

Corticosterone mediated costs of reproduction link current to future breeding



Glenn T. Crossin^{a,*}, Richard A. Phillips^b, Christine R. Lattin^c, L. Michael Romero^c, Tony D. Williams^d

^a Department of Biology, Dalhousie University, Halifax, Nova Scotia B3H 4R2, Canada

^b British Antarctic Survey, Natural Environment Research Council, High Cross, Madingley Road, Cambridge CB3 0ET, United Kingdom

^c Department of Biology, Tufts University, Medford, MA 02155, United States

^d Department of Biological Sciences, Simon Fraser University, Burnaby, British Columbia V5A 1S6, Canada

ARTICLE INFO

Article history:

Received 6 December 2012

Revised 4 July 2013

Accepted 8 July 2013

Available online 26 July 2013

Keywords:

Breeding-moult overlap

Macronectes

Chick rearing

Feather corticosterone

Foraging behaviour

ABSTRACT

Life-history theory predicts that costs are associated with reproduction. One possible mediator of costs involves the secretion of glucocorticoid hormones, which in birds can be measured in feathers grown during the breeding period. Glucocorticoids mediate physiological responses to unpredictable environmental or other stressors, but they can also function as metabolic regulators during more predictable events such as reproduction. Here we show that corticosterone ("Cort") in feathers grown during the breeding season reflects reproductive effort in two Antarctic seabird species (giant petrels, *Macronectes* spp.). In females of both species, but not males, feather Cort ("fCort") was nearly 1.5-fold higher in successful than failed breeders (those that lost their eggs/chicks), suggesting a cost of successful reproduction, i.e., high fCort levels in females reflect the elevated plasma Cort levels required to support high metabolic demands of chick-rearing. Successful breeding also led to delayed moult prior to winter migration. The fCort levels and pre-migration moult score that we measured at the end of current breeding were predictive of subsequent reproductive effort in the following year. Birds with high fCort and a delayed initiation of moult were much more likely to defer breeding in the following year. Cort levels and the timing of moult thus provide a potential mechanism for the tradeoff between current and future reproduction.

Crown Copyright © 2013 Published by Elsevier Inc. All rights reserved.

1. Introduction

Life-history theory asserts that natural selection cannot simultaneously maximize both survival and reproductive activity, and assumes that reproduction has costs (Stearns, 1972). The physiological basis of these costs is not well understood, but glucocorticoid hormones (e.g., corticosterone) may be involved in mediating such costs. Well known for their role in allostasis and the physiological responses to unpredictable environmental stressors (Blas et al., 2007; MacDougall-Shackleton et al., 2009; Romero and Wikelski, 2001; Wingfield et al., 1998), glucocorticoids can trigger breeding desertion and failure if threshold levels are exceeded, as individuals must re-direct resources towards self-maintenance in order to cope with stressor at hand (Breuner et al., 2008). However, glucocorticoids also function as metabolic regulators during more predictable events, like migration and seasonal breeding, and can be positively correlated with measures of current reproductive effort (Crossin et al., 2012a; Hau and Beebe, 2011). Studies detailing the role of corticosterone in the regulation

of parental care, via positive effects on foraging behaviour and provisioning effort, underscore its ubiquitous role in organismal fitness (Bonier et al., 2011; Crossin et al., 2012a; Love et al., 2004).

Not surprisingly, the relationships between glucocorticoids and fitness measures are complex and influenced by factors like age, breeding status, physiological condition, population, species, and ecology. Two recent reviews illustrate how variation in plasma levels of corticosterone (hereafter called "Cort"), at any single point in time, can be either positively, negatively, or non-significantly related to fitness or surrogates thereof (Bonier et al., 2009a; Breuner et al., 2008). Understanding the role of Cort in fitness, and predicting the direction of the relationship between the two, may depend on whether baseline or stress-induced plasma levels are examined. However, in studies where no relationship is observed (typically in studies examining seasonal phenomena), plasma Cort offers only a "snapshot" view of an individual's physiological condition (hours to day), which may be insufficient for resolving events operating over longer time-frames. A longer-term, integrated measure of Cort secretion and hypothalamo–pituitary–adrenal axis (HPA) activity has the potential to resolve Cort's role in broad temporal processes like seasonal migrations, reproduction, or transitions between these life-history stages, especially in situations where plasma Cort does not reveal any associations. Analysis of Cort in keratin-based

* Corresponding author. Address: Department of Biology, Life Science Centre, Dalhousie University, 1355 Oxford Street, Halifax, Nova Scotia B3H 4R2, Canada.

E-mail address: gtc@dal.ca (G.T. Crossin).

tissues (feathers, hair), which accumulate glucocorticoid hormones during growth (weeks to months), has been used recently to provide a more useful means for interpreting variation in broad-scale life-history events (birds Bortolotti et al., 2008, 2009; mammals Fourie and Bernstein, 2011; Macbeth et al., 2010). The analysis of Cort in feathers (hereafter called “fCort”) has the potential to increase our understanding of the mechanisms controlling avian life-histories (Bortolotti et al., 2008, 2009; Lattin et al., 2011).

Experimental studies of captive birds have confirmed that fCort levels can reflect high, or stress-induced, plasma Cort levels during the period when feathers are grown (Bortolotti et al., 2008; Lattin et al., 2011). Furthermore, variation in fCort has been linked to reproductive effort in captive partridges (*Alectoris rufa*) (Bortolotti et al., 2008). But only a small number of studies have linked natural variation in fCort to fitness measures (e.g., reproductive success) in free-living birds. Fairhurst et al. (2012) found that variation in parental investment by a seabird species (Cory’s shearwaters, *Calonectris diomedea*) was reflected in fCort levels in the feathers of chicks. Specifically, they found that low foraging efficiency by parents led to a suppression or down-regulation of Cort secretion by chicks, which they interpreted as a buffer against nutritional stress and the deleterious effects of long-term HPA activation, resulting in lower fCort levels. Although fCort levels were not measured in the parent shearwaters, we might expect these to correlate positively with foraging effort (Angelier et al., 2008; Crossin et al., 2012a), and with chick growth (Bonier et al., 2011; Crossin et al., 2012a). More recently, Kouwenberg et al. (2013) show positive correlations between pre-breeding fCort levels and egg sizes in Atlantic puffins (*Fratercula arctica*), providing a link between winter foraging effort and reproductive effort.

Collectively, the studies by Bortolotti et al. (2008), Fairhurst et al. (2012), and Kouwenberg et al. (2013) provide growing evidence for a positive link between variation in fCort and fitness components in birds, but there is also an example of a negative fitness link. Koren et al. (2011) examined fCort levels in feathers grown during a post-breeding moult in sparrows (*Passer domesticus*) and found that elevated levels were related to lower overwinter survival. This study did not identify the underlying mechanism, but recent studies have shown that elevated Cort can both slow the rate of moult and negatively affect feather quality (DesRochers et al., 2009; Romero et al., 2005; Storchlic and Romero, 2008), including reductions in the insulating properties of feathers critical for survival at low winter temperatures.

