures, cultivated fields, roadsides, grasslands, sedge bogs, salt marsh boundaries, and tundra (Wheelwright and Rising, 2020). Upon their return to the breeding grounds, males establish territories and engage in courtship displays and birdsong to secure a mate. Males will demonstrate agonistic displays toward other males to maintain territories, and both males and females will aggressively defend their nests (Wheelwright and Rising, 2020). Females lay their eggs (average clutch size of 4) in nests built on the ground in open fields (Wheelwright and Rising, 2020). Eggs hatch after ~12 days of incubation and fledging occurs ~9–11 days post-hatching (Wheelwright and Rising, 2020).

We collected hormone samples during the breeding season (May – July) from 2009 to 2018 and from 2021 to 2022 on Kent Island, New Brunswick, Canada (44.58254°N, 66.75604°W). Kent Island is an 80-ha island located 9 km southeast of Grand Manan Island in the Bay of Fundy and is the traditional territory of the Abenaki, the Passamaquoddy, and the Wabanaki Confederacy. The main study area consists of two open fields, measuring 1 and 6 ha that are split into 50 × 50 m quadrats by mowed pathways (Pakkala et al., 2016). Breeding behaviour has been monitored annually in these two fields since 1987, including individually marking all adults, daily territory mapping, and finding and monitoring nests (Woodworth et al., 2017a). Annual predation rates of Savannah sparrow nests on the island ranged from 6 to 54 % (Woodworth et al., 2017a), with the most common predator being the American crow (*Corvus brachyrhynchos*; Wheelwright et al., 1997). Breeding pairs build replacement nests following the failure of a previous brood. If the breeding pair successfully fledges a first brood, the female will sometimes initiate a second brood (Wheelwright and Rising, 2020). In this study, the term second brood is only used to refer to a double-brood nest produced after fledging of a first brood. All protocols were approved and complied with the guidelines of the Canadian Council on Animal Care and the University of Guelph Institutional Animal Care and Use Committee.

2.2. Banding, territory mapping, and nest monitoring

Each year at the beginning of the season, unbanded adults were captured in mist nets and tagged with a unique combination of a U.S. Fish and Wildlife Service/Canadian Wildlife Service (USFWS/CWS) aluminum leg band and three coloured leg bands. Coloured bands enabled visual identification of each adult within the study area. Male territories were mapped by observing behaviours such as singing from perches and territory defense (for details see Sharma et al., 2024). Mating pairs were identified by observing social interactions of the pair within the male's territory. Nests were found by observing female nest

building and incubation behaviours. Once found, nests were monitored every other day for hatching, and nestlings were banded with a USFWS/CWS aluminum leg band and one coloured leg band on post-hatch day 7. To prevent researchers from inducing premature fledging, nests were not visited following day 7. Nesting areas were monitored for fledging every other day by observing parental defense or feeding behaviours following the 9th day after hatching. After the fledging of the first brood, breeding pairs were monitored at least once every three days to determine whether they would initiate a second brood (double-brood) or remain single-brooded.

2.3. Nest enclosures

In 2 of 12 years of the study (2021 and 2022), a subset of females had their nests protected by predator enclosures (Fig. 1) to increase the sample size of birds that successfully fledged young from their first nest. In addition, efforts to collect blood samples from females for corticosterone analysis were also increased in these two years (Suppl. Fig. 1). Enclosures enabled Savannah sparrows to enter and exit the nests while preventing avian predators from accessing the nests. Enclosures were built from sheets of steel mesh and were 22" × 22" × 22" with 2-in. square sizes. Five enclosures were built to be 22" × 22" × 36" to enable enclosures to be placed over tall vegetation. Enclosures were first used in a subset of nests in 2021 ($n = 31/44$ first broods, $n = 10/12$ s broods). Of the 13 first nests that did not have an enclosure, 10 were predated and both second broods without enclosures also were unsuccessful (one predated, one abandoned). All 31 first broods and 10 s broods with enclosures fledged one or more offspring. In 2022, $n = 41/43$ first broods and $n = 25/26$ s broods had enclosures. Of the two first broods that did not have an enclosure, one was predated. Of the 66 nests with enclosures, all but three fledged one or more offspring (one abandoned first brood and two abandoned second broods).

In the current dataset, corticosterone concentrations were obtained from 27 females each in 2021 and 2022. In 2021, 81 % (22/27) of females' first broods were protected with enclosures. In 2022, 96 % (26/27) of females' first broods were protected with enclosures. Predation events still occurred on nests that were not protected in years when predator enclosures were used and predators remained active in the study area (H.A.S., D.R.N., S.D.M., *personal observation*). There was no evidence that having a predator enclosure affected female baseline corticosterone levels (Suppl. Table 1a).

2.4. Sample collection and corticosterone measurement

To determine corticosterone levels in Savannah Sparrows, we obtained blood samples from breeding females. Females were captured by mist-net once during the first brood period, either during the incubation



Fig. 1. Images of predator enclosures covering Savannah sparrow nests in South Field, Kent Island, NB. a) enclosure (red arrow) protecting a Savannah sparrow nest from aerial predators. b) close up view of an enclosure protecting a nest with Savannah sparrow nestlings (red arrow).

(post-laying to hatching, a window of 12 days) or nestling stage of breeding (day one post-hatch to fledging, a window of 10 days). Upon capture, females were measured for mass, tarsus length, and fat scores (methods in LPBO, 2019). If previously unbanded, females were aged by tail feather shape (Pyle, 2022). A baseline blood sample was obtained within 3 min. of capture. Blood samples (~100 μ L, <1 % body weight; Kramer and Harris, 2010) were extracted using brachial venipuncture with a 26-gauge needle and blood was collected into microhematocrit capillary tubes. Brachial venipuncture is the recommended technique when collecting small volumes of blood from small birds (Owen, 2011). Blood was transferred to microcentrifuge tubes and stored on ice for 1–4 h, then centrifuged at 2200 xg for 10 min. Serum was collected and stored at -20°C until processing the following autumn. Total corticosterone concentrations were measured using I125 Corticosterone Double Antibody RIA Kits (MP Biomedicals Ltd., Solon, OH), modified for avian plasma (Washburn et al., 2002; Newman et al., 2008; Pakkala et al., 2016). The optimal plasma volume (1 μ L) was previously determined in song sparrow (*Melospiza melodia*) plasma through validation with serially diluted plasma to confirm parallelism with the standard curve (Newman et al., 2008). In that study, inter-assay variation for the RIA kit was determined to be 8.0 % (low control) and 6.8 % (high control) ($n = 10$ assays; Newman and Soma, 2009). In our study, all samples were run in duplicate. The minimum detection limit was 3.1 ng/mL, and intra-assay variation was <4 %.

