Title	Moulting season corticosterone correlates with winter season bodyweight in an Arctic migrant bird
Author(s)	Doyle, Susan; Cabot, David; Furlong, John; Liu, Yixin; Colhoun, Kendrew; Walsh, Alyn J.; McMahon, Barry J.
Publication date	2020-08-11
Original citation	Doyle, S., Cabot, D., Furlong, J., Liu, Y., Colhoun, K., Walsh, A. J. and McMahon, B. J. (2020) 'Moulting season corticosterone correlates with winter season bodyweight in an Arctic migrant bird', Ibis. doi: 10.1111/ibi.12876
Type of publication	Article (peer-reviewed)
Link to publisher's version	http://dx.doi.org/10.1111/ibi.12876 Access to the full text of the published version may require a subscription.
Rights	**© 2020, British Ornithologists' Union. Published by John Wiley & Sons, Inc. This is the peer reviewed version of the following article: Doyle, S., Cabot, D., Furlong, J., Liu, Y., Colhoun, K., Walsh, A. J. and McMahon, B. J. (2020) 'Moulting season corticosterone correlates with winter season bodyweight in an Arctic migrant bird', Ibis, doi: 10.1111/ibi.12876, which has been published in final form at https://doi.org/10.1111/ibi.12876. This article may be used for non-commercial purposes in accordance with Wiley Terms and Conditions for Use of Self-Archived Versions.**
Embargo information	Access to this article is restricted until 12 months after publication by request of the publisher.
Embargo lift date	2021-08-11
Item downloaded from	http://hdl.handle.net/10468/10665

2

3 **Moulting season corticosterone correlates with winter season body-weight in**

4 **an Arctic migrant bird**

5

6 SUSAN DOYLE,[1] DAVID CABOT,[2] JOHN FURLONG,[3] YIXIN LIU,[1] KENDREW

7 COLHOUN,[1] ALYN J. WALSH[4] & BARRY J. MCMAHON[1*]

8

9 *[1]UCD School of Agriculture and Food Science, University College Dublin, Ireland*

10 *[2]School of Biological, Earth and Environmental Sciences, University College Cork,*

11 *Ireland*

12 *[3]UCD School of Veterinary Medicine, University College Dublin, Ireland*

13 *[4]National Parks and Wildlife Service, Department of Culture, Heritage and the*

14 *Gaeltacht, Wildfowl Reserve, North Slobland, Wexford, Ireland*

15

16 *Corresponding author.

17 Email: barry.mcmahon@ucd.ie

18

In vertebrates, the endocrine system translates environmental changes into physiological responses on which natural selection can act to regulate individual fitness and, ultimately, population dynamics. Corticosterone (CORT) and dehydroepiandrosterone (DHEA) are important regulators of the avian endocrine system but relatively few studies have investigated their downstream effects on key morphological fitness-related traits in free-living populations. This study quantified endocrine-morphology relationships in free-living Greenland Barnacle Geese *Branta leucopsis* that breed in the high Arctic. CORT and DHEA were extracted from feather and blood samples and tested for relationships with three morphological traits associated with survival and reproduction: body weight, body size and facial plumage coloration. We expected CORT concentration to be higher in birds with less favourable morphological traits (i.e. lighter, smaller and less attractive) and DHEA to be higher in birds with more favourable traits (i.e. heavier, bigger and more attractive). As expected, individuals with higher CORT during the post-breeding moult (July/August) had significantly lower body weight during the following winter (November - April). In contrast, we found no robust DHEA-morphology relationships and no statistically significant relationship between CORT and body size or facial plumage. Overall, this study provides evidence of a negative relationship between CORT and body-weight extending across different seasons of the annual cycle in a long-distance migrant. This is of particular interest because body-weight fluctuates rapidly in response to environmental resources and is closely linked to both survival and reproductive success in this species. Understanding the relationship between CORT and key morphological traits is important because endocrine-disrupting contaminants in the Arctic increasingly interfere with CORT function in birds,

2

43 including Barnacle Geese, and based on the results of this study may have

44 consequences for body-weight regulation.

45

46 **Keywords:** Barnacle Goose, *Branta leucopsis*, CORT, DHEA, plumage

47

3

48 The endocrine system is an important modulator of fitness in vertebrates.

49 Environmental conditions affect fitness - an individual's ability to survive and

50 reproduce - which in turn regulates population dynamics (Orr 2009). Hormones play

51 a key role in this sequence by translating environmental information into individual

52 physiological responses that have positive or negative fitness consequences (Norris

53 2018, Wingfield 2018). In birds, hormones modulate morphology such as body-

54 weight (Awerman & Romero 2010), body size (Müller *et al.* 2009) and plumage

55 coloration (Jawor & Breitwisch 2003, Grunst *et al.* 2015) – morphological traits that

56 are strongly connected with survival and reproductive success (e.g. Poisbleau *et al.*

57 2006, Tombre *et al.* 2012, Badás *et al.* 2018). Importantly, natural selection acts on

58 the downstream physiological effects of hormones (i.e. weight, size and plumage)

59 rather than the circulating hormones themselves. As such, identifying endocrine-

60 morphology relationships can provide insight into the sequence between

61 environment, individuals and population. Although the major functions of the

62 endocrine system are highly conserved among vertebrates, endocrine-morphology

63 relationships are not always consistent among taxa or even within a species' life-

64 history (Bonier *et al.* 2009), therefore studies on novel species or relationships can

65 provide new insights.

66

67 The glucocorticoid hormone corticosterone (CORT) and the androgen hormone

68 dehydroepiandrosterone (DHEA) are principal modulators of the avian endocrine

69 system (Hau *et al.* 2010, Wingfield *et al.* 2018). CORT principally modulates

70 homeostasis, energy balance and stress response via release by the hypothalamic-

71 pituitary-adrenal axis in response to changing conditions (Sapolsky *et al.* 2000, Creel

72 *et al.* 2013). Mildly elevated CORT in the short term can be an adaptive mechanism

4

73 for coping with environmental or physiological challenges, such as parasite infection

74 or increased energetic demand (Kouwenberg *et al.* 2013, Rivers *et al.* 2017, Grace &

75 Anderson 2018, Schoenle *et al.* 2018, Vágási *et al.* 2018). In contrast, chronically

76 elevated CORT can negatively affect overall body condition (Harms *et al.* 2015, Latta

77 *et al.* 2016). Common Kestrel *Falco tinnunculus* nestlings experimentally implanted

78 with CORT (to represent an environmental stressor) were smaller, with shorter tarsi

79 and flight feathers before fledging compared to control nestlings (Müller *et al.* 2009).

80 American Yellow Warblers *Setophaga petechia* with higher CORT had lower

81 carotenoid hues in plumage associated with sexual signalling (Grunst *et al.* 2015).

82 Thus, chronic elevated CORT relative to an individual's conspecifics may be a

83 fitness disadvantage (Bonier *et al.* 2009).