Plasma Cort is usually down-regulated at the onset of post-breeding moult. As a catabolic steroid, this is presumably to avoid the breakdown of structural proteins at a time when anabolic processes are needed (e.g., new feather synthesis), as well as the Cort-dependent inhibition of new protein synthesis during feather growth (DesRochers et al., 2009). For species that moult during chick-rearing, how is the potential need to up-regulate baseline corticosterone in support of foraging and parental effort reconciled against the need to down-regulate it to avoid negative effects on feather growth? More importantly, what are the functional consequences of breeding activity and Cort secretion on the rate of new feather growth, fCort levels, future survival, and future breeding?

In this study, we test the hypothesis that fCort in breeding adults can provide an integrated measure of HPA activity in relation to current reproductive effort. We also hypothesize that fCort would be correlated with future reproductive effort. To address this, we took advantage of the unusual characteristic of giant petrels (*Macronectes* spp.), which unlike most birds (but see Bridge, 2006) begin moulting during incubation or early chick-rearing, and continue growing new feathers until the end of the breeding season (Crossin et al., 2012b; Hunter, 1984a), i.e., they exhibit a breeding-moult overlap, coincident with parental care. We

measured both plasma Cort and fCort in feathers grown by parents during chick-rearing in year x (their current reproductive attempt), and compared levels in parent birds who lost their eggs or chicks earlier in the season. Comparisons between successful and failed breeders should reveal the HPA activity and Cort secretion needed to support investment in chick rearing. We therefore sampled successful and failed individuals from two species of giant petrels at the end of a breeding season (northern *Macronectes halli* and southern *Macronectes giganteus*). In addition to plasma and fort, we compared moult scores in successful and failed breeders. We then followed and recorded the survival and breeding activity of the same individuals over successive breeding seasons. By doing so, we could examine associations between fCort, plasma Cort, and feather moult during the current reproductive effort (year x), with future survival and reproductive decisions (breeding vs. deferral in year $x + 1$).

We predicted three things. (1) That reproductive success in year x would be positively related to fCort levels, based on an assumption that baseline Cort is up-regulated in successfully breeding birds, relative to failed breeders, to support the higher metabolic demands of parental care. Given its role in the stress response however, we expect the relationship between Cort and parental care measures to fit an inverse U function, wherein low levels of parental care can result from very low or very high levels (e.g., a stress response). But at the middle of this curve, high levels should be positively linked to parental care. (2) We also predicted that the relationship between Cort and reproductive effort would be more readily detected in feathers than in plasma, as feathers should integrate the hormonal signal over the extended period of feather growth coincident with incubation and chick-rearing. Finally (3) we predicted that a relationship between fCort levels and future breeding probability in year $x + 1$ would indicate a long-term cost of reproductive investment. Given that successfully breeding giant petrels in year x have a delayed or slower rate of moult and feather growth than failed breeders (Crossin et al., 2012b; Hunter, 1984a), we predicted that high fCort and delayed moult would provide a potential mechanism underlying the tradeoff between current and future reproduction.

2. Materials and methods

2.1. Study site and sample collection

Data were collected from northern and southern giant petrels breeding on Bird Island, South Georgia (54°00'S, 38°02'W) during chick-rearing in the the austral summer of 2008/09. Blood samples and moult scores were collected in 2008/09 only, but survival and subsequent breeding histories for all individuals were monitored closely during the 2009/10, 2010/11, and 2011/12 breeding seasons. Our principal aim was to link current breeding activity (2008/09 season) to future reproduction (2009/10 season). Given the high breeding site fidelity of giant petrels, we assumed that birds unobserved after the 2008/09 season had died at sea. Although we recognize that there could be alternative reasons for birds being unobserved, a precedent for this assumption was established by Foote et al.’s study of giant petrels (Foote et al., 2011).

Giant petrels lay only one egg, and parents share egg incubation and chick feeding duties nearly equally (Hunter, 1984b). Northern giant petrels laid from mid September to mid October, chicks hatched from mid November to mid December, and fledged from mid March to early April (Fig. 1). Southern giant petrels began laying in November, about 6 weeks later than northern giant petrels, and chicks hatched in January and fledged in late April to May. Birds with chicks were sampled on 5–13 March 2009, when chicks

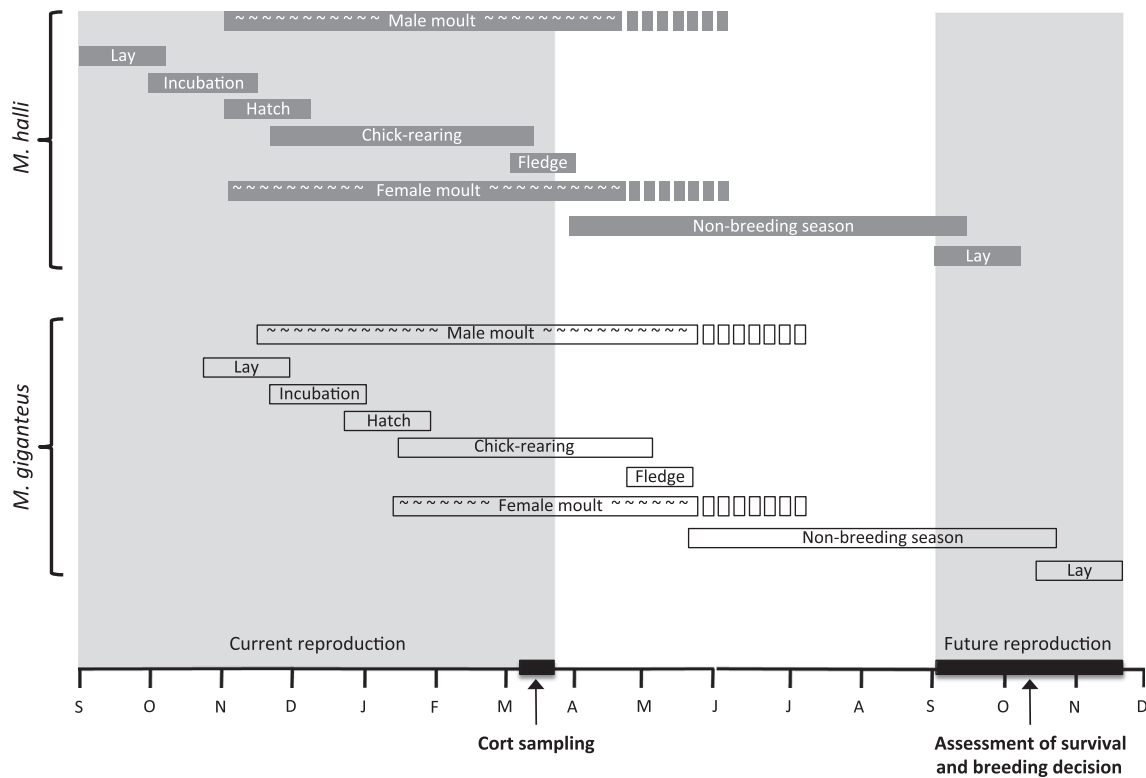


Fig. 1. Schematic of breeding activity of northern (*Macronectes halli*) and southern (*M. giganteus*) giant petrels at Bird Island, South Georgia during the austral summer of 2008/09. Broken bars indicate a particular event that continues once adult petrels have completed reproduction. The black bars along the date axis indicates when individuals were sampled for newly grown feathers and blood plasma, and when assessments of future survival and future breeding decisions were made.

