

# Assessing baseline stress physiology as an integrator of environmental quality in a wild avian population: Implications for use as a conservation biomarker



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## ABSTRACT

Stress hormones (i.e., glucocorticoids such as corticosterone and cortisol) have been widely proposed as biomarkers of habitat quality and disturbance. However, there is growing evidence that baseline glucocorticoid (GC) levels are highly context-dependent, potentially confounding their utility for inferring population-level disturbance depending on the life history stage and the duration, severity, and type of environmental change being measured. Determining which aspects of an organism's environment are consistently reflected by baseline GC levels is therefore of paramount importance to establishing how they may be best suited to conservation monitoring goals. We investigated the relationship between baseline GC levels and three extrinsic (food availability, inter-specific nest competition, intra-specific competition) and two intrinsic (reproductive investment, body condition) environmental contexts in breeding female tree swallows (*Tachycineta bicolor*) at two reproductive stages. We combined this with a manipulation of energetic demand (i.e., a decrease in foraging profitability) to determine whether baseline GCs reflect the extrinsic or intrinsic environment when females are faced with an unexpected disturbance. Baseline GC levels were not reflective of any environmental component in control females, regardless of reproductive stage. However, levels increased and were reflective of a decrease in body mass when females were challenged during the offspring provisioning period. Our findings suggest that baseline GCs may not always be indicative of the environmental contexts we associate with variation in habitat quality, particularly when individuals are operating within their expected energetic demand. In a conservation sense, baseline GCs may be more valuable in reflecting unexpected perturbations, which could limit their applicability as sensitive, predictive biomarkers across a diversity of systems.

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## 1. Introduction

The ability to detect anthropogenic disturbances in wildlife populations is of paramount importance to monitoring and conservation management (Nichols and Williams, 2006). As traditional demographic measures are often labor-intensive and require extended time spans to detect population trends, many conservation biologists have begun to employ more sensitive, labile physiological measures to monitor the health and condition of wildlife systems of interest (Cooke et al., 2013; Seebacher and Franklin, 2012 and Wikelski and Cooke, 2006). The growing field of conservation physiology offers many potential traits spanning energetics, immune function, toxicology, reproductive biology and nutrition, each with their own optimal conditions for use and considerations for interpretation (Cooke et al., 2013). However, for a given physiological measure to be a sensitive biomarker, it must

be reflective of the environmental changes that can influence condition, population health, and viability (Cooke and O'Connor, 2010). Glucocorticoids (i.e., cortisol, corticosterone) represent potential biomarkers due to their function in the maintenance of energetic balance (Landys et al., 2006), mediation of life history trade-offs (Crespi et al., 2013), and role in allowing individuals to respond behaviourally to perturbations in their environment (Wingfield, 2013).

Glucocorticoids (GCs) can be measured at baseline and stress-induced levels through blood samples (plasma or serum), and over more integrated time periods in feces and keratinized outer integuments such as hair and feathers (Sheriff et al., 2011). Baseline measures are appealing because they are obtained less invasively when compared with the handling protocol required to achieve stress-induced samples and although still more invasive than fecal collection, blood samples always allow GC levels to be tied unambiguously to individuals and time periods. Perhaps most importantly, baseline GCs have been theoretically viewed as integrators of an individual's internal and external environment (Fig. 1) due to their role in maintenance of energetic balance through the promotion of foraging and the mobilization of stored

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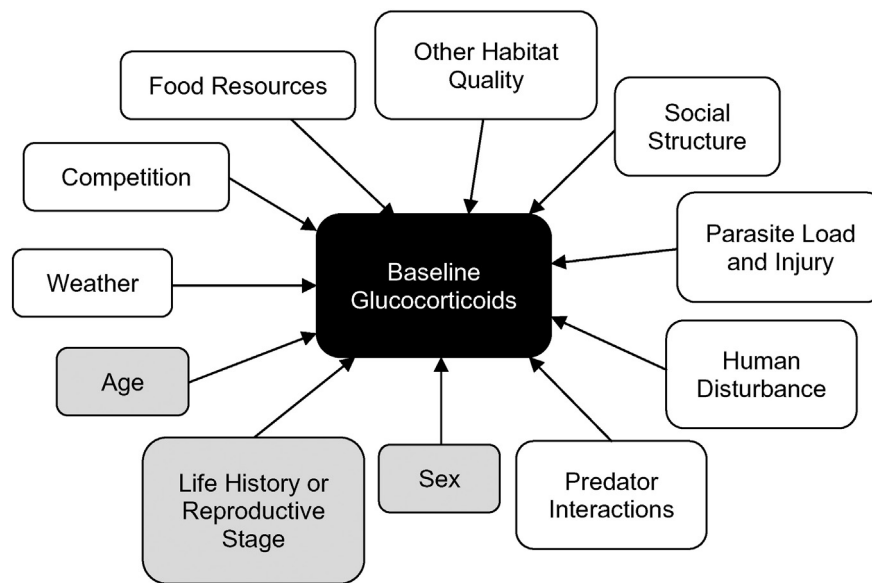


Fig. 1. Intrinsic (gray) and extrinsic (white) environmental variables expected to influence baseline glucocorticoid levels.

energy reserves (Dantzer et al., 2014; McEwen and Wingfield, 2010, and Shultz and Kitaysky, 2008). We would therefore expect an observable increase in baseline GC levels with any change in the environment that necessitates increased energetic expenditure or decreased access to food resources (i.e., increased energy expense or allostatic load; McEwen and Wingfield, 2010). Indeed, more energetically demanding life history stages are characterized by increased baseline GC levels (Romero, 2002), and on a finer temporal scale, more demanding stages of reproduction have been associated with higher baseline GC levels (e.g., Bonier et al., 2009; Reedy et al., 2014 and Rubenstein and Wikelski, 2005).

