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PHYSIOLOGICAL ECOLOGY - ORIGINAL RESEARCH

An evaluation of feather corticosterone as a biomarker of fitness and an ecologically relevant stressor during breeding in the wild

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Abstract Feather corticosterone (CORT) levels are increasingly employed as biomarkers of environmental stress. However, it is unclear if feather CORT levels reflect stress and/or workload in the wild. We investigated whether feather CORT represents a biomarker of environmental stress and reproductive effort in tree swallows (*Tachycineta bicolor*). Specifically, we examined whether individual state and investment during reproduction could predict feather CORT levels in subsequently moulted feathers and whether those levels could predict future survival and reproductive success. Through a manipulation of flight cost during breeding, we also investigated whether an increase in stress level would be reflected in subsequently grown feathers, and whether those levels could predict future success. We found that CORT levels of feathers grown during moult did not (1) reflect past breeding experience ($n = 29$), (2) predict reproductive output ($n = 18$), or (3) respond to a manipulation of flight effort during reproduction (10 experimental, 14 control females). While higher feather CORT levels predicted higher return rate (a proxy for survival), they did so only in the manipulated group ($n = 36$), and this relationship was opposite to expected. Overall, our results add to the mixed literature reporting that feather CORT levels can be positively, negatively, or not related to proxies of within-season and longer-term fitness (i.e., carryover effects). In addition, our results indicate that CORT levels or disturbances experienced during one time (e.g., breeding) may not carry over to subsequent stages (e.g., moult). We, therefore, petition for directed research investigating whether feather CORT represents exposure to chronic stress in feathers grown during moult.

Keywords fCORT · Feather clipping · Glucocorticoid · Moult · Tree swallow

Introduction

The fields of ecological and conservation physiology seek to employ physiological biomarkers of health and environmental quality as tools to provide context and mechanism across a variety of applications and taxa (Stevenson et al. 2005). To be relevant for conservation purposes, physiological biomarkers must be sensitive enough to accurately reflect an individual's capacity to manage daily challenges, while also providing the mechanistic capacity to causally predict how organisms will respond to future increases in these challenges (Cooke and O'Connor 2010). In addition, a biomarker will be most useful if it reflects disturbance in advance of traditional demographic measures, providing researchers with the predictive capacity necessary to proactively manage threats to wildlife of interest (Carey 2005; Wikelski and Cooke 2006; Ellis et al. 2011). Although a number of physiological traits have been proposed and studied, the concept and measurements of 'stress' are some of the most heavily investigated because of their ability to incorporate the balance between an organism's energy resources and demands (McEwen and Wingfield 2010; Dantzer et al. 2014; Madliger and Love 2014). In particular, the measurement of glucocorticoid (GC) hormones has long been proposed as an important tool for assessing the current state of individuals and populations given their role

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in regulating homeostasis (Landys et al. 2006; Busch and Hayward 2009).

The method and medium of glucocorticoid sampling also has a large influence on their use and interpretation (Sheriff et al. 2011). For example, the standard quantification of circulating baseline levels of GCs in blood is often difficult to obtain and interpret in free-living species since levels respond rapidly to the capture and handling required to collect samples, and as such are further subject to the organism's experiences in the hours prior to capture (Romero and Reed 2005). A number of alternative sampling media have been developed (e.g., fecal, salivary) to potentially remedy this. Sampling in keratinized outer integuments (i.e., hair and feathers) is an attractive, although poorly understood, emerging technique (Sheriff et al. 2011). Since GC levels in feathers are thought to primarily be the result of deposition during feather growth, it has been proposed that feather GCs should reflect circulating levels over a period of weeks (Bortolotti et al. 2008). If correct, feather GCs could represent a cumulative measure of the challenges faced over this time period, which would make them valuable biomarkers of exposure to chronic environmental stressors (Bortolotti et al. 2008). In addition, feathers provide potential advantages compared with other sampling media because: (1) a relatively large amount of material can be obtained from feathers and, (2) individually identifiable flight and covert feathers make consistent, standardized sampling possible. Finally, feathers are normally only moulted at strict periods and are only vascularized during growth (Howell et al. 2004). Therefore, if deposition is limited to this vascularized period then feather-based measures of corticosterone (CORT) could provide a lasting measure of GC activity of a target period for the life of the integument.

