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### Baseline corticosterone does not reflect iridescent plumage traits in female tree swallows

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REGULAR ARTICLE

**Baseline corticosterone does not reflect iridescent plumage traits in female tree swallows**

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1 **Abstract**

2 The production of high quality secondary sexual traits can be constrained by trade-offs in the  
3 allocation of energy and nutrients with other metabolic activities, and is mediated by  
4 physiological processes. In birds, the factors influencing male plumage quality have been well  
5 studied; however, factors affecting female plumage quality are poorly understood. Furthermore,  
6 it remains uncertain which physiological traits mediate the relationship between body condition  
7 and ornaments. In this three-year study of after-second-year female tree swallows (*Tachycineta*  
8 *bicolor*), we investigated (1) the relationship between baseline corticosterone near the end of  
9 the brood-rearing period (CORT<sub>BR</sub>) and feather colour characteristics (hue, saturation,  
10 brightness) the following year, and (2) the relationship between baseline corticosterone  
11 measured during incubation (CORT<sub>I</sub>) and brood rearing (CORT<sub>BR</sub>), and feather colour in the same  
12 year. To control for reproductive effort, we included reproductive parameters as covariates in all  
13 analyses. In this first study between CORT and the plumage colour characteristics of a species  
14 bearing iridescent feathers, we did not find any relationship between CORT<sub>BR</sub> and the colour of  
15 subsequently-produced feathers, nor did we find any relationship between CORT<sub>I</sub> and the colour  
16 of feathers displayed during that breeding season. If CORT levels at the end of breeding carry  
17 over to influence the immediately subsequent moult period as we expect, our results generally  
18 indicate that structural plumage quality may not be as sensitive to circulating CORT levels  
19 compared to carotenoid-based colouration. Future studies, particularly those employing  
20 experimental manipulations of CORT during moult in species with iridescent traits, are necessary  
21 to fully determine the role glucocorticoids play in mediating the quality of secondary sexual  
22 characteristics.

Keywords: Glucocorticoid, Corticosterone, Reproduction, Secondary sexual characteristic,

Honest signal, Mate choice, Iridescence

## 1. Introduction

23 The honest signaling function of secondary sexual ornaments has been well established in a  
24 variety of species. Studies have shown that individuals bearing quality ornaments are in better  
25 body condition (e.g., Kodric-Brown and Brown, 1984), defend better/larger territories (e.g., Hill,  
26 1988; Keyser and Hill, 2000; Wolfenbarger, 1999), provide better parental care (e.g., Hill, 1991;  
27 Linville et al., 1998; Siefferman and Hill, 2003), and have greater reproductive success and  
28 potential fitness (e.g., Bitton et al., 2007; McGraw et al., 2001). Signal honesty is likely to be  
29 maintained because individuals in better condition pay relatively lower costs for the  
30 development of sexually selected traits (Grafen, 1990; Rubenstein and Hauber, 2008), and costs  
31 of production are constrained by trade-offs with other metabolic activities and mediated  
32 through physiological processes (Tibbetts, 2014). In birds, most of the evidence for condition-  
33 dependent signaling has been provided through the study of male plumage (Dale, 2006; Griffith  
34 and Pryke, 2006; Hill, 2006; Senar, 2006). In contrast, relatively little is known about the  
35 condition-dependence of plumage colouration and the factors that may influence the  
36 production of quality feathers in females. This is of particular interest since male mate choice  
37 has been demonstrated in a large number of species (Amundsen, 2000; Amundsen and Pärn,  
38 2006; Clutton-Brock, 2007), especially in cases where males and females both possess elaborate  
39 ornaments, and because females of most avian species invest more than males in reproduction  
40 (Kokko and Jennions, 2012). As a result, the plumage quality of females should reflect past  
41 reproductive investment and/or the potential for future reproductive effort, thereby influencing  
42 their ability to attract high quality mates (Clutton-Brock, 2009; Doutrelant et al., 2012).