of southern giant petrels were ~2 months old, and those of northern giant petrels 6 weeks older and close to fledging. These dates were at the end of the breeding season, shortly before out-migrations from the colony. On each of these dates, failed breeders (those known to have lost their eggs/chicks) were also sampled. From the colony database, it became apparent that nearly all failed birds had failed during the incubation stage. Thus, at the season's end, we compared the physiological state and condition of successfully breeding giant petrels with those who lost their eggs months earlier during incubation. All birds were part of a long-term study population, were identifiable by alpha-numeric plastic leg bands, and were of known sex and breeding status. Also known were the dates of breeding failure (loss of eggs or chicks).

Blood samples (2 ml) were collected from the tarsal vein using syringes with 25 gauge needles, returned to the lab, and the plasma was separated by centrifugation and stored at -20°C . In all cases, blood was collected in less than 3 min from first approach to the bird. Bill measurements were made with calipers to nearest mm. Body mass was measured to the nearest 10 g with a Pesola spring balance. Five newly grown breast feathers, which are easily distinguished from older feathers by their darker colour and unweathered condition (e.g., lack of abrasions at the tips), were plucked and stored in labeled bags. Hunter (Hunter, 1984a) noted that in *Macronectes* spp. body feathers grow continuously throughout the breeding season. Feathers were stored in the dark, at 4°C until analysis. In addition to our breeding and failed-breeding birds, we opportunistically sampled feathers from two non-breeding female giant petrels (a northern and a southern) which did not lay but deferred reproduction altogether. This was to provide a qualitative point of reference to the breeding birds, especially the failed breeders which should be similar to non-breeders as they were not providing parental care in any form.

Giant petrels have 10 primary flight feathers and a minute 11th primary feather, which can be difficult to check in a live bird and was thus ignored, as in previous studies (Crossin et al., 2012b; Hunter, 1984a). Mass-corrected primary feather moult in our birds was scored according to Dawson & Newton (2004), which involves scoring the growth of new primary feathers on a scale of 0–4: 0 = a dropped feather but no new feather yet present; 1 = approximately one-quarter grown (i.e., eruption from the follicle to one-quarter grown); 2 = approximately half-grown; 3 = approximately three-quarters grown; and 4 = fully grown. The score (0–4) for each primary position was divided by 4 and multiplied by the proportion that the final mass of that particular primary contributes to the total mass of all 10 primaries. Summing the scores of individual feathers thus produces a total moult score (synonymous with new feather growth) ranging from 0.0 before moult to 1.0 in a fully moulted bird. Data on individual primary feather mass were collected from fresh giant petrel carcasses (from both species), which were found in the breeding colonies and which died of natural causes.

2.2. Hormone assays

Feather Cort was determined by a standard radioimmunoassay following Bortolotti et al. (2008) and modified by Lattin et al. (2011). The calami were removed from all five breast feathers before their total lengths and weights were recorded. Feathers were then minced into pieces $<5\text{ mm}^2$. Bortolotti et al. (2008, 2009) make a clear case for standardizing fCort by length, rather than mass, due to the way feathers grow and elongate along their radial circumference. Although sample mass can affect fCort concentrations (Lattin et al., 2011), this effect only occurs with samples of

low mass (≤ 50 mg). All samples used in this study were far above this threshold (range: 145–238 mg).

We added 7 ml of methanol to each sample before a 30 min incubation all in a sonicating water bath for 30 min. Samples were then placed into a shaking water bath at 50 °C overnight. The next day, feather fragments were separated from samples using vacuum filtration, and the methanol extracts dried using nitrogen gas at 50 °C. Samples were reconstituted in phosphate-buffered saline with glucose and run in duplicate through a standard radioimmunoassay as described in Wingfield et al. (1992) using an anti-corticosterone antibody suitable for feather extracts (Sigma–Aldrich C8784, Saint Louis, MO, USA, lot 57K4791). We determined the intra-assay coefficient of variation by measuring differences between duplicates, and the inter-assay variation by measuring differences between standard samples (we used two standards: a known amount of Cort, and pulverized, homogenized feathers). Intra-assay variation was 2.2%; inter-assay variation was 5.2%. Assay parallelism was previously demonstrated by Bortolotti et al. (2008).

Plasma Cort was determined by double antibody radioimmunoassay (^{125}I -RIA, MP Biomedicals, 07-120103), according to Crossin et al. (2012b). The assay detection limit was 3.13 pg Cort per tube (i.e., the lowest Cort standard, 12.5 ng ml $^{-1}$, using a 50 μ l assay volume). The low Cort control and a consistent native plasma sample were analyzed in each assay to determine an inter-assay variation (5.1%). Serially diluted native plasma samples were parallel to the corticosterone standard curve. Coefficients of variation for final concentrations were 9.9% for northern giant petrels ($N = 3$) and 7.5% for southern giant petrels ($N = 3$). These plasma Cort data have been previously published (Crossin et al., 2012b), but are presented here for comparison with fCort levels.

2.3. Statistics

Analyses were run with the JMP 9.0 and R software packages. Data transformations were applied when the distribution of residuals from models were non-normal, as determined by Shapiro–Wilk tests. For the failed breeders, we used analysis of covariance (ANCOVA) to examine whether there were species or sex differences in fCort and plasma Cort while controlling for variation in fail date (e.g., date when breeding failure occurred, which ranged from 8.9 to 20.9 weeks before our sampling dates and illustrates how failure by giant petrels usually occurs at the egg stage). Then, because giant petrels are sexually size-dimorphic (Hunter, 1984b), we used linear regression to explore the relationship between body mass and fCort in each species and sex independently. Because these were related, variation in fCort and plasma Cort were therefore examined by three main effects: species, sex, and breeding outcome (failed or successful), while also controlling for allometric variation in body mass (ANCOVA). Interactions between all main effects were included in the models, as was a sex*body mass term (due to sexual dimorphism). A similar model was used to analyze variation in moult score. Finally, ANOVA was used to explore differences in body mass between species and breeding outcome.