To date, studies have suggested that elevated CORT levels can be detected following a large experimental elevation of circulating CORT during feather growth (Lattin et al. 2011; Fairhurst et al. 2013; Jenni-Eiermann et al. 2015). To complicate matters, these elevated levels can also appear in already grown sections of feather (Lattin et al. 2011), possibly because the vascularized section of feather extends beyond the skin (Maderson et al. 2009; Jenni-Eiermann et al. 2015). However, the mechanism of deposition remains inconclusive since researchers often look for this signal only where they assume it will be deposited and therefore omit feather sections where it is not expected. In addition, manipulated circulating CORT levels are often elevated beyond stress-induced levels and are high enough to cause changes in feather structure (Bortolotti et al. 2009a; DesRochers et al. 2009; Jenni-Eiermann et al. 2015), confounding the understanding of how these levels may relate to typical deposition and feather capacity. Indeed, feather CORT does not appear to reflect the relatively small daily variation in baseline circulating CORT levels (Bortolotti et al. 2008; Fairhurst et al. 2013). This complexity may be due to the instantaneous nature of circulating measures from blood sampling; however, experiments utilizing stressors to increase endogenous CORT also do not generate correlations to feather CORT (Bortolotti et al. 2009b; Fairhurst et al. 2011; Hõrak et al. 2013; Gow and Wiebe 2014a; Lattin et al. 2014; Cruz-Martinez et al. 2015; Patterson et al. 2015). Instead, it may be that only large, prolonged increases in CORT levels are sufficient to be deposited accurately naturally.

Overall, it is currently unclear to what extent feather CORT levels of feathers grown during natural moult reflect past and future success or other disturbances faced by wild birds even though these validations are fundamentally important to using feather CORT as a biomarker of environmental stress. To address this, we assessed whether feather CORT could reflect environmental and reproductive stress in wild tree swallows (*Tachycineta bicolor*) in two ways. First, we examined whether individual state during the breeding season and reproductive investment could predict feather CORT levels in subsequently moulted feathers and whether those feather CORT levels could predict future survival and reproductive success. Second, through a manipulation of increased flight cost during breeding, we experimentally investigated whether a biologically relevant increase in stress level would be reflected in subsequently naturally grown feathers, and whether those levels could, in turn, predict future success. Tree swallows begin moulting flight feathers immediately after (or, in some individuals, during) breeding, making them an excellent candidate for determining how challenges faced during the breeding season may carry over to CORT levels in naturally moulted feathers. In addition, we chose one of the first feathers to be moulted as our target for feather CORT analyses to ensure that the energetic challenge imposed by the clipping manipulation could remain as relevant as possible while the feather was being re-grown.

If feather CORT is indeed a robust and reliable measure of stress, we would predict that integument hormone levels reflect individual state and reproductive investment immediately preceding moult and indicate future capacity to survive and invest in offspring. Further, after a manipulation which raised circulating CORT (Madliger et al. 2015) and increased body mass loss over reproduction (Madliger and Love 2016) in our population, and has been shown to decrease survival to the following year in this species (Winkler and Allen 1995; Ardia and Clotfelter 2006), feather CORT levels should reflect a manipulated female's diminished state, increased costs, and reduced capacity for future fitness. These arguments are particularly imperative when considering the logistical ease of this tool. As many bird species are most accessible during the breeding season, confirming that feathers sampled during this time can reliably be interpreted as a biomarker of disturbance or fitness is of great importance to the refinement of the method.

Materials and methods

Study system

Fieldwork was carried out on a nestbox-breeding population of tree swallows at Ruthven Park National Historic Site and Taquanyah Conservation Area in southern Ontario, Canada. The sites' 175 nestboxes have been scientifically monitored daily through the breeding period (late April to early July) since 2010. We weighed and numbered all eggs on the day of laying, recorded incubation length and nestling hatch date, weighed nestlings at 6 and 12 days post-hatching (i.e., linear phase of post-natal growth: Quinney et al. 1986; McCarty 2001), banded nestlings with a federal aluminum band, and obtained morphological measurements at day 12 post-hatching. We also determined fledging date and success (number of nestlings successfully leaving the nest). We trapped all adult females in their nestboxes to band (Canadian Wildlife Service Permit 10808), weigh, and obtain blood samples from the brachial vein for baseline circulating levels of CORT at 10 days into incubation and again at day 12 of nestling–rearing.

