Functional Ecology 2008, **22**, 494–500 doi: 10.1111/j.1365-2435.2008.01387.x

Corticosterone in feathers is a long-term, integrated measure of avian stress physiology

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Summary

1. Stress has pervasive consequences for the well-being of animals. Currently, understanding how individuals cope with stressors is typically accomplished via short-term quantification of blood glucocorticoids released after activation of the hypothalamic–pituitary–adrenal (HPA) axis.
2. We investigated whether the amount of corticosterone (CORT) deposited in growing feathers provides a long-term, integrated measure of HPA activity in birds using captive red-legged partridges *Alectoris rufa* as a model species.
3. We examined CORT levels in primary feathers induced to grow at the same time as stress series were performed with a capture and restraint protocol. Plasma CORT titres after stress-induced stimulation, but not baseline values, correlated with feather CORT. Feather levels showed the same pattern as plasma of decline across the breeding season, but more severely.
4. For females, CORT in naturally moulted flank feathers was highly and positively correlated with the number of eggs laid in the previous few months, but not clutch size of the following year. For males, the amount of black on a feather, known to be a social signal, was positively correlated with its CORT level.
5. The analysis of feather CORT is a novel methodology that allows for meaningful interpretations of how individuals respond to environmental perturbations and adjust to life-history stages.
6. The analysis of feather hormones has the unique advantages of allowing for experimentation and sampling at any time of the year with minimal investigator-induced impacts and artefacts, and shows the HPA activity of an individual with a flexible time frame from days to months depending on the length of time taken to grow the feather. As this technique can be applied to living or dead birds, or feathers picked up after moult, it provides the ultimate non-invasive physiological measure of considerable benefit in terms of animal welfare and sampling effort.

Key-words: hypothalamic–pituitary–adrenal axis, glucocorticoids, non-invasive technique, reproductive effort, seasonal variation, social signal

Introduction

When faced with environmental perturbations, the vertebrate hypothalamic–pituitary–adrenal (HPA) axis responds by releasing circulating glucocorticoids, which then elicit a suite of behavioural and physiological changes known as the stress response (Astheimer, Buttemer & Wingfield 1992; Holberton 1999; Sapolsky, Romero & Munck 2000; Koch, Wingfield & Buntin 2002; Blas *et al*. 2007). This response redirects animals to a life-saving state or 'emergency life-history stage' allowing them to overcome stress and re-establish homeostasis in the best possible physical condition (Wingfield & Ramenofsky 1999; Wingfield & Silverin 2002). However, despite the adaptive value of short-term elevation of glucocorticoids, chronically elevated levels have detrimental consequences to cognitive ability, growth, immune defence, body condition, reproduction and survival (Wingfield & Ramenofsky 1999; Sapolsky *et al*. 2000; Kitaysky *et al*. 2003).

Shortcomings of blood measures of glucocorticoids are well-known, not the least of which is the investigator-induced stress of capture and handling (Romero & Reed 2005; Hinson & Raven 2006). The alternative of determining glucocorticoid metabolites in urine and faeces is species limited, can be problematic in terms of sample collection and investigator-induced corticosterone (CORT) release, and ultimately represents the glucocorticoid profile from a relatively short time frame (Creel 2001; Cabezas *et al*. 2007). Although dead upon maturity, the cells of a growing feather are highly

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vascularized and numerous compounds which have no function in the grown feather (e.g. heavy metals, trace elements, xenobiotics) are deposited incidentally in the keratin structure (Bortolotti & Barlow 1988; Dauwe *et al*. 2003). In this study, we tested the hypothesis that the amount of immunoreactive CORT deposited in feathers provides an historical record of an individual's HPA activity during the period of feather growth. As feathers develop relatively slowly (many days or weeks), these measurements would evaluate a time interval far longer than any other available measure for this hormone, and with the potential to integrate different aspects of HPA activity, including variation in baseline levels and responses to stressors. We propose that feather CORT should integrate not only the intensity of the physiological response, but also how long CORT is elevated within the bloodstream, and the frequency of exposure to stressors. Such a measure, coupled with other ecological and physiological data, would provide a unique window into both causes and consequences of stress.