Baseline GCs have also been shown to be representative of a variety of conservation-relevant variables (Busch and Hayward, 2009) including vegetation cover (Bauer et al., 2013; Janin et al., 2012 and Stabach et al., 2015), parasite load (Bauer et al., 2013), urbanization (Bonier, 2012 and French et al., 2008), predation pressure (Clinchy et al., 2011), forestry practices (Leshyk et al., 2012), pollution (Nordstad et al., 2012), traffic intensity (Strasser and Heath, 2013), tourism (French et al., 2010) and food availability (Fokidis et al., 2012). However, whether and in which direction GC levels change has not been consistent despite the predominant assumption that any disturbance will lead to an increase in GC levels (Bonier et al., 2011; Dickens and Romero, 2013 and Madliger and Love, 2014, 2015). As a result, baseline GC levels may respond to environmental variation in a context-dependent manner that necessitates the careful consideration of underlying reproductive, demographic, or conditional parameters (Madliger and Love, 2014).

Investigating multiple aspects of environmental quality (both internal and external) may help to determine which components most sensitively correlate to GC levels. Unfortunately, few studies have investigated how multiple components of environmental quality may influence baseline GC levels simultaneously, particularly across gradients (Bauer et al., 2013; Grunst et al., 2014; Strasser and Heath, 2013 and Zhang et al., 2011). In addition, we currently have limited information on how baseline GCs may integrate environmental contexts differently depending on reproductive stage. This is particularly important given that timing of sampling could be highly relevant when considering GCs as physiological biomarkers since even short time periods (e.g., those separating different stages of reproduction) can have profound influences on underlying baseline GC levels (e.g., Pereyra and Wingfield, 2003; Kern et al., 2005; Goymann et al., 2006; Rector et al., 2012 and Williams et al., 2008). From a practical perspective, this type of information is

necessary for conservation managers to determine whether certain time periods may be better-suited to the sampling of GCs, or whether contexts that vary within a population (e.g., reproductive status) could influence the ability of GCs to represent disturbances or environmental quality.

We combined three years of reproductive monitoring data, an experimental manipulation of energetic demand, and an assessment of multiple components of habitat quality to determine whether baseline GC levels represent a relevant biomarker of the intrinsic state and extrinsic environmental quality experienced by breeding female tree swallows (*Tachycineta bicolor*). Tree swallows are a member of the aerial insectivore guild which has been experiencing dramatic population declines in North America (Nebel et al., 2010); as a result, investigating how stress physiology relates to underlying variation in body condition and habitat quality can also contribute to our understanding of how future changes may influence this species and others in the guild. We specifically focused on two reproductive stages that differ in their parental energetic demand (Tatner and Bryant, 1993): incubation (lower demand) and offspring provisioning (higher demand). We chose environmental variables that represent major extrinsic and intrinsic factors that individuals of this species would experience during reproduction (Table 1), and that would therefore be expected to influence overall energetic management through changes in activity level or body reserves: 1) food availability; 2) inter-specific nest competition; 3) intra-specific nest competition; 4) reproductive investment (i.e., clutch size and brood mass); and 5) intrinsic state (i.e., body condition). We also experimentally increased energetic demand through feather clipping to test whether baseline GC levels are responsive to, and differentially influenced by, these environmental contexts when individuals are faced with an unexpected and prolonged disturbance while raising offspring (i.e., a decrease in foraging profitability and therefore the overall quality of their environment). Importantly, our manipulation forced individuals outside of preferred (optimal) investment decisions, but not past their capacity to successfully raise offspring. If baseline GCs are to be used as conservation-relevant biomarkers, we would predict that levels would be correlated with intrinsic and extrinsic environmental factors at both stages of reproduction. We also predicted that due to an increase in energetic demand (Tatner and Bryant, 1993), baseline GC levels would increase over the reproductive period, and levels of birds facing an unexpected decrease in environmental quality (feather clipping) would be elevated in comparison to control individuals.

**Table 1**

Descriptions of extrinsic and intrinsic environmental variables measured at incubation and offspring provisioning stages. Abbreviations for each variable used in AIC models are provided.

Environmental context	AIC model abbreviation	Description	Incubation stage		Nestling provisioning stage	
			Range		Range	
Intra-specific competition	<i>Density</i>	Number of tree swallow pairs within 200 m radius	7–29		Number of tree swallow pairs within 200 m radius	7–29
Nest site competition	<i>Disturb</i>	Distance to wooded area (forest or hedgerow) (m)	0–145		Distance to building (m)	20–604
Food availability	<i>Food</i>	Average insect biomass over incubation period (mg)	7.6–71.3		Average insect biomass over days 5–10 of chick rearing (mg)	7.9–154.3
Investment	<i>Invest</i>	Clutch size (number of eggs)	1–8		Brood mass (total mass of nestlings at day 12) (g)	19.7–147.9
Intrinsic state	<i>State</i>	Size-corrected body mass at blood sampling (g)	0.17–0.23		Percent loss in body mass over chick-rearing	3.9–27.1