Nominal logistic regression was used to explore whether costs of reproduction (breeding outcome, fCort, moult score) influenced the tradeoff between current and future reproduction. Species, sex, breeding outcome (failed or successful in 2008/09), fCort, and moult score were main effects (including all interactions) in the highest order model examining the probability of deferred reproduction in the following year (year 2009/10).

3. Results

We sampled 80 giant petrels at or near their nests in 2008/09 (or year x ; see Table 1 for numbers sampled from each species

and sex). No partner birds were sampled, meaning that the birds we sampled represent 80 independent successful or failed breeding attempts. Of these 80 birds, 73 were re-sighted in subsequent years (91%; range 83–100% depending on species and sex; Table 1). From these re-sighting data, we determined overwinter survival rates and future breeding activity.

Before comparing fCort and plasma Cort in successful versus failed breeders, we first examined whether date of nest failure influenced these variables in the failed breeders, but there was no significant effect (fCort, $F_{3,20} = 0.232$, $P = 0.873$; Cort, $F_{3,21} = 0.600$, $P = 0.622$). Of the birds breeding in the 2008/09 season (year x), there were no significant differences in body mass between species or between successful and failed breeders (females, $F_{2,37} = 0.444$, $P = 0.645$; males, $F_{2,37} = 0.069$, $P = 0.934$), but linear regression revealed significant negative relationships between body mass and fCort in both sexes, independently of species and breeding outcome (both $P < 0.029$). Body mass was therefore used as a covariate when exploring differences in fCort between species, sexes, and breeding groups.

An ANCOVA with species, sex, and current breeding outcome as main effects, and body mass as a covariate, revealed that fCort was significantly related to all of these effects and to the body mass covariate (full model: $F_{8,65} = 8.136$, $P < 0.001$) (see Table 2 for detailed statistical output and effects sizes). Interactions terms were included in the model; only the sex*breeding outcome was significant (Table 2). This interaction suggests that mean (least squares) fCort was higher in successfully breeding females of both species than in failed breeders, but this was not the case in males, which partially supports our first prediction (Table 2, Fig. 2). In contrast, plasma Cort levels did not differ by species, sex, or breeding outcome, and did not vary with body mass (full model: $F_{8,67} = 0.593$, $P = 0.780$), which supports our second prediction (Table 2, Fig. 2). By way of contrast, fCort levels in the two non-breeding (deferring) female giant petrels that we opportunistically sampled were similar to the mean value measured in all failed giant petrels (1.78 and 1.91 versus a mean of 2.13 pg mm $^{-1}$).

Patterns of primary moult varied according to species, sex, and breeding outcome (Fig. 3). By the end of the breeding, failed birds had progressed further in primary moult than successful birds, and generally, moult was more advanced in males than in females. Successfully breeding southern giant petrel females had initiated only about 5% of moult by the end of the 2008/09 season, which was significantly lower than all other successfully breeding birds (ANCOVA, full model- $F_{8,71} = 22.17$, $P < 0.001$). This means that successful southern females must defer moult to a greater extent than other species/sexes, and must therefore grow the majority of their primaries at sea during the winter non-breeding period (see Hunter, 1984a).

A full nominal logistic regression model ($\chi^2 = 55.17$, $P < 0.001$, $N = 74$) examining the probability of future breeding deferral revealed no significant effect of species ($P = 0.223$), but significant effects of sex ($P < 0.001$), breeding outcome ($P = 0.017$), and their interaction (sex*breeding outcome, $P < 0.001$). There was also a significant species*breeding outcome interaction ($P = 0.008$). Due to this latter interaction, we ran subsequent species-by-sex models to reveal that the main effects of fCort and moult score significantly predicted the probability of future breeding deferral in female southern giant petrels only (see Table 3 for statistical output of species-by-sex models). The effect of current breeding outcome did not predict future breeding deferral. In other words, the combination of high fCort levels and low moult score at the end of the breeding seasons increased the probability of deferred breeding the next year, independent of breeding outcome in the current year. The only other significant effect was current breeding outcome on future breeding deferral in male southern giant petrels, which suggests that successful breeding increases the tendency

Table 1

Breeding activity of giant petrels (*Macronectes* spp.) at Bird Island, South Georgia in the 2008/09 and 2009/10 breeding seasons. The number of successful and of failed breeders sampled for blood plasma and feathers in 2008/09 are indicated in parentheses in the year x column. Breeding activity for the same individual was then monitored the next year in 2009/10, after winter migrations. Moulting scores and feather samples were collected at the end of year x to provide measures of current reproductive effort, and links to subsequent, future reproduction are explored in this study. Northern giant petrels lay in mid September to mid October, on average 6 weeks earlier than southern giant petrels, which lay in November.

Species	Sex	Year x	Year $x + 1$				
		No. sampled	No. re-sighted ^a	Proportion surviving winter	No. breeding	No. deferring	Proportion breeding
<i>M. halli</i>	F	17 (9,8)	16	0.94	16	0	1.00
	M	22 (17,5)	22	1.00	18	4	0.82
<i>M. giganteus</i>	F	23 (15,8)	20	0.87	13	10	0.65
	M	18 (14,4)	15	0.83	8	10	0.44

^a Re-sighted birds were those sampled in year x and observed again at the colony in subsequent years, i.e., the 2009/10 or 2010/11 breeding seasons. These re-sightings allowed us to calculate winter survival rates from year x to year $x + 1$.

for future deferral irrespective of fCort or moult scores (Table 3). Using a similar logistic regression modeling approach, the probability of future survival (to year $x + 1$) was unrelated to species, sex, breeding outcome, fCort levels, or moult scores (full model, $\chi^2 = 14.05$, $P = 0.446$, $N = 64$), which is not surprising given the high survival and return rates of giant petrels noted here (Table 1) and elsewhere (Brooke, 2004).

4. Discussion

Despite years of study, our understanding of the physiological mechanisms controlling life-history events and individual fitness is rudimentary. In this study, we describe how natural variation in feather Cort (or fCort) is linked to two important life-history events in free-living birds. Both current reproductive success and future reproductive effort (breeding vs. deferral) were significantly linked to fCort, such that high fCort levels were indicative of successful breeding in female giant petrels. Conversely, high fCort was predictive of deferred breeding in the following year, but in a context dependent manner which we describe in greater detail below. Based on our results we propose that circulating Cort, as indexed by fCort, mediates a trade-off between current and future reproduction.