84

85 DHEA principally modulates aggression and dominance behaviour (Soma *et al.*

86 2008, 2015). It is produced by the adrenal glands and other organs, then taken up by

87 the brain and converted into sex steroids that generate physiological changes (Soma

88 *et al.* 2015, Wingfield *et al.* 2018). Hau *et al.* (2004) found that aggressive

89 vocalisations by Spotted Antbirds *Hylophylax naevioides naevioides* during

90 simulated territorial intrusions were positively correlated with DHEA levels. Similarly,

91 male Song Sparrows *Melospiza melodia morphna* experimentally implanted with

92 DHEA showed greater aggression in simulated territory intrusions than control

93 sparrows (Wacker *et al.* 2016). In contrast, Poisbleau *et al.* (2009) found a negative

94 correlation between DHEA and dominance score (the percentage of competitive

95 interactions "won" by an individual) in wintering Brent Geese *Branta bernicla*

96 *bernicla*, indicating uncertainty in the role of DHEA in aggression among taxa.

97 However, DHEA also modulates potential negative impacts of glucocorticoids and

5

98   has positive neuroprotective properties (Newman *et al.* 2010, 2013). It has also been

99   linked to alternative plumage colour morphs (Spinney *et al.* 2006). Based on the

100   literature, one could hypothesise that elevated DHEA relative to an individual's

101   conspecifics may offer a fitness advantage, both through its protective properties and

102   as dominant individuals would be more likely to access resources with positive

103   outcomes for body condition (Poisbleau *et al.* 2006).

104

105   Although the relationship between CORT and fitness is well studied in wild bird

106   populations, fewer studies concern its relationship with morphology. Additionally, the

107   relationship between DHEA and fitness or morphology in wild bird populations is not

108   well studied. Quantifying endocrine-morphology relationships in free-living

109   populations can reveal patterns that may not be apparent in laboratory investigations

110   (Schoech *et al.* 2011, Wingfield 2018). Thus, this study aimed to quantify endocrine-

111   morphology links in a free-living population of Greenland Barnacle Goose *Branta*

112   *leucopsis.* The majority of this population breeds in high-Arctic north-east Greenland

113   (70-78°N), with a small emerging breeding population in south-east Iceland (64°N).

114   Barnacle Geese here undergo a post-breeding synchronous flight feather moult in

115   July/August before migrating to Ireland and Scotland for winter (October – April)

116   (Cabot *et al.* 1984). Increased body weight is a fitness advantage in geese, as more

117   reserves are available for migration, egg formation and nesting (Poisbleau *et al.*

118   2006, Hahn *et al.* 2011, Tombre *et al.* 2012). Increased body size is also a fitness

119   advantage as it improves social status and access to resources (Choudhury *et al.*

120   1996, Poisbleau *et al.* 2006, Kurvers *et al.* 2010). Barnacle Geese also have

121   prominent white patches extending across the face contrasting with black plumage

122   around the eye and bill. These patches are an important social signal (including

6

123 during aggressive encounters) and, although the role is not fully understood, it

124 appears that, at least among females, individuals with darker faces are more

125 attractive as mates (Black *et al.* 2014). Hence, we expect hormone concentrations

126 associated with increased body weight, body size and smaller cheek patches to

127 improve overall fitness in Barnacle Geese.

128

129 We analysed the strength of relationships between these three morphological traits

130 and CORT and DHEA. The analysis determines whether meaningful endocrine-

131 morphology relationships are present but cannot confirm causality. We hypothesised

132 that there would be a negative correlation between body weight and CORT and

133 between body size and CORT, but a positive correlation between cheek patch size

134 and CORT (i.e. individuals with higher CORT are lighter, smaller and less attractive).

135 We hypothesised that DHEA would correlate in the opposite way (i.e. individuals with

136 higher DHEA are heavier, bigger and more attractive). In addition, we used long-term

137 data on body-weight, body size and cheek patch size to investigate if these traits

138 have changed over time, which could signal changing selective pressures. CORT

139 and DHEA play a critical regulatory role in the avian endocrine system, so

140 determining consistent relationships with morphological traits associated with

141 survival and reproduction will improve our understanding of these systems and how

142 birds are affected by and adapt to their changing environment.

143

144 **METHODS**

145 **Study outline**

146 During winter 2017/2018, 55 adult Barnacle Geese were captured on their wintering

147 grounds in Ireland using cannon-netting. Four flocks were captured at four different

7

148　locations in November 2017 (n = 5 at 54.12, -10.21), January 2018 (n = 33 at 55.29,

149　-7.28) and April 2018 (n = 8 at 55.28, -7.31 and n = 9 at 55.38, -7.37). Birds were

150　sexed by cloacal examination and morphological measurements were collected.

151　Feather and blood samples were then collected to extract CORT and DHEA,

152　respectively. Morphological measurements from an additional 53 Barnacle Geese

153　were collected by one author (D.C.) using the same methods from 1970-2018 (see

154　Table S1 for annual breakdown). Capture and sampling of birds was carried out

155　under licences by the National Parks and Wildlife Service of the Government of

156　Ireland and the British Trust of Ornithology. All feather and blood sampling

157　procedures were approved by the University College Dublin Animal Research Ethics

158　Committee and the Health Products Regulatory Authority of Ireland (AE18982/P115).

159

160　**Feather CORT**

161　CORT is deposited into segments of the feather vane and rachis containing the

162　blood quill during growth and can be measured from feather trimmings subsequently

163　(Jenni-Eiermann *et al.* 2015). Because CORT concentration in the feather is fixed

164　after passing the blood quill, assuming a constant growth rate, measurements from

165　feathers provide a smoothed average baseline of an individual Barnacle Goose's

166　hypothalamo-pituitary-adrenal activity during the synchronous growth period in

167　July/August (Jenni-Eiermann *et al.* 2015, Taff *et al.* 2018). Studies of Baltic Barnacle

168　Geese found that primary feather growth rate was 8 ± 1.58 mm/day (n = 52) in

169　females and 7.3 ± 1.33 mm/day (n = 50) in males, indicating relatively consistent

170　growth rates (Larsson 1996). As such, CORT recovery from feathers is preferable to

171　recovery from blood when cannon-netting as CORT will not be affected by the long

172　and variable handling times associated with this capture procedure.

8

173

174 Approximately 25 mm was trimmed from the distal tip of the fifth secondary flight

175 feather using a scissors and stored in paper envelopes until CORT extraction. CORT

176 was extracted from feathers using a methanol-based extraction technique based on

177 Bortolotti *et al.* (2008). The feather sample was trimmed to a weight of ~15.5 g and

178 minced into <5 mm^2 pieces using a blade, then shaken for 2 minutes with 1.5 ml

179 HPLC grade 99.9% methanol in a Qiagen Tissue Lyser. This sample weight was a

180 compromise between maximising and standardising the number of samples while

181 removing as little feather from the bird as possible (whole feathers were not plucked

182 for ethical reasons). The sample was transferred to a flask with an additional 8.5ml

183 methanol and incubated overnight in a shaking water bath at 50 °C to extract CORT.

184 The sample was run through a 0.45 μm syringe filter to remove the feather particles

185 and then heated in a sand block at 50 °C until the liquid fraction evaporated

186 completely. The dried extract was reconstituted in 500 μl phosphate-buffered saline

187 liquid and frozen at -20 °C until CORT concentration analysis. From the 55 birds

188 captured, we extracted CORT from 53 samples (2 samples excluded as they were

189 below the threshold weight of 15.5 g). As CORT was extracted in two batches, inter-

190 extraction variation was measured as 0.06% using two pool samples from a single

191 homogenous pool created by mixing a number of feathers.