2.5. Statistics

We tested whether corticosterone concentrations were associated with other measures of female quality (body condition, fat scores) using Pearson's product-moment correlations and whether corticosterone concentrations predicted double-brooding using a generalized linear mixed-effects model (GLMM; glmmTMB Package; Brooks et al., 2017) fit to a binomial distribution. Analyses were performed in R (v. 4.2; R Core Team, 2023). All means are reported \pm SD. For each continuous variable included within the GLMM, normality of residuals was assessed using visual diagnostic tools, including QQ plots and histograms, as well as by Shapiro-Wilk tests. To reduce the positive skew of the distribution of baseline corticosterone, to reduce the impact of outlying values, and to linearize the relationships between baseline corticosterone and measures of female condition (body condition and fat score), baseline corticosterone values were log transformed prior to analysis (Hoyt et al., 2016). Model validation was assessed using the DHARMA package (Hartig, 2022). We only included females whose complete breeding history for the season of sample collection was known (i.e., excluding females whose first nest was not found). Details regarding each analysis are reported below.

We assessed whether baseline concentrations were correlated with female body condition and fat score within a subset of individuals for whom we had these measurements ($n = 118$ body condition, $n = 97$ fat score). Body condition was a continuous variable measured as the residuals of the relationship between mass and tarsus length (Mitchell et al., 2011). Fat score was determined by assigning a numerical rank (0–7) to visible subcutaneous fat (LPBO, 2019). Because we did not observe birds with fat scores higher than 3, fat score in this analysis was categorized as a four-level factor (0–3).

We assessed whether baseline corticosterone concentrations in breeding females predicted double-brooding ($n = 82$ females remained single-brooded and $n = 47$ females produced a second brood) using a GLMM. Previous research in our study system using a larger dataset (1987–2005 and 2007–2016) determined that double-brooding was associated with female age (older females were more likely to double-brood), timing of breeding (early-breeding females were more likely to double-brood), predation of the first brood (females that had their first attempts depredated rarely reared two broods to fledging), and local density (double-brooding was more likely when the population density was low; Woodworth et al., 2017a). Thus, we included female

age, timing of breeding, and predation of the first brood in the model. For this dataset, we were unable to calculate local density as some individuals nested in areas where their neighbours were not monitored. To account for known differences in reproductive performance between experienced and first-time breeders (Wheelwright and Schultz, 1994; Woodworth et al., 2017a), we included female age (classified as a two-level factor: 1 = one year, and 2 = two years or older). Timing of breeding (continuous variable) was the ordinal date on which females initiated laying for the season. For females that successfully fledged their first brood, lay date was calculated by subtracting 12d from the hatch date (average incubation duration; Dixon, 1978) and one extra day for each egg laid up to the penultimate egg. For females whose first broods failed before hatching, hatch date was estimated by calculating the average difference (in days) between when a nest was found and when it hatched and then subtracting this value from the found date of the failed nest. The estimated hatch date was then used to determine lay date, as was done with successful first broods. Predation was classified as a two-level factor: 0 = first attempt not predated, and 1 = first attempt predated. To test predictions from the hypothesis that the relationship between corticosterone and double-brooding would depend on breeding stage, we also included the interaction between corticosterone concentration and the female's breeding stage at capture. Breeding stage was included as a two-level factor: 0 = incubation stage, and 1 = nestling stage (females sampled after her first brood hatch date but before first brood fledging). Estimated trends for the interaction between baseline corticosterone and breeding stage were carried out using the emmeans package (emmeans function; Lenth, n.d). We included individual ID as a random effect to account for repeated sampling of individuals across multiple seasons ($n = 19$ individuals). We included sample year as a categorical random variable to account for year effects.

In an effort to move away from reporting results with the language of significance (based on an arbitrary p -value cut off), we have used the language of evidence, as suggested by Muff et al., (2022). We report no evidence when p -values were between 1 and 0.1, weak evidence when p -values were between 0.09 and 0.05, moderate evidence when p -values were between 0.04 and 0.01, and strong evidence when p -values were <0.01 (Muff et al., 2022).

3. Results

We explored whether baseline corticosterone concentrations during the first brood correlated with other metrics of female condition in breeding females. We found weak evidence that baseline corticosterone was negatively correlated with female body condition ($r = -0.16$, $t = -1.77$, $df = 116$, $p = 0.08$; Fig. 2a) and found strong evidence that baseline corticosterone was negatively correlated with fat score ($r = -0.35$, $t = -3.66$, $df = 95$, $p < 0.001$; Fig. 2b).

We assessed whether female baseline corticosterone concentrations that were sampled during either the incubation or nestling stage of the first brood predicted the probability of double-brooding that season. Baseline corticosterone levels differed between breeding stages (Pearson's product-moment correlation: $r = 0.30$, $t = 3.59$, $df = 127$, $p < 0.001$). Mean baseline corticosterone levels were 10.6 ± 8.0 ng/mL during the incubation stage ($n = 63$) and 14.6 ± 9.2 ng/mL during the nestling stage ($n = 66$). After controlling for the fixed effects of age ($\beta = -0.34$, $z = -0.65$, $p = 0.52$), lay date ($\beta = -0.20$, $z = -3.53$, $p < 0.001$), and predation ($\beta = -4.40$, $z = -3.77$, $p < 0.001$), there was strong evidence that an interaction between baseline corticosterone and breeding stage predicted double-brooding ($\beta = 2.80$, $z = 3.12$, $p = 0.002$; Fig. 3; Suppl. Table 2a). We found moderate evidence that when they were sampled during the nestling stage of their first brood, females with low baseline corticosterone were more likely to double-brood compared to females with high baseline corticosterone ($\beta = -1.59$, $z = -2.52$, $p = 0.01$; Fig. 3; Suppl. Table 2b). However, we found weak evidence that when they were sampled during the incubation stage of their first brood, females with high corticosterone were more likely to double-brood ($\beta =$