To test whether a collective measure of these components of stress physiology is biologically meaningful, we examined variation in feather CORT in red-legged partridges (*Alectoris rufa*) held under the controlled conditions of a captive breeding facility. We investigated the degree to which the levels of hormone in feathers correlate with circulating levels in plasma, and parallel the seasonal regulation of plasma CORT. Furthermore, we explored potential associations between HPA activity as recorded in feathers and reproductive effort, as well as a known social signal in this species, that is, feather colour (Bortolotti *et al*. 2006). Our objective in this paper is to introduce the novel technique of analysing feather CORT, and through examples demonstrate how this integrated, long-term measure of stress hormone offers a unique and valuable perspective about the ecophysiology of birds.

Material and methods

STUDY SPECIES

We studied captive red-legged partridges at the Lugar Nuevo breeding centre, Andujar, southern Spain. A mixed-sex flock of 33 adult birds was maintained in an outdoor aviary ($30 \times 15 \times 4$ m), with natural vegetation, water and food provided *ad libitum*, and with natural temperature and photoperiod, from August 2003 until January 2004. We created an experimental setting comparable in phenology and social circumstance for the species in the wild. In January 2004, 28 birds were paired and housed in independent, outdoor breeding aviaries of wired mesh walls ($4 \times 3 \times 3$ m), visually isolated from humans and conspecifics. Pairing date was decided upon by observation of wild red-legged partridges dispersing from winter flocks in the natural surroundings of the facility. Each aviary was provided with food and water *ad libitum*, and two nesting structures lined with natural vegetation (two clutches are often laid, one for each sex, see Cramp & Simmons 1980). Laying period and clutch size were within the natural variability reported for the species in the wild. These birds were used to investigate the relationship between CORT in the plasma during stress series and CORT in feathers grown concurrently (see sampling protocols below).

A second set of paired birds were housed in 1997 and 1998 in smaller breeding pens ($120 \times 80 \times 50$ cm). Eggs were collected in both years as they were laid so incubation and brood rearing were not possible. These pairs were used to study the association between feather CORT and coloration used as a social signal, and also the relationship between CORT and reproduction (see below). Red-legged partridges have a large and conspicuous black banding pattern on the flanks of their bodies that is important in social displays (Goodwin 1953; Cramp & Simmons 1980). The pattern is created by the alignment of black bars that run across individual feathers, and the area of black, especially on the ventral (i.e. most visible) portion of each feather, is a condition-dependent trait (Bortolotti *et al*. 2006). We analysed CORT in the same display feathers (see sampling details below) that were used in a previous study of the function of coloration (Bortolotti *et al*. 2006).

PLASMA COLLECTION AND SAMPLING PROTOCOL

To determine the adrenocortical response to stress (i.e. circulating baseline and stress-induced CORT levels) we used the classical capture-restraint protocol whereby several blood samples are collected at fixed intervals of time following capture (i.e. stress series) (Wingfield & Ramenofsky 1999). Birds were captured with a handheld net and a baseline blood sample (50–100 μL) was collected within 5 min following capture (mean time from capture to blood sampling 2·78 ± 0·77 min). Within this interval, there was no association between handling time and circulating CORT (Pearson $r = 0{\cdot}135$; $P = 0{\cdot}138$, $n = 123$), suggesting that our protocol was effective for the estimation of baseline (i.e. unstressed) HPA activity. After collecting a baseline blood sample, birds were subjected to 60 min of physical restraint in independent cardboard boxes, and then a second 50–100 μL blood sample indicative of the stress-induced response was collected. The optimal handling time for the stress-induced samples was determined by performing a pilot assessment of the response to human capture and handling in a set of birds not used for the main study (G.R. Bortolotti *et al.*, unpublished data). The stress series were performed as described above, starting in December 2003 before pairing and then monthly from March through July 2004.