192

193 Analysis of CORT concentration was conducted in a single batch by

194 radioimmunoassay using the ImmuChem Corticosterone Double Antibody kit (MP

195 Biomedicals, Orangeburg, NY). The frozen samples, including pool samples, were

196 brought to room temperature and vortexed to mix before aliquoting 100 μl of each for

197 analysis. A standard calibration curve was created by serial dilution of a 1000 ng/ml

9

198 CORT calibrator and 100 µl of high and low CORT control reconstituted in de-ionised

199 water were aliquoted to provide reference value samples. We added 200 µl of

200 ^{125}Iodine labelled CORT tracer to all samples followed by 200 µl of rabbit anti-CORT.

201 The tracer was allowed to compete with CORT for a fixed amount of antibody sites

202 for 2 h. The antibody-tracer complex was then separated by adding 500 µl

203 precipitation solution and centrifuging at 2 000 rpm at 4 °C for 20 mins. Unbound

204 tracer was removed by aspiration with a vacuum pump. A 60 s gamma count was

205 conducted using a Wallac 1470 gamma counter, with the quantity of radiation

206 measured inversely proportionate to the quantity of CORT. CORT concentrations in

207 each sample were determined by interpolation from the standard calibration curve.

208 All samples were analysed in duplicate and the mean final hormone concentration of

209 feather extracted samples was expressed as a function of feather length (ng/mm)

210 following Bortolotti *et al.* (2008). The intra-assay coefficient of variation (CV) was

211 4.8%, within the range of other similar studies (e.g. Bortolotti *et al.* 2008, Legagneux

212 *et al.* 2013, Johns *et al.* 2018).

213

214 **Blood DHEA**

215 DHEA concentration can be measured accurately from avian blood and, unlike other

216 hormone modulators of aggression such as testosterone, does not appear to be

217 affected by capture or variable handling time in geese (Poisbleau *et al.* 2009) making

218 it a suitable measurement in a cannon-netting procedure with long and variable

219 handling times. No literature was available on DHEA levels in geese during the

220 breeding season (when feathers are grown) therefore we chose to measure DHEA

221 from blood. However, because Newman *et al.* (2013) found that 30 min restraint of

10

222 Song Sparrows altered DHEA concentration, we took the opportunity to include an

223 analysis of the effect of handling time on DHEA levels in our study.

224

225 A 1.5 ml blood sample was collected from the metatarsial vein using a syringe (23

226 G). Blood was centrifuged at 3000 rcf for 15 min to separate the extracellular fluid

227 from red blood cells and 50 µl serum was drawn off using a micropipette and sealed

228 in a culture tube. Tubes were frozen at -18 °C as soon as possible and transferred to

229 -20 °C within 48 h until DHEA concentration analysis. From the 55 birds captured,

230 we recovered DHEA from 35 samples due to time constraints on blood sampling, as

231 all birds in the captured flock must be released together within the maximum

232 permitted time following initial capture according to ethical guidelines.

233

234 DHEA concentration was analysed at NationWide Laboratories (Cambridge, UK) by

235 enzyme immunoassay using the DHEA ELIZA kit (IBL International GmbH,

236 Hamburg, Germany). All samples were checked for gross haemolysis prior to

237 analysis. The frozen samples were brought to room temperature and 20 µl was

238 aliquoted into wells on a microtiter plate coated with polyclonal rabbit anti-DHEA. A

239 20 µl high and low control sample were also aliquoted along with serial dilution of a

240 30 ng/ml DHEA calibrator to produce a standard calibration curve. Next, 100 µl of

241 DHEA-horseradish peroxidase conjugate was added to each well. The conjugate

242 was allowed to compete with DHEA for a fixed amount of antibody sites for 1 hr.

243 Unbound conjugate was then washed off with phosphate buffer. Next, 100 µl TMB

244 Substrate Solution was added to each well and incubated for 30 min at room

245 temperature before stopping the reaction with 100 µl TMB Stop Solution. The optical

246 density of the wells was measured using a microtiter plate reader at 450 nm, with the

11

247   intensity of colour developed during the TMB reaction inversely proportional to DHEA

248   concentration. DHEA concentrations in each sample were determined by

249   interpolation from the standard calibration curve. All samples were analysed in

250   duplicate, with the final hormone concentration expressed as a function of blood

251   volume (nmol/L).

252

253   **Morphology**

254   Measurements of body-weight, body size and cheek patch size were collected from

255   the 55 birds in 2017/18 (18 males and 37 females) and from an additional 53 birds in

256   1970-2018 (20 males and 33 females). Each individual was weighed to the nearest

257   10.0 g using a spring scale. The length of the tarsus was measured to the nearest

258   0.1 mm with a callipers and used as a proxy for body size because it remains fixed in

259   adults (Choudhury *et al.* 1996). One observer (D.C.) carried out all measurements to

260   minimise observer bias.

261

262   To measure cheek patch size, we took a photograph of the head at a 90° lateral

263   angle. The area of brilliant white pigmentation on the forehead and lower mandible

264   was measured in pixels using the polygon tool in the ImageJ program

265   (https://imagej.net/Welcome; Abràmoff *et al.* 2004). One observer (Y.L.) carried out

266   all measurements to minimise observer bias. Because the data included 1970-2018

267   photographs, images could not be standardised at the time of collection. To address

268   this, the eye, which differs little among individuals, was used as a standard reference

269   to calibrate photographs. A correction factor was calculated as the ratio of the eye

270   area in each photograph to the mean eye area of all photographs. This correction

12

271 factor was used to scale white patch area, providing a relative patch size for each

272 bird.

273

274 **Statistical analysis**

275 Preliminary analysis of the data indicated that body-weight, tarsus length and cheek

276 patch size were continuous data of a normal distribution. CORT was a continuous

277 measurement with a strong positive skew, as there were relatively few birds with

278 high CORT concentrations. DHEA was a continuous measurement limited to a lower

279 boundary of 0.1 because the enzyme immunoassay detected concentrations to a

280 lower limit of 0.1 nmol/L. To account for physiological fluctuations over winter, we

281 included the day of capture as a parameter in the data by counting the number of

282 days elapsed between the beginning of winter (November 1; marks the end of the

283 autumn migration period for this species) and the day of capture. We also included

284 sex as a parameter in the data because many physiological traits are known to differ

285 between males and females. Finally, we included handling time on the day of

286 capture, defined as minutes elapsed between initial capture and blood sampling, to

287 account for potential effects of handling on DHEA concentration.

