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Moulting season corticosterone correlates with winter season body-weight in an Arctic migrant bird

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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,

including Barnacle Geese, and based on the results of this study may have consequences for body-weight regulation.

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

The endocrine system is an important modulator of fitness in vertebrates. Environmental conditions affect fitness - an individual's ability to survive and reproduce - which in turn regulates population dynamics (Orr 2009). Hormones play a key role in this sequence by translating environmental information into individual physiological responses that have positive or negative fitness consequences (Norris 2018, Wingfield 2018). In birds, hormones modulate morphology such as body-weight (Awerman & Romero 2010), body size (Müller *et al.* 2009) and plumage coloration (Jawor & Breitwisch 2003, Grunst *et al.* 2015) – morphological traits that are strongly connected with survival and reproductive success (e.g. Poisbleau *et al.* 2006, Tombre *et al.* 2012, Badás *et al.* 2018). Importantly, natural selection acts on the downstream physiological effects of hormones (i.e. weight, size and plumage) rather than the circulating hormones themselves. As such, identifying endocrine-morphology relationships can provide insight into the sequence between environment, individuals and population. Although the major functions of the endocrine system are highly conserved among vertebrates, endocrine-morphology relationships are not always consistent among taxa or even within a species' life-history (Bonier *et al.* 2009), therefore studies on novel species or relationships can provide new insights.

The glucocorticoid hormone corticosterone (CORT) and the androgen hormone dehydroepiandrosterone (DHEA) are principal modulators of the avian endocrine system (Hau *et al.* 2010, Wingfield *et al.* 2018). CORT principally modulates homeostasis, energy balance and stress response via release by the hypothalamic-pituitary-adrenal axis in response to changing conditions (Sapolsky *et al.* 2000, Creel *et al.* 2013). Mildly elevated CORT in the short term can be an adaptive mechanism

for coping with environmental or physiological challenges, such as parasite infection or increased energetic demand (Kouwenberg *et al.* 2013, Rivers *et al.* 2017, Grace & Anderson 2018, Schoenle *et al.* 2018, Vágási *et al.* 2018). In contrast, chronically elevated CORT can negatively affect overall body condition (Harms *et al.* 2015, Latta *et al.* 2016). Common Kestrel *Falco tinnunculus* nestlings experimentally implanted with CORT (to represent an environmental stressor) were smaller, with shorter tarsi and flight feathers before fledging compared to control nestlings (Müller *et al.* 2009). American Yellow Warblers *Setophaga petechia* with higher CORT had lower carotenoid hues in plumage associated with sexual signalling (Grunst *et al.* 2015). Thus, chronic elevated CORT relative to an individual's conspecifics may be a fitness disadvantage (Bonier *et al.* 2009).

DHEA principally modulates aggression and dominance behaviour (Soma *et al.* 2008, 2015). It is produced by the adrenal glands and other organs, then taken up by the brain and converted into sex steroids that generate physiological changes (Soma *et al.* 2015, Wingfield *et al.* 2018). Hau *et al.* (2004) found that aggressive vocalisations by Spotted Antbirds *Hylophylax naevioides naevioides* during simulated territorial intrusions were positively correlated with DHEA levels. Similarly, male Song Sparrows *Melospiza melodia morphna* experimentally implanted with DHEA showed greater aggression in simulated territory intrusions than control sparrows (Wacker *et al.* 2016). In contrast, Poisbleau *et al.* (2009) found a negative correlation between DHEA and dominance score (the percentage of competitive interactions "won" by an individual) in wintering Brent Geese *Branta bernicla bernicla*, indicating uncertainty in the role of DHEA in aggression among taxa. However, DHEA also modulates potential negative impacts of glucocorticoids and

has positive neuroprotective properties (Newman *et al.* 2010, 2013). It has also been linked to alternative plumage colour morphs (Spinney *et al.* 2006). Based on the literature, one could hypothesise that elevated DHEA relative to an individual's conspecifics may offer a fitness advantage, both through its protective properties and as dominant individuals would be more likely to access resources with positive outcomes for body condition (Poisbleau *et al.* 2006).