FEATHER COLLECTION

On 1 March 2004, we collected baseline and stress-induced blood samples, and then pulled primary eight of the left wing. One month later (6 April) during mid-feather growth, and also 2 months later (6 May) just after full feather regeneration, we performed additional stress series on the same birds. The regenerated feathers were collected after the May stress series. The feathers were cut in half after removal of the calamus so that the distal portion could be compared to other time periods (see below), but the mean CORT of the two-halves was also used as an estimate for the entire feather. A second induced feather was pulled on 7 June, and the feather that grew after that, that is, the third induced, was pulled on 12 July. As the latter two feathers were not always fully grown (or in a few cases not replaced at all, so sample size varies) only the composition of the distal half of the feather was compared for the analysis of seasonal trends. Egg laying was initiated between 28 March and 3 May, and if birds incubated they started between 19 May and 8 June. The first induced feather was representative of the prelaying and laying periods. The second sample was grown in May, principally the month of laying, while the next two feathers in June and July represent incubation and brood-rearing, respectively, although not all birds successfully produced young. The final number of eggs varied from 11 to 47 (mean = 26·8, SE = 1·92, $n = 26$). While the latter seems excessively

large, the red-legged partridge may lay more eggs than any species of bird, and two nests were often made (one for each sex to incubate, see Cramp & Simmons 1980; Green 1984). Feathers were stored by taping the calamus to a sheet of paper in a binder kept on a shelf at ambient indoor temperature in Saskatoon, Canada, before analysis in 2006.

Contour feathers with the black bars were collected from the flanks of the pairs used in the earlier study (see Bortolotti *et al*. 2006) to test how feather CORT may be associated with reproduction and the social signal. The feathers were collected in early April 1998 before any pairs began laying, but they had grown as a result of natural moult in late summer of the previous year. These samples had been stored as described above until hormone analysis in 2006.

PLASMA CORT MEASUREMENTS

Blood samples were collected in heparinized tubes and kept in coolers until centrifuged the same day. Plasma was stored at –20 °C until radioimmunoassay (RIA) determination of circulating CORT following standard methods (Blas *et al*. 2005). Antiserum and purified CORT for the standards were purchased from Sigma-Aldrich (Oakville, Canada); ^{3}H-corticosterone was purchased from New England Nuclear (Woodbridge, Canada). Measurements were performed on reconstituted organic ethyl ether extracts of the plasma samples; hormone recovery during this extraction was > 90%. Each sample was measured in duplicate within the RIA. The minimum detection limit of the assay was 16·7 pg per tube. Samples were measured into nine separate assays, with average intra- and interassay coefficients of variation of 6·6% and 11·7%, respectively.

FEATHER CORT MEASUREMENT

Buchanan & Goldsmith (2004) have emphasized the need to fully validate techniques where alternative tissues are utilized to monitor the endocrine status of animals. To our knowledge, we are the first to report that feathers can be used as a non-invasive measure of endocrine status in birds. Consequently, all of the methods used in the present study were validated from a number of perspectives following the recommendations of (Buchanan & Goldsmith 2004) (see Supplementary Appendix S1).

A methanol-based extraction technique was used to extract CORT from feathers (Thieme *et al*. 2003; Kintz 2004). The calamus was removed and feather vanes minced into pieces of < 5 mm^2 with scissors. Ten millilitres of methanol (HPLC grade, VWR International, Mississauga, ON) was added and the samples were placed in a sonicating water bath at room temperature for 30 min, followed by incubation at 50 °C overnight in a shaking water bath. The methanol was then separated from feather material by vacuum filtration, using a plug of synthetic polyester fibre in the filtration funnel. The feather remnants, original sample vial and filtration material were washed twice with *c.* 2·5 mL of additional methanol; the washes were added to the original methanol extract. The methanol extract was placed in a 50 °C water bath and subsequently evaporated in a fume hood under air. Evaporation of the samples was completed within a few hours and the extract residues were reconstituted in a small volume of the phosphate buffer system (PBS; 0·05 M, pH 7·6) used in the CORT RIA (Blas *et al*. 2005). The filtration step was generally found to be sufficient to remove feather particulates but, if needed, further particulate material could be removed by centrifugation of the PBS-reconstituted samples. Reconstituted samples were frozen at –20 °C until analysed for CORT. The efficiency of methanol extraction was assessed by including feather samples spiked with a small amount (*c.* 4000 DPM) of ^{3}H-corticosterone in each extraction. Greater than 90% of the radioactivity was recoverable in the reconstituted samples. All feathers from an experiment were extracted in the same batch to minimize the effects of the small differences in extraction recoveries between batches and hormone data are presented without correction for extraction recovery (see also Supplementary Appendix S1).