Although our study is correlative by design, and so we can only speculate about the role of Cort as a mediator between current and future reproduction, they nevertheless (1) show the utility of fCort for resolving long-term patterns of HPA activity and glucocorticoid secretion with links to the fitness-related events of current reproductive success and future breeding investment, (2) highlight the potential of using fCort as an integrated signal of metabolic up-regulation in support of successful current reproductive investment rather than solely as an indicator of cumulative physiological “stress”, and (3) reveal a potential cost of reproduction via an interaction between fCort and the timing of moult that affects future breeding activity. Collectively, these results suggest that a Cort-mediated mechanism underlies the trade-off between current and future reproduction, thus lending support to the corticosterone-adaptation hypothesis (Bonier et al., 2009a) which predicts positive associations between Cort secretion and fitness measures. Interestingly, this link was not discernable via plasma Cort levels, which seemed insufficient for resolving broad-scale associations between current and future breeding activity in this study. It was only through analysis of feather Cort levels that we could resolve these links (Bortolotti et al., 2008, 2009). Finally, we also show that fCort was unrelated to long-term survival in giant petrels, which is perhaps not surprising given the high annual survival observed in these long-lived species (see Table 1). Clearly, further work is needed to more rigorously test the role of Cort (measured in an

integrated manner using fCort) as the causal agent responsible for variation in these life-history events. Additionally, fCort and plasma Cort are only two elements in a complex HPA regulatory network. Relating variation in Cort to fitness thus requires an assumption that all other aspects of the HPA axis were expressed similarly among individuals. Although we did not measure the activity of other elements of this axis, we nevertheless demonstrate how individual variation in fCort links breeding success to future breeding investment.

Key to the interpretation of our data was knowledge of species' ecology, which puts into context the role of Cort in reproductive effort. During the breeding season, giant petrels exhibit marked sexual and spatial segregation of foraging behaviours (González-Solís et al., 2008; Phillips et al., 2011). That we observed a significant link between variation in fCort levels in female petrels and not in males we attribute to this segregation. Female giant petrels forage in pelagic waters, flying hundreds to thousands of kilometers during trips to productive areas throughout the southern latitudes (e.g., continental shelf breaks, Antarctic Convergence zone). These foraging trips are much longer in duration and range than those made by males, particularly during incubation and early chick-rearing when the latter feed to a much larger extent on local carrion (González-Solís et al., 2008; Phillips et al., 2011). Experimental up-regulation of plasma Cort has recently been shown to increase foraging effort and provisioning rates in seabirds, with positive effects on chick growth (Angelier et al., 2008; Bonier et al., 2009b; Crossin et al., 2012a; but see also Angelier et al., 2009). By demonstrating that fCort levels were higher in successful than in unsuccessful females, our results suggest that the higher metabolic demands of parental care in females requires HPA up-regulation to sustain higher provisioning effort and meet the demands of growing chicks. In the failed females, nearly all of whom failed at the incubation stage before the initiation of moult (female petrels begin moulting at around the time of egg hatching; Hunter, 1984a,b), fCort levels were significantly lower than in breeders. Presumably, these low fCort levels reflect the lack of parental commitment that results from failed breeding; free from the demands of parenthood and a need to feed chicks, females were free to forage with less urgency, on their own schedules. Indeed, failed females had fCort values that were similar to those of two non-breeding (e.g., non-parenting) females that we opportunistically sampled. In contrast, the lack of relationship between fCort and breeding activity in the males was presumably due to the abundant carrion found on the beaches close to breeding sites (which are usually dominated by males). With a food source so readily available, the urgency to forage is perhaps less intense in breeding males than in breeding females, and might not require HPA up-regulation in support of foraging effort. We should caution however that the lack of relationship between fCort and current breeding

Table 2

Statistical output from analyses of covariance comparing feather corticosterone (fCort) and plasma corticosterone by species, sex, and breeding outcome. Bold *P*-values are significant with $\alpha = 0.05$. See Fig. 2 for mean fCort and plasma Cort values.

Response	Model effects	<i>F</i>	<i>P</i>	Effect size (η_p^2)
fCort	Whole model (<i>N</i> = 74)	8.136	<0.001	
	Species	6.813	0.011	0.091
	Sex	20.704	<0.001	0.233
	Breeding outcome	8.058	0.006	0.106
	Mass	26.344	<0.001	0.279
	Species*sex	2.399	0.126	0.034
	Species*breeding outcome	2.934	0.092	0.041
	Sex*breeding outcome	4.440	0.039	0.061
	Sex*mass	2.939	0.083	0.046
Plasma Cort	Whole model (<i>N</i> = 76)	0.593	0.780	
	Species	0.989	0.324	0.016
	Sex	2.797	0.099	0.043
	Breeding outcome	1.170	0.283	0.018
	Mass	2.931	0.092	0.045
	Species*sex	0.155	0.695	0.002
	Species*breeding outcome	0.055	0.815	0.001
	Sex*breeding outcome	0.126	0.723	0.002
	Sex*mass	0.547	0.462	0.009

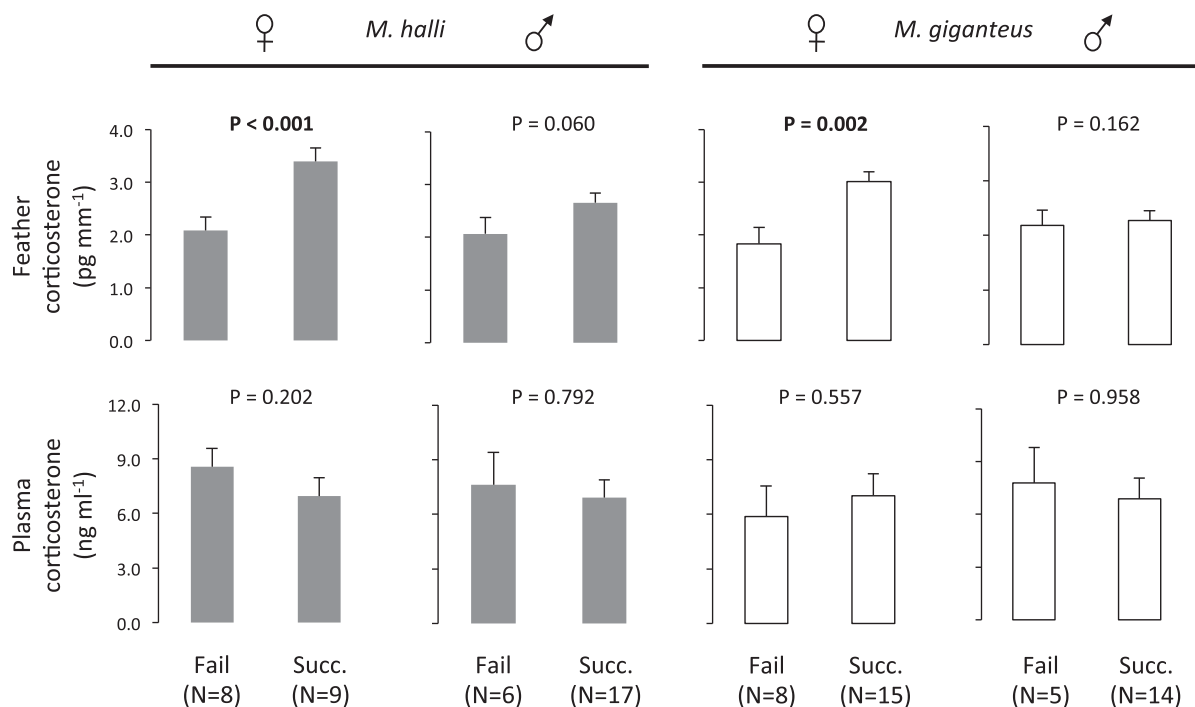


Fig. 2. Feather and baseline plasma corticosterone concentrations in male and female northern and southern giant petrels sampled late in the breeding season, according to breeding outcome (failed or successful). Columns represent least squares means which account for allometric variation in body mass (ANCOVA). Error bars signify + SEM. Statistical significance was assessed at $\alpha = 0.05$.