Although the relationship between CORT and fitness is well studied in wild bird populations, fewer studies concern its relationship with morphology. Additionally, the relationship between DHEA and fitness or morphology in wild bird populations is not well studied. Quantifying endocrine-morphology relationships in free-living populations can reveal patterns that may not be apparent in laboratory investigations (Schoech *et al.* 2011, Wingfield 2018). Thus, this study aimed to quantify endocrine-morphology links in a free-living population of Greenland Barnacle Goose *Branta leucopsis.* The majority of this population breeds in high-Arctic north-east Greenland (70-78°N), with a small emerging breeding population in south-east Iceland (64°N). Barnacle Geese here undergo a post-breeding synchronous flight feather moult in July/August before migrating to Ireland and Scotland for winter (October – April) (Cabot *et al.* 1984). Increased body weight is a fitness advantage in geese, as more reserves are available for migration, egg formation and nesting (Poisbleau *et al.* 2006, Hahn *et al.* 2011, Tombre *et al.* 2012). Increased body size is also a fitness advantage as it improves social status and access to resources (Choudhury *et al.* 1996, Poisbleau *et al.* 2006, Kurvers *et al.* 2010). Barnacle Geese also have prominent white patches extending across the face contrasting with black plumage around the eye and bill. These patches are an important social signal (including

during aggressive encounters) and, although the role is not fully understood, it appears that, at least among females, individuals with darker faces are more attractive as mates (Black *et al.* 2014). Hence, we expect hormone concentrations associated with increased body weight, body size and smaller cheek patches to improve overall fitness in Barnacle Geese.

We analysed the strength of relationships between these three morphological traits and CORT and DHEA. The analysis determines whether meaningful endocrine-morphology relationships are present but cannot confirm causality. We hypothesised that there would be a negative correlation between body weight and CORT and between body size and CORT, but a positive correlation between cheek patch size and CORT (i.e. individuals with higher CORT are lighter, smaller and less attractive). We hypothesised that DHEA would correlate in the opposite way (i.e. individuals with higher DHEA are heavier, bigger and more attractive). In addition, we used long-term data on body-weight, body size and cheek patch size to investigate if these traits have changed over time, which could signal changing selective pressures. CORT and DHEA play a critical regulatory role in the avian endocrine system, so determining consistent relationships with morphological traits associated with survival and reproduction will improve our understanding of these systems and how birds are affected by and adapt to their changing environment.

METHODS

Study outline

During winter 2017/2018, 55 adult Barnacle Geese were captured on their wintering grounds in Ireland using cannon-netting. Four flocks were captured at four different

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.

Approximately 25 mm was trimmed from the distal tip of the fifth secondary flight feather using a scissors and stored in paper envelopes until CORT extraction. CORT was extracted from feathers using a methanol-based extraction technique based on Bortolotti *et al.* (2008). The feather sample was trimmed to a weight of ~15.5 g and minced into <5 mm^2 pieces using a blade, then shaken for 2 minutes with 1.5 ml HPLC grade 99.9% methanol in a Qiagen Tissue Lyser. This sample weight was a compromise between maximising and standardising the number of samples while removing as little feather from the bird as possible (whole feathers were not plucked for ethical reasons). The sample was transferred to a flask with an additional 8.5ml methanol and incubated overnight in a shaking water bath at 50 ℃ to extract CORT. The sample was run through a 0.45 µm syringe filter to remove the feather particles and then heated in a sand block at 50 ℃ until the liquid fraction evaporated completely. The dried extract was reconstituted in 500 µl phosphate-buffered saline liquid and frozen at -20 ℃ until CORT concentration analysis. From the 55 birds captured, we extracted CORT from 53 samples (2 samples excluded as they were below the threshold weight of 15.5 g). As CORT was extracted in two batches, inter-extraction variation was measured as 0.06% using two pool samples from a single homogenous pool created by mixing a number of feathers.

Analysis of CORT concentration was conducted in a single batch by radioimmunoassay using the ImmuChem Corticosterone Double Antibody kit (MP Biomedicals, Orangeburg, NY). The frozen samples, including pool samples, were brought to room temperature and vortexed to mix before aliquoting 100 µl of each for analysis. A standard calibration curve was created by serial dilution of a 1000 ng/ml

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 ℃ 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

Song Sparrows altered DHEA concentration, we took the opportunity to include an analysis of the effect of handling time on DHEA levels in our study.