Tree swallows are an ideal candidate for feather CORT biomarker validations as they are a well-studied free-living model species (Jones 2003), meaning that important life history factors such as moult timing and order are understood (Stutchbury and Rohwer 1990). Both prebasic moult and migration occur soon after the breeding season, beginning in July (Stutchbury and Rohwer 1990). Also, their flight feathers are relatively uniform in color, reducing any confounding effects of pigment differences on feather CORT capacity or affinity (Jenni-Eiermann et al. 2015). In addition, they are highly philopatric to their breeding location (Winkler et al. 2004) and respond well to human intrusion and manipulation (Jones 2003). Finally, their widespread distribution and willingness to breed in nestboxes in a variety of habitat types has led to their frequent use in assessing impacts of habitat change, anthropogenic disturbance, and habitat reclamation where the principles and tools of ecological and conservation physiology aspire to assist (Ghilain and Bélisle 2008; Harms et al. 2010; Custer, 2011; Paquette et al. 2013; Cruz-Martinez et al. 2015).

Manipulation of stress and feather collection

In 2011, we conducted a primary feather clipping manipulation (previously validated in this and other passerine species: Winkler and Allen 1995; Ardia and Clotfelter 2006; Love and Williams 2008; Patterson et al. 2011) designed to increase the energetic cost of flight and therefore baseline plasma CORT levels throughout the nestling–rearing stage. All experimental methods were approved by the University of Windsor's Animal Care Committee (AUPP#10-10) and the Canadian Wildlife Service (Permit CA0266). Treatment birds ($n = 36$) had every other primary feather (P8,6,4,2) clipped using scissors at the level of the covert feathers (Winkler and Allen 1995; Ardia and Clotfelter 2006) immediately after being blood sampled and processed just prior to the nestling–rearing period (i.e., at 10 days into incubation). As feathers were clipped rather than plucked, they were not replaced immediately and birds remained handicapped until moult. In the eastern region of North America, over 93% of adult tree swallows begin moult in July, starting with the innermost primary and moving outwards (Stutchbury and Rohwer 1990). Indeed, some birds nesting late in the season (i.e., still raising offspring in early July) begin moult while still provisioning their nestlings (Hussell 1983a; Stutchbury and Rohwer 1990). Females in our colony finish raising offspring in mid-June to early July. As a result, the feather clipping manipulation was expected to continue to influence flight performance and energetic demand while the feather we collected for analysis was being grown during natural moult directly following the breeding season. Control birds ($n = 41$) were blood sampled, handled and processed as above, leaving all primary feathers intact. Treatment females were paired with control females to ensure spatial, temporal, and reproductive investment balance across the manipulation. Second year females (i.e., first-time breeders as identified by plumage colouration; Hussell 1983b) were excluded from the clipping manipulation due to low sample size.

In the following breeding season (2012), we again collected a feather sample from returning birds to assess whether the manipulation of stress in 2011 affected subsequent CORT levels in feathers grown after the breeding season. As the goal in 2012 was to sample rather than induce a handicap, we clipped only the right 2nd primary feather at the level of the coverts following blood sampling. Since tree swallows moult primary feathers from primary 1 outwards (Stutchbury and Rohwer 1990), the 2nd primary represents the first feather from the manipulation to be replaced. To facilitate the maximum number of comparisons between treatments, feather samples were taken from returning birds from both the clipped and control groups. All feathers were stored in brown paper envelopes in a sealed container until lab processing.

Feather preparation and hormone analysis

To remove surface contaminants (Bortolotti et al. 2008), feathers were washed before analysis by immersion and swirling in a 50 mL falcon tube filled with a dilute (1%)

soap solution made from Dawn™ and ultrapure water for 30 s. We then rinsed feathers briefly with ultrapure water. After feathers were allowed to dry overnight, the length of the feather sample was measured with calipers and extracted according to the protocol outlined in Bortolotti et al. (2008). To summarize, feathers were minced into fine pieces using scissors in a weighed glass scintillation vial. Hormone was extracted using 10 mL of HPLC grade methanol. Samples were sonicated for 30 min and then placed in a 50 °C water bath overnight. We separated feather pieces from the extract by vacuum filtration, after which the methanol was evaporated. We reconstituted samples using kit-provided assay buffer and assayed samples using a previously optimized commercial ELISA (Enzo Life Sciences CORT Enzyme Immunoassay—ADI-901-097). Intra- and inter-assay variation was 6.52 and 10.66%, respectively, and all samples were of a similar mass to minimize any potential issues of mass-dependency of the extraction (Lattin et al. 2011).