## 2. Materials and methods

### 2.1. Study site

Our study was completed between April and July of 2010–2012 in a wild population of nest box-breeding tree swallows in Haldimand County, Ontario, Canada. Tree swallows represent an ideal study species for this investigation as they are easily accessible, settle in a variety of habitat types, and have been well-studied in the context of reproductive biology and ecological requirements (Jones, 2003). Our study area consists of 175 nest boxes in the Grand River watershed within Ruthven Park National Historic Site (42°58' N, 79°52' W) and Taquanyah Conservation Area (42°57' N, 79°54' W) (approximately four kilometers apart). The study area is a matrix of landuse types including riparian vegetation, fallow and livestock fields, active agricultural fields, Carolinian forest, and wetlands. Boxes are located in lines along roadways and in groups within fallow fields. For this study, we focused on the 96 boxes that were clustered in fallow fields to allow for quantification of food resources (see below). Boxes differed in terms of surrounding landuse type (which has been shown to influence food availability in this species; Paquette et al., 2013), intra-specific density, and distance to features that dictate the presence of primary nest site competitors such as house wrens (*Troglodytes aedon*) and house sparrows (*Passer domesticus*) (Table 1).

### 2.2. Nest monitoring and blood sampling

All experimental methods were approved by the University of Windsor's Animal Care Committee (AUPP #10–10) and the Canadian Wildlife Service (Permit CA 0266). Over the three years, we monitored 292 reproductive attempts of female tree swallows by checking boxes once daily to record the date of the first egg (lay date), mass of each egg on the day it was laid, clutch size, hatching success (number of chicks successfully hatched), nestling mass at six and 12 days post-hatching, and breeding productivity (number of offspring that successfully left the nest box). We focused on adult females because they are the sole incubators and the more accessible sex overall, allowing us to obtain necessary sample sizes for subsequent analyses. We captured females by plugging the nest hole at two time periods during the reproductive season: 10 days after clutch completion (incubation stage) and 12 days post-hatching (peak offspring provisioning stage). At each capture, we obtained a small blood sample from each female representing less than 10% of total blood volume (i.e., <150 µl) in heparinized microcapillary tubes through puncture of the brachial vein. We collected all blood samples between 0800 and 1200 h to control for diel variation in baseline GC levels (i.e., birds had been actively foraging for approximately two hours prior to sampling). We obtained all samples within two minutes of covering the nest hole to ensure sampling of circulating baseline levels (Romero and Reed, 2005). The amount of time required to trap a bird did not correlate with GC levels at either reproductive stage (linear model: incubation:  $F = 1.82, P = 0.07$ ; nestling provisioning:  $F = -0.66, P = 0.42$ ). In addition, we recorded body mass, wing length, and age and gave unbanded birds a numbered aluminum leg band (Canadian Wildlife Service – Permit 10808).

### 2.3. Experimental manipulation

In 2011, we experimentally increased energetic demand via a primary feather-clipping manipulation (as per Ardia and Clotfelter, 2007 and Winkler and Allen, 1995) on a random subset of females ( $n = 33$ ) temporally and spatially matched (i.e., on the same day and within the same site) to controls ( $n = 38$ ). More specifically, we cut off every other primary flight feather (four feathers per wing) at its base during the incubation stage capture (10 days after clutch completion). The manipulation creates an increase in the workload associated with flight and a decrease in foraging profitability in this species (Winkler and Allen, 1995) for the remainder of the breeding season until new feathers are naturally molted in the months following breeding (Stutchbury and Rohwer, 1990). As aerial insectivores, tree swallows catch all of their food resources for self-maintenance and offspring provisioning on the wing (Robertson et al., 1992) so this manipulation causes a decrease in realized habitat quality by increasing the effort required to access food resources. In addition, this handicap (Ardia and Clotfelter, 2007; Hasselquist et al., 2001 and Winkler and Allen, 1995) was anticipated to cause a concomitant increase in baseline GC levels compared to control individuals.

### 2.4. Extrinsic habitat variables

We recorded geographic coordinates for each nest box and completed all spatial calculations in ArcGIS 10.1 (Esri) using a 2010 orthorectified SWOOP (Southwestern Ontario Orthoimagery Project – 30 cm resolution) satellite image as a base layer. We calculated three extrinsic habitat variables to represent intra-specific competition, inter-specific nest site competition, and food availability for each reproductive stage. At both the incubation and offspring provisioning stage, we calculated the number of occupied nest boxes within a 200 m foraging radius (McCarty and Winkler, 1999) for each nest box to represent breeding density. Female tree swallows display territoriality and prefer to nest as far as possible from conspecifics, most likely to decrease intraspecific brood parasitism and limit nest usurpation (Dunn and Hannon, 1991 and Muldal et al., 1985), so we used density as a proxy of intra-specific competition. In addition, we calculated a proxy of inter-specific nest site competition at each reproductive stage. During the incubation stage, tree swallows compete for nest sites with native house wrens and must defend their nest to prevent their eggs from being pierced and removed (Rendell and Robertson, 1990 and Quinn and Holroyd, 1989). As house wrens are associated with the edges of forests and hedgerows (Rendell and Robertson, 1990), we calculated the distance of each box from a wooded edge (forest or hedgerow) to provide a proxy of inter-specific nest site competition (i.e., house wren-associated risk) during incubation. At the nestling provisioning stage, tree swallows face strong competition from non-native house sparrows which can injure or kill adults and nestlings (Robertson et al., 1992 and Robinson, 1927). As house sparrows are associated with human residences and outbuildings such as barns (Summers-Smith, 1963 and Summers-Smith, 1988), we calculated the distance from the nearest building as a proxy for inter-specific nest site competition (i.e., house sparrow-associated risk) during offspring provisioning.