A 1.5 ml blood sample was collected from the metatarsial vein using a syringe (23 G). Blood was centrifuged at 3000 rcf for 15 min to separate the extracellular fluid from red blood cells and 50 µl serum was drawn off using a micropipette and sealed in a culture tube. Tubes were frozen at -18 ℃ as soon as possible and transferred to -20 ℃ within 48 h until DHEA concentration analysis. From the 55 birds captured, we recovered DHEA from 35 samples due to time constraints on blood sampling, as all birds in the captured flock must be released together within the maximum permitted time following initial capture according to ethical guidelines.

DHEA concentration was analysed at NationWide Laboratories (Cambridge, UK) by enzyme immunoassay using the DHEA ELIZA kit (IBL International GmbH, Hamburg, Germany). All samples were checked for gross haemolysis prior to analysis. The frozen samples were brought to room temperature and 20 µl was aliquoted into wells on a microtiter plate coated with polyclonal rabbit anti-DHEA. A 20 µl high and low control sample were also aliquoted along with serial dilution of a 30 ng/ml DHEA calibrator to produce a standard calibration curve. Next, 100 µl of DHEA-horseradish peroxidase conjugate was added to each well. The conjugate was allowed to compete with DHEA for a fixed amount of antibody sites for 1 hr. Unbound conjugate was then washed off with phosphate buffer. Next, 100 µl TMB Substrate Solution was added to each well and incubated for 30 min at room temperature before stopping the reaction with 100 µl TMB Stop Solution. The optical density of the wells was measured using a microtiter plate reader at 450 nm, with the

intensity of colour developed during the TMB reaction inversely proportional to DHEA concentration. DHEA concentrations in each sample were determined by interpolation from the standard calibration curve. All samples were analysed in duplicate, with the final hormone concentration expressed as a function of blood volume (nmol/L).

Morphology

Measurements of body-weight, body size and cheek patch size were collected from the 55 birds in 2017/18 (18 males and 37 females) and from an additional 53 birds in 1970-2018 (20 males and 33 females). Each individual was weighed to the nearest 10.0 g using a spring scale. The length of the tarsus was measured to the nearest 0.1 mm with a callipers and used as a proxy for body size because it remains fixed in adults (Choudhury *et al.* 1996). One observer (D.C.) carried out all measurements to minimise observer bias.

To measure cheek patch size, we took a photograph of the head at a 90° lateral angle. The area of brilliant white pigmentation on the forehead and lower mandible was measured in pixels using the polygon tool in the ImageJ program (https://imagej.net/Welcome; Abràmoff *et al.* 2004). One observer (Y.L.) carried out all measurements to minimise observer bias. Because the data included 1970-2018 photographs, images could not be standardised at the time of collection. To address this, the eye, which differs little among individuals, was used as a standard reference to calibrate photographs. A correction factor was calculated as the ratio of the eye area in each photograph to the mean eye area of all photographs. This correction

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

Statistical analysis

Preliminary analysis of the data indicated that body-weight, tarsus length and cheek patch size were continuous data of a normal distribution. CORT was a continuous measurement with a strong positive skew, as there were relatively few birds with high CORT concentrations. DHEA was a continuous measurement limited to a lower boundary of 0.1 because the enzyme immunoassay detected concentrations to a lower limit of 0.1 nmol/L. To account for physiological fluctuations over winter, we included the day of capture as a parameter in the data by counting the number of days elapsed between the beginning of winter (November 1; marks the end of the autumn migration period for this species) and the day of capture. We also included sex as a parameter in the data because many physiological traits are known to differ between males and females. Finally, we included handling time on the day of capture, defined as minutes elapsed between initial capture and blood sampling, to account for potential effects of handling on DHEA concentration.

We performed six models to test endocrine-morphology relationships using the R statistical language and environment 3.5.1 (R Core Team 2018). Linear regression was used to test body weight as a function of (1) CORT and (2) DHEA. These models included the additional parameters sex, tarsus (to account for bigger birds being inherently heavier) and the interaction between hormone and linear and quadratic effects of day of capture. Linear regression was also used to test patch size as a function of (3) CORT and (4) DHEA. These models included the additional

parameters sex and the interaction between hormone and linear and quadratic effects of day of capture. A generalized linear model with gamma errors and a log link was used to test (5) CORT as a function of tarsus length. This model included the additional parameter sex. Finally, a Tobit regression with a lower boundary of 0.1 (VGAM package 1.1-1; Yee 2015) was used to test (6) DHEA as a function of tarsus length. This model included the additional parameters sex, linear and quadratic effects of day of capture and the effect of handling time. Table 1 provides a summary of the six initial models.