43           The condition-dependence of plumage quality arises because of trade-offs in the  
44 allocation of energy and nutrients between the production of feathers and other costly  
45 metabolic and physiological maintenance activities. Exactly how these trade-offs are controlled  
46 is still largely debated (Morehouse, 2014). Because they are involved in the regulation and  
47 balance of energetic demands, physiological traits are thought to mediate the expression of  
48 traits advertising quality, including plumage colouration in birds (Bortolotti et al., 2009; Kimball,  
49 2006; McGlothlin et al., 2008). Glucocorticoids, such as corticosterone (CORT) in birds,  
50 demonstrate great potential for this role: they are involved in the maintenance of whole-  
51 organism energetic balance (Landys et al., 2006), and mediate other key life history trade-offs  
52 (Crespi et al., 2013). Indeed, exogenously administered CORT has been found to slow the rate of  
53 feather growth (Romero et al., 2005), and higher levels of circulating CORT during feather  
54 growth can result in poorer quality feathers in terms of colouration, barbule density, strength,  
55 and micro-structure (DesRochers et al., 2009; Lattin et al., 2011; Roulin et al., 2008).  
56 Furthermore, levels of CORT in feathers, a potentially integrated measure of baseline CORT,  
57 have been found to correlate with carotenoid-based plumage colour (Kennedy et al., 2013;  
58 Lendvai et al., 2013), and baseline and stress-induced plasma CORT levels have been linked to  
59 measures of reproductive investment (Bonier et al., 2009a; Bonier et al., 2009b; Breuner et al.,  
60 2008; Love et al., 2014). Since moult often occurs immediately following breeding, plumage  
61 quality is likely to be influenced by past reproductive investment and success (Norris et al.,  
62 2004); however, whether CORT can mediate the longer-term trade-off between reproductive  
63 investment and feather quality is largely unexplored (Tibbetts, 2014), and has never been  
64 assessed for iridescent structural colouration.

65           The iridescent plumage of tree swallows (*Tachycineta bicolor*) is coloured by a single  
66 layer of keratin overlaying multiple layers of melanosomes in the barbules, which produces

67 colour through thin-film interference (Maia et al., 2009; Prum, 2006). The mechanisms  
68 promoting the condition-dependence of iridescent plumage remain poorly studied (Doucet and  
69 Meadows, 2009; Maia and Macedo, 2011), and nanostructural differences leading to among-  
70 individual variation in colouration has been almost completely overlooked (but see Doucet et  
71 al., 2006). In satin bowerbirds (*Ptilonorhynchus violaceus*), a species in which structural colours  
72 are produced by the same nanostructural mechanism, the hue of the feather is associated with  
73 the average thickness of the keratin layer (Doucet et al., 2006), which is influenced by the size,  
74 quantity, and homogeneous distribution of melanosomes during feather keratinization (Maia et  
75 al., 2012). Colour purity (saturation) should be influenced by the homogeneousness of the  
76 keratin layer, and brightness has been correlated with barbule density (Doucet, 2002; Maia and  
77 Macedo, 2011). Because higher levels of CORT during feather growth have been demonstrated  
78 to reduce barbule density, strength, and micro-structure (DesRochers et al., 2009; Lattin et al.,  
79 2011; Roulin et al., 2008), and because physiological and nutritional stressors are known to  
80 induce keratin deposition abnormalities (e.g., fault bars; Jovani and Blas, 2004), baseline CORT  
81 levels during moult should influence iridescent plumage colouration. However, no studies have  
82 examined the potential relationship between iridescent plumage quality and circulating CORT.

83         In this study, we investigated whether the quality of mantle plumage colouration in  
84 after-second-year (ASY) female tree swallows reflects past or current year baseline plasma  
85 CORT. Using data collected over three years, we specifically test (1) the relationship between  
86 circulating CORT near the end of the brood rearing period ( $CORT_{BR}$ ) and feather colour  
87 characteristics (hue, saturation, brightness) the following year, and (2) the relationship between  
88 circulating CORT measured during the incubation ( $CORT_I$ ) and brood rearing period ( $CORT_{BR}$ ),  
89 and feather colour in the same year (**Figure 1**). We predicted that individuals with higher

90 baseline  $CORT_{BR}$  would subsequently produce lower quality plumage, and that  $CORT_I$  and  $CORT_{BR}$   
91 levels would be negatively correlated with current-year plumage quality attributes.

92

## 93 **2. Methods**

### 94 **2.1 Species and study design**

95 The tree swallow is a Neotropical migrant passerine that breeds across most of North America  
96 (Winkler et al., 2011). While second-year (SY) females possess dull grey plumage, males and ASY  
97 females bear iridescent upperparts which range in colour from green to blue among individuals  
98 (Hussell, 1983a). Delayed plumage maturation has been demonstrated to reduce conspecific  
99 aggression towards SY females during territorial intrusions, an adaptive trait in a species for  
100 which nest site competition is very high (Coady and Dawson, 2013). Even in iridescent adult  
101 females, reduced plumage brightness may reduce aggressive interactions (Berzins and Dawson,  
102 2016). Male tree swallows with brighter plumage are older (Bitton and Dawson, 2008), and have  
103 greater extra-pair fertilization success (observational study in Bitton et al., 2007, and  
104 experimentally confirmed in Whittingham and Dunn, 2016). Bluer males (lower hue value) are  
105 older, have increased survival rates (Bitton and Dawson, 2008) and greater immune response  
106 (Beck et al., 2015) compared to greener males. In females, bluer feathers are associated with  
107 older females and greater fledging success (Bitton et al., 2008), and plumage brightness is  
108 positively correlated with total clutch egg mass (Bitton et al., 2008). Furthermore, plumage  
109 colouration seems to play an important role in intersexual interactions as there is assortative  
110 mating based on plumage brightness (Bitton et al., 2008), and individuals paired with greener  
111 mates (indicative of lower quality) feed their own nestlings at a greater rate (Dakin et al., 2016).