effort in especially northern males was marginal ($P = 0.060$, Fig. 2), so we cannot say with certainty that fCort is not linked to or correlated with their breeding fate. Nevertheless, given what we know about the positive role of elevated baseline Cort on foraging behaviour and parental care in birds (Bonier et al., 2009a,b, 2011; Crossin et al., 2012a), we propose that the observed differences in fCort reflect differences in parental effort. Direct telemetric measurement of foraging behaviour and estimates of activity budgets, and sampling for fCort, would be needed to explore causal relationships (Angelier et al., 2008, 2009; Crossin et al., 2012a).

Another unusual feature of giant petrel ecology that may have bearing on our results is their breeding-moult overlap (Crossin et al., 2012b). Breeding and moult are both assumed to be energetically expensive (Murphy and Taruscio, 1995; Schieltz and Murphy,

1995), and when moult is extensive and occurring simultaneously with reproductive activity, a trade-off between adult somatic maintenance and parental care is predicted, which could potentially reduce chick provisioning rates, chick fledging mass, or the number of chicks fledged (Morton and Morton, 1990; Svensson and Nilsson, 1997). Although, some studies have shown little to no effect of moult status on current breeding effort (Hemborg and Merilä, 1998; Morales et al., 2007; Siikamäki, 1998), Hemborg (1999) suggested that in breeding-moult overlap species, where males initiate moult before females, females must increase reproductive effort to compensate for the reduced parental care provided by their moulting partner. Coupled with the routinely higher foraging effort required of female birds, we might anticipate longer-term costs of breeding-moult overlap for females which

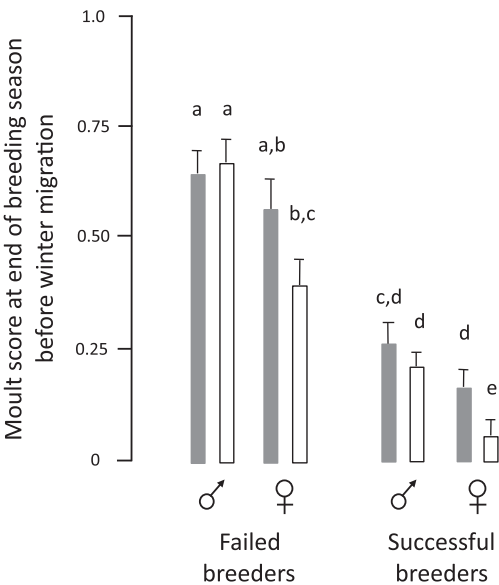


Fig. 3. Proportion of total primary flight feather moult in northern (gray bars) and southern (white bars) giant petrels completed by the end of the breeding season prior to winter migrations at sea. Moult scores were collected late in the breeding season, and data are grouped by breeding outcome and sex. Bars signify least squares means + SEM. Differing letters indicate statistically significant contrasts ($\alpha = 0.05$).

would explain why, in this study, fCort was related to breeding success in females but not males. However, we must caution again that the lack of relationship in males was only marginally non-significant.

Cort secretion appeared to have some cost to giant petrels, given the negative correlation between fCort and body mass in both males and females that we observed. Despite this apparent cost, there were no significant differences in body mass between failed and successful individuals, so the relationship between fCort and body mass might indicate a commitment to current reproduction but without significant consequence for subsequent survival (Golet

et al., 1998). Indeed, fCort was not related to overwinter survival in giant petrels in this study. This contrasts with Koren et al. (2011) who showed a link between fCort and winter survival in house sparrows, but sparrows are of course much smaller and shorter-lived than seabirds. We did however document significant association between fCort levels during current breeding with future reproductive effort (breeding decision), such that high fCort in successfully breeding southern giant petrel females increased the probability for deferred breeding in the next year. This suggests that the Cort up-regulation in support of current reproductive effort exacts a cost on future effort.

There is an intuitive appeal to the idea that stress, or the mechanisms underlying responses to stress, can have negative effects on reproduction, and there is no shortage of studies that document negative relationships between circulating Cort and the likelihood of breeding successfully. The increased likelihood for deferred future breeding when fCort is high could be interpreted as a potential “stress” effect. Certainly over-stimulation of the Cort-response due to chronic stress can have long-term costs to reproduction (Romero et al., 2009). In brooding king penguins *Aptenodytes patagonicus* for example, plasma Cort was 2- to 4-fold higher in individuals that abandoned eggs or chicks compared with those who were relieved by their partners (Groscolas et al., 2008). However, barring chronic over-stimulation, an appropriate or adaptive physiological stress response should allow an individual to cope with challenges over time without breaching a threshold level beyond which deleterious effects occur (Crossin et al., 2012a; Hau et al., 2010; Love et al., 2004). Labeling Cort as a “stress hormone” is thus overly simplistic, and obscures its essential role in regulating metabolism, activity levels, and energy use (Buttemer et al., 1991; Landys et al., 2006). We do not interpret the relationship between high fCort levels and deferred future breeding to be a stress effect. We suggest that there is an interaction between Cort levels, feather quality (Langston and Rohwer, 1996; Rohwer et al., 2011), and the timing of moult that underlies the deferred breeding of southern giant petrel females.

To summarize, our data support a role of Cort secretion in reproductive effort, and support predictions from the corticosterone-adaptation hypothesis (*sensu* Bonier et al., 2009a). Successful breeding in female giant petrel was associated with high fCort lev-

Table 3
Nominal logistic regression models examining relationships between metrics of current reproductive effort in 2008/09 (success or failure, feather corticosterone levels, and moult score), and the probability of breeding deferral in the subsequent year (2009/10). A highest order model that included species and sex as main effects was initially run, and both variables were significant (see Results). Based on this, and on known differences in life-history, physiology, and pattern of breeding activity in giant petrels, final models were run independently for each species by sex combination, and are presented here. Significant effects are indicated by bold text. Note that interaction terms were non-significant in all models and were thus removed from the final models to increase statistical power.