Finally, we quantified the daily availability of flying insects (i.e., the primary food resource of tree swallows; [Hussell and Quinney, 1987](#)) at each reproductive stage. Within each of five grid systems, we placed a centrally located four-sided malaise trap (110 × 110 × 110 cm SLAM traps, MegaView Science Co.), which caught insects passively in ethanol and was changed daily between 1300 and 1700 h. Traps were raised 60 cm above the ground to better quantify a section of the air column frequented by foraging tree swallows ([McCarty and Winkler, 1999](#)). Other passive traps at this height have been used previously to estimate food availability for this species during the breeding season ([Hussell and Quinney, 1987](#); [Mengelkoch et al., 2004](#) and [Paquette et al., 2013](#)). We identified all insects to order, with the exception of Dipterans, which were further classified into sub-order *Nematocera* or *Brachycera* (midges and heavy-bodied flies, respectively) due to their large difference in size and mass. As per [Hussell and Quinney \(1987\)](#), we measured body lengths to place individual insects into 2 mm size categories. Within each order or suborder and each size class a sub-sample of randomly chosen, intact insects were used to determine biomass conversion factors for all other samples. We calculated the combined average daily biomass (mg) of six orders of insects that constitute the majority of tree swallow diet (*Diptera*, *Coleoptera*, *Hymenoptera*, *Hemiptera*, *Tichoptera*, and *Ephemeroptera*; [Quinney and Ankney, 1985](#) and [Johnson and Lombardo, 2000](#)). We limited our calculation based on size category to insects under 10 mm based on previous findings that 99% of prey items are under this length and larger insects heavily bias biomass estimates ([Madliger and Love, unpublished data](#) and [Quinney and Ankney, 1985](#)). For the incubation stage, we calculated the average daily biomass over the 12 days prior to hatching (focal incubation period) for each female (similar to [Nooker et al., 2005](#)). For the nestling provisioning stage, we calculated the average daily biomass for each female over the time when nestlings were 5–10 days old (similar to [Nooker et al., 2005](#)) and therefore in their most demanding and fastest growth phase ([McCarty, 2001](#)) as this should represent an integral food availability period during parental care.

### 2.5. Hormone analysis

Blood samples were stored on ice for up to five hours prior to being centrifuged to separate plasma and then stored at  $-80^{\circ}\text{C}$  until analysis. We determined plasma levels of total baseline corticosterone, the primary GC in birds, in non-extracted plasma using a commercially-available Corticosterone Enzyme-linked Immunoabsorbent Assay (EIA – Assay Designs Inc., Michigan USA, catalog #901-097). We ran samples in triplicate at a total volume of 100  $\mu\text{l}$  with 1:40 dilution and 1.5% steroid displacement buffer. Each assay plate contained a six-point standard curve created by serial dilution from 20 000  $\text{pg}^{-1}\text{ml}$  to 15.63  $\text{pg}^{-1}\text{ml}$  fitted with a four parameter logistic fit ([Love and Williams, 2008](#)). The detection limit of the assay was 0.74  $\text{ng}^{-1}\text{ml}$ , calculated as per the manufacturer's method as the concentration of CORT that was two standard deviations from zero along our standard curves. Of a total of 291 samples, 12 fell below this limit and were therefore assigned the value of the detection limit. Intra-assay variation was 7.7%, 8.0%, and 10.3% in 2010, 2011, and 2012, respectively. Inter-assay variation was 6.7%, 13.3%, and 6.0% in 2010, 2011, and 2012, respectively.

### 2.6. Statistical analyses

We used separate linear mixed-effect models at each stage of reproduction (incubation and offspring provisioning) to determine the relationship between intrinsic and extrinsic environmental variables and baseline GC levels in female tree swallows. We log transformed GC values prior to analysis to achieve normality (GC levels prior to transformation were 0.74–9.47  $\text{ng/ml}$  at incubation and 0.74–12.17  $\text{ng/ml}$  at nestling provisioning). As baseline GC levels did not differ between our five grid sites (ANOVA:  $P > 0.05$ ), we pooled sites in all analyses. We had instances of the same individual being present in our dataset for multiple years and therefore limited our dataset so that each

female was included only once by randomly choosing one year. In addition, we excluded any individuals in their first year of reproduction (i.e., second-year birds) as we did not have a sufficient sample size to analyze this age class separately. This yielded a sample size of 127 females at the incubation stage and 93 females at the nestling provisioning stage across three years (2010–2012). Five environmental (independent), fixed-effect variables were included in each analysis to represent intra-specific competition, inter-specific nest site competition, food availability, reproductive investment, and intrinsic state ([Table 1](#)). None of the variables were highly inter-correlated as Variance Inflation Factors (VIFs) were all less than 1.30 ([O'Brien, 2007](#)). Since we had strong a priori reasons why each of the five environmental variables could correlate with GC levels, and no reason to eliminate any specific combination of variables, we used an all sub-sets approach that yielded 32 models in each analysis. We did not have a priori reasons why any interaction terms would be more biologically relevant than others so to avoid model over-fitting we did not include any interaction effects in our models. We included year as a random effect and relative lay date (individual lay date relative to the intra-annual population mean) as a fixed effect in all models to take into account potential influences of seasonality or individual quality ([Stutchbury and Robertson, 1988](#) and [Winkler and Allen, 1996](#)) on baseline GC levels.