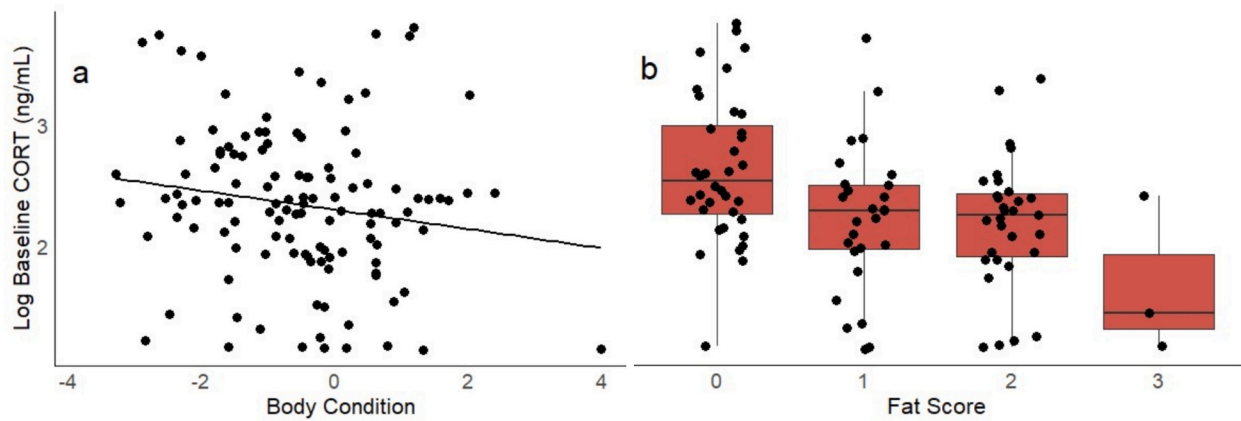


Fig. 2. Correlations between female Savannah sparrow corticosterone concentrations and other physiological condition metrics: body condition (a) and fat score (b). a) weak evidence demonstrated that baseline corticosterone negatively correlated with body condition, b) strong evidence demonstrated that baseline corticosterone negatively correlated with fat scores. Error bars represent $1.5\times$ interquartile range.

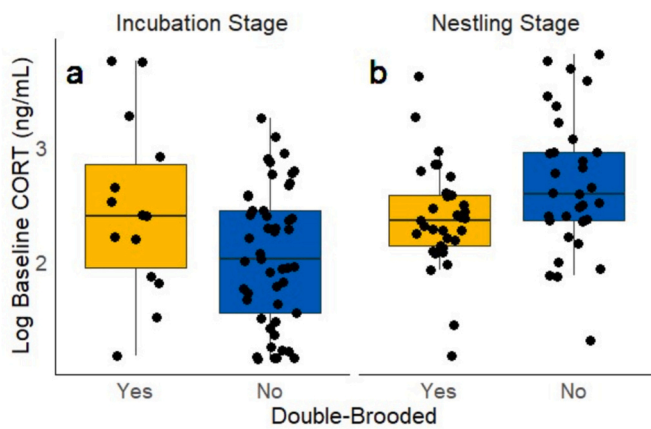


Fig. 3. Relationships between double-brooding and corticosterone between breeding stages in female Savannah sparrows. a) Weak evidence suggested baseline corticosterone was higher in females that produced a second brood that season when corticosterone was measured during the incubation stage of their first brood. b) Moderate evidence suggested baseline corticosterone was lower in females that produced a second brood that season when corticosterone was measured during the nestling stage of their first brood. Error bars represent $1.5\times$ interquartile range.

1.20, $z = 1.98$, $p = 0.05$; Fig. 3; Suppl. Table 2b).

4. Discussion

We present the first evidence for a relationship between baseline corticosterone concentrations and double-brooding in free-living passerines. We found moderate evidence for a negative relationship between corticosterone levels sampled during the first brood nestling stage and double-brooding probability that season and weak evidence for a positive relationship between corticosterone levels sampled during the first brood incubation stage and double-brooding probability that season. We discuss how these results provide support for the Cort-Fitness Hypothesis that predicts a negative relationship between baseline corticosterone and fitness (Bonier et al., 2009b) and postulate about the mechanisms driving a positive relationship between baseline corticosterone during the incubation stage and double-brooding.

Double-brooding can be a highly profitable breeding strategy for Savannah sparrows because females that produce second broods can double their annual fecundity (Woodworth et al., 2017a) and increase their lifetime reproductive success (unpublished data). Double-brooded

females may have the ability to produce a second brood because they are higher quality individuals relative to other females in the population (Verboven and Verhulst, 1996). We hypothesized that, because corticosterone is involved in energy regulation and mobilization of glucose, it may reflect other measures of condition and be related to double-brooding. We found weak and strong evidence, respectively, that body condition and fat scores were negatively correlated with baseline corticosterone concentrations. Thus, lower baseline corticosterone may be reflective of higher female condition. Because we also demonstrated a negative relationship between baseline corticosterone sampled during the first brood nestling stage and females attempting second broods, baseline corticosterone may be able to be used as a predictive biomarker for the female's capacity for double-brooding that season. Future experimental studies manipulating resource availability and assessing the impact on baseline corticosterone and double-brooding could assess whether the mechanism is driven by resource-based condition versus other corticosterone-related processes.

While baseline corticosterone may predict double-brooding because it reflects resource-based condition, an alternative mechanism could be that high corticosterone levels at the end of the first-brood period directly suppress a second round of reproduction. In female birds, breeding onset involves a cascade of hormone secretion by the hypothalamic-pituitary-gonadal (HPG) axis: an increase in gonadotropin releasing hormone stimulates the release of luteinizing hormone and follicle stimulating hormone from the pituitary, leading to the release of sex steroid hormones including estradiol and testosterone (Dawson et al., 2001; Sharp et al., 1998). Following the activation of the HPG axis, reproductive behaviours including mating, nest building, and egg laying occur (Austin et al., 2021; Cheng, 1975). Multiple studies on birds have demonstrated that high baseline corticosterone levels or exogenous corticosterone treatment may suppress hormone release from the HPG axis (Goutte et al., 2010a; Wilson and Follett, 1975) and delay or halt breeding or egg laying (Goutte et al., 2011; Goutte et al., 2010b; Salvante and Williams, 2003; Schoech et al., 2009). Future studies could manipulate corticosterone concentrations in females during early-brooding and the late-nestling stage to determine whether suppressing or increasing circulating corticosterone concentrations increases or decreases double-brooding rates, respectively. Because high levels of corticosterone could simulate an acute stress response, studies manipulating corticosterone should limit an experimental increase to fall within typical physiological range for baseline corticosterone levels within the population.