288

289 We performed six models to test endocrine-morphology relationships using the R

290 statistical language and environment 3.5.1 (R Core Team 2018). Linear regression

291 was used to test body weight as a function of (1) CORT and (2) DHEA. These

292 models included the additional parameters sex, tarsus (to account for bigger birds

293 being inherently heavier) and the interaction between hormone and linear and

294 quadratic effects of day of capture. Linear regression was also used to test patch

295 size as a function of (3) CORT and (4) DHEA. These models included the additional

13

296 parameters sex and the interaction between hormone and linear and quadratic

297 effects of day of capture. A generalized linear model with gamma errors and a log

298 link was used to test (5) CORT as a function of tarsus length. This model included

299 the additional parameter sex. Finally, a Tobit regression with a lower boundary of 0.1

300 (VGAM package 1.1-1; Yee 2015) was used to test (6) DHEA as a function of tarsus

301 length. This model included the additional parameters sex, linear and quadratic

302 effects of day of capture and the effect of handling time. Table 1 provides a summary

303 of the six initial models.

304

305 In all models, numerical explanatory variables were standardised by subtracting the

306 mean and dividing by the standard deviation to allow for meaningful comparisons

307 between variables on different scales. Likelihood ratio tests (LRT) were used to

308 determine whether each parameter significantly improved the model fit. Parameters

309 were only retained in the final model if the LRT value was significant (<0.05) or

310 borderline significant (<0.099). Table S2 provides all LRT results.

311

312 In addition to the above analyses, we combined 2017/18 and 1970-2018

313 morphological data to test for long-term changes in the three Barnacle Goose

314 morphological traits over time. Firstly, we performed two linear mixed models (lme4

315 package 1.1-21; Bates *et al.* 2015) to test body-weight and patch size as a function

316 of year. A random intercept for the month of measurement was included in these

317 models to account for seasonal variation in physiology. Secondly, we performed a

318 linear regression to test tarsus length as a function of year. All three models included

319 sex as an additional parameter. Table 1 provides a summary of the three models.

320

14

RESULTS

Mean CORT concentration was 3.51ng/mm (range 0.54 - 13.95ng/mm, n = 52; we excluded one sample with an individual CV >20% from the analysis, as recommended by Reed *et al.* (2002)). It should be noted that some bird species suppress CORT during moult (Romero 2002), therefore CORT concentration in these samples could be suppressed relative to concentration during other life history stages. Interestingly, above-average values of CORT were only recorded among females, although LRT indicated that there was not statistically significant effect of sex. Broader variation in CORT was also recorded in early winter (January and November) compared to late winter (April).

The final body-weight model included additive effects of CORT, tarsus (i.e. body size), sex and day of capture (Table 2). There was a significant negative relationship between body weight and CORT (Fig. 1(a)). After accounting for sex, body size and day of capture, a 1 sd (standard deviation) increase in CORT was associated with a ~60 g decrease in body-weight (almost 5% of the body-weight of an average bird). Bigger birds were heavier; a 1 sd increase in tarsus length was associated with a ~40 g increase in weight. Males were, on average, ~120 g heavier than females. Finally, body weight increased linearly between November and April. The final patch size model included only the variable day of capture, indicating no relationship between patch size and CORT (Table 2). Similarly, the final tarsus length model was the null model, indicating no relationship between tarsus length and CORT.

Mean blood DHEA concentration was 0.75 nmol/L (range 0.10 nmol/L - 4.65 nmol/L, n = 35). The final body-weight model included additive effects of DHEA, sex, tarsus

15

346 (i.e. body size) and day of capture (Table 2). After accounting for sex, body size and

347 day of capture, a 1 sd increase in DHEA was associated with a ~50 g decrease in

348 body weight, but this relationship was not statistically significant (Fig. 1(b)). As

349 before, bigger birds were heavier (a 1 sd increase in tarsus length was associated

350 with a ~55 g increase in body weight), males were, on average, ~115 g heavier than

351 females and body weight increased linearly between November and April. The final

352 patch size model included the variables DHEA and day of capture. A 1 sd increase in

353 DHEA was associated with a ~3.5 decrease in patch size (pixels), but this

354 relationship was not significant (Fig. 1(c)). Patch size decreased linearly between

355 November and April. As before, the final tarsus length model was the null model,

356 indicating no relationship between tarsus length and DHEA. Note that the LRT for

357 this model also indicates no significant effect of handling time on blood DHEA

358 concentrations.

359

360 The models combining 1970-2018 and 2017/18 morphological data (*n* = 108)

361 detected no change in mean Barnacle Goose body weight, patch size or tarsus

362 length since 1970. After accounting for variation between the sexes and seasonal

363 fluctuations, none of the three models detected a significant relationship between

364 morphological trait and year (Table 2).

365

366

367 **DISCUSSION**

368 This is the first study to quantify endocrine-morphology relationships in Barnacle

369 Geese, to our knowledge. Individuals with higher CORT during body moult had a

370 lower body weight early the following winter. It is one of few studies to demonstrate

16

371 this effect in a free-living population outside controlled laboratory conditions. In

372 contrast, we did not find robust evidence of DHEA-morphology relationships or

373 CORT-tarsus and CORT-patch relationships. We also show that mean Barnacle

374 Goose body weight, tarsus length and patch size have not varied significantly since

375 1970.

376

377 Our CORT-weight results are consistent with experimental observations from other

378 bird species and vertebrate taxa. A negative CORT-weight relationship has been

379 observed in aviary enclosure tests of captive House Sparrow *Passer domesticus*

380 (Vágási *et al.* 2018), Gambel's White-crowned Sparrow *Zonotrichia leucophrys*

381 *gambelii* (Busch *et al.* 2008) and Starling *Sturnus vulgaris* (Awerman & Romero

382 2010). Farm-bred Japanese Quail *Coturnix coturnix japonica* treated with CORT had

383 lower body weights than controls (Hull *et al.* 2007). Among mammals, stressed

384 laboratory Striped Field Mice *Apodemus agrarius* had higher cortisol (the mammalian

385 equivalent of CORT) and lower body weight than their unstressed counterparts

386 (Wang *et al.* 2011). Simulated chronic stress tests on laboratory rats showed that a

387 surge in CORT caused a sustained decrease in body weight (Scherer *et al.* 2011). A

388 negative CORT-weight relationship in free-living Common Eider *Somateria*

389 *mollissima borealis* has been observed (Harms *et al.* 2015), but further examples

390 using free-living populations are limited.

391

392 Because this study is cross-sectional rather than longitudinal, it is not possible to

393 confirm whether body weight affects CORT or visa-versa. Heavier birds could

394 experience fewer homeostatic challenges or could be better able to cope with such

395 challenges and maintain lower CORT (López-Jiménez *et al.* 2017). Nutritional stress

17

396 could both reduce body weight and increase allostatic load and increased allostatic

397 load has been shown to upregulate CORT (Johns *et al.* 2018). Alternatively, the

398 results could indicate carry-over effects of stressful environmental conditions during

399 moult (Legagneux *et al.* 2013, Latta *et al.* 2016). For instance, unusually arid

400 conditions were associated with increased CORT in House Sparrows (Treen *et al.*

401 2015). Awerman and Romero (2010) and Busch *et al.* (2008) show that elevated

402 CORT increases protein metabolism and muscle-wasting, providing a physiological

403 mechanism for weight loss. Research on other Arctic-breeding waterfowl suggests

404 that external environmental factors have a stronger influence on CORT than intrinsic

405 factors (Legagneux *et al.* 2013). If the same is true for Barnacle Geese, CORT could

406 provide a medium for carry-over effects across the annual cycle. By the same

407 process, the results could indicate a cost of reproduction: at the end of the breeding

408 season, successful breeders may have higher CORT following the stress of raising

409 offspring compared to failed or sabbatical breeders (Crossin *et al.* 2017, Ibañez *et al.*

410 2018, Ramos *et al.* 2018). Although Barnacle goslings are precocial, offspring

411 typically remain with the parents for their first year, requiring some form of care

412 (Black *et al.* 2014). Ramos *et al.* (2018) show that the cost of reproduction can be

413 physiologically mediated by CORT from breeding to winter season in Cory's

414 Shearwater *Calonectris borealis*. However, due to the remote breeding sites of

415 Barnacle Geese, it was not possible to know if individuals in this study successfully

416 reared offspring that were lost prior to sampling (i.e. prior to arrival on the winter

417 grounds), therefore the relationship between breeding success and CORT could not

418 be tested.