To determine the effect of the clipping manipulation performed in 2011, we compared GC levels of birds assigned to control ( $n = 38$ ) and treatment ( $n = 35$ ) groups prior to manipulation (incubation) using a t-test and after manipulation (offspring provisioning) using an ANCOVA to control for prior (incubation) GC level. We also performed a repeated measures ANCOVA to determine whether the two groups (control and clipped) changed GC levels differently from the incubation to the nestling provisioning stage (i.e., to test for a time × treatment interaction). We used general linear models to assess the influence of intrinsic and extrinsic variables on the GC levels of clipped and control birds separately at the offspring provisioning stage. As in the correlational analyses, five environmental (independent) variables were included ([Table 1](#)) using an all sub-sets approach. We did not detect any collinearity among variables (VIFs < 1.40). Lay date was included as a fixed effect in all models.

For all general linear model analyses, we used the Akaike Information Criterion corrected for small sample size ( $AIC_c$ ) as a basis to perform model selection ([Burnham and Anderson, 2002](#)). We calculated  $AIC_c$ ,  $\Delta AIC_c$  (difference between each model's  $AIC_c$  and that of the lowest model), Akaike weights and cumulative weights for each model ([Burnham and Anderson, 2002](#)). Akaike weights can be viewed as the probability that a given model is the best approximating model to describe the data out of the full candidate set of models ([Symonds and Moussalli, 2011](#)). Cumulative weights allow for the determination of a 95% confidence set of models (i.e., a set of models in which we are 95% certain that the best model among the candidate set of models is included). Model uncertainty occurs when no single model can be identified as best (i.e., the Akaike weight of the top model is < 0.90) ([Burnham and Anderson, 2002](#)). Where this was evident, we used multi-model inference based on the 95% confidence set to obtain model-averaged parameter estimates ( $\beta$ ), unconditional standard errors and 95% confidence intervals ([Burnham and Anderson, 2002](#) and [Johnson and Omland, 2004](#)). Model-averaged  $\beta$ -values and unconditional standard errors are weighted by the Akaike weights of the models in the confidence set. All analyses were completed in JMP 10 (SAS Institute), except for the calculation of marginal and conditional  $R^2$  which was completed in R 3.1.1 (R Development Core Team, 2015) with the rsquared.glm package ([Barton, 2015](#)).

## 3. Results

### 3.1. Natural environmental variability

The top model in our analysis to determine which environmental variables explained variation in baseline GC levels at the incubation



stage included only body mass (*state*) with a model weight of 0.77 (Table 2). However, there was some model uncertainty, with four models comprising the 95% confidence set (Table 2). In other words, we can be 95% certain that a model within this confidence set represents the AIC best model out of the full candidate set. The null model (with lay date as a fixed effect and year as a random effect) was also included in the confidence set. The parameter estimates and unconditional error rates indicate that food availability (*food*) had a very weak positive relationship with GC levels while reproductive investment (*invest*) and body mass (*state*) had poor parameter estimation and 95% confidence intervals that cross zero (Table 3). The marginal and conditional  $R^2$  of the global model were 0.15 and 0.16, respectively. At the nestling provisioning stage, the null model, (with lay date as a fixed effect and year as a random effect), represented the best model with an Akaike weight of 0.98 (Table 2). The marginal and conditional  $R^2$  of the global model were 0.02 and 0.09, respectively.

3.2. Unexpected environmental challenge

Feather clipped females displayed a lower number of foraging trips based on a 1-hour observation period at day 8 or 9 of offspring provisioning as compared to control females while controlling for brood size and date (linear model:  $t_{71} = 2.68, P = 0.009$ ; control (mean  $\pm$  SE) =  $9.7 \pm 0.7$ , clipped =  $6.8 \pm 0.8$ ).

Birds assigned to control and treatment groups did not differ in baseline GC levels prior to the manipulation (t-test, t-ratio = -1.04;  $P = 0.30$ ; Fig. 2). GC levels of control birds and those with experimentally decreased foraging profitability (via feather clipping) responded differently from the incubation to nestling provisioning stage (repeated measures ANCOVA, time $\times$  treatment: t-ratio = 2.95,  $P = 0.004$ ). Specifically, feather clipped birds increased baseline GC levels over the reproductive season and had significantly higher levels of baseline GCs at the nestling provisioning stage compared to control birds (ANCOVA, t-ratio = -2.69;  $P = 0.009$ ; Fig. 2). When determining whether GC levels represented environmental variables in the control group, there was considerable model uncertainty with 21 models included in the 95% confidence set. Only the model with food availability (*food*) as the sole independent variable ranked higher than the null model. However, the  $R^2$  of this model was 0.09 indicating a poor fit to the data overall. In addition, model-averaged unconditional standard errors and 95% confidence intervals of all environmental variables cross zero indicating poor precision in parameter estimation, coinciding with results from the multi-year analysis.