Species and sex	Effects	n	χ^2	LR χ^2	P
<i>M. halli</i> Females	Whole model	16		Inestimable ^a	
	Current breeding outcome				
	Feather corticosterone				
	Moult score				
Males	Whole model	18	4.067	<0.001	0.254
	Current breeding outcome				0.991
	Feather corticosterone				0.230
	Moult score				0.426
<i>M. giganteus</i> Females	Whole model	19	13.143		0.004
	Current breeding outcome			1.694	0.193
	Feather corticosterone			8.177	0.004
	Moult score			4.035	0.045
Males	Whole model	16	9.543		0.023
	Current breeding outcome			5.813	0.016
	Feather corticosterone			0.535	0.465
	Moult score			0.876	0.349

^a All surviving female northern giant petrels sampled in 2008/09 bred again in 2009/10, i.e., none deferred and so there was no variation in breeding outcome.

els, which we've interpreted as a commitment to, and an increase in, current breeding effort compared to failed females. This commitment had presumed costs in the form of delayed moult, which for the late breeding southern species requires that they replace the majority of new feathers at sea during the non-breeding, migration season, which contrasts with the earlier breeding northern species. Coupled with a possible negative effect of elevated fCort on feather quality, this could lead to increases in flying effort and to energetic shortfalls in southern females that result in future breeding deferral (Langston and Rohwer, 1996; Rohwer et al., 2011). High fCort, reduced body mass, and slower moult may thus influence the decision to breed in the following year. Because many of these variables are correlated, our models examining the relationships with future breeding should be interpreted with some caution, especially as the relatively low samples sizes (Table 3) may limit our ability to determine whether it is only one variable that exerts the most influence on future breeding effort (e.g. fCort), or whether there is a synergistic effect of a suite of variables. Future work would benefit from experimental manipulations of Cort, repeated fCort and plasma Cort measurements, and detailed (e.g., telemetric) observation of current and future breeding effort.

Acknowledgments

We thank Stacey Adlard, Fabrice Le Bouard, Ruth Brown, Kristen Gorman, and Andy Wood for assistance. Financial support was provided by the British Antarctic Survey through their Antarctic Funding Initiative, by a National Science and Engineering Research Council (Canada) Post-doctoral Fellowship and E-BIRD funding to GTC, by a NSERC Discovery Grant to TDW, and by a National Science Foundation (U.S.) grant (IOS-1048529) to LMR. GTC was also supported by an Antarctic Science Bursary.