419

18

420 Understanding endocrine-morphology relationships is useful in the current period of

421 rapid global change. Endocrine-disrupting contaminants released by agricultural and

422 industrial activity are increasingly known to interfere with natural endocrinological

423 function and are exacerbated by weather extremes associated with climate change

424 (Jenssen 2006, Norris 2018). Climate change and accumulation of endocrine-

425 disrupting contaminants are amplified in the Arctic compared to lower latitudes as

426 heat and contaminants are transported northward on natural currents (Bekryaev *et*

427 *al.* 2010, AMAP 2015, Praetorius *et al.* 2018). Glucocorticoids (including CORT) that

428 regulate adaptation to stress are among the most susceptible hormones to

429 contaminant disruption (Jenssen 2006). Indeed, there is already evidence that

430 exposure to endocrine-disrupting contaminants interferes with the normal

431 behavioural and physiological responses to acute stress in Svalbard Barnacle

432 Geese; individuals exposed to trace metals at coal-mining sites were found to

433 erratically increase CORT secretion during subsequent stress tests (Scheiber *et al.*

434 2018). The effects of environmental change are evident in other systems: Carolina

435 Chickadees *Poecile carolinensis* in forest disturbed by logging had elevated CORT

436 and lower body mass compared to chickadees from undisturbed forest (Lucas *et al.*

437 2006). Adélie Penguins *Pygoscelis adeliae* confronted with unusual sea ice

438 conditions in the Antarctic had elevated CORT and lower body-weight (Cockrem *et*

439 *al.* 2006). Barn Owl *Tyto alba* nestlings growing up in intensive agricultural areas in

440 Switzerland also had elevated baseline CORT and lower body-weight (Almasi *et al.*

441 2015). Upregulation of CORT could be an adaptive response to maintain condition in

442 the face of stressors (e.g. Rivers *et al.* 2017, Vágási *et al.* 2018) but could also

443 negatively impact, for example, future reproductive investment (Kouwenberg *et al.*

444 2013, Crossin *et al.* 2017) and survival (Koren *et al.* 2012).

19

445

446 This study is one of few to investigate DHEA-morphology relationships in birds.

447 Although a small number of studies have established DHEA-morphology links in

448 birds and mammals (e.g. Spinney *et al.* 2006, de Heredia *et al.* 2007) and we

449 hypothesised that DHEA-morphology relationships might manifest via dominant

450 behaviour and improved access to resources, our analysis did not provide robust

451 evidence to relate winter DHEA to winter body-weight, size or facial plumage in

452 Barnacle Geese. Poisbleau *et al.* (2009) also found no correlation between DHEA

453 and body condition in Brent Geese, adding to evidence that DHEA is not strongly

454 associated with these morphological traits. DHEA may have a stronger relationship

455 with behavioural traits or other morphological traits not tested in this study. DHEA

456 may also play different roles depending on the territoriality or gregariousness of a

457 species (compare Hau *et al.* 2004 to Poisbleau *et al.* 2009). Future studies on a

458 greater taxonomic range of birds are warranted to shed light on this. The analysis

459 also detected no relationship between CORT and tarsus length or cheek patch size,

460 providing no support for a link between CORT and body size or a role of CORT in

461 social signalling in Barnacle Geese, despite strong evidence of such links in other

462 bird species (e.g. Grunst et al. 2015, López-Jiménez et al. 2017).

463

464 Understanding the mechanistic role hormones such as CORT and DHEA play in

465 free-living birds provides insight into the links between environment, individual and

466 population. These hormones are principal regulators of the avian endocrine system.

467 However, the downstream effects of CORT on morphological traits on which natural

468 selection can act are less well studied in free-living populations and the downstream

469 effects of DHEA on both morphology and fitness are not well studied. This cross-

20

sectional study provides evidence of a correlation between moulting season feather

CORT and subsequent winter body weight in free-living Barnacle Geese. It is widely

accepted that chronically elevated CORT has negative effects on fitness in birds, but

our results suggest that, in Barnacle Geese, this effect could be associated with

variation in body-weight. It may also broaden our understanding of some of the ways

in which bird physiology may be affected by or adapt to environmental change.

Funding

Conflict of interest

The authors declare they have no conflicts of interest.

Author contributions

SD, DC and BM conceived ideas and designed methodology. SD, DC, BM, AW and

KC captured birds and collected samples, with DC responsible for all historic data.

SD and JF conducted corticosterone measurements and SD and YL conducted

plumage measurements. SD analysed the data, with assistance from BM, and led

21

497 writing of the manuscript. All authors contributed critically to drafts and gave final

498 approval for publication.

499

500

501 **Data availability statement**

502 The data that support the findings of this study are available from the corresponding

503 author upon reasonable request.

504

22

REFERENCES

505

506 **Abràmoff, M.D., Magalhães, P.J. & Ram, S.J.** 2004. Image processing with

507 ImageJ. *Biophotonics Int.* **11**: 36–41.

508 **Almasi, B., Béziers, P., Roulin, A. & Jenni, L.** 2015. Agricultural land use and

509 human presence around breeding sites increase stress-hormone levels and

510 decrease body mass in barn owl nestlings. *Oecologia* **179**: 89–101.

511 **AMAP**. 2015. *Temporal trends in persistent organic pollutants in the Arctic*.

512 **Awerman, J.L. & Romero, L.M.** 2010. Chronic psychological stress alters body-

513 weight and blood chemistry in European starlings (Sturnus vulgaris). *Comp.*

514 *Biochem. Physiol. - A Mol. Integr. Physiol.* **156**: 136–142.

515 **Badás, E.P., Martínez, J., Rivero-de Aguilar, J., Ponce, C., Stevens, M. &**

516 **Merino, S.** 2018. Colour change in a structural ornament is related to individual

517 quality, parasites and mating patterns in the blue tit. *Sci. Nat.* **105**: 17.

518 **Bates, D., Maechler, M., Bolker, B. & Walker, S.** 2015. Fitting linear mixed-effects

519 models using lme4. *J. Stat. Softw.* **67**: 1–48.