112 In general, tree swallows produce a single brood per season (Winkler et al., 2011). They  
113 are semi-colonial secondary cavity nesters that breed readily in artificial cavities and are highly



114 philopatric (Winkler et al., 2004), which has made them an ideal study species for inter-annual  
115 investigations (Jones, 2003). In southern Ontario, breeding generally occurs from late April to  
116 mid-July and is usually immediately followed by moult (though moult can begin before nestlings  
117 fledge; see Hussell, 1983b). This complete prebasic moult begins with primary 1 and proceeds  
118 outward with body moult beginning with the back, breast, and belly regions when primary 2 to 4  
119 are actively being replaced (Stutchbury and Rohwer, 1990). Moult continues through migration  
120 and is normally completed by mid-November (Stutchbury and Rohwer, 1990).

121 We studied two populations of tree swallows ~4 km apart in Haldimand County,  
122 Ontario: Ruthven Park National Historic Site (42° 58' N, 79° 52' W) supports 140 nest boxes, and  
123 Taquanyah Conservation area (42° 57' N, 79° 54' W) supports 35 nest boxes. Both areas are  
124 characterized by a matrix of riparian, agricultural, fallow field, and deciduous forest land use  
125 types. Tree swallows breeding at these locations have been comprehensively banded since  
126 2010, and data for this study were collected during the breeding seasons (April to July) of 2011,  
127 2012, and 2013. In each year, we monitored nest boxes daily to record nest-building activity,  
128 onset of laying, clutch size, and fledging success for all breeding pairs.

129 We captured ASY females inside their nest boxes during late incubation (10 days  
130 following clutch completion) and recorded body mass, wing length, and age, and banded  
131 unbanded birds with a standard Fish and Wildlife Survey aluminum leg band. Females were  
132 recaptured during the brood-rearing period (day 12 of nestling provisioning) and body mass was  
133 re-measured. This time period (approximately 7-9 days before fledging) coincides with  
134 maximum nestling mass (McCarty, 2001; Quinney et al., 1986), near maximum parental feeding  
135 effort (Leffelaar and Robertson, 1986), and the end of the safe period for handling nestlings  
136 without pre-fledging or affecting fledge date (Burt, 1977). In 2011 and 2012, we also obtained a  
137 small blood sample from each female at each capture (Incubation - CORT<sub>i</sub>, Brood rearing -

138 CORT<sub>BR</sub>) through puncture of the brachial vein (less than 10 % of total blood volume i.e., < 150  
139 µl). We collected all blood samples between 0800 and 1200 hr to control for diel variation in  
140 CORT levels, and obtained all samples within two minutes of capture to ensure sampling of  
141 circulating baseline levels (Romero and Reed, 2005). Blood samples were stored on ice for up to  
142 five hours prior to centrifugation to separate plasma. Plasma samples were stored at -80° until  
143 assay. In 2012 and 2013, we also obtained five to six mantle feathers from each female during  
144 the incubation capture. Feathers were stored in opaque envelopes at room temperature until  
145 colour assessment.

146

## 147 **2.2 Baseline corticosterone quantification**

148 We determined plasma levels of total baseline CORT in non-extracted plasma using a  
149 Corticosterone Enzyme-linked-Immunosorbent Assay (ADI-900-097, EIA - Assay Designs,  
150 Michigan, USA; Madliger and Love, 2016a) with a 4-parameter logistic fit. This standard,  
151 commercially-available assay kit uses a sheep polyclonal antibody to corticosterone and 96-well  
152 plates coated with donkey anti-sheep IgG. Samples were run in triplicate at a total volume of  
153 100 µl with 1:40 dilution and 1.5 % steroid displacement buffer. When measured concentrations  
154 fell below the detection limit of the assay (0.74 ng/ml), we assigned this detection limit to those  
155 samples (< 10 % of samples). The intra-assay variation was 8.0 % in 2011 and 10.3 % in 2012.  
156 Inter-assay variation was 13.3 % in 2011 and 6.0 % in 2012.

157

## 158 **2.3 Feather characteristics**

159 Five mantle feathers from each individual were overlaid as naturally found on a live bird and  
160 secured onto low-reflectance black velvet. Surface reflectance data was acquired using a  
161 USB2000 spectrophotometer and PX-2 Pulsed Xenon light source (Ocean Optics, Dunedin,