The amount of CORT in the reconstituted feather extracts was measured with the same RIA system utilized for the plasma samples. Samples were measured in six separate assays, with intra- and interassay coefficients of variation of 8·3% and 10·5%, respectively. All feather hormone values were expressed as a function of feather length (pg mm^{-1}) as we found this to be more suitable than expressing CORT as a function of feather mass (i.e. concentration, see Supplementary Appendix S1).

Results

Initially, ANOVA's were performed with the CORT of the entire, initial induced feather as the dependent variable, with the independent variable being plasma CORT averaged over the three sample episodes (March, April and May) that spanned the entire growing period of the induced feather. Sex was included as a factor but was never significant (ps > 0·1), so a Pearson correlation with sexes lumped was used instead. Baseline plasma titre had no significant relationship with feather CORT (r = –0·013, P = 0·960, n = 19); however, stimulated plasma levels were significantly related to feather CORT (r = 0·466, P = 0·044, n = 19, Fig. 1).

Patterns over time were examined to further investigate the potential concordance of feather and circulating CORT. The HPA axis in birds may be down-regulated during breeding or depending on the season (Sapolsky *et al*. 2000; Wingfield & Sapolsky 2003; Love *et al*. 2004). For analyses of plasma CORT we used a GLMM (GLIMMIX, SAS Institute). The dependent variable presented a normal type error distribution and we modelled data accordingly, using the identity-link function. We used individual bird identity as a random variable and also considered the bird's sex as an independent factor. When examining trends over time from December to July, baseline titres showed a significant effect of month (n = 123 observations corresponding to 33 individuals sampled along 6 months, month: $F_{5,80}$ = 11·05, P < 0·0001; sex: $F_{1,80}$ = 2·38, P = 0·127; month × sex: $F_{5,80}$ = 2·460 P = 0·040) (Fig. 2a); however, when only the breeding season was examined, month was no longer significant (n = 107 observations corresponding to 28 individuals sampled along 5 months, month: $F_{4,71}$ = 0·45, P = 0·774; sex: $F_{1,71}$ = 4·48, P = 0·038; month × sex: $F_{4,71}$ = 0·88, P = 0·480). For the stimulated CORT levels in plasma there was a significant seasonal decline including December (n = 123 observations corresponding to 33 individuals sampled along 6 months, month: $F_{5,80}$ = 3·95, P = 0·003; sex: $F_{1,80}$ = 0·00, P = 0·956; month × sex: $F_{4,80}$ = 2·18, P = 0·064) (Fig. 2b), but unlike baseline values there was also a decline considering only the breeding season (n = 107 observations corresponding to 28 individuals sampled along 5 months, month: $F_{4,71}$ = 4·29, P = 0·004; sex: $F_{1,71}$ = 0·00,