In all models, numerical explanatory variables were standardised by subtracting the mean and dividing by the standard deviation to allow for meaningful comparisons between variables on different scales. Likelihood ratio tests (LRT) were used to determine whether each parameter significantly improved the model fit. Parameters were only retained in the final model if the LRT value was significant (<0.05) or borderline significant (<0.099). Table S2 provides all LRT results.

In addition to the above analyses, we combined 2017/18 and 1970-2018 morphological data to test for long-term changes in the three Barnacle Goose morphological traits over time. Firstly, we performed two linear mixed models (lme4 package 1.1-21; Bates *et al.* 2015) to test body-weight and patch size as a function of year. A random intercept for the month of measurement was included in these models to account for seasonal variation in physiology. Secondly, we performed a linear regression to test tarsus length as a function of year. All three models included sex as an additional parameter. Table 1 provides a summary of the three models.

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

(i.e. body size) and day of capture (Table 2). After accounting for sex, body size and day of capture, a 1 sd increase in DHEA was associated with a ~50 g decrease in body weight, but this relationship was not statistically significant (Fig. 1(b)). As before, bigger birds were heavier (a 1 sd increase in tarsus length was associated with a ~55 g increase in body weight), males were, on average, ~115 g heavier than females and body weight increased linearly between November and April. The final patch size model included the variables DHEA and day of capture. A 1 sd increase in DHEA was associated with a ~3.5 decrease in patch size (pixels), but this relationship was not significant (Fig. 1(c)). Patch size decreased linearly between November and April. As before, the final tarsus length model was the null model, indicating no relationship between tarsus length and DHEA. Note that the LRT for this model also indicates no significant effect of handling time on blood DHEA concentrations.

The models combining 1970-2018 and 2017/18 morphological data (n = 108) detected no change in mean Barnacle Goose body weight, patch size or tarsus length since 1970. After accounting for variation between the sexes and seasonal fluctuations, none of the three models detected a significant relationship between morphological trait and year (Table 2).

DISCUSSION

This is the first study to quantify endocrine-morphology relationships in Barnacle Geese, to our knowledge. Individuals with higher CORT during body moult had a lower body weight early the following winter. It is one of few studies to demonstrate

this effect in a free-living population outside controlled laboratory conditions. In contrast, we did not find robust evidence of DHEA-morphology relationships or CORT-tarsus and CORT-patch relationships. We also show that mean Barnacle Goose body weight, tarsus length and patch size have not varied significantly since 1970.

Our CORT-weight results are consistent with experimental observations from other bird species and vertebrate taxa. A negative CORT-weight relationship has been observed in aviary enclosure tests of captive House Sparrow *Passer domesticus* (Vágási *et al.* 2018), Gambel's White-crowned Sparrow *Zonotrichia leucophrys gambelii* (Busch *et al.* 2008) and Starling *Sturnus vulgaris* (Awerman & Romero 2010). Farm-bred Japanese Quail *Coturnix coturnix japonica* treated with CORT had lower body weights than controls (Hull *et al.* 2007). Among mammals, stressed laboratory Striped Field Mice *Apodemus agrarius* had higher cortisol (the mammalian equivalent of CORT) and lower body weight than their unstressed counterparts (Wang *et al.* 2011). Simulated chronic stress tests on laboratory rats showed that a surge in CORT caused a sustained decrease in body weight (Scherer *et al.* 2011). A negative CORT-weight relationship in free-living Common Eider *Somateria mollissima borealis* has been observed (Harms *et al.* 2015), but further examples using free-living populations are limited.

Because this study is cross-sectional rather than longitudinal, it is not possible to confirm whether body weight affects CORT or visa-versa. Heavier birds could experience fewer homeostatic challenges or could be better able to cope with such challenges and maintain lower CORT (López-Jiménez *et al.* 2017). Nutritional stress