162 Florida, USA) combined with a bifurcated fibre-optic probe. The probe was fitted with a rubber  
163 stopper at the tip to exclude ambient light, standardize the distance between the probe and the  
164 feathers, and allow each measurement to be taken at 90° from the surface (normal incidence).  
165 Five measurements were obtained from each sample; spectral data were recorded for  
166 wavelengths between 300 – 700 nm as the proportion of light reflected relative to the  
167 reflectance of a Spectralon pure white standard (Ocean Optics). Each spectral curve was  
168 smoothed using a locally-weighted scatterplot smoothing algorithm to prevent colour metrics  
169 from being influenced by effects of electrical noise. We subsequently averaged the five spectral  
170 curves, and calculated three colour metrics for the feathers of each individual. We calculated  
171 hue as the wavelength at maximum reflectance, mean brightness as the average reflectance  
172 over the 300 – 700 nm visual range, and chroma as the maximum reflectance minus the  
173 minimum reflectance divided by mean brightness (Andersson and Prager, 2006; Montgomerie,  
174 2006). This measure of chroma generates increasing scores with increasing peak height while  
175 controlling for overall brightness (Andersson et al., 2002). Spectral curve processing and the  
176 extraction of colour metrics was conducted with the package ‘pavo’ (Maia et al., 2013) for the  
177 statistical language R (R Development Core Team, 2016). All data and scripts will be made  
178 available upon request.

179

## 180 **2.4 Analyses**

181 To investigate the relationship between  $CORT_{BR}$  levels and plumage characteristics following  
182 moult, we compared linear mixed models fit by restricted maximum likelihood. We used  
183 measures of  $CORT_{BR}$  and condition measured during the brood rearing period from year X  
184 (either 2011 or 2012) and measures of plumage colour from year X+1 (either 2012 or 2013;  
185 **Figure 1**). While  $CORT$  levels were not measured during moult, females in our population with

186 higher baseline CORT during nestling provisioning returned the following year with higher  
187 baseline CORT during incubation (Madliger and Love, 2016a), indicating long-term repeatability  
188 of individual CORT levels. As a measure of body condition, we used body mass, because the  
189 residual of a linear regression of mass over wing length was not significant (year included as  
190 covariate:  $F_{2,32} = 1.06$ ,  $p = 0.37$ ; Schulte-Hostedde et al., 2005). To avoid pseudoreplication by  
191 including values for females that had been captured during more than one inter-annual  
192 breeding attempt, all models included female identity as the within-subject random factor. For  
193 each of the three plumage characteristics included as the dependent variable (hue, brightness,  
194 chroma), we generated a set of eight biologically relevant candidate models (and an additional  
195 intercept-only ninth model) which were compared using Akaike's information criterion  
196 corrected for small sample size (AICc; Akaike, 1973; Burnham and Anderson, 2002; Hurvich and  
197 Tsai, 1989). All models except for the intercept-only model included  $CORT_{BR}$  and year of data  
198 collection. Seven of the models also included as independent factors body mass, laying date,  
199 and clutch size in all possible combinations (See Supplemental Material for the complete set of  
200 models). We did not include female age because the full study site was initiated the year before  
201 this study began, and we had incomplete age data for too many individuals. The best fitting  
202 models were considered equally plausible when the AICc value differed by no more than 2.00  
203 ( $\Delta AICc < 2.00$ ) compared with the model with lowest value (Burnham and Anderson 2002).  
204 Variance explained by the best fitting models was calculated using marginal and conditional  $R^2$   
205 (Nakagawa and Schielzeth 2013) using the 'rsquared' function in the R package 'piecewiseSEM'  
206 (Lefcheck, 2016).

207 To investigate the predictive value of plumage characteristics on  $CORT_I$  and  $CORT_{BR}$   
208 levels, we compared linear models using measures of plumage characteristics, condition, and  
209 CORT collected within the same year (2012 only). For each of the two CORT measures we

210 compared a set of candidate models that all included in various combinations at least one of the  
211 three plumage characteristics (hue, brightness, chroma). In half of the models, two measures of  
212 condition, laying date (Verhulst and Nilsson 2008) and body mass, were also included. The  
213 regression of mass over wing length was also not significant for this group of females  
214 (incubation mass:  $p = 0.08$ ; brood rearing mass:  $p = 0.20$ ). All sets of models included a global  
215 model and an intercept model (See Supplemental Material for the complete set of models), and  
216 the best fitting models were selected based on their AICc scores as above.

217

### 218 **3. Results**

219 When investigating the relationships between  $CORT_{BR}$  levels and plumage characteristics  
220 following moult, we used 37 observations obtained from 33 different females (four females  
221 captured in both years). To satisfy assumptions of normal distribution, we log-transformed  
222  $CORT_{BR}$ . We treated clutch size as an ordinal factor, including only nests that had between four  
223 and seven eggs to avoid strong imbalance in sample size between groups. Before comparing  
224 models, we ensured that they did not suffer from multicollinearity of independent factors  
225 through the calculation of variance inflation factors (VIFs). The set of models that best fit the  
226 data for plumage brightness and chroma had the intercept-only model indicating that none of  
227 the models fit the data well (**Table 1**). A single model fit the data for plumage hue, and included  
228 the predictors CORT, clutch size and mass (marginal  $R^2 = 0.132$ , Conditional  $R^2 = 0.132$ ).  
229 However, only mass (estimate: -10.01; 95 % CI: -19.21 – -0.81) but neither CORT (estimate: -  
230 0.88; 95 % CI: -10.40 – 8.63) nor clutch size (estimate: -1.25; 95 % CI: -22.66 – 20.16), were  
231 significant predictors.