Within the clipped treatment, a single best model could not be resolved; 19 models comprised the 95% confidence set and all five environmental variables were found within the set (Table 4). Model-averaged parameter estimates and unconditional standard errors indicated that *state* (i.e., loss of body mass over the nestling provisioning period) was positively associated with GC levels (Table 5). In addition, the top three best supported models (with  $\Delta AIC_c < 2$ ) all included *state* and displayed  $R^2$  values greater than 0.20, with the top model having

Table 2

Confidence set (95%) of linear mixed-effect models used to predict baseline glucocorticoid levels at the incubation (n = 127) and nestling provisioning (n = 93) stages. All models included year as a random effect and relative lay date as a fixed effect. Variables in each model, number of parameters (K), Akaike Information Criterion adjusted for small sample size ( $AIC_c$ ), difference between each model and the model with the smallest  $AIC_c$  ( $\Delta AIC_c$ ), model weights ( $\omega$ ) and cumulative weights (cum.  $\omega$ ) are provided for each model.

Models	K	$AIC_c$	$\Delta AIC_c$	$\omega$	cum. $\omega$
Incubation					
<i>State</i>	5	47.26	0.00	0.77	0.77
<i>Null</i>	4	51.96	4.70	0.07	0.85
<i>State, food</i>	6	52.11	4.85	0.07	0.92
<i>State, invest</i>	6	53.39	6.12	0.04	0.95
Nestling provisioning					
<i>Null</i>	4	51.79	0.00	0.98	0.98

Table 3

Model-averaged parameter estimates, unconditional standard errors (SE) and 95% confidence intervals from linear mixed-effect models used to predict baseline glucocorticoid levels at the incubation stage. Values were calculated with models included in the confidence set by using Akaike weights as weighting factors (see Materials and methods).

Variable	Estimate	Unconditional SE	95% confidence interval lower, upper
<i>State</i>	-4.21	2.3	-8.72, 0.29
<i>Food</i>	0.005	0.002	0.002, 0.01
<i>Invest</i>	-0.16	0.65	-1.43, 1.11
<i>Lay date</i>	-0.0004	0.003	-0.006, 0.005

an  $R^2$  of 0.27. All other environmental variables had 95% confidence intervals that crossed zero indicating poor parameter estimation and a lack of association with GC levels.

4. Discussion

4.1. Natural environmental variability

Under natural conditions, baseline GC levels did not reflect the external or internal environment at either stage of reproduction (incubation or offspring provisioning), indicating that baseline GCs were not representative of any component of habitat quality, individual condition, or reproductive investment that we measured, regardless of underlying parental demand. We do not believe that this is a consequence of the environmental gradients not representing sufficiently variable conditions, as similar environmental variation has been shown to result in fitness consequences in this species (proximity to wooded areas: Robertson and Jones, 2002; food availability: Ghilain and Bélisle, 2008). Additionally, our study eliminated other factors known to influence baseline GC levels such as sex (Homan et al., 2003; Lormee et al., 2003 and Rector et al., 2012), age (Angelier et al., 2006 and Riechert et al., 2012), reproductive stage (Bonier et al., 2009; Rubenstein and Wikelski, 2005 and Williams et al., 2008), and time of day (Breuner et al., 1999). More importantly, the variables we measured represent a broad assessment of the environmental variation faced by tree swallows during the reproductive season and are comparable to variables that practitioners are able to measure within their wildlife systems. It is possible that unmeasured factors such as parasite load (Bauer et al., 2013; St Juliana et al., 2014 and Raouf et al., 2006) or predatory interactions (Clinchy et al., 2013) or a particularly harsh year with unexpected conditions (e.g., drought, excessive heat,

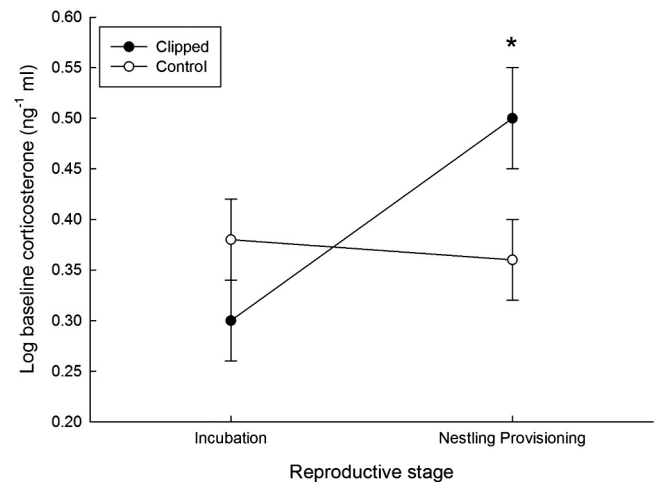


Fig. 2. Baseline glucocorticoid (i.e., corticosterone) levels ( $\pm$  SEM) of individuals in control (n = 38) and clipped (n = 33) treatment groups at the incubation (pre-clipping) and nestling provisioning (post-clipping) stage. Baseline glucocorticoids in clipped birds increased from the incubation to the nestling provisioning stage, while controls remained unchanged.

**Table 4**

Confidence set (95%) of general linear models used to predict baseline glucocorticoid levels at the nestling provisioning stage for clipped ( $n = 33$ ) and control ( $n = 38$ ) treatment groups (2011). All models included relative lay date as a fixed effect. Variables in each model, number of parameters (K), Akaike Information Criterion adjusted for small sample size ( $AIC_c$ ), difference between each model and the model with the smallest  $AIC_c$  ( $\Delta AIC_c$ ), model weights ( $\omega$ ), cumulative weights (cum.  $\omega$ ) and  $R^2$  are provided for each model.