Statistical analyses

Previous investment as a predictor of feather corticosterone

We first investigated potential sources of inter-individual variation in feather CORT levels in unmanipulated birds. We used a general linear model with measures of individual female state during breeding and reproductive effort from the previous year immediately prior to moult to predict current year feather CORT levels (i.e., those moulted after the previous breeding season and collected during the current reproductive attempt). Individuals were included in this analysis only if state and reproductive data for these birds was available from both the previous and current year, as well as feather CORT levels in the current year ($n = 29$). We looked at relationships across two sets of years (2010–2011, and 2011–2012). Since this first goal was to illuminate correlative relationships in unmanipulated birds, individuals returning in 2012 from the clipping manipulation in 2011 were excluded to avoid any confounding effects. The percent change in female body mass from late incubation to the 12th day of nestling–rearing, baseline circulating plasma CORT levels at peak nestling provisioning (log transformed), clutch initiation date (expressed as days since May 1 and square-root transformed), and total brood mass at day 12 of nestling–rearing were used as measures of individual state and reproductive effort, since they represent energy invested in self-maintenance, GC function before moult, reproductive timing, and energy invested in the clutch, respectively. Feather CORT was log transformed and all other variables were transformed to normality where appropriate. Year was included in the model as a fixed effect. The least significant variables were removed from the model in a backwards stepwise manner ($\alpha = 0.05$).

Feather corticosterone as a predictor of future success

To investigate whether feather CORT levels were indicative of future reproductive capacity or carry-over effects, we evaluated its ability to predict future reproductive success and survival using separate generalized linear models. As above, individuals were only included in these analyses if they were not manipulated ($n = 18$), as clipped individuals may have had their current and future success altered. All individuals used were sampled in 2012 negating the inclusion of year in the models. As a historical record of CORT activity cannot directly affect success, it is likely to have a large number of subtle mechanisms beyond the sample size and scope of this study. We, therefore, focused on ultimate measures of success to limit comparisons. We did not include covariates from the current year (i.e., lay date, clutch size, etc.) as our sample size was more suited to detecting a relationship between feather CORT and success rather than the mechanisms by which it occurs. The relationship between feather CORT measured in the current year and the number of nestlings fledged in that year was assessed using a generalized linear model with a Poisson distribution and log link function. The relationship between feather CORT measured in the current year and survival to the next year was assessed using a generalized linear model with a binomial distribution and logit link function. Survival was defined as the recapture of the individual at any time within the next 2 years subsequent to sampling. As tree swallows are highly philopatric (Winkler et al. 2004) and our study sites represent the largest concentration of nestboxes across a broad spatial area, this local return rate provides a proxy for survival. Of the 18 birds analyzed, 7 individuals returned in subsequent years.

Effort as a predictor of feather corticosterone under energetic challenge

To assess the impact of the feather clipping manipulation on feather CORT, we compared CORT levels in the feathers of clipped birds from 2011 and those of both returning clipped and control birds in 2012 using separate t tests (taking into account repeated measures within individuals where appropriate—see below). Since it was not possible to collect feathers from control individuals in the manipulation year (2011) without compromising their ability to act as controls, a fully balanced repeated-measures approach was not possible. To account for this, we first tested for inter-annual consistency in feather CORT levels by comparing CORT levels in the feathers removed from the clipped females in 2011 to those of returned control birds in 2012 ($n = 36$, 14, respectively). This interpretation is relevant because the manipulation was balanced by lay date and clutch size between the clipped and control group throughout the breeding season, meaning that clipped birds were as similar as possible to controls until the moment of the handicap. We then tested whether the experimental manipulation increased CORT levels in subsequently moulted feathers by comparing CORT levels of feathers collected from clipped individuals in 2011 with those of females returning from the clipping manipulation in 2012. We conducted this analysis at 2 scales by comparing across the 2 years in the entire group ($n = 36$, 10), as well as within individuals using a paired comparison ($n = 10$). Finally, to determine whether manipulated individuals could be differentiated from controls using feather CORT as a biomarker of exposure of stress during reproduction, feather CORT levels of returned clipped females were compared with those of returned control birds ($n = 10$, 14, respectively). Feather CORT values were log transformed to ensure normality and all groups showed equal variance as indicated by a Levene's test.