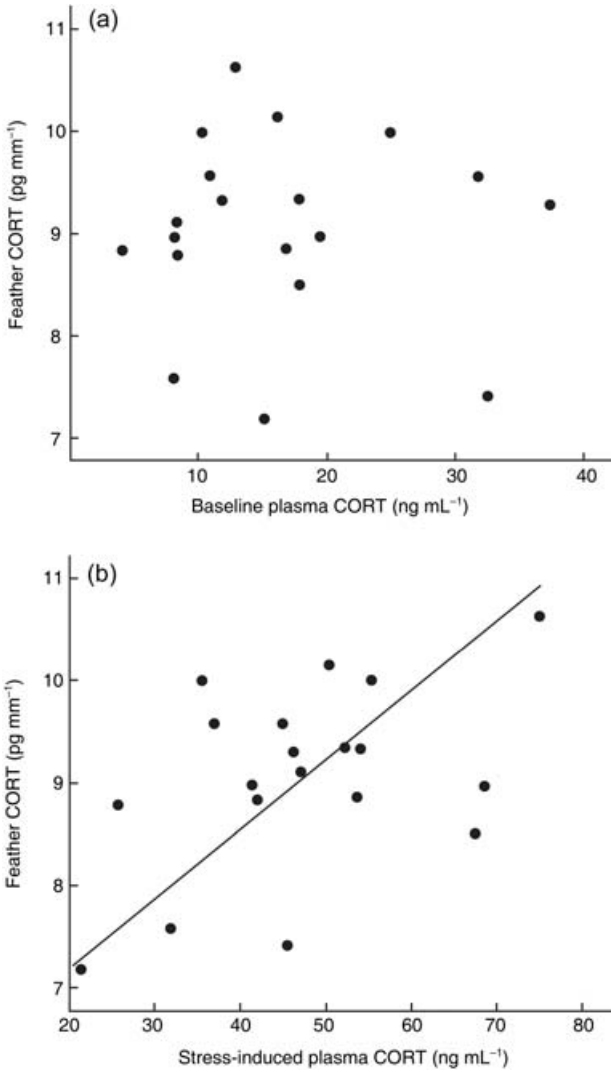

Fig. 1. Mean (a) baseline and (b) stress-induced corticosterone in plasma for stress series conducted in March, April and May in relation to corticosterone in a primary feather induced to grow at the same time (line fit by reduced major axis).

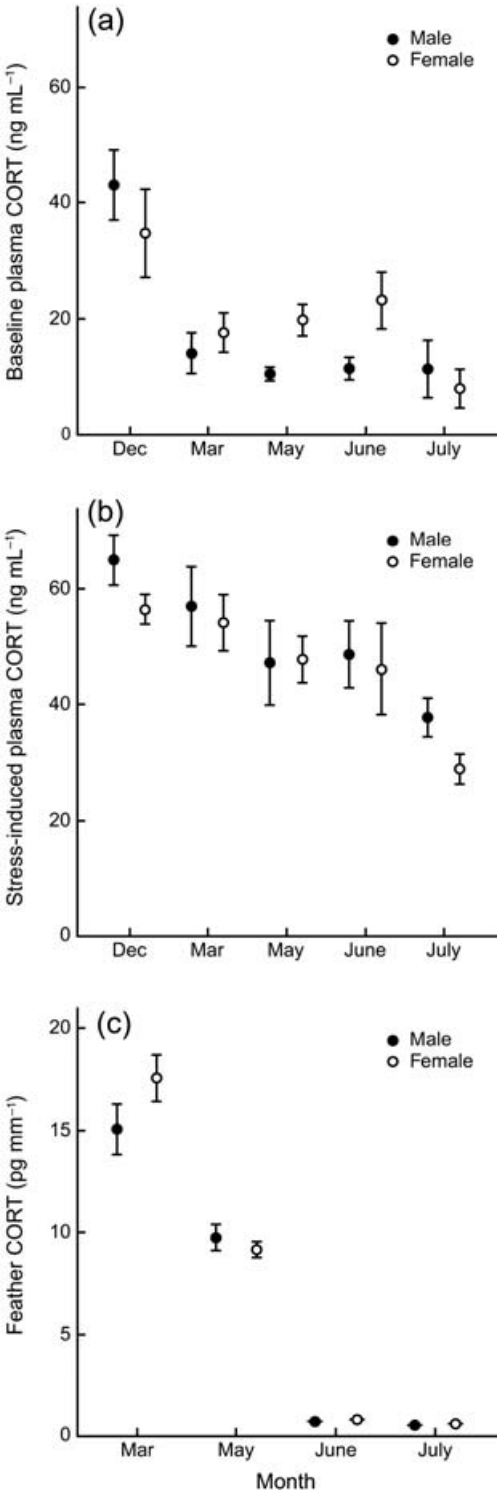

Fig. 2. Monthly mean ± SE of (a) baseline and (b) stress-induced corticosterone in plasma for stress series conducted in December, and during the breeding season from March to July, and (c) corticosterone in primary feathers induced to grow corresponding to each month during the breeding season.