Models	K	$AIC_c$	$\Delta AIC_c$	$\omega$	cum. $\omega$	$R^2$
<b>Clipped</b>						
Density, state	5	-94.00	0.00	0.18	0.18	0.27
State	4	-93.92	0.08	0.17	0.35	0.20
State, disturb	5	-92.89	1.11	0.10	0.45	0.24
State, invest	5	-91.99	2.01	0.06	0.51	0.22
Density, state, disturb	6	-91.90	2.10	0.06	0.58	0.29
Density, state, invest	6	-91.80	2.20	0.06	0.64	0.29
State, food	5	-91.43	2.57	0.05	0.68	0.21
Density, state, food	6	-91.29	2.71	0.05	0.73	0.28
Density	4	-90.90	3.10	0.04	0.77	0.13
State, disturb, invest	6	-90.62	3.38	0.03	0.80	0.26
State, food, disturb	6	-90.03	3.97	0.02	0.83	0.25
Density, state, disturb, invest	7	-89.38	4.62	0.02	0.84	0.31
NULL	3	-89.34	4.66	0.02	0.86	0.01
State, food, invest	6	-89.24	4.76	0.02	0.88	0.23
Density, disturb	5	-89.22	4.79	0.02	0.89	0.16
Disturb	4	-89.05	4.95	0.01	0.91	0.08
Density, state, food, disturb	7	-88.86	5.14	0.01	0.92	0.29
Density, state, food, invest	7	-88.81	5.19	0.01	0.93	0.29
Density, food	5	-88.68	5.32	0.01	0.95	0.14
<b>Control</b>						
Food	4	-89.55	0.00	0.19	0.19	0.09
Null	3	-89.23	0.32	0.16	0.36	0.02
Food, invest	5	-87.23	2.33	0.06	0.42	0.10
State, food	5	-87.11	2.45	0.06	0.47	0.10
Density, food	5	-87.02	2.53	0.05	0.53	0.10
Food, disturb	5	-86.98	2.57	0.05	0.58	0.09
State	4	-86.98	2.57	0.05	0.63	0.03
Invest	4	-86.95	2.60	0.05	0.68	0.03
Density	4	-86.88	2.67	0.05	0.74	0.03
Disturb	4	-86.83	2.72	0.05	0.78	0.02
State, food, invest	6	-84.86	4.70	0.02	0.80	0.11
State, invest	5	-84.76	4.79	0.02	0.82	0.04
Food, disturb, invest	6	-84.50	5.05	0.02	0.84	0.10
Density, food, invest	6	-84.49	5.06	0.02	0.85	0.10
Density, state	5	-84.45	5.10	0.01	0.87	0.03
State, disturb	5	-84.43	5.12	0.01	0.88	0.03
Density, invest	5	-84.40	5.15	0.01	0.90	0.03
Density, state, food	6	-84.39	5.16	0.01	0.91	0.10
Disturb, invest	5	-84.39	5.16	0.01	0.92	0.03
State, food, disturb	6	-84.38	5.17	0.01	0.94	0.10
Density, disturb	5	-84.29	5.26	0.01	0.95	0.03

**Table 5**

Model-averaged parameter estimates, unconditional standard errors (SE) and 95% confidence intervals from linear mixed-effect models predicting baseline glucocorticoid levels in clipped and control treatment females at the nestling provisioning stage. Values were calculated with models included in the confidence set using Akaike weights as weighting factors (see Materials and methods).

Variable	Estimate	Unconditional SE	95% confidence interval lower, upper
<b>Clipped</b>			
Food	-0.0006	0.0010	-0.003, 0.002
Invest	-0.001	0.0010	-0.004, 0.002
State	0.02	0.01	0.002, 0.05
Density	0.01	0.007	-0.003, 0.03
Disturb	0.0004	0.0003	-0.0003, 0.001
Lay date	-0.004	0.008	-0.02, 0.01
<b>Control</b>			
Food	0.003	0.002	-0.0006, 0.007
Invest	-0.0008	0.002	-0.004, 0.003
State	0.006	0.01	-0.019, 0.03
Density	-0.003	0.008	-0.02, 0.01
Disturb	-0.0001	0.0003	-0.0007, 0.0005
Lay date	-0.004	0.007	-0.02, 0.01

excessive rainfall) could result in greater energy requirements and therefore greater responsiveness of GC levels, our findings indicate that baseline GCs did not represent a reliable integrator of body condition and environmental quality under natural variability that is considered relevant for our study species.

It is also possible that the underlying extrinsic and intrinsic variation we measured constitutes a predictable component of an individual's environment to which baseline GC levels are relatively insensitive. During habitat selection, individuals may have established expectations of the features that will be present during the subsequent breeding season and may be able to maintain sufficient intrinsic resources to cope with expected challenges or adjust reproductive decisions accordingly (Doligez et al., 2003; Doligez et al., 2008 and Sih et al., 2011). As a result, small within-season changes in environmental quality may not be sufficient to cause pronounced changes in baseline GC levels, particularly in years with predictable conditions. This could also explain our finding that baseline GC levels did not increase from the incubation stage to the nestling provisioning stage in control females. While it is possible that HPA sensitivity may be modulated (down-regulated) during the most demanding stages of reproduction, allowing individuals to progress through the breeding season despite changes in environmental quality (Holberton and Wingfield, 2003; Love et al., 2004; Wilson and Holberton, 2004 and Wingfield et al., 1995), GC levels may have maintained consistency between stages because they are representative of overall reproductive investment decisions when females are working within expected conditions (Love et al., 2014). Indeed, consistency (i.e., repeatability) in baseline GC levels between incubation and nestling provisioning stages has been shown previously in this species (Ouyang et al., 2011), with differences in GC levels only detectable when brood size is enlarged (Bonier et al., 2011). These findings reinforce that it may be difficult to detect gradual alterations in environmental conditions with baseline GCs in some species.