Feather corticosterone as a predictor of success under energetic challenge

To assess whether CORT deposited into feathers following a prolonged reproductive (energetic) stressor predicted future reproductive investment or survival, we used generalized linear models as above to investigate the effects of the 2011 manipulation on both the number of nestlings fledged in 2012 and subsequent adult survival ($n = 36$). In this case, survival was defined as recapture of the same individual any time within the next 3 years subsequent to sampling. All analyses described herein were performed in JMP 10 (SAS Institute).

Results

Previous investment as a predictor of feather corticosterone

None of the chosen variables representing individual female state, reproductive investment or effort from the previous breeding season predicted feather CORT levels upon return the next year (Table 1). More specifically, baseline CORT levels, lay date, total brood weight, and the change in female body mass over breeding were not significant predictors of feather CORT levels. In addition, none of the variables achieved significance through stepwise removal.

Feather corticosterone as a predictor of future success

Under natural, unmanipulated conditions, CORT levels of feathers collected in the current year from female tree swallows did not predict the number of nestlings fledged in the current breeding season ($n = 18$, $df = 1$, $\chi^2 = 0.85$, $P = 0.36$, 95% CI [−1.56, 4.10]). In addition, CORT levels of feathers collected in the current year (2012) did not predict survival to the following breeding seasons ($n = 18$, $df = 1$, $\chi^2 = 0.65$, $P = 0.42$, 95% CI [−7.00, 20.3]).

Effort as a predictor of feather corticosterone under energetic challenge

Overall, we found no significant difference between the CORT levels of feathers taken for the clipping manipulation in 2011 and the feathers of returning control birds in 2012 ($t(48) = 1.95$, $P = 0.06$; Fig. 1), indicating that feather CORT levels did not differ between years. In addition, the feather clipping manipulation during reproduction in 2011 did not increase the CORT levels of subsequently grown feathers collected in 2012; there was no significant difference between feather CORT levels of feathers from birds clipped in 2011 and feathers from those same birds returning in 2012 both as a group ($t(44) = 1.68$, $P = 0.11$; Fig. 1) or within individuals (i.e., pairwise) ($t(9) = 0.20$, $P = 0.84$; Fig. 2). Importantly, birds which had undergone the manipulation in 2011 could not be differentiated from the rest of the population by feather CORT alone as there was no significant difference between feather CORT levels from returning clipped birds and those of returning control birds in 2012 ($t(22) = 0.11$, $P = 0.91$; Fig. 1).

Table 1 Global model summary for general linear mixed-effects model between current year feather corticosterone levels and previous year measures of circulating corticosterone levels, reproductive effort, and timing in female tree swallows ($n = 29$)

Previous year variable	β	SE	t Ratio	P	95% CI lower, upper
Baseline corticosterone level at provisioning (ng ml^{-1})	−0.01	0.07	−0.16	0.87	−0.15, 0.13
Percent change in female body mass	0.004	0.005	0.68	0.50	−0.01, 0.02
Total brood mass (g)	−0.0004	0.0009	−0.41	0.68	−0.002, 0.001
Clutch initiation date	0.001	0.002	0.63	0.53	−0.003, 0.006
Year	−0.04	0.03	−1.54	0.14	−0.10, 0.01

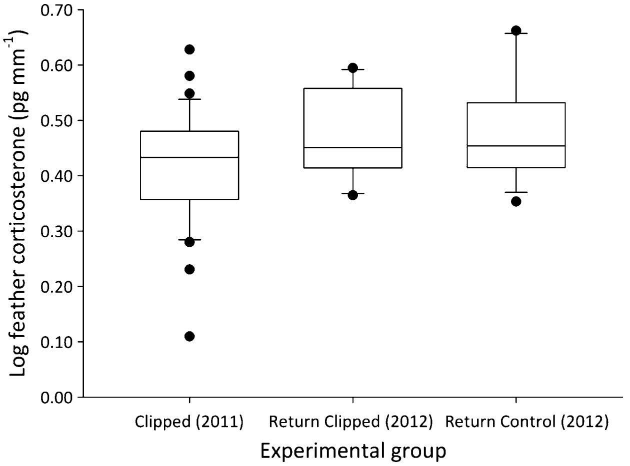

Fig. 1 Feather corticosterone levels in female tree swallows before feather clipping ($n = 36$) and after their return the following year ($n = 10$), as well as levels in control birds (not manipulated) ($n = 14$) after their return the following year