A positive relationship between baseline corticosterone sampled during the first brood incubation stage and the probability of double-brooding could support the Cort-Adaptation Hypothesis (Bonier et al.,

2009b). Differences in baseline corticosterone between single- and double-brooded birds during incubation could be reflective of differences in the costs of incubating and raising young as environmental conditions change across the breeding season (Bonier et al., 2009b). In this population, double-brooded females are often the earliest breeding females (Woodworth et al., 2017a) and temperatures are typically cooler earlier in the season in the Martimes region. Thus, it might be more difficult for females to accumulate sufficient resources for egg laying and incubation earlier in the season (te Marvelde et al., 2012). In our sample, first brood lay dates were on average 4 days earlier in double-brooded females compared to single-brooded females (single-brooded female first brood lay date: mean = ordinal date 148.5 ± 5.7 , double-brood female first brood lay date: mean = ordinal date 144.5 ± 5.1). Average daily temperature values for the period between laying and hatching for double-brooders in this dataset were 0.4°C lower than those for single-brooders (single-brooded females mean temperature between lay date and hatch date = $12.8 \pm 1.4^\circ\text{C}$; double-brooded females mean temperature between lay date and hatch date = $12.4 \pm 1.0^\circ\text{C}$; weather data taken from an Environment and Climate Change Canada weather station approximately 110 km northeast of Kent Island; Burant et al., 2022; Woodworth et al., 2017b). However, while baseline corticosterone levels have been shown to be negatively related to environmental temperature in other songbird populations (Hau et al., 2022), in our population, baseline corticosterone increased with temperature during the incubation period and decreased with temperature during the nestling period (Suppl. Table 1b). Thus, the hypothesis that double-brooders have higher corticosterone during the incubation period because they require more energy to breed when temperatures are lower earlier in the season seems unlikely. Alternatively, the finding that females with higher baseline corticosterone during the incubation stage were more likely to be double-brooded that season could be a spurious relationship. This relationship was weakly positive and appeared to be driven by a small number of individuals with highly elevated baseline corticosterone (Fig. 3). Thus, the positive relationship between baseline corticosterone sampled during the first brood incubation stage and the probability of double-brooding should be interpreted with caution.

Similar to the findings in tree swallows (Bonier et al., 2009b), we observed that the relationships between corticosterone and double-brooding in Savannah sparrows depended on breeding stage. However, while Bonier et al. (2009b) found a negative relationship between baseline corticosterone sampled during early incubation and metrics of fitness (the number of fledglings produced and clutch mass), we found a negative relationship between baseline corticosterone and the probability of double-brooding only during the nestling stage. Bonier et al. (2009b) also observed that the corticosterone-fitness relationship reversed in direction during the nestling period, where total baseline corticosterone was positively related to clutch mass. One reason for differences in the direction of the relationship between corticosterone and components of fitness in our study compared to Bonier et al. (2009b) could be that the energetic requirements of each breeding stage differ between species. As aerial insectivores, tree swallows have been shown to expend less energy during the incubation period compared to the feeding period (Williams, 1988), while ground-foraging Savannah sparrows did not differ in the amount of energy expenditure during incubation compared to feeding (Williams, 1987). Thus, elevated corticosterone concentrations observed in Bonier et al. (2009b) may be reflective of increased energetic requirements in females provisioning large broods whilst foraging on the wing. Since energetic requirements remain relatively consistent across the breeding season in Savannah sparrows, we speculate that a negative corticosterone-fitness relationship may have only been observed during the nestling stage due to the proximity in time between when sample measurements and the decision to double brood occurred. Because the decision to double-brood occurs either before or soon after the first brood fledges, a female's physiological condition during the nestling stage may be more reflective of the

energy a female has available to initiate a second round of reproduction and brood rearing.

While we did not set out to test whether the relative change in individual corticosterone concentrations between breeding stages may be an important predictor of double-brooding, our results suggest that this may be an interesting pattern to explore in future studies. Comparing corticosterone levels within single-brooded females, mean baseline corticosterone appeared to be higher in females sampled during the nestling stage compared to females sampled during the incubation stage (Fig. 3). Comparing corticosterone levels within double-brooded females, baseline corticosterone levels appeared similar between females sampled during the incubation and nestling stages (Fig. 3). Because high-quality females may have sufficient resources to mitigate the energetic costs of providing parental care (Mitchell et al., 2012; van Noordwijk and de Jong, 1986), an increase in corticosterone across the parental care period may only be expected in lower quality individuals. Thus, an increase in corticosterone during the period of parental care would be predicted to occur in single- but not double-brooded females. In this population, there was no evidence for a difference between single- and double-brooded females in terms of how baseline corticosterone levels changed across the season (Suppl. Table 1a). However, because we did not sample the same females repeatedly during the first brood, it remains possible that changes in corticosterone levels within individuals across the season could differ between single- and double-brooders. Future studies could resample individuals throughout the breeding season to observe whether the relative change of baseline corticosterone across different parental care stages can predict double-brooding probability.

In conclusion, our results contribute to the understanding of intra-population variation in double-brooding behaviour and fecundity. We demonstrated that a female's baseline corticosterone concentrations may reflect her internal energy stores and her capacity for double-brooding. Our study provides further support to the hypothesis that corticosterone-fitness relationships differ across life-history stages (Bonier et al., 2009b) and highlights the importance of limiting timing of sample collection to one breeding stage or controlling for the timing of sample collection within the statistical models.

CRediT authorship contribution statement

Hayley A. Spina: Writing – review & editing, Writing – original draft, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **D. Ryan Norris:** Writing – review & editing, Supervision, Methodology, Investigation, Data curation, Conceptualization. **Linda Nong:** Writing – review & editing, Investigation. **Sarah L. Dobney:** Writing – review & editing, Investigation. **Sarah D. Mueller:** Writing – review & editing, Investigation. **Nikole E. Freeman:** Writing – review & editing, Investigation. **Stéphanie M. Doucet:** Writing – review & editing, Investigation. **Daniel J. Mennill:** Writing – review & editing, Investigation. **Amy E.M. Newman:** Writing – review & editing, Supervision, Methodology, Investigation, Funding acquisition, Data curation, Conceptualization.