could both reduce body weight and increase allostatic load and increased allostatic load has been shown to upregulate CORT (Johns *et al.* 2018). Alternatively, the results could indicate carry-over effects of stressful environmental conditions during moult (Legagneux *et al.* 2013, Latta *et al.* 2016). For instance, unusually arid conditions were associated with increased CORT in House Sparrows (Treen *et al.* 2015). Awerman and Romero (2010) and Busch *et al.* (2008) show that elevated CORT increases protein metabolism and muscle-wasting, providing a physiological mechanism for weight loss. Research on other Arctic-breeding waterfowl suggests that external environmental factors have a stronger influence on CORT than intrinsic factors (Legagneux *et al.* 2013). If the same is true for Barnacle Geese, CORT could provide a medium for carry-over effects across the annual cycle. By the same process, the results could indicate a cost of reproduction: at the end of the breeding season, successful breeders may have higher CORT following the stress of raising offspring compared to failed or sabbatical breeders (Crossin *et al.* 2017, Ibañez *et al.* 2018, Ramos *et al.* 2018). Although Barnacle goslings are precocial, offspring typically remain with the parents for their first year, requiring some form of care (Black *et al.* 2014). Ramos *et al.* (2018) show that the cost of reproduction can be physiologically mediated by CORT from breeding to winter season in Cory's Shearwater *Calonectris borealis*. However, due to the remote breeding sites of Barnacle Geese, it was not possible to know if individuals in this study successfully reared offspring that were lost prior to sampling (i.e. prior to arrival on the winter grounds), therefore the relationship between breeding success and CORT could not be tested.

Understanding endocrine-morphology relationships is useful in the current period of rapid global change. Endocrine-disrupting contaminants released by agricultural and industrial activity are increasingly known to interfere with natural endocrinological function and are exacerbated by weather extremes associated with climate change (Jenssen 2006, Norris 2018). Climate change and accumulation of endocrine-disrupting contaminants are amplified in the Arctic compared to lower latitudes as heat and contaminants are transported northward on natural currents (Bekryaev *et al.* 2010, AMAP 2015, Praetorius *et al.* 2018). Glucocorticoids (including CORT) that regulate adaptation to stress are among the most susceptible hormones to contaminant disruption (Jenssen 2006). Indeed, there is already evidence that exposure to endocrine-disrupting contaminants interferes with the normal behavioural and physiological responses to acute stress in Svalbard Barnacle Geese; individuals exposed to trace metals at coal-mining sites were found to erratically increase CORT secretion during subsequent stress tests (Scheiber *et al.* 2018). The effects of environmental change are evident in other systems: Carolina Chickadees *Poecile carolinensis* in forest disturbed by logging had elevated CORT and lower body mass compared to chickadees from undisturbed forest (Lucas *et al.* 2006). Adélie Penguins *Pygoscelis adeliae* confronted with unusual sea ice conditions in the Antarctic had elevated CORT and lower body-weight (Cockrem *et al.* 2006). Barn Owl *Tyto alba* nestlings growing up in intensive agricultural areas in Switzerland also had elevated baseline CORT and lower body-weight (Almasi *et al.* 2015). Upregulation of CORT could be an adaptive response to maintain condition in the face of stressors (e.g. Rivers *et al.* 2017, Vágási *et al.* 2018) but could also negatively impact, for example, future reproductive investment (Kouwenberg *et al.* 2013, Crossin *et al.* 2017) and survival (Koren *et al.* 2012).

This study is one of few to investigate DHEA-morphology relationships in birds. Although a small number of studies have established DHEA-morphology links in birds and mammals (e.g. Spinney *et al.* 2006, de Heredia *et al.* 2007) and we hypothesised that DHEA-morphology relationships might manifest via dominant behaviour and improved access to resources, our analysis did not provide robust evidence to relate winter DHEA to winter body-weight, size or facial plumage in Barnacle Geese. Poisbleau *et al.* (2009) also found no correlation between DHEA and body condition in Brent Geese, adding to evidence that DHEA is not strongly associated with these morphological traits. DHEA may have a stronger relationship with behavioural traits or other morphological traits not tested in this study. DHEA may also play different roles depending on the territoriality or gregariousness of a species (compare Hau *et al.* 2004 to Poisbleau *et al.* 2009). Future studies on a greater taxonomic range of birds are warranted to shed light on this. The analysis also detected no relationship between CORT and tarsus length or cheek patch size, providing no support for a link between CORT and body size or a role of CORT in social signalling in Barnacle Geese, despite strong evidence of such links in other bird species (e.g. Grunst et al. 2015, López-Jiménez et al. 2017).