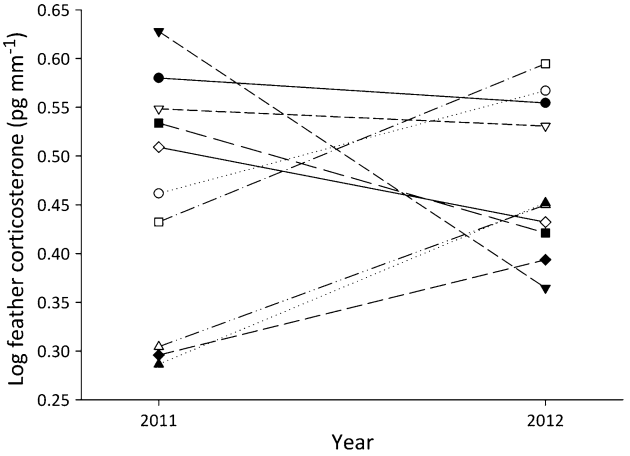

Fig. 2 Individual feather corticosterone response to the feather clipping manipulation ($n = 10$) in female tree swallows. Levels in 2011 are hormone levels from clipped feathers while 2012 levels are those of feathers re-grown during moult following the manipulation

Feather corticosterone as a predictor of success under energetic challenge

The CORT levels of feathers collected in the current year from manipulated females failed to predict the number of offspring fledged in the manipulation season ($n = 36$, $df = 1$, $\chi^2 = 0.27$, $P = 0.60$). In contrast to results from reproductive success and the previous investigation of unmanipulated females, feather CORT levels of feather-clipped birds in 2011 significantly predicted survival following the manipulation year ($n = 36$, $df = 1$, $\chi^2 = 4.70$, $P = 0.03$, $e^\beta = 4.08$; Fig. 3), indicating that manipulated birds with higher feather CORT levels had a higher chance of survival.

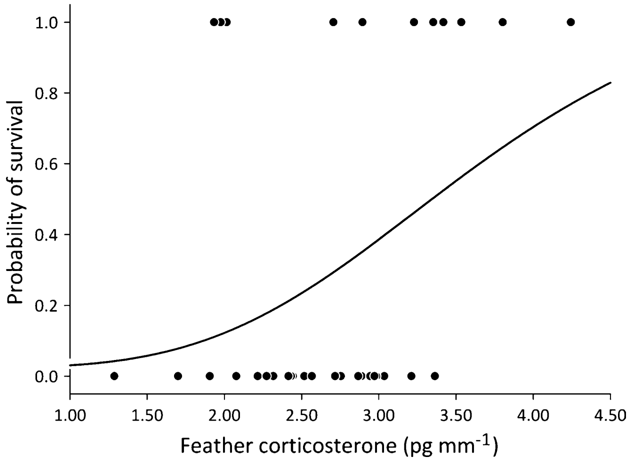

Fig. 3 Survival of female tree swallows to future breeding seasons by current feather corticosterone level ($n = 36$, $df = 1$, $\chi^2 = 4.70$, $P = 0.03$, $e^\beta = 4.08$). The relationship shows probability of survival after clipping of primary flight feathers

Discussion

Feather corticosterone after natural moult

The current understanding of feather CORT deposition hypothesizes that CORT levels in feathers represent an integrative measure of circulating levels over the period of feather growth (Bortolotti et al. 2008). Further, it is thought that these levels remain relatively fixed after the end of vascularization, meaning that researchers would be able to reliably measure CORT in feathers collected any time before the following moult (Bortolotti et al. 2009a). It therefore follows that individual birds undergoing chronic elevated stress immediately prior to moult, or those still working to mitigate carry-over costs at the time of moult, should exhibit elevated CORT levels in subsequently grown feathers. Despite this reasoning, circulating CORT levels in unmanipulated tree swallows measured during the peak of the nestling provisioning stage and female mass loss from incubation to peak provisioning did not predict the CORT levels of subsequently moulted feathers. Moreover, neither the timing of reproduction nor the degree of reproductive effort appeared to impact the CORT levels of subsequently moulted feathers in these birds. Similarly, feather CORT deposited in the feather during the previous moult did not predict reproductive success or survival in the current year in unmanipulated females. It is important to note that our sample size for these final analyses was conservative ($n = 18$) due to sampling constraints and we, therefore, only had the ability to detect relatively strong relationships between feather CORT and current reproductive success and survival. In particular, the 95% confidence interval associated with the survival analysis was wide, indicating that this result should be verified with future study. Nonetheless, to represent a robust biomarker, especially in conservation situations where sample sizes are often constrained and decisions will effect management priorities within the context of limited budgets and manpower, it will be important to validate that strong relationships exist where a larger proportion of the variation in success can be explained by the measurement of a physiological variable such as feather CORT.