232 When investigating the relationships between our two corticosterone measures ( $CORT_I$   
233 and  $CORT_{BR}$ ) and same year plumage characteristics, we used 32 observations, all from different

234 females.  $CORT_I$  and  $CORT_{BR}$  were log transformed, only nests that included between four and  
235 seven eggs were used, and clutch size was treated as an ordinal factor. We also ensured that our  
236 models did not suffer from multicollinearity. The set of models that best fit the data for  $CORT_I$   
237 included the intercept-only model indicating that none of our models fit the data well (**Table 2**).  
238 In contrast, two models fit the data for  $CORT_{BR}$ . The first model included brightness, female body  
239 mass and clutch size as predictors (adjusted  $R^2$ : 0.27); the second included brightness, hue,  
240 female body mass and clutch size (adjusted  $R^2$ : 0.27; **Table 2**). We calculated the parameter  
241 estimates by weighting the parameter values and associated standard deviations of the two  
242 equally likely models by their respective model weight. This indicated that neither brightness  
243 (estimate: -0.065; 95 % CI: -0.139 – 0.008), hue (estimate: 0.005; 95 % CI: -0.005 – 0.015), nor  
244 body mass (estimate: -0.125; 95 % CI: -0.352 – 0.103), were significant predictors. In these  
245 models only clutch size (estimate: 0.96; 95 % CI: 0.41 – 1.51) was a significant predictor.

246

#### 247 **4. Discussion**

248 There is mounting evidence in some species that female plumage colouration is not a genetic  
249 carryover from male plumage colouration, that female plumage characteristics are associated  
250 with quality and reproductive success, and that males select females based on secondary sexual  
251 characteristics (Amundsen, 2000; Amundsen and Pärn, 2006). However, we know very little  
252 about which physiological and/or metabolic processes link the cost of reproduction to plumage  
253 quality in females (Moore et al., 2016). In this study, we used data collected over three years on  
254 breeding ASY female tree swallows to investigate the relationship between individual variation  
255 in baseline plasma corticosterone (CORT) and the characteristics of iridescent plumage. We  
256 predicted that females with high baseline CORT levels just prior to moulting would produce low  
257 quality plumage, and that females with high quality plumage would have low baseline CORT

258 upon returning to the breeding site. In contrast to our predictions, plumage characteristics were  
259 not associated with previous or subsequent circulating CORT levels.

260

#### 261 **4.1 Baseline corticosterone as a predictor of plumage quality**

262 Because moult occurs immediately following breeding in tree swallows (Winkler et al., 2011),  
263 the large investment in reproduction is expected to limit the energy and resources available for  
264 the production of high quality feathers (e.g., Hemborg and Lundberg, 1998; Norris et al., 2004).  
265 Indeed, observational and experimental studies have demonstrated the influence of breeding  
266 effort on structural plumage quality in other species. For example, male, but not female, eastern  
267 bluebirds (*Sialia sialis*) raising artificially enlarged broods produced lower quality non-iridescent  
268 plumage (Siefferman and Hill, 2005, 2008). Similarly, the plumage hue of male and female blue  
269 tits (*Cyanistes caeruleus*) shifted towards shorter (bluer) wavelengths, an indication of higher  
270 quality plumage, after fledging regular size broods, but not enlarged broods (Doutrelant et al.,  
271 2012). Because reproductive effort has also been associated with increased baseline CORT in  
272 several studies (Crespi et al., 2013), we predicted that females with higher levels of CORT  
273 immediately preceding feather moult (i.e., CORT<sub>BR</sub>) would produce lower quality plumage.  
274 Unlike these previous studies, our research failed to find any relationship between CORT<sub>BR</sub> and  
275 subsequent plumage colouration. However, it is important to note that all studies presented  
276 above were experimental in design, whereas we did not manipulate the reproductive effort of  
277 the females included in our study. Ours is but a first step in identifying the signaling mechanisms  
278 that mediate plumage colour in female tree swallows, but experiments that either directly  
279 increase CORT levels during moult or manipulate reproductive effort could arrive at different  
280 conclusions. Other non-mutually exclusive reasons may explain our results.