$P = 0{\cdot}950$; month × sex: $F_{4,71} = 2{\cdot}80$, $P = 0{\cdot}032$). For analyses of feather CORT we also used a generalised linear mixed model but the dependent variable was modelled following a γ-type error distribution and a log-link function. We used individual bird identity as a random variable and considered the bird's sex as an independent factor. In contrast to the gradual seasonal change in plasma, CORT in feathers showed large declines from one sample period to the next ($n = 79$ observations corresponding to 24 individuals sampled along 4 months, month: $F_{3,49} = 2112{\cdot}47$, $P < 0{\cdot}0001$; sex: $F_{1,49} = 1{\cdot}97$, $P = 0{\cdot}167$; month × sex: $F_{3,49} = 1{\cdot}79$, $P = 0{\cdot}161$) (Fig. 2c).

The samples of induced primary feathers above show how feather CORT varied over the breeding season. The naturally moulted flank feathers collected in spring 1998 provided a different perspective on how CORT may potentially be associated with reproduction, that is, as a predictor of egg laying (breeding following sampling in 1998), or as an indicator

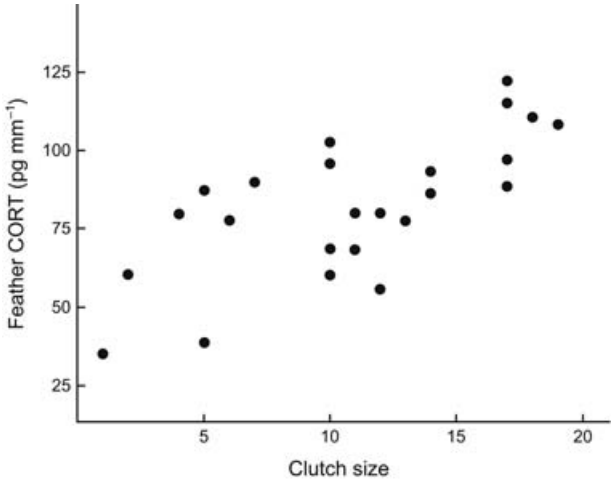

Fig. 3. Number of eggs laid by females in spring in relation to the corticosterone in the flank feathers naturally grown in late summer and fall of the same year.

of the past reproductive performance (laying just prior to feather growth in 1997). For testing potential consequences of HPA activity on the number of eggs laid in 1998 (response variable), ANOVAs incorporated male feather CORT, female feather CORT, laying date of the first egg, and area of the ventral, black bar of each member of the pair. The only significant effects were laying date ($F_{1,18} = 24{\cdot}644$, $P < 0{\cdot}0001$) and area of the male's bar ($F_{1,18} = 6{\cdot}402$, $P = 0{\cdot}021$) consistent with previous results (Bortolotti *et al.* 2006). In contrast, when examining how clutch size and laying date in 1997 was related to feather hormones, female CORT was explained solely and to a great extent by how many eggs were laid previously ($F_{1,23} = 56{\cdot}978$, $P < 0{\cdot}0001$, Fig. 3). Male CORT was not significantly explained by either previous laying date or clutch size ($ps > 0{\cdot}4$).

Earlier work showed that males with wide black bands negatively influenced the development of that signal in their mates, and that narrow bands in females were associated with stress (high H : L ratio from an increase in heterophils) (Bortolotti *et al.* 2006). Therefore, we examined the relationship between a feather's display colour and its CORT level while controlling for the colour and CORT of the bird's mate. The black area and CORT of males were significantly and positively correlated ($r = 0{\cdot}474$, $n = 18$, $P = 0{\cdot}035$) when controlling for female colour and CORT. However, female colour and CORT showed no such relationship ($r = -0{\cdot}188$, $n = 18$, $P = 0{\cdot}429$), no doubt because of the large effect of egg laying on CORT (Fig. 3).