520 **Bekryaev, R. V., Polyakov, I. V. & Alexeev, V.A.** 2010. Role of polar amplification

521 in long-term surface air temperature variations and modern Arctic. *J. Clim.* **23**:

522 3888–3906.

523 **Black, J.M., Prop, J. & Larsson, K.** 2014. *The Barnacle Goose*, 1st ed. T & AD

524 Poyser, London.

525 **Bonier, F., Martin, P.R., Moore, I.T. & Wingfield, J.C.** 2009. Do baseline

526 glucocorticoids predict fitness? *Trends Ecol. Evol.* **24**: 634–642.

527 **Bortolotti, G.R., Marchant, T.A., Blas, J. & German, T.** 2008. Corticosterone in

528 feathers is a long-term, integrated measure of avian stress physiology. *Funct.*

529 *Ecol.* **22**: 494–500.

23

530 **Busch, D.S., Sperry, T.S., Peterson, E., Do, C.-T., Wingfield, J.C. & Boyd, E.H.**

531   2008. Impacts of frequent, acute pulses of corticosterone on condition and

532   behavior of Gambel's white-crowned sparrow (Zonotrichia leucophrys gambelii).

533   *Gen. Comp. Endocrinol.* **158**: 224–233.

534 **Cabot, D., Nairn, R., Newton, S. & Viney, M.** 1984. *Biological Expedition of*

535   *Jameson Land Greenland 1984*, 1st ed. Barnacle Books, Dublin, Ireland.

536 **Choudhury, S., Black, J.M. & Owen, M.** 1996. Body size, fitness and compatibility

537   in Barnacle Geese Branta leucopsis. *Ibis (Lond. 1859).* **138**: 700–709.

538 **Cockrem, J.F., Potter, M.A. & Candy, E.J.** 2006. Corticosterone in relation to body

539   mass in Adelie penguins (Pygoscelis adeliae) affected by unusual sea ice

540   conditions at Ross Island, Antarctica. *Gen. Comp. Endocrinol.* **149**: 244–252.

541 **Creel, S., Dantzer, B., Goymann, W. & Rubenstein, D.R.** 2013. The ecology of

542   stress: effects of the social environment. *Funct. Ecol.* **27**: 66–80.

543 **Crossin, G.T., Phillips, R.A., Lattin, C.R., Romero, L.M., Bordeleau, X., Harris,**

544   **C.M., Love, O.P. & Williams, T.D.** 2017. Costs of reproduction and carry-over

545   effects in breeding albatrosses. *Antarct. Sci.* **29**: 155–164.

546 **de Heredia, F.P., Cerezo, D., Zamora, S. & Garaulet, M.** 2007. Effect of

547   dehydroepiandrosterone on protein and fat digestibility, body protein and

548   muscular composition in high-fat-diet-fed old rats. *Br. J. Nutr.* **97**: 464–470.

549 **Grace, J.K. & Anderson, D.J.** 2018. Early-life maltreatment predicts adult stress

550   response in a long-lived wild bird. *Biol. Lett.* **14**: 20170679.

551 **Grunst, M.L., Grunst, A.S., Parker, C.E., Romero, L.M. & Rotenberry, J.T.** 2015.

552   Pigment-specific relationships between feather corticosterone concentrations

553   and sexual coloration. *Behav. Ecol.* **26**: 706–715.

554 **Hahn, S., Loonen, M.J.J.E. & Klaassen, M.** 2011. The reliance on distant

24

resources for egg formation in high Arctic breeding barnacle geese Branta

leucopsis. *J. Avian Biol.* **42**: 159–168.

Harms, N.J., Legagneux, P., Gilchrist, H.G., Bêty, J., Love, O.P., Forbes, M.R.,

Bortolotti, G.R. & Soos, C. 2015. Feather corticosterone reveals effect of

moulting conditions in the autumn on subsequent reproductive output and

survival in an Arctic migratory bird. *Proc. R. Soc. B Biol. Sci.* **282**: 20142085.

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.

Hau, M., Stoddard, S.T. & Soma, K.K. 2004. Territorial aggression and hormones

during the non-breeding season in a tropical bird. *Horm. Behav.* **45**: 40–49.

Hull, K.L., Cockrem, J.F., Bridges, J.P., Candy, E.J. & Davidson, C.M. 2007.

Effects of corticosterone treatment on growth, development, and the

corticosterone response to handling in young Japanese quail (Coturnix coturnix

japonica). *Comp. Biochem. Physiol. - A Mol. Integr. Physiol.* **148**: 531–543.

Ibañez, A.E., Graña Grilli, M., Figueroa, A., Pari, M. & Montalti, D. 2018. Declining

health status of Brown Skua (Stercorarius antarcticus lonnbergi) parents and

their offspring during chick development. *Polar Biol.* **41**: 193–200.

Jawor, J.M. & Breitwisch, R. 2003. Melanin ornaments, honesty, and sexual

selection. *Auk* **120**: 249–265.

Jenni-Eiermann, S., Helfenstein, F., Vallat, A., Glauser, G. & Jenni, L. 2015.

Corticosterone: effects on feather quality and deposition into feathers. *Methods*

Ecol. Evol. **6**: 237–246.

Jenssen, B.M. 2006. Endocrine-disrupting chemicals and climate change: A worst-

case combination for arctic marine mammals and seabirds? *Environ. Health*

25

580   *Perspect.* **114**: 76–80.

581 **Johns, D.W., Marchant, T.A., Fairhurst, G.D., Speakman, J.R. & Clark, R.G.**

582   2018. Biomarker of burden: feather corticosterone reflects energetic expenditure

583   and allostatic overload in captive waterfowl. *Funct. Ecol.* **32**: 345–357.

584 **Koren, L., Nakagawa, S., Burke, T., Soma, K.K., Wynne-Edwards, K.E. & Geffen,**

585   **E.** 2012. Non-breeding feather concentrations of testosterone, corticosterone

586   and cortisol are associated with subsequent survival in wild house sparrows.

587   *Proc. R. Soc. B Biol. Sci.* **279**: 1560–1566.

588 **Kouwenberg, A.L., Mark Hipfner, J., McKay, D.W. & Storey, A.E.** 2013.

589   Corticosterone and stable isotopes in feathers predict egg size in Atlantic Puffins

590   Fratercula arctica. *Ibis (Lond. 1859).* **155**: 413–418.

591 **Kurvers, R.H.J.M., Prins, H.H.T., van Wieren, S.E., van Oers, K., Nolet, B.A. &**

592   **Ydenberg, R.C.** 2010. The effect of personality on social foraging: Shy barnacle

593   geese scrounge more. *Proc. R. Soc. B Biol. Sci.* **277**: 601–608.

594 **Larsson, K.** 1996. Genetic and environmental effects on the timing of wing moult in

595   the barnacle goose. *Heredity (Edinb).* **76**: 100–107.

596 **Latta, S.C., Cabezas, S., Mejia, D.A., Paulino, M.M., Almonte, H.,**

597   **Miller-Butterworth, C.M. & Bortolotti, G.R.** 2016. Carry-over effects provide

598   linkages across the annual cycle of a Neotropical migratory bird, the Louisiana

599   Waterthrush Parkesia motacilla. *Ibis (Lond. 1859).* **158**: 395–406.

600 **Legagneux, P., Harms, N.J., Gauthier, G., Chastel, O., Gilchrist, H.G., Bortolotti,**

601   **G., Bêty, J. & Soos, C.** 2013. Does feather corticosterone reflect individual

602   quality or external stress in arctic-nesting migratory birds? *PLoS One* **8**: e82644.