Funding

We thank the Natural Sciences and Engineering Research Council of Canada (NSERC) for scholarship support to H.A.S., and for Discovery Grants to D.R.N., S.M.D., D.J.M., and A.E.M.N. We also thank the Society for Canadian Ornithologists (Taverner Award), and the American Ornithological Society (Student Research Grant) for research support to H.A.S.

Acknowledgements

We are grateful for the many field researchers and field assistants that were involved in banding and nest monitoring over the 12 years of

this study. We thank Bowdoin Scientific Station for ongoing logistical support. We thank Brendan Delehanty for providing writing support on the original article.

Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.yhbeh.2025.105679>.

References

- Angelier, F., Wingfield, J.C., Weimerskirch, H., Chastel, O., 2010. Hormonal correlates of individual quality in a long-lived bird: A test of the "corticosterone-fitness hypothesis." *Biol. Lett.* 6, 846–849.
- Austin, S.H., Krause, J.S., Viernes, R., Farrar, V.S., Booth, A.M., Harris, R.M., Angelier, F., Lee, C., Bond, A., Wingfield, J.C., MacManes, M.M., Calisi, R.M., 2021. Uncovering the sex-specific endocrine responses to reproduction and parental care. *Front. Endocrinol. (Lausanne)* 12, 1–17. <https://doi.org/10.3389/fendo.2021.631384>.
- Bókony, V., Lendvai, A.Z., Likér, A., Angelier, F., Wingfield, J.C., Chastel, O., 2009. Stress response and the value of reproduction: are birds prudent parents? *Am. Nat.* 173, 589–598.
- Bonier, F., Martin, P.R., Moore, I.T., Wingfield, J.C., 2009a. Do baseline glucocorticoids predict fitness? *Trends Ecol. Evol.* 24, 634–642. <https://doi.org/10.1016/j.tree.2009.04.013>.
- Bonier, F., Moore, I.T., Martin, P.R., Robertson, R.J., 2009b. The relationship between fitness and baseline glucocorticoids in a passerine bird. *Gen. Comp. Endocrinol.* 163, 208–213. <https://doi.org/10.1016/j.ygcen.2008.12.013>.
- Breuner, C.W., Patterson, S.H., Hahn, T.P., 2008. In search of relationships between the acute adrenocortical response and fitness. *Gen. Comp. Endocrinol.* 157, 288–295. <https://doi.org/10.1016/j.ygcen.2008.05.017>.
- Breuner, C.W., Wingfield, J.C., Romero, L.M., 1999. Diel rhythms of basal and stress-induced corticosterone in a wild, seasonal vertebrate, Gambel's white-crowned sparrow. *J. Exp. Zool.* 284, 334–342. [https://doi.org/10.1002/\(sici\)1097-010x\(19990801\)284:3<334::aid-jez11>3.0.co;2-%26](https://doi.org/10.1002/(sici)1097-010x(19990801)284:3<334::aid-jez11>3.0.co;2-%26).
- Brooks, M.E., Kristensen, K., van Benthem, K.J., Magnusson, A., Berg, C.W., Nielsen, A., Skaug, H.J., Maechler, M., Bolker, B.M., 2017. glmmTMB balances speed and flexibility among packages for zero-inflated generalized linear mixed modeling. *The R Journal* 9, 378–400.
- Bukor, B., Seress, G., Pipoly, I., Sándor, K., Sinkovics, C., Vincze, E., Likér, A., 2021. Double-brooding and annual breeding success of great tits in urban and forest habitats. *Curr. Zool.* 517–525. <https://doi.org/10.1093/cz/zoab096>.
- Bulluck, L., Huber, S., Viverette, C., Blem, C., 2013. Age-specific responses to spring temperature in a migratory songbird: older females attempt more broods in warmer springs. *Ecol. Evol.* 3, 3298–3306. <https://doi.org/10.1002/ece3.673>.
- Burant, J.B., Heisey, E.W., Wheelwright, N.T., Newman, A.E.M., Whelan, S., Mennill, D. J., Doucet, S.M., Mitchell, G.W., Woodworth, B.K., Norris, D.R., 2022. Natal experience and pre-breeding environmental conditions affect lay date plasticity in Savannah sparrows. *Ecology* 103, 1–11. <https://doi.org/10.1002/ecy.3575>.
- Carro, M.E., Mermoz, M.E., Fernández, G.J., 2014. Factors affecting the probability of double brooding by southern house wrens. *J. F. Ornithol.* 85, 227–236. <https://doi.org/10.1111/jof.12064>.
- Cheng, M. fang, Silver, R., 1975. Estrogen-progesterone regulation of nest-building and incubation behavior in ovariectomized ring doves (*Streptopelia risoria*). *J. Comp. Physiol. Psychol.* 88, 256–263. <https://doi.org/10.1037/h0076181>.
- Clark, G., 1979. Body weights of birds: a review. *Condor* 81, 193–202.
- Cornell, A., Williams, T.D., 2016. Individual quality and double-brooding in a highly synchronous songbird population. *Auk* 133, 251–260. <https://doi.org/10.1642/AUK-15-165.1>.
- Crespi, E.J., Williams, T.D., Jessop, T.S., Delehanty, B., 2013. Life history and the ecology of stress: how do glucocorticoid hormones influence life-history variation in animals? *Funct. Ecol.* 27, 93–106. <https://doi.org/10.1111/1365-2435.12009>.
- Dallman, M., Strack, A., Akana, S., Bradbury, M., Hanson, E., Scribner, K., Smith, M., 1993. Feast and famine: critical role of glucocorticoids with insulin in daily energy flow. *Front. Neuroendocrinol.* 14, 303–347.
- Dallman, M.F., La Fleur, S.E., Pecoraro, N.C., Gomez, F., Houshyar, H., Akana, S.F., 2004. Mini-review: Glucocorticoids - food intake, abdominal obesity, and wealthy nations in 2004. *Endocrinology* 145, 2633–2638. <https://doi.org/10.1210/en.2004-0037>.
- Dawson, A., King, V.M., Bentley, G.E., Ball, G.F., 2001. Photoperiodic control of seasonality in birds. *J. Biol. Rhythm.* 16, 365–380. <https://doi.org/10.1177/074873001129002079>.
- Den Boer-Hazewinkel, J., 1987. On the costs of reproduction: parental survival and production of second clutches in the great tit. *Ardea* 75, 99–110.
- Dixon, C.L., 1978. Breeding biology of the Savannah sparrow on Kent Island. *Auk* 95, 235–246.
- Duckworth, B.M., Jawor, J.M., 2018. Corticosterone profiles in northern cardinals (*Cardinalis cardinalis*): do levels vary through life history stages? *Gen. Comp. Endocrinol.* 263, 1–6. <https://doi.org/10.1016/j.ygcen.2018.04.021>.
- Evans Oden, L.J., Stutchbury, B.J.M., 1996. Constraints on double brooding in a neotropical migrant, the hooded warbler. *Condor* 98, 736–744. <https://doi.org/10.2307/1369855>.
- Fowler, M.A., Paquet, M., Legault, V., Cohen, A.A., Williams, T.D., 2018. Physiological predictors of reproductive performance in the European starling (*Sturnus vulgaris*). *Front. Zool.* 15, 1–17.