Discussion

Our results indicate that the level of CORT in feathers offers a measure of HPA activity in the red-legged partridge. The presence of CORT in feathers is not unique to partridges, as we have analysed hundreds of individuals from dozens of species covering a wide range of phylogeny and ecology (G.R. Bortolotti, T.A. Marchant, J. Blas & T. German, unpublished data, and see Supplementary Appendix S1 for some examples). Importantly, we provide evidence that feather CORT reflects plasma hormone levels during the period of feather growth, and so it should be integrating variation in baseline level, magnitude of the stimulated response, time course for the stress response and the number of stressors. The significant correlation between the stimulated, but not baseline, plasma CORT and feathers (Fig. 1) may be expected, as stimulated CORT levels are considerably higher than baseline levels and so should have a disproportional effect on feather CORT deposition during feather growth. However, such a correlation may not always be evident, especially in birds studied in the wild exposed to uncontrolled and a variable number of stressors. Because our birds were in captivity, the number of stressful events (e.g. bad weather, feeding by keepers) were likely very similar among individuals, and hence the magnitude of the stress-induced CORT response explained a significant amount of the variance.

We also found that CORT deposition in feathers parallels modulation in HPA activity assessed from plasma hormone levels in accordance with different life-history stages. Birds may down-regulate the adrenocortical response to stress during the breeding season as a strategy to maximize reproductive success (e.g. Sapolsky *et al.* 2000; Wingfield & Sapolsky 2003; Love *et al.* 2004). Both male and female red-legged partridges incubate eggs and care for young (Cramp & Simmons 1980; Gaudioso *et al.* 2002), and seasonal declines in plasma and feather CORT levels were similar in both sexes in our study. Stimulated levels in plasma, in particular, illustrate how birds in our study decreased their maximum response to handling stress over the breeding season. However, such a decline in plasma CORT was modest by comparison to the change we found in feather CORT levels. These findings suggest that birds respond less vigorously in terms of HPA activation during each individual stress event and that they find fewer stimuli stressful during later breeding stages. It is possible that these findings could be explained in part by habituation to the repeated handling (e.g. see Love *et al.* 2003; Walker, Boersma & Wingfield 2006); however, our samples were separated over a period of months so habituation may be less likely, and such an effect does not negate our results on feather CORT. Our observation of the behaviour of red-legged partridges is consistent with the interpretation that the change in responsiveness was linked to a particular life stage. During prelaying and laying in April and May, when considerable CORT could still be measured in feathers (Fig. 2c), partridges showed clear fright and escape behaviours when we entered their cages. However, as soon as incubation began we could routinely enter the cage and pick up a bird from its nest by hand. This change in behaviour corresponded to the time when CORT levels in the feathers were very low (Fig. 2c). It is very likely that feather CORT presents a more natural and meaningful picture of HPA activity than the extreme protocol of capture and restraint.

Our analyses of display feathers reveal that feather CORT provides a stable record of past physiology, and with regards to reproduction and coloration the findings are unique for

birds. For females, reproductive investment in eggs in spring and summer was strongly correlated with CORT in feathers in summer and fall. One possible interpretation for this positive relationship is that elevated HPA activity during egg laying in female birds is a cost of reproduction – a measure of considerable importance to the study of life-history evolution (Williams 2005; Harshman & Zera 2007). In other words, CORT could be viewed as showing that egg production is stressful, or that current reproductive investment trades off subsequent resistance to stress. In fact, CORT is a hormone with a wide range of critical physiological functions, including the modulation of whole body energy usage (Romero 2004) that may be needed in the normal recovery from egg production. Regardless, the strong correlation between clutch size and feather CORT could prove to be invaluable to avian ecologists as a relative measure of reproductive output.

In males, feather CORT levels were not associated with the reproductive effort of their mates. This is expected given males did not have the metabolic demands of egg laying and were not allowed to express parental behaviours under the design of this study (i.e. eggs were collected after laying). However, the area of black on the conspicuous display feathers of males was positively related to feather CORT levels. This feather banding pattern is known to be an important social signal (Goodwin 1953; Cramp & Simmons 1980) and is a condition-dependent trait in this species (Bortolotti *et al*. 2006). The relationship between stress and social position in birds is complex, and increased HPA activity may be a consequence of subordination or a cost of dominance (Creel 2001). The finding of an elevated feather CORT level in male partridges with the largest black bands would support the latter interpretation. Feather CORT may thus be helpful in understanding social relationships, even those occurring prior to the arrival of the investigator, as well as revealing potential consequences of dominance.