281 Baseline CORT as assessed in this study is a temporally-specific measure which can vary  
282 based on a number of factors including social interactions (Creel, 2001), food availability (Jenni-  
283 Eiermann et al., 2008), and predation risk (Cockrem and Silverin, 2002). Because this measure of  
284 energetic stress is known to vary on a short temporal scale, it may not be an accurate reflection  
285 of the condition of the individual at the time of feather production. Although it was not possible  
286 to obtain CORT measures from moulting birds, and we compared plumage characteristics to  
287 CORT measurements obtained as close as possible to the production of the new feathers, a  
288 change in the phase of the annual cycle from breeding to migration could influence the levels of  
289 plasma CORT during the production of feathers (Romero, 2002). However, birds with higher  
290 CORT levels in one life history stage (e.g., breeding) could be expected to also have higher CORT  
291 levels during subsequent stages (e.g., moult). While baseline CORT levels generally show low  
292 repeatability between years, they can show moderate within-individual repeatability over  
293 shorter time scales (weeks to months) (Ouyang et al., 2011; Romero and Reed, 2008;  
294 Schoenemann and Bonier, 2018; Taff et al., 2018). Even over a longer time frame, in our  
295 population of tree swallows, females with higher baseline CORT during nestling provisioning  
296 return the following year with higher baseline CORT during incubation (Madliger and Love,  
297 2016a). Therefore, we believe it is possible that females with elevated CORT at the end of the  
298 nestling provisioning stage could continue to experience elevated CORT during the moult period,  
299 which occurs immediately following the cessation of breeding (i.e., females that find one stage  
300 energetically-demanding may also face higher physiological stress in subsequent stages).

301 If the production of quality plumage is important for females, as would be implied by  
302 assortative mating found in this species (Bitton et al., 2008), female tree swallows could be  
303 down-regulating their CORT levels during feather replacement to avoid the negative effects on  
304 protein production and therefore feather quality. CORT levels in some species are known to be



305 at their lowest towards the end of the breeding season and during moult (e.g. Done et al., 2011;  
306 Romero, 2002), and CORT-implanted European starlings (*Sturnus vulgaris*) have been shown to  
307 decrease feather growth leading to the production of feathers with quality independent of CORT  
308 levels (Romero et al., 2005). Indeed, CORT levels measured in feathers are not always associated  
309 with plasma CORT (Patterson et al., 2015), suggesting the existence of a mechanism that would  
310 control CORT levels in follicles. As a result, there may be inter-individual variation in the ability  
311 to down-regulate CORT production during moult (Romero, 2002) or in the release of CORT into  
312 the growing feather (Harris et al., 2016), which could lead to the lack of relationship between  
313 plasma CORT at nestling provisioning and feather quality indices.

314 While the feathers were produced soon after the collection of the CORT<sub>BR</sub> sample, they  
315 were not collected for characterization until about nine months later, once the females had  
316 returned to the breeding grounds. During this time, feather characteristics may have changed.  
317 The brightness of plumage is known to be reduced through abrasion in carotenoid-based  
318 (Figuerola and Senar, 2005; McGraw and Hill, 2004) and non-iridescent structurally coloured  
319 feathers (Örnberg et al., 2002), and it is thought that wear may also be responsible for changes  
320 in hue and saturation of non-iridescent structural colouration (Örnberg et al., 2002). If changes  
321 in feather colour characteristics were non-random in such a way that higher quality feathers  
322 were more likely to be degraded, a relationship between CORT<sub>BR</sub> and feather quality the  
323 subsequent year would be more difficult to detect. However, it may be more likely that  
324 individuals that produced high quality plumage can allocate more energy to feather  
325 maintenance, thus reducing degradation, which would increase the perceived effect of CORT<sub>BR</sub>  
326 on feather quality.

327 Finally, our inability to accurately age females may have added confounding variation to  
328 our study. Since reproductive investment and reproductive success are influenced by age in tree

329 swallows (Robertson and Rendell, 2001), and that these factors (including age) have been  
330 associated with plumage colouration (Bitton and Dawson, 2008; Bitton et al., 2008), it may be  
331 difficult to disentangle the relationships between reproduction-induced stress, age, and  
332 plumage attributes without specific age information. For instance, it is possible that younger  
333 females have more difficulty managing stress or energetic demand than older females  
334 (Wingfield and Sapolsky, 2003), which could lead to relationships between plumage traits and  
335 CORT in young females but not in older females. A longitudinal study, or experiment in which  
336 CORT levels are manipulated while blocking treatments on female age, could help resolve these  
337 questions.