- Geupel, G.R., DeSante, D.F., 1990. Incidence and determinants of double brooding in Wrentits. *Condor* 92, 67–75.
- Goutte, A., Angelier, F., Chastel, C.C., Trouvé, C., Moe, B., Bech, C., Gabrielsen, G.W., Chastel, O., 2010a. Stress and the timing of breeding: glucocorticoid-luteinizing hormones relationships in an arctic seabird. *Gen. Comp. Endocrinol.* 169, 108–116. <https://doi.org/10.1016/j.ygcen.2010.07.016>.
- Goutte, A., Antoine, É., Weimerskirch, H., Chastel, O., 2010b. Age and the timing of breeding in a long-lived bird: a role for stress hormones? *Funct. Ecol.* 24, 1007–1016. <https://doi.org/10.1111/j.1365-2435.2010.01712.x>.
- Goutte, A., Clément-Chastel, C., Moe, B., Bech, C., Gabrielsen, G.W., Chastel, O., 2011. Experimentally reduced corticosterone release promotes early breeding in blacklegged kittiwakes. *J. Exp. Biol.* 214, 2005–2013. <https://doi.org/10.1242/jeb.051979>.
- Green, A.J., 2001. Mass/length residuals: measures of body condition or generators of spurious results? *Ecology* 82, 1473–1483. [https://doi.org/10.1890/0012-9658\(2001\)082\[1473:MLRMOB\]2.0.CO;2](https://doi.org/10.1890/0012-9658(2001)082[1473:MLRMOB]2.0.CO;2).
- Hartig, F., 2022. DHARMA: residual diagnostics for hierarchical (multi-level / mixed) regression models. R package version 0 (4), 6.
- Hau, M., Deimel, C., Moiron, M., 2022. Great tits differ in glucocorticoid plasticity in response to spring temperature. *Proc. R. Soc. B Biol. Sci.* 289, 14–16. <https://doi.org/10.1098/rspb.2022.1235>.
- Hau, M., Ricklefs, R.E., Wikelski, M., Lee, K.A., Brawn, J.D., 2010. Corticosterone, testosterone and life-history strategies of birds. *Proc. R. Soc. B Biol. Sci.* 277, 3203–3212. <https://doi.org/10.1098/rspb.2010.0673>.
- Hoffmann, J., Postma, E., Schaub, M., 2015. Factors influencing double brooding in Eurasian Hoopoes *Upupa epops*. *Ibis (Lond. 1859)* 157, 17–30. <https://doi.org/10.1111/ibi.12188>.
- Holberton, R.L., 1999. Changes in patterns of corticosterone secretion concurrent with migratory fattening in a neotropical migratory bird. *Gen. Comp. Endocrinol.* 116, 49–58. <https://doi.org/10.1006/gcen.1999.7336>.
- Holmes, W.N., Phillips, J.G., 1976. The adrenal cortex of birds. In: Chester-Jones, I., Henderson, I.W. (Eds.), *General, comparative, and clinical endocrinology of the adrenal cortex*. Academic Press, London, pp. 293–420.
- Horner, H.C., Munck, A., Lienhard, G.E., 1987. Dexamethasone causes translocation of glucose transporters from the plasma membrane to an intracellular site in human fibroblasts. *J. Biol. Chem.* 262, 17696–17702. [https://doi.org/10.1016/s0021-9258\(18\)45435-x](https://doi.org/10.1016/s0021-9258(18)45435-x).
- Hoyt, L.T., Ehrlich, K.B., Cham, H., Adam, E.K., 2016. Balancing scientific accuracy and participant burden: testing the impact of sampling intensity on diurnal cortisol indices. *Stress* 19, 476–485. <https://doi.org/10.1080/10253890.2016.1206884>.
- Jackson, P., Cresswell, W., 2017. Factors determining the frequency and productivity of double brooding of Barn Owls *Tyto alba*. *Bird Study* 64, 353–361. <https://doi.org/10.1080/00063657.2017.1363716>.
- Jacobs, A.C., Reader, L.L., Fair, J.M., 2013. What determines the rates of double brooding in the Western Bluebird? *Condor* 115, 386–393. <https://doi.org/10.1525/cond.2013.120085>.
- Jenni, L., Jenni-Eiermann, S., Spina, F., Schwabl, H., 2000. Regulation of protein breakdown and adrenocortical response to stress in birds during migratory flight. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* 278, R1182–R1189. <https://doi.org/10.1152/ajpregu.2000.278.5.r1182>.
- Johns, M.E., Warzybok, P., Bradley, R.W., Jahncke, J., Lindberg, M., Breed, G.A., 2018. Increased reproductive investment associated with greater survival and longevity in Cassin's auklets. *Proc. R. Soc. B Biol. Sci.* 285, 1–9. <https://doi.org/10.1098/rspb.2018.1464>.
- Kluijver, H.N., 1951. The population ecology of the great tit. *Parus m. major L. Ardea* 39, 1–135.
- Kramer, M.H., Harris, D.J., 2010. Avian blood collection. *J. Exot. Pet Med.* 19, 82–86. <https://doi.org/10.1053/j.jepm.2010.01.006>.
- Laet, J.F., Dhont, A.A., 1989. Weight loss of the female during the first brood as a factor influencing second brood initiation in Great Tits *Parus major* and Blue Tits *P. caeruleus*. *Ibis (Lond. 1859)* 131, 281–289. <https://doi.org/10.1111/j.1474-919X.1989.tb02771.x>.
- Landys, M.M., Ramenofsky, M., Wingfield, J.C., 2006. Actions of glucocorticoids at a seasonal baseline as compared to stress-related levels in the regulation of periodic life processes. *Gen. Comp. Endocrinol.* 148, 132–149. <https://doi.org/10.1016/j.ygcen.2006.02.013>.
- Landys-Ciannelli, M.M., Ramenofsky, M., Piersma, T., Jukema, J., Wingfield, J.C., 2002. Baseline and stress-induced plasma corticosterone during long-distance migration in the bar-tailed godwit, *Limosa lapponica*. *Physiol. Biochem. Zool.* 75, 101–110. <https://doi.org/10.1086/338285>.
- Lenth, R., emmeans: Estimated Marginal Means, aka Least-Squares Means. R package version 1.8.5. <https://CRAN.R-project.org/package=emmeans>.
- Lindén, M., 1988. Reproductive trade-off between first and second clutches in the Great Tit *Parus major*: An experimental study. *Oikos* 51, 285–290.
- Long, J.A., Holberton, R.L., 2004. Corticosterone secretion, energetic condition, and a test of the migration modulation hypothesis in the Hermit Thrush (*Catharus guttatus*), a short-distance migrant. *Auk* 121, 1094–1102. <https://doi.org/10.1093/auk/121.4.1094>.
- LPBO, 2019. Long Point Bird Observatory: Migration monitoring protocol <www.bsc-ecoc.org/download/LPBOMigrationProtocol.pdf> (accessed 24.10.24.).
- MacDougall-Shackleton, S.A., Bonier, F., Romero, L.M., Moore, I.T., 2019. Glucocorticoids and "stress" are not synonymous. *Integr. Org. Biol.* 1. <https://doi.org/10.1093/iob/obz017>.
- MacDougall-Shackleton, S.A., Schmidt, K.L., Furlonger, A.A., MacDougall-Shackleton, E. A., 2013. HPA axis regulation, survival, and reproduction in free-living sparrows:

- functional relationships or developmental correlations? *Gen. Comp. Endocrinol.* 190, 188–193. <https://doi.org/10.1016/j.ygcen.2013.05.026>.
- Marra, P.P., Holberton, R.L., 1998. Corticosterone levels as indicators of habitat quality: Effects of habitat segregation in a migratory bird during the non-breeding season. *Oecologia* 116, 284–292. <https://doi.org/10.1007/s004420050590>.
- Middletton, A.L.A., 1979. Influence of age and habitat on reproduction by the American goldfinch. *Ecology* 60, 418–432.
- Mitchell, G.W., Guglielmo, C.G., Wheelwright, N.T., Freeman-Gallant, C.R., Norris, D.R., 2011. Early life events carry over to influence pre-migratory condition in a free-living songbird. *PLoS One* 6, 1–10. <https://doi.org/10.1371/journal.pone.0028838>.
- Mitchell, G.W., Wheelwright, N.T., Guglielmo, C.G., Norris, D.R., 2012. Short- and long-term costs of reproduction in a migratory songbird. *Ibis (Lond. 1859)* 154, 325–337. [doi:https://doi.org/10.1111/j.1474-919X.2012.01212.x](https://doi.org/10.1111/j.1474-919X.2012.01212.x).
- Muff, S., Nilsen, E.B., O'Hara, R.B., Nater, C.R., 2022. Rewriting results sections in the language of evidence. *Trends Ecol. Evol.* 37, 203–210.
- Müller, C., Jenni-Eiermann, S., Blondel, J., Perret, P., Caro, S.P., Lambrechts, M., Jenni, L., 2006. Effect of human presence and handling on circulating corticosterone levels in breeding blue tits (*Parus caeruleus*). *Gen. Comp. Endocrinol.* 148, 163–171. <https://doi.org/10.1016/j.ygcen.2006.02.012>.
- Munck, A.U., Koritz, S.B., 1962. Studies on the mode of action of glucocorticoids in rats. *Biochim. Biophys. Acta* 57, 1–23.
- Newman, A.E.M., Pradhan, D.S., Soma, K.K., 2008. Dehydroepiandrosterone and corticosterone are regulated by season and acute stress in a wild songbird: jugular versus brachial plasma. *Endocrinology* 149, 2537–2545. <https://doi.org/10.1210/en.2007-1363>.
- Newman, A.E.M., Soma, K.K., 2009. Corticosterone and dehydroepiandrosterone in songbird plasma and brain: effects of season and acute stress. *Eur. J. Neurosci.* 29, 1905–1914. <https://doi.org/10.1111/j.1460-9568.2009.06748.x>.
- Nomi, D., Yuta, T., Koizumi, I., 2018a. Male feeding contribution facilitates multiple brooding in a biparental songbird. *Ibis (Lond. 1859)* 160, 293–300. <https://doi.org/10.1111/ibi.12540>.
- Nomi, D., Yuta, T., Koizumi, I., 2018b. Facultative multiple breeding as a female conditional strategy in Japanese Tits: Partner's quality affects the initiation of second clutches. *Ecol. Res.* 33, 479–485. <https://doi.org/10.1007/s11284-018-1565-5>.
- Owen, J.C., 2011. Collecting, processing, and storing avian blood: a review. *J. F. Ornithol.* 82, 339–354.
- Pakkala, J.J., Norris, D.R., Sedinger, J.S., Newman, A.E.M., 2016. Experimental effects of early-life corticosterone on the hypothalamic–pituitary–adrenal axis and migratory behaviour in a wild songbird. *Funct. Ecol.* 30, 1149–1160. <https://doi.org/10.1111/1365-2435.12603>.
- Patterson, S.H., Winkler, D.W., Breuner, C.W., 2011. Glucocorticoids, individual quality and reproductive investment in a passerine bird. *Anim. Behav.* 81, 1239–1247. <https://doi.org/10.1016/j.anbehav.2011.03.012>.
- Pilastro, A., Griggio, M., Matessi, G., 2003. Male Rock Sparrows adjust their breeding strategy according to female ornamentation: Parental or mating investment? *Anim. Behav.* 66, 265–271. <https://doi.org/10.1006/anbe.2003.2203>.
- Pyle, P., 2022. Identification guide to North American birds, Part 1, 2nd Edition. Slate Creek Press, California.
- R Core Team, 2023. R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna.
- Rodenhouse, N.L., Holmes, R.T., 1992. Results of experimental and natural food reductions for breeding black-throated blue warblers. *Ecology* 73, 357–372.
- Romero, L.M., 2002. Seasonal changes in plasma glucocorticoid concentrations in free-living vertebrates. *Gen. Comp. Endocrinol.* 128, 1–24. [https://doi.org/10.1016/S0016-6480\(02\)00064-3](https://doi.org/10.1016/S0016-6480(02)00064-3).
- Romero, L.M., Dickens, M.J., Cyr, N.E., 2009. The reactive scope model: a new model integrating homeostasis, allostasis, and stress. *Horm. Behav.* 55, 375–389. <https://doi.org/10.1016/j.yhbeh.2008.12.009>.
- Salvante, K.G., Williams, T.D., 2003. Effects of corticosterone on the proportion of breeding females, reproductive output and yolk precursor levels. *Gen. Comp. Endocrinol.* 130, 205–214. [https://doi.org/10.1016/S0016-6480\(02\)00637-8](https://doi.org/10.1016/S0016-6480(02)00637-8).