Our study clearly indicates that measurement of feather CORT levels is feasible and can provide unique insights about stress physiology in birds. As with the interpretation of data collected using other techniques, it is a challenge to determine if changes in stress-induced CORT are best understood as an adaptation or as a disease (Carr & Summers 2002). CORT redirects animals to a life-saving state ('emergency life-history stage', Wingfield *et al*. 1998) allowing them to overcome environmental perturbations; however, elevated and chronic exposure to CORT may be detrimental (reviewed in Sapolsky *et al*. 2000; Kitaysky *et al*. 2003). Measurement of blood levels of this stress hormone, by virtue of its characteristic snapshot in time, has been unable to significantly advance our understanding of these costs and benefits. Feather CORT provides a novel temporal perspective on CORT that will facilitate future research of cause vs. effect and the significance of chronic vs. short-term exposure to elevated CORT.

The flexible time frame for studying stress is one of the most significant advantages of analysing feather CORT, with profound ramifications for avian ecology and endocrinology. The temporal sample is very flexible, ranging from days to months depending on the growth rate of the type of feather and how much of the feather is to be analysed. The investigator may cut up a single feather to different lengths (e.g. representing a few days of growth), and then examine changes in CORT over time, that is, from the most temporally distant sample at the distal end of the complete feather, to the most recent sample proximally. Given that CORT is expressed per millimetre or other length unit measure of a feather, the hormone values will vary with the rate of elongation of feathers (see Supplementary Appendix S1). Variation in growth rate of one type of feather (e.g. contours of the breast) within or among individuals is relatively minor considering there will be measurement error; differences in growth rates for different types of feathers (e.g. body vs. flight feathers). It is thus imperative to compare feathers similar in morphology.

Analysis of feather CORT is the only method available to obtain a long-term and retrospective measure of stress. It appears that feather CORT is measurable and gives meaningful results even after years of storage as we showed with the analysis of flank feathers kept for nearly a decade. Therefore, it may be possible to conduct an historical analysis of populations, or even individuals, in a changing environment – a particularly powerful approach in conservation biology. Similarly, population-level analyses of species whose numbers cycle, hormone modulation by xenobiotics and the cost of developing sexually selected traits are just a few of many topics where assessment of CORT in feathers should prove fruitful. Another advantage of the technique is that experiments can be initiated at any time, as plucked feathers are soon replaced and the new growth can be related to any manipulated or natural source of variation in the bird's external environment or endogenous physiology. Birds can be studied at times they are normally inaccessible, for example, pelagic birds in winter, and without the direct and undesirable investigator-induced effects and artefacts that plague the analysis of blood hormones. As moulted feathers can be also analysed, the capture of birds can be avoided, providing the ultimate non-invasive physiological measure of considerable benefit in terms of animal welfare and sampling effort. The handling, transport and storage of samples are convenient and safe from the many avian, blood-borne biohazards. Collectively, our results and these methodological advantages confirm that evaluation of feather CORT adds a powerful tool with novel insights in the study of stress – one of the most profound and pervasive factors influencing the well-being and fitness of animals.

Acknowledgements

The first two authors contributed equally to the paper. We thank J.J. Negro, J.L. Tella, S. Poland, P. Prieto, J.M. Terrero and S. Cabezas for helping to collect data. S. Cabezas kindly assisted with statistical analyses. J.J. Negro, F. Mougeot, J.E. Smits, S. Cabezas and G. Fairhurst provided helpful comments on the manuscript. Funding was provided by NSERC (GRB), the Stuart and Mary Houston Professorship in Ornithology (GRB), and the Isabel Maria López Martínez Memorial Scholarship (JB) and CSIC-Fondo Social Europeo (in an I3P contract to JB). We are grateful to the Consejeria de Medio Ambiente of the Junta de Andalucia for providing access to the Lugar Nuevo facilities. This work was conducted under permit by Consejeria de Medio Ambiente, and approved by animal care committees in Canada and Spain.

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Received 26 September 2007; accepted 15 January 2008
Handling Editor: Keith Sockman

Supplementary material

The following supplementary material is available for this article:

Appendix S1. Validation of the technique for quantifying corticosterone in feathers

This material is available as part of the online article from: http://www.blackwell-synergy.com/doi/full/10.1111/j.1365-2435.2008.01387.x
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