Feather corticosterone after energetic challenge before moult

Our feather clipping manipulation, designed to increase energetic workload during an already energetically demanding life history stage (i.e., nestling provisioning), successfully increased circulating CORT levels two weeks post-manipulation, increased female body mass loss, and decreased total brood mass (Madliger et al. 2015; Madliger and Love 2016). Importantly, moult in this species occurs directly following breeding (i.e., in July) and can even begin prior to the cessation of reproduction in late breeders (Hussell 1983a; Stutchbury and Rohwer 1990). In addition, feathers were clipped rather than plucked and the feather used for hormone quantification (primary 2) would have been the first of the clipped feathers to be replaced in the normal moult order (Stutchbury and Rohwer 1990). As a result, this handicap would have persisted in these aerial insectivores throughout the growth of that feather. Finally, other flight feathers (secondaries and rectrices) do not begin to be moulted in this species until after primary 2 (Stutchbury and Rohwer 1990), giving us confidence that only manipulated females would be facing the constraint of reduced wing area at this time.

Despite the energetic constraint, there was no change to CORT levels in subsequently moulted feathers collected from birds returning the following year. Furthermore, a within-female analysis in feather-clipped females failed to detect an increase in feather CORT across years; females undergoing the manipulation the year before could not be distinguished from returning control birds by feather CORT levels (despite fledging the same number of offspring the year before). As with unmanipulated females, feather CORT levels also did not predict reproductive success after a significant manipulation of workload during reproduction. In particular, the significant relationship between feather CORT and survival for the feather-clipped females alone suggests that feather CORT levels may only be useful in predicting susceptibility to mortality after a large-scale change or challenge rather than small-scale environmental variability. Interestingly, and somewhat counter-intuitively (i.e., see Koren et al. 2011), higher feather CORT predicted a higher probability of survival following the challenge. These results suggest two possibilities. First, it is possible that challenges experienced during reproduction may not carry over substantially enough to the subsequent moulting period to be reflected in feather CORT, even in species with a short temporal window between breeding and moult. Alternatively, feather CORT in feathers grown during natural moult may not always respond to elevated stress as expected by the current understanding of deposition; however, feather CORT might be useful to forecast which individuals will be best able to weather a serious challenge.

Sensitivity of feather corticosterone in moulted feathers to a prior ecological stressor

While our feather clipping manipulation caused significant changes in female body mass and circulating CORT, it did not cause increased rates of nest abandonment and females raised a similar number and quality of offspring (Madliger and Love 2016). As such, clipped females did not substantially reduce their investment in the current brood. Furthermore, clipped females returned the following year with higher baseline plasma levels of CORT compared to their control counterparts (Madliger and Love 2016), indicating that the manipulation likely resulted in long-term influences on CORT physiology that were relevant during moult and over-wintering. As such, it is difficult to suggest that our manipulation was not harsh enough to reach the sensitivity required to alter feather CORT as a more drastic manipulation may have reduced fledging success or caused abandonment of reproduction. This lack of response at this level of disturbance suggests that the sensitivity threshold of feather CORT may limit its relevance as a biologically relevant biomarker as it would be unable to indicate stress or disturbance with more sensitivity than demographics. However, we acknowledge that our inability to measure circulating CORT levels during moult limits our capacity to explicitly conclude that feather CORT is insensitive to energetic challenge or environmental disturbance. Future research imposing experimental challenges during moult in the wild will be necessary to confirm this interpretation.

As a group, feather CORT levels should have been higher in birds returning from manipulation if for no reason other than birds that survived generally had higher feather CORT levels. It is possible that with a larger sample size the mean of the distribution could have been increased because low feather CORT birds were more susceptible to death and fewer returned from the manipulation, removing them from the distribution. However, in this scenario, the increased average feather CORT levels would have been a result of selection instead of a response to chronic stress. If researchers simply collected feathers from these birds without knowing anything about the birds' previous history, those higher-level individuals would incorrectly be considered to be of lower individual quality despite their survival in the face of a large unexpected perturbation.