- Schoech, S.J., Rensel, M.A., Bridge, E.S., Boughton, R.K., Wilcoxon, T.E., 2009. Environment, glucocorticoids, and the timing of reproduction. *Gen. Comp. Endocrinol.* 163, 201–207. <https://doi.org/10.1016/j.ygcen.2008.09.009>.
- Schwabl, P., Bonaccorso, E., Goymann, W., 2016. Diurnal variation in corticosterone release among wild tropical forest birds. *Front. Zool.* 13, 1–11. <https://doi.org/10.1186/s12983-016-0151-3>.
- Sharma, S.P.S., Dobney, S.L., Norris, D.R., Doucet, S.M., Newman, A.E.M., Burant, J.B., Moran, I.G., Mueller, S.D., Spina, H.A., Mennill, D.J., 2024. Effects of age, breeding strategy, population density, and number of neighbors on territory size and shape in *Passerculus sandwichensis* (Savannah sparrow). *Ornithology* 1–18.
- Sharp, P.J., Dawson, A., Lea, R.W., 1998. Control of luteinizing hormone and prolactin secretion in birds. *Comp. Biochem. Physiol. - C Pharmacol. Toxicol. Endocrinol.* 119, 275–282. [https://doi.org/10.1016/S0742-8413\(98\)00016-4](https://doi.org/10.1016/S0742-8413(98)00016-4).
- Smith, H.G., Kallander, H., Nilsson, J., 1987. Effect of experimentally altered brood size on frequency and timing of second clutches in the Great Tit. *Auk* 104, 700–706.
- Sterling, P., Eyer, J., 1988. Allostasis: a new paradigm to explain arousal pathology. In: Fisher, S., Reason, J. (Eds.), *Handbook of Life Stress Cognition and Health*. John Wiley and Sons, Inc., New York, pp. 629–650.
- Taborsky Jr., G.J., Porte Jr., D., 1981. Endogenous hyperglycemia restores insulin release impaired by somatostatin analogue. *Am. J. Physiol. Endocrinol.* 240, E407–E413.
- te Marvelde, L., Webber, S.L., Meijer, H.A.J., Visser, M.E., 2012. Energy expenditure during egg laying is equal for early and late breeding free-living female great tits. *Oecologia* 168, 631–638. <https://doi.org/10.1007/s00442-011-2122-x>.
- Tinbergen, J.M., Verhulst, S., 2000. A fixed energetic ceiling to parental effort in the Great Tit? *J. Anim. Ecol.* 69, 323–334. <https://doi.org/10.1046/j.1365-2656.2000.00395.x>.
- van Noordwijk, A.J., de Jong, G., 1986. Acquisition and allocation of resources: their influence on variation in life history tactics. *Am. Nat.* 128, 137–142.
- Verboven, N., Verhulst, S., 1996. Seasonal variation in the incidence of double broods: the date hypothesis fits better than the quality hypothesis. *J. Anim. Ecol.* 65, 264. <https://doi.org/10.2307/5873>.
- Washburn, B.E., Morris, D.L., Millsbaugh, J.J., Faaborg, J., Schulz, J.H., 2002. Using a commercially available radioimmunoassay to quantify corticosterone in avian plasma. *Condor* 104, 558–563.
- Wheelwright, N.T., Mauck, R.A., 1998. Philopatry, natal dispersal, and inbreeding avoidance in an island population of Savannah sparrows. *Ecology* 79, 755–767.
- Wheelwright, N.T., Rising, J.D., 2020. Savannah Sparrow (*Passerculus sandwichensis*), version 1.0. In: *Birds of the World*. Cornell Lab of Ornithology, New York.
- Wingfield, J.C., Romero, L.M., 2015. Tempests, poxes, predators, and people: Stress in wild animals and how they cope. Oxford University Press, Oxford.
- Wheelwright, N.T., Schultz, C.B., 1994. Age and reproduction in Savannah sparrows and tree swallows. *J. Anim. Ecol.* 63, 686–702.
- Wheelwright, N.T., Lawler, J.J., Weinstein, J.H., 1997. Nest-site selection in Savannah sparrows: using gulls as scarecrows? *Anim. Behav.* 53, 197–208. <https://doi.org/10.1006/anbe.1996.0288>.
- Williams, J.B., 1987. Field metabolism and food consumption of Savannah sparrows during the breeding season. *Auk* 104, 277–289.
- Williams, J.B., 1988. Field metabolism of tree swallows during the breeding season. *Auk* 105, 706–714.
- Wilson, F.E., Follett, B.K., 1975. Corticosterone-induced gonadosuppression in photostimulated tree sparrows. *Life Sci.* 17, 1451–1456. [https://doi.org/10.1016/0024-3205\(75\)90166-6](https://doi.org/10.1016/0024-3205(75)90166-6).
- Woodworth, B.K., Wheelwright, N.T., Newman, A.E.M., Norris, D.R., 2017a. Local density regulates migratory songbird reproductive success through effects on double-brooding and nest predation. *Ecology* 98, 2039–2048. <https://doi.org/10.1002/ecy.1911>.
- Woodworth, B.K., Wheelwright, N.T., Newman, A.E., Schaub, M., Norris, D.R., 2017b. Winter temperatures limit population growth rate of a migratory songbird. *Nat. Commun.* 8, 1–9. <https://doi.org/10.1038/ncomms14812>.
- Zabala, J., Lambin, X., Soufflot, J., Soufflot, P., Chenesseau, D., Millon, A., 2020. Proximate causes and fitness consequences of double brooding in female barn owls. *Oecologia* 192, 91–103. <https://doi.org/10.1007/s00442-019-04557-z>.