338

#### 339 **4.2 Plumage characteristics representing current-year corticosterone**

340 The role of plumage colouration in inter- and intra-sexual competition has been well  
341 documented (Griffith and Pryke, 2006; Senar, 2006). A common premise is that plumage  
342 characteristics are representative of an individual's quality even if those feathers were produced  
343 months before the arrival to the breeding grounds. Since baseline CORT levels have been  
344 experimentally shown to mediate reproductive investment in birds (e.g., Bonier et al., 2011;  
345 Hennin et al., 2016; Love et al., 2014), we predicted a negative relationship between same year  
346 plumage colour attributes and CORT during reproduction. Our lack of significant results may not  
347 be surprising as a few studies have found a potential role for CORT as a mediator between  
348 condition and plumage colouration (e.g., Fairhurst et al., 2014; Grindstaff et al., 2012; Lendvai et  
349 al., 2013), while several have not (e.g., Jenkins et al., 2013; Merrill et al., 2014). Even among  
350 studies that have found relationships between CORT and secondary sexual characteristics, the  
351 direction of the relationship is not always as predicted (negative association between CORT  
352 levels and plumage quality), and may be context-dependent (Fairhurst et al., 2014; Lendvai et

353 al., 2013). Furthermore, given the correlative nature of the current study it is possible that the  
354 individuals were not subjected to sufficient stress to lead to effects on ornament quality. Direct  
355 or indirect manipulation of circulating CORT right at the end of the breeding season or during  
356 moult, or handicapping the birds to increase their work load, would help determine its  
357 relationship with iridescent plumage quality.

358         Unlike the large majority of studies investigating relationships between CORT and  
359 plumage attributes, tree swallows display structurally-coloured plumage, not pigment-coloured  
360 plumage (see Grindstaff et al., 2012 for an example of non-iridescent structural study). Keratin  
361 and melanin, which produce the nanostructures responsible for the colours in tree swallow  
362 feathers, are metabolically produced *de novo* at the follicle during feather development  
363 (McGraw, 2006b), unlike carotenoids which must be acquired from the diet and used by other  
364 physiological processes (e.g., anti-oxidants, McGraw, 2006a). Therefore, keratin and melanin  
365 may not be influenced by metabolic processes in the same way as carotenoids (Fairhurst et al.,  
366 2015). Indeed, studies investigating the link between CORT and plumage attributes have more  
367 often found relationships in species bearing carotenoid-based plumage, not melanin-based  
368 plumage. In mallards, *Anas platyrhynchos*, feather CORT in ducklings was positively correlated  
369 with carotenoid-based signals in adults, but not correlated with melanin-based signals (Fairhurst  
370 et al., 2015). Similarly, in yellow warblers, *Setophaga petechia*, feather CORT negatively  
371 correlated with several measures of carotenoid-based plumage signals (hue, chroma), but not  
372 phaeomelanin-based plumage attributes (Grunst et al., 2015). Several other studies have failed  
373 to find relationships between CORT and melanin or structurally based plumage colouration (e.g.,  
374 Jenkins et al., 2013, but see Grindstaff et al., 2012 and Henderson et al., 2013 for contrary  
375 examples). The potential for energetic and physiological costs to influence different aspects of  
376 plumage colouration likely depends on the underlying physical and pigmentary structures that

377 are responsible for the colours, how their organization is controlled in the developing feathers,  
378 and feather maintenance behaviours.

379 It is possible that the level of reproductive demand and baseline CORT affects aspects  
380 of plumage colouration that we did not measure. Iridescent plumage, by definition, changes in  
381 colour with changes in angle between the viewer and the light source. Iridescent feathers could  
382 therefore be assessed for the range of angles over which colour is displayed (called *directionality*  
383 in Van Wijk et al., 2016b), and the hues at these angles. These metrics may reflect different  
384 feather properties than those obtained from taking measurements at normal incidence (hue,  
385 saturation, and brightness calculated at 90° from the feather surface), and *directionality* better  
386 predicts some aspect of male reproductive success in this species. Future studies on the  
387 proximate mechanism of iridescent plumage colouration should assess these changes in colour  
388 with viewing angle and better evaluate the functionality of iridescence itself (Meadows et al.,  
389 2011; Van Wijk et al., 2016a).

390

### 391 **4.3 Conclusion**

392 The role of variation in baseline CORT in mediating the relationship between condition and  
393 secondary sexual characteristics in birds is far from being well understood. A large number of  
394 factors can influence CORT levels at any time (Moore et al., 2016), and measures of stress are  
395 not always repeatable within individuals between seasons and years (Legagneux et al., 2013;  
396 Perez et al., 2016; Schoenemann and Bonier, 2018; Taff et al., 2018). In an attempt to best  
397 capture a temporally valid measure of stress, we collected blood samples to assess baseline  
398 plasma CORT during the incubation period and during the brood rearing period. This allowed us  
399 to compare the plumage characteristics to the most relevant levels of CORT: CORT<sub>BR</sub> to plumage  
400 traits produced soon after leaving the nest, CORT<sub>I</sub> to plumage colouration upon arrival at the

401 breeding site. However, we did not find any relationship between CORT levels and plumage  
402 traits in female tree swallows. While multiple explanations can be invoked to rationalize our  
403 findings, the interpretation of our results is difficult because there is a lack of studies  
404 investigating the link between CORT and the production of ornaments in females, and a paucity  
405 of studies investigating the link between CORT and the production of structural colouration,  
406 both iridescent and non-iridescent. It is generally unknown how trade-offs between plumage  
407 traits and metabolism are mediated differently in males and females, and it is not clear what  
408 relationship we should expect between CORT and the expression of colours produced from  
409 nanostructures. This invites future studies on the topic, particularly ones that would manipulate  
410 CORT levels (or workload that would elevate baseline CORT; Madliger and Love, 2016b) in  
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412