Understanding the mechanistic role hormones such as CORT and DHEA play in free-living birds provides insight into the links between environment, individual and population. These hormones are principal regulators of the avian endocrine system. However, the downstream effects of CORT on morphological traits on which natural selection can act are less well studied in free-living populations and the downstream effects of DHEA on both morphology and fitness are not well studied. This cross-

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.

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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

writing of the manuscript. All authors contributed critically to drafts and gave final approval for publication.

Data availability statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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Table 1. Table 1. Summary of the six endocrine-morphology models and three long-term change models. Models include additive (+), synergistic (*), quadratic (^2) or 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

Table 2. The formula of each final model is provided, along with the percentage variance/deviance explained (%) and the parameter estimates (β ± standard error) for each explanatory variable.

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

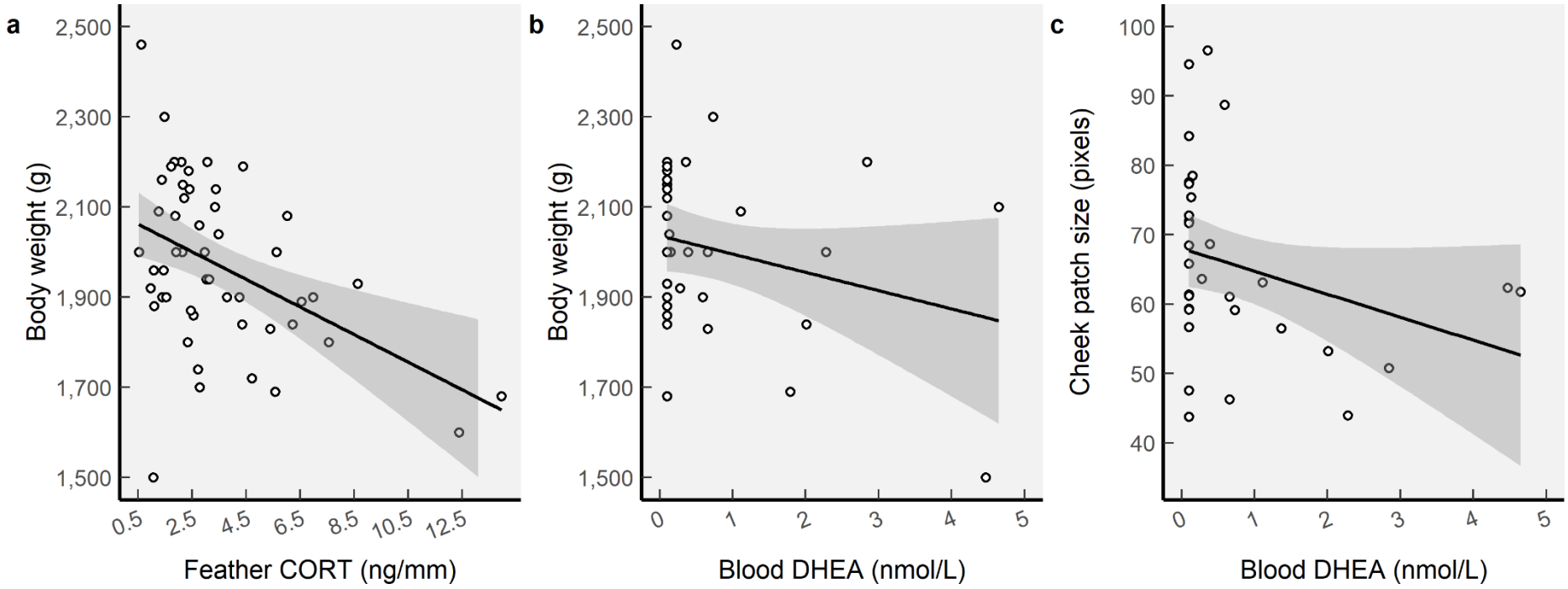

Figure 1. Endocrine-morphology relationships in Barnacle Geese. (a) There is a significant negative relationship between feather CORT (ng/mm) and body weight (g). There are negative relationships between blood DHEA (nmol/L) and (b) body weight and (c) cheek patch size (measured in pixels), but these relationships are not statistically significant. The solid line represents least-squares fit with the shaded area representing standard error around the mean.

SUPPORTING INFORMATION

Table S1. Annual breakdown of the number of Barnacle Geese captured from 1970-2018.

Table S2. Results of likelihood ratio tests