603 **López-Jiménez, L., Blas, J., Tanferna, A., Cabezas, S., Marchant, T., Hiraldo, F.**

604   **& Sergio, F.** 2017. Lifetime variation in feather corticosterone levels in a long-

26

605   lived raptor. *Oecologia* **183**: 315–326.

606   **Lucas, J.R., Freeberg, T.M., Egbert, J. & Schwabl, H.** 2006. Fecal corticosterone,

607   body mass, and caching rates of Carolina chickadees (Poecile carolinensis)

608   from disturbed and undisturbed sites. *Horm. Behav.* **49**: 634–643.

609   **Müller, C., Jenni-Eiermann, S. & Jenni, L.** 2009. Effects of a short period of

610   elevated circulating corticosterone on postnatal growth in free-living Eurasian

611   kestrels Falco tinnunculus. *J. Exp. Biol.* **212**: 1405–1412.

612   **Newman, A.E.M., MacDougall-Shackleton, S.A., An, Y.-S, Kriengwatana, B. &**

613   **Soma, K.K.** 2010. Corticosterone and dehydroepiandrosterone have opposing

614   effects on adult neuroplasticity in the avian song control system. *J. Comp.*

615   *Neurol.* **518**: 3662–3678.

616   **Newman, A.E.M., Zanette, L.Y., Clinchy, M., Goodenough, N. & Soma, K.K.**

617   2013. Stress in the wild: chronic predator pressure and acute restraint affect

618   plasma DHEA and corticosterone levels in a songbird. *Stress* **16**: 363–367.

619   **Norris, D.O.** 2018. Comparative endocrinology: past, present, and future. *Integr.*

620   *Comp. Biol.* **58**: 1033–1042.

621   **Orr, H.A.** 2009. Fitness and its role in evolutionary genetics. *Nat. Rev. Genet.* **10**:

622   531–539.

623   **Poisbleau, M., Fritz, H., Valeix, M., Perroi, P.-Y., Dalloyau, S. & Lambrechts,**

624   **M.M.** 2006. Social dominance correlates and family status in wintering dark-

625   bellied brent geese, Branta bernicla bernicla. *Anim. Behav.* **71**: 1351–1358.

626   **Poisbleau, M., Lacroix, A. & Chastel, O.** 2009. DHEA levels and social dominance

627   relationships in wintering brent geese (Branta bernicla bernicla). *Behav.*

628   *Processes* **80**: 99–103.

629   **Praetorius, S., Rugenstein, M., Persad, G. & Caldeira, K.** 2018. Global and Arctic

27

630 climate sensitivity enhanced by changes in North Pacific heat flux. *Nat.*

631 *Commun.* **9**: 3124.

632 **R Core Team**. 2018. *R: A language and environment for statistical computing*. R

633 Foundation for Statistical Computing, Vienna.

634 **Ramos, R., Llabrés, V., Monclús, L., López-Béjar, M. & González-Solís, J.** 2018.

635 Costs of breeding are rapidly buffered and do not affect migratory behavior in a

636 long-lived bird species. *Ecology* **99**: 2010–2024.

637 **Reed, G.F., Lynn, F. & Meade, B.D.** 2002. Use of coefficient of variation in

638 assessing variability of quantitative assays. *Clin. Diagn. Lab. Immunol.* **9**: 1235–

639 1239.

640 **Rivers, J.W., Newberry, G.N., Schwarz, C.J. & Ardia, D.R.** 2017. Success despite

641 the stress: violet-green swallows increase glucocorticoids and maintain

642 reproductive output despite experimental increases in flight costs. *Funct. Ecol.*

643 **31**: 235–244.

644 **Romero, M.L.** 2002. Seasonal changes in plasma glucocorticoid concentrations in

645 free-living vertebrates. *Gen. Comp. Endocrinol.* **128**: 1–24.

646 **Sapolsky, R.M., Romero, L.M. & Munck, A.U.** 2000. How do glucocorticoids

647 influence stress responses? Integrating permissive, suppressive, stimulatory,

648 and preparative actions. *Endocr. Rev.* **21**: 55–89.

649 **Scheiber, I.B.R., Weiß, B.M., de Jong, M.E., Braun, A., van den Brink, N.W.,**

650 **Loonen, M.J.J.E., Millesi, E. & Komdeur, J.** 2018. Stress behaviour and

651 physiology of developing Arctic barnacle goslings (Branta leucopsis) is affected

652 by legacy trace contaminants. *Proc. R. Soc. B Biol. Sci.* **285**: 20181866.

653 **Scherer, I., Holmes, P. V & Harris, R.B.S.** 2011. The importance of corticosterone

654 in mediating restraint-induced weight loss in rats. *Physiol. Behav.* **102**: 225–233.

28

655 **Schoech, S.J., Rensel, M.A. & Heiss, R.S.** 2011. Short- and long-term effects of

656 developmental corticosterone exposure on avian physiology, behavioral

657 phenotype, cognition, and fitness: a review. *Curr. Zool.* **57**: 514–530.

658 **Schoenle, L.A., Schoepf, I., Weinstein, N.M., Moore, I.T. & Bonier, F.** 2018.

659 Higher plasma corticosterone is associated with reduced costs of infection in

660 red-winged blackbirds. *Gen. Comp. Endocrinol.* **256**: 89–98.

661 **Soma, K.K., Rendon, N.M., Boonstra, R., Albers, H.E. & Demas, G.E.** 2015.

662 DHEA effects on brain and behavior: insights from comparative studies of

663 aggression. *J. Steroid Biochem. Mol. Biol.* **145**: 261–272.

664 **Soma, K.K., Scotti, M.A.L., Newman, A.E.M., Charlier, T.D. & Demas, G.E.** 2008.

665 Novel mechanisms for neuroendocrine regulation of aggression. *Front.*

666 *Neuroendocrinol.* **29**: 476–489.

667 **Spinney, L.H., Bentley, G.E. & Hau, M.** 2006. Endocrine correlates of alternative

668 phenotypes in the white-throated sparrow (Zonotrichia albicollis). *Horm. Behav.*

669 **50**: 762–771.

670 **Taff, C.C., Schoenle, L.A. & Vitousek, M.N.** 2018. The repeatability of

671 glucocorticoids: a review and meta-analysis. *Gen. Comp. Endocrinol.* **260**: 136–

672 145.

673 **Tombre, I.M., Erikstad, K.E. & Bunes, V.** 2012. State-dependent incubation

674 behaviour in the high arctic barnacle geese. *Polar Biol.* **35**: 985–992.

675 **Treen, G.D., Hobson, K.A., Marchant, T.A. & Bortolotti, G.R.** 2015. Large-scale

676 spatial variation in feather corticosterone in invasive house sparrows (Passer

677 domesticus) in Mexico is related to climate. *Ecol. Evol.* **5**: 3808–3817.