References

- Angelier, F., Bost, C.A., Giraudeau, M., Bouteloup, G., Dano, S., Chastel, O., 2008. Corticosterone and foraging behavior in a diving seabird: the Adélie penguin, *Pygoscelis adeliae*. *Gen. Comp. Endocrinol.* 156, 134–144.
- Angelier, F., Giraudeau, M., Bost, C.A., Le Bouard, F., Chastel, O., 2009. Are stress hormone levels a good proxy for foraging success? An experiment with king penguins, *Aptenodytes patagonicus*. *J. Exp. Biol.* 212, 2824–2829.
- Blas, J., Bortolotti, G.R., Tella, J.L., Baos, R., Marchant, T.A., 2007. Stress response during development predicts fitness in a wild, long lived vertebrate. *Proc. Natl. Acad. Sci. USA* 104, 8880–8884.
- Bonier, F., Martin, P.R., Moore, I.T., Wingfield, J.C., 2009a. Do baseline glucocorticoids predict fitness? *Trends Ecol. Evol.* 24, 634–642.
- Bonier, F., Moore, I.T., Martin, P.R., Robertson, R.J., 2009b. The relationship between fitness and baseline glucocorticoids in a passerine bird. *Gen. Comp. Endocrinol.* 163, 208–213.
- Bonier, F., Moore, I.T., Robertson, R.J., 2011. The stress of parenthood? Increased glucocorticoids in birds with experimentally enlarged broods. *Biol. Lett.* 7, 944–946.
- Bortolotti, G.R., Marchant, T.A., Blas, J., German, T., 2008. Corticosterone in feathers is a long-term integrated measure of avian stress physiology. *Funct. Ecol.* 22, 494–500.
- Bortolotti, G.R., Marchant, T.A., Blas, J., Cabezas, S., 2009. Tracking stress: localisation, deposition and stability of corticosterone in feathers. *J. Exp. Biol.* 212, 1477–1482.
- Breuner, C.W., Patterson, S.H., Hahn, T.P., 2008. In search of relationships between the acute adrenocortical response and fitness. *Gen. Comp. Endocrinol.* 157, 288–295.
- Bridge, E.S., 2006. Influences of morphology and behavior on wing-moult strategies in seabirds. *Mar. Ornithol.* 34, 7–19.
- Brooke, M., 2004. *Albatrosses and Petrels Across the World*. Oxford University Press, Oxford.
- Buttemer, W.A., Astheimer, L.B., Wingfield, J.C., 1991. The effect of corticosterone on standard metabolic rates of small passerine birds. *J. Comp. Physiol. B* 161, 427–431.
- Crossin, G.T., Trathan, P.N., Phillips, R.A., Gorman, K.B., Dawson, A., Sakamoto, K.Q., Williams, T.D., 2012a. Corticosterone predicts foraging behaviour and parental care in macaroni penguins. *Am. Nat.* 182, E31–E41.
- Crossin, G.T., Dawson, A., Phillips, R.A., Trathan, P.N., Gorman, K.B., Adlard, S., Williams, T.D., 2012b. Seasonal patterns of prolactin and corticosterone secretion in an Antarctic seabird that moults during reproduction. *Gen. Comp. Endocrinol.* 175, 74–81.
- Dawson, A., Newton, I., 2004. Use and validation of a moult score index corrected for primary-feather mass. *Auk* 121, 372–379.
- DesRochers, D.W., Reed, J.M., Awerman, J., Kluge, J.A., Wilkinson, J., van Griethuisen, L.I., Aman, J., Romero, L.M., 2009. Exogenous and endogenous corticosterone alter feather quality. *Comp. Biochem. Physiol. A* 152, 46–52.
- Fairhurst, G.D., Navarro, J., González-Solis, J., Marchant, T.A., Bortolotti, G.R., 2012. Feather corticosterone of a nestling seabird reveals consequences of sex-specific parental investment. *Proc. R. Soc. B* 279, 177–184.
- Foote, C.G., Daunt, F., González-Solis, J., Nasir, L., Phillips, R.A., Monaghan, P., 2011. Individual state and survival prospects: age, sex and telomere length in a long-lived seabird. *Behav. Ecol.* 22, 156–161.
- Fourie, N.H., Bernstein, R.M., 2011. Hair cortisol levels track phylogenetic and age related differences in hypothalamic–pituitary–adrenal (HPA) axis activity in non-human primates. *Gen. Comp. Endocrinol.* 174, 150–155.
- Golet, G.H., Irons, D.B., Estes, J.A., 1998. Survival costs of chick rearing in black legged kittiwakes. *J. Anim. Ecol.* 67, 827–841.
- González-Solis, J., Croxall, J.P., Afanasyev, V., 2008. Offshore spatial segregation in giant petrels *Macronectes* spp.: differences between species, sexes and seasons. *Aquat. Conserv. Mar. Freshwater Ecosyst.* 17, S22–S36.
- Groscolas, R., Lacroix, A., Robin, J.-P., 2008. Spontaneous egg or chick abandonment in energy-depleted king penguins: a role for corticosterone and prolactin? *Horm. Behav.* 53, 51–60.
- Hau, M., Beebe, K., 2011. Plastic endocrine regulation of year-round territorial aggression in tropical male spotted antbirds. *Gen. Comp. Endocrinol.* 172, 305–313.
- 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* 277, 3203–3212.
- Hemborg, C., 1999. Sexual differences in moult-breeding overlap and female reproductive costs in pied flycatchers, *Ficedula hypoleuca*. *J. Anim. Ecol.* 68, 429–436.
- Hemborg, C., Merilä, J., 1998. A sexual conflict in collared flycatchers, *Ficedula albicollis*: early male moult reduces female fitness. *Proc. R. Soc. B* 265, 2003–2007.
- Hunter, S., 1984a. Moult of the giant petrels *Macronectes halli* and *M. giganteus* at South Georgia. *Ibis* 126, 119–132.
- Hunter, S., 1984b. Breeding biology, population dynamics of giant petrels *Macronectes* at South Georgia (Aves: Procellariiformes). *J. Zool. (London)* 203, 441–460.
- Koren, L., Nakagawa, S., Burke, T., Soma, K.K., Wynne-Edwards, K.E., Geffen, E., 2011. Non-breeding feather concentrations of testosterone, corticosterone and cortisol are associated with subsequent survival in wild house sparrows. *Proc. R. Soc. B* 279, 1560–1566.
- Kouwenberg, A.-L., Hipfner, J.M., McKay, D.W., Storey, A.E., 2013. Corticosterone and stable isotopes in feathers predict egg size in Atlantic puffins. *Ibis* 155, 413–418.
- Landys, M.M., Ramenofsky, M., Wingfield, J.C., 2006. Actions of glucocorticoids at a seasonal baseline as compared to stress-related levels in the regulation of periodic life processes. *Gen. Comp. Endocrinol.* 148, 132–149.
- Langston, N.E., Rohwer, S., 1996. Molt-breeding tradeoffs in albatrosses: life history implications for big birds. *Oikos* 76, 498–510.
- Lattin, C.R., Reed, J.M., DesRochers, D.W., Romero, L.M., 2011. Elevated corticosterone in feathers correlates with corticosterone-induced decreased feather quality: a validation study. *J. Avian Biol.* 42, 247–252.
- Love, O.P., Breuner, C.W., Vézina, F., Williams, T.D., 2004. Mediation of a corticosterone-induced reproductive conflict. *Horm. Behav.* 46, 59–65.
- Macbeth, B.J., Cattet, M.R.L., Stenhouse, G.B., Gibeau, M.L., Janz, D.M., 2010. Hair cortisol concentration as a noninvasive measure of long-term stress in free-ranging grizzly bears (*Ursus arctos*): considerations with implications for other wildlife. *Can. J. Zool.* 88, 935–949.
- MacDougall-Shackleton, S.A., Dindia, L., Newman, A.E.M., Potvin, D.A., Stewart, K.A., MacDougall-Shackleton, E.A., 2009. Stress, song and survival in sparrows. *Biol. Lett.* 5, 746–748.
- Morales, J., Moreno, J., Merino, S., Sanz, J.J., Tomas, G., Arriero, E., Lobato, E., Martínez-de la Puente, J., 2007. Early moult improves local survival and reduces reproductive output in female pied flycatchers. *Ecoscience* 14, 31–39.
- Morton, G.A., Morton, M.L., 1990. Dynamics of postnuptial molt in free-living mountain white-crowned sparrows. *Condor* 92, 813–828.
- Murphy, M.E., Taruscio, T.G., 1995. Sparrows increase their rates of tissue and whole-body protein synthesis during the annual molt. *Comp. Biochem. Physiol.* A 111, 385–396.
- Phillips, R.A., McGill, R.A., Dawson, D.A., Bearhop, S., 2011. Sexual segregation in distribution, diet and trophic level of seabirds: insights from stable isotope analysis. *Mar. Biol.* 158, 2199–2208.
- Rohwer, S., Viggiano, A., Marzluff, J.M., 2011. Reciprocal tradeoffs between moult and breeding in albatrosses. *Condor* 113, 61–70.
- Romero, L.M., Wikelski, M., 2001. Corticosterone levels predict survival probabilities of Galapagos marine iguanas during El Niño events. *Proc. Natl. Acad. Sci. USA* 98, 7366–7370.
- Romero, L.M., Strohlic, D., Wingfield, J.C., 2005. Corticosterone inhibits feather growth: potential mechanism explaining seasonal down regulation of corticosterone during molt. *Comp. Biochem. Physiol. A* 142, 65–73.
- Romero, L.M., Dickens, M.J., Cyr, N.E., 2009. The reactive scope model: a new model integrating homeostasis, allostasis, and stress. *Horm. Behav.* 55, 375–389.
- Schieltz, P.C., Murphy, M.E., 1995. Diurnal variation in oxygen consumption by molting and nonmolting sparrows. *Comp. Biochem. Physiol.* A 112, 265–272.
- Siikamäki, P., 1998. Limitation of reproductive success by food availability and breeding time in pied flycatchers. *Ecology* 79, 1789–1796.

- Stearns, S.C., 1972. Life-history tactics: a review of the ideas. *Q. Rev. Biol.* 51, 3–47.
- Strochlic, D., Romero, L.M., 2008. The effects of chronic psychological and physical stress on feather replacement in European starlings (*Sturnus vulgaris*). *Comp. Biochem. Physiol. A* 149, 68–79.
- Svensson, E., Nilsson, J.-A., 1997. The trade-off between molt and parental care: a sexual conflict in the blue tit? *Behav. Ecol.* 8, 92–98.
- Wingfield, J.C., Vleck, C.M., Moore, M.C., 1992. Seasonal changes in the adrenocortical response to stress in birds of the Sonoran desert. *J. Exp. Zool.* 264, 419–428.
- Wingfield, J.C., Breuner, C.W., Honey, P., Jacobs, J., Lynn, S., Maney, D., Ramenofsky, M., Richardson, R., 1998. Ecological bases of hormone-behavior interactions: the “emergency life history stage”. *Am. Zool.* 38, 191–206.