A potential explanation for why feather CORT did not reflect reproductive effort in unmanipulated birds, or change after a large perturbation, could be due to differences between natural moult and induced feather replacement (the primary source for validation of this technique to date; e.g., Bortolotti et al. 2008; Lattin et al. 2011; Jenni-Eiermann et al. 2015). Moult is an energetically demanding period involving the regeneration of a number of tissues and partially compromises flight and thermoregulation (Howell 2010). It is thought that the observed decrease in both baseline and acute levels of circulating CORT at this in comparison to other life history stages is a result of down-regulation of CORT release to minimize the amount of time required to re-grow quality feathers (Romero 2002; Strochlic and Romero 2008). In addition, while exogenous CORT causes slower feather growth rates, it appears to do so differently in natural compared with induced moult (Romero et al. 2005). In contrast, physical and psychological stress can result in much lower increases in circulating CORT levels due to the down-regulation of the hypothalamic–pituitary–adrenal (stress) axis, and only the nutritional stress of food deprivation has been shown to slow feather re-growth rate (Strochlic and Romero 2008). We would, therefore, expect that the moult characteristics and feather CORT levels of the feather-clipped birds should not be different from the controls if clipped birds were still able to find sufficient food for proper feather growth while also suppressing their elevated circulating CORT levels. This possible explanation is particularly relevant to our study as we were unable to observe moult and are, therefore, unable to account for potential modifications of timing or rate of moult in clipped individuals. However, the same compensatory changes would likely occur in any similar use of this technique, meaning that feather CORT would not be useful as a sole biomarker of exposure to stress.

A further possibility is that feather CORT levels under free-living conditions are not as stable over the long term as currently assumed. If feather CORT levels are not stable in the feather once it has been grown, even if feather CORT levels were increased appropriately at moult in the feather-clipped group, the potential increase and the differences between clipped and control females may not persist until sampling during the following breeding season. A related issue would be if feather CORT levels are responsive to changes in stress physiology but the differences caused in the feathers are not great enough to be detected without large sample sizes due to the variation introduced by feather wear, washing, extraction, and assaying. If the effect of these issues was species-specific due to differences in feather structure, pigmentation, and environmental exposure, it would induce even further variation in interpreting feather CORT levels. Finally, feather CORT relationships may also depend on the feather sampled as feathers from other body regions may have different signals or differ in environmental and preening exposure.

Conclusion

We found that feather CORT levels of tree swallow feathers grown during natural moult did not (1) reflect past breeding experience, (2) predict reproductive output, or (3) respond to a significant manipulation of flight effort during the height of reproduction. Furthermore, while high feather CORT levels predicted survival, they did so only in the group undergoing the manipulation and the positive relationship was opposite to what would be expected. These combined results join the growing number of studies reporting that high feather CORT levels can be positively (Kouwenberg et al. 2013; Sild et al. 2014) or negatively (Koren et al. 2011; Gow and Wiebe 2014b) related to proxies of individual state or fitness, or that they do (Crossin et al. 2013) or do not (Bourgeon et al. 2014) represent carry-over effects. Taken together, this growing body of results suggests that feather CORT may be no less context-dependent than measures of circulating levels (Madliger and Love 2014). These results further suggest that the current understanding of deposition and stability of CORT in naturally grown feathers may be inadequate to allow the type of interpretation necessary for use as a biomarker. Without a stronger understanding of how feather CORT relates to circulating CORT and stress, interpretation of high feather CORT as negative is problematic, as even in circulating levels, a “higher is worse” interpretation may not be enough (Dantzer et al. 2014; Madliger and Love 2014). Overall, our results call to attention the need for further validation prior to the use of feather CORT as a simplistic indicator of exposure to chronic stress in feathers of free-living birds (Berk et al. 2016; Harris et al. 2016).

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Author contribution statement CMH conceived and designed the experiments, with input from CLM and OPL. CMH and CLM collected and analyzed the data. CMH wrote the manuscript; CLM and OPL provided editorial advice.

Compliance with ethical standards

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Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval All applicable institutional and/or national guidelines for the care and use of animals were followed (see "Materials and methods" section).

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