678 **Vágási, C.I., Pătraş, L., Pap, P.L., Vincze, O., Mureşan, C., Németh, J. &**

679 **Lendvai, Á.Z.** 2018. Experimental increase in baseline corticosterone level

680 reduces oxidative damage and enhances innate immune response. *PLoS One*

681 **13**: e0192701.

682 **Wacker, D.W., Khalaj, S., Jones, L.J., Champion, T.L., Davis, J.E., Meddle, S.L.**

683 **& Wingfield, J.C.** 2016. Dehydroepiandrosterone heightens aggression and

684 increases androgen receptor and aromatase mRNA expression in the brain of a

685 male songbird. *J. Neuroendocrinol.* **28**: 10.1111/jne.12443.

686 **Wang, Z., Wang, B. & Lu, J.** 2011. Behavioral and physiological responses of

687 striped field mice (Apodemus agrarius) to predator odor. *Integr. Zool.* **6**: 334–

688 340.

689 **Wingfield, J.C.** 2018. Environmental endocrinology: insights into the diversity of

690 regulatory mechanisms in life cycles. *Integr. Comp. Biol.* **58**: 790–799.

691 **Wingfield, J.C., Wacker, D.W., Bentley, G.E. & Tsutsui, K.** 2018. Brain-derived

692 steroids, behavior and endocrine conflicts across life history stages in birds: a

693 perspective. *Front. Endocrinol. (Lausanne).* **9**: 270.

694 **Yee, T.W.** 2015. *Vector Generalized Linear and Additive Models: with an*

695 *implementation in R*. Springer, New York, USA.

696

697 **Table 1.** Table 1. Summary of the six endocrine-morphology models and three long-

698 term change models. Models include additive (+), synergistic (*), quadratic (^2) or

699 random (1|) effects.

Model	Response variable	Explanatory variables
endocrine-morphology relationships		
1	weight	~ sex + tarsus + CORT * days + CORT * days^2
2	patch	~ sex + CORT * days + CORT * days^2
3	CORT	~sex + tarsus
4	weight	~ sex + tarsus + DHEA * days + DHEA * days^2
5	patch	~sex + DHEA * days + DHEA * days^2
6	DHEA	~sex + handling + days + days^2 + tarsus
long-term changes in morphological traits		
	weight	~sex + years + (1 | month)
	patch	~sex + years + (1 | month)
	tarsus	~sex + years

700

Model	Final model	%	Variable	Estimate
1	weight ~ sex + tarsus + CORT + days	36	sex	118.69 ± 47.19g
			tarsus	40.86 ± 23.42g
			CORT	-59.81 ± 22.79g
			days	53.38 ± 23.65g
2	patch ~ days	14	days	-5.71 ± 1.96
4	weight ~ sex + tarsus + DHEA + days	26	sex	115.93 ± 67.87g
			tarsus	55.46 ± 32.96g
			DHEA	-52.09 ± 28.56g
			days	79.02 ± 30.97g
5	patch ~ DHEA + days	17	DHEA	-3.56 ± 2.13
			days	-4.97 ± 2.15
	weight ~ sex + years + (1 | month)	35	sex	159.94 ± 33.72g
			years	0.70 ± 1.08g
	patch ~ sex + years + (1 | month)	19	sex	6.55 ± 2.95
			years	-0.05 ± 0.09
	tarsus ~ sex + years	11	sex	3.16 ± 0.85mm
			years	-0.03 ± 0.03mm

704
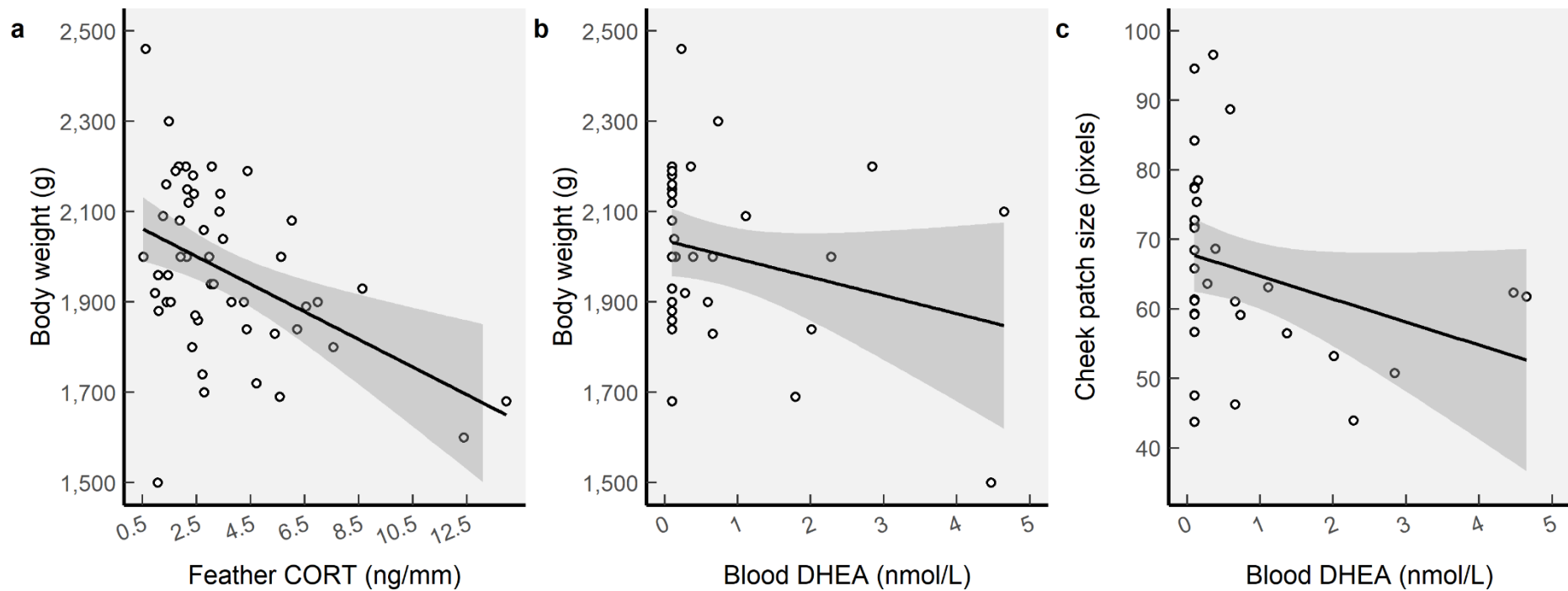
Figure 1. Endocrine-morphology relationships in Barnacle Geese. (a) There is a significant negative relationship between feather

706 CORT (ng/mm) and body weight (g). There are negative relationships between blood DHEA (nmol/L) and (b) body weight and (c)

707 cheek patch size (measured in pixels), but these relationships are not statistically significant. The solid line represents least-

708 squares fit with the shaded area representing standard error around the mean.

33

709   **SUPPORTING INFORMATION**

710

711   **Table S1.** Annual breakdown of the number of Barnacle Geese captured from 1970-

712   2018.

713

714   **Table S2.** Results of likelihood ratio tests

715

34