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427

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429

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432

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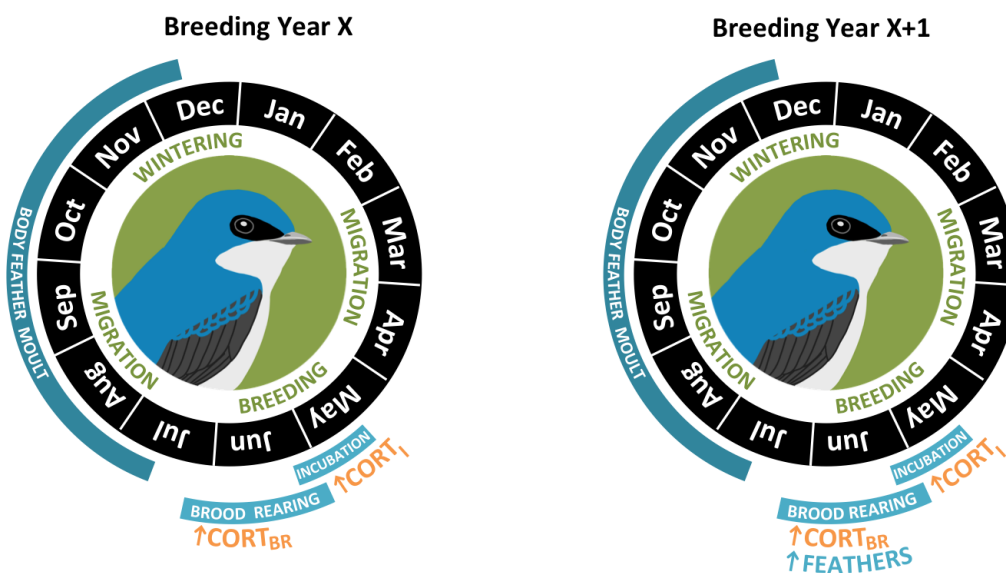
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727 **Figure 1.** Sampling scheme for this study. Using a three year data set we tested the relationship  
728 between circulating CORT near the end of the brood rearing period ( $CORT_{BR}$ ; Year X) and feather  
729 colour characteristics (hue, saturation, brightness) the following year (Year X+1), and the  
730 relationship between circulating CORT measured during incubation ( $CORT_I$ ) and brood-rearing  
731 period ( $CORT_{BR}$ ) feather colour in the same year (e.g., Year X+1).



732

733 **Table 1.** Summary of the linear mixed models that best fit the data when investigating the  
734 relationship between baseline corticosterone sampled during the brood-rearing period ( $CORT_{BR}$ )  
735 in year X and plumage characteristics sampled in year X+1 (these feathers would have grown in  
736 shortly after brood rearing when corticosterone was sampled the previous year). All models also  
737 included the year of feather collection as covariate (not show for brevity). From all candidate  
738 models, only those with a  $\Delta AICc < 2.0$  are presented.

739

Dependent variable	Parameters	k	AIC	AICc	$\Delta AICc$	Evidence ratio	Weight
Plumage brightness	$CORT_{BR}$ Clutch size	5	195.76	197.83	0.00	1.00	0.34
	Intercept	3	197.24	198.01	0.18	0.91	0.31
	$CORT_{BR}$	4	198.19	199.52	1.70	0.43	0.15
Plumage chroma	Intercept	3	-33.23	-32.46	0.00	1.00	0.98
Plumage hue	$CORT_{BR}$ Clutch size Mass	6	285.38	288.38	0.00	1.00	0.81

740

741 **Table 2.** Summary of the linear mixed models that best fit the data when investigating the  
742 relationship between same-year corticosterone levels collected during the incubation period  
743 (CORT<sub>I</sub>) and brood-rearing period (CORT<sub>BR</sub>), and plumage characteristics. From all candidate  
744 models, only those with a  $\Delta\text{AICc} < 2.0$  are presented.

745

Dependent variable	Parameters	k	AIC	AICc	$\Delta\text{AICc}$	Evidence ratio	Weight
CORT <sub>I</sub>	Intercept	2	62.04	62.45	0.00	1.00	0.37
	Chroma	3	63.36	64.21	1.76	0.41	0.16
CORT <sub>BR</sub>	Brightness Body mass Clutch size	5	57.70	60.00	0.00	1.00	0.39
	Brightness Hue Body mass Clutch size	6	58.55	61.91	1.90	0.39	0.15

746 CORT<sub>I</sub>: Corticosterone during egg incubation; CORT<sub>BR</sub>: Corticosterone during brood rearing