Our findings are consistent with others that have found insensitivity in baseline GC levels in relation to intrinsic and extrinsic variation. For example, baseline GC levels in western fence lizards (*Sceloporus occidentalis*) did not vary based on temperature, humidity, or condition across a range of sites (Dunlap and Wingfield, 1995). In addition, there is growing evidence that baseline GCs may respond to environmental variation in a context-dependent manner; a recent review by Bonier (2012) indicates that avian responses to urbanization can result in increases, decreases, or no change in baseline GC levels, likely in part due to the differential ability of species to avoid, persist in, or exploit urban conditions. Moreover, Nordstad et al. (2012) found a positive relationship between baseline GC levels and concentrations of polychlorinated biphenyls (PCBs) at the pre-laying, but not other stages of reproduction in black-legged kittiwakes (*Rissa tridactyla*). Similarly, Clinchy et al. (2011) showed that greater predation threat can increase baseline GC levels in male, but not female song sparrows (*Melospiza melodia*). Finally, based on a meta-analysis of laboratory and field studies across diverse species, Dickens and Romero (2013) concluded that a generalized GC profile for chronically stressed wild animals is currently unsupported. Overall, these results indicate that baseline GC levels may respond to environmental variation in a context-dependent manner that necessitates the careful consideration of underlying reproductive, demographic, or conditional parameters (Madliger and Love, 2014), and suggests that there may be limited circumstances when baseline GCs are useful biomarkers of intrinsic condition or the external environment in some species.

#### 4.2. Unexpected environmental challenge

The feather clipping manipulation led to a decrease in the frequency of feeding trips as compared to control females, likely due to a decrease in overall foraging efficiency (Patterson et al., 2011 and Winkler and Allen, 1995). In contrast to natural underlying environmental variability, we found that this unpredictable environmental challenge increased

the baseline GC levels of female tree swallows. Furthermore, baseline GC levels were also indicative of intrinsic state (change in body mass over offspring provisioning) when females were exposed to this environmental challenge, with individuals with higher baseline GC levels post-manipulation experiencing greater losses in body mass. Our manipulation represented a perturbation that extended over a two week time period and it is therefore possible that this unexpected or elongated disturbance more easily manifested into an energetic deficit that led to a measurable change in baseline GCs. Indeed, baseline GCs have previously been found to be representative of other unexpected, or severe perturbations in habitat quality including oil spills (Wikelski et al., 2002), severe food restrictions (Romero and Wikelski, 2001), presence of a novel invasive species (Graham et al., 2012), and logging (Leshyk et al., 2012). Our findings reinforce the role of baseline GCs in the context of energetic management and provide further indication that environmental alterations that manifest as dramatic changes to resource availability or energy expenditure will more likely be represented by changes in baseline GCs (Madliger and Love, 2014).

In immediate response to the clipping manipulation, baseline GCs would likely have risen (i.e., a stress response would have been triggered). This initial stress response is adaptive in the face of short, acute stressors as it allows individuals to respond to a perturbation (e.g., predator, weather event) by causing short-term changes in behavior, immunity, and the mobilization energy resources (Wingfield et al., 1998). However, if a perturbation is long-lived, this normally adaptive system can be pushed past its adaptive capacity (Dickens and Romero, 2013) and animals can experience chronically elevated GC levels that can lead to negative consequences for health, reproduction, or survival (Wingfield, 2003). Given that baseline GC levels of feather clipped individuals were higher than controls two weeks following the initiation of the manipulation, it is likely that these individuals were experiencing a chronic elevation, albeit within baseline levels, of GCs over that time period. As a result, we would expect that the elevation in circulating GC levels in manipulated birds would impart fitness consequences, with trade-offs likely manifesting between current reproductive success and survival (Crossin et al., 2015). Moreover, it is likely that baseline GC levels of clipped individuals may relate to how they responded to the manipulation. Specifically, individuals that more greatly value their current brood are expected to sacrifice self-maintenance in favor of increasing workload for their offspring and would therefore likely exhibit greater increases in baseline GC levels and therefore stronger negative longer-term (i.e., survival) consequences than individuals favoring somatic maintenance at the expense of reproductive output (Bokony et al., 2009). Although outside of the scope of the current study, future studies investigating the fitness costs of ecologically-relevant experimentally-manipulated baseline GCs have been cited as a pressing need for determining the usefulness of GCs as biomarkers (Madliger and Love, 2014) and to the field of Conservation Physiology as a whole (Cooke, 2014).

#### 4.3. Implications for glucocorticoids as biomarkers

Our results suggest that baseline GCs may be more consistently useful in detecting whether certain populations have been exposed to unexpected or more severe extended impacts, rather than monitoring gradual changes in environmental quality that may be useful in predicting future population changes. We have confidence that these negative results are not due to low sample size as the associated confidence intervals for individual environmental variables are relatively narrow and cross zero. Overall, baseline plasma GCs may represent a trait that is too labile to easily be implemented as a sensitive indicator of habitat quality or disturbance in many species. For example, recent temperature, wind, precipitation, time since last feeding, or whether an interaction with a conspecific or competitor has recently occurred may be more influential on short-term baseline GC levels. If this is the case, baseline plasma GCs will be harder to interpret for practitioners

and a more integrative measure that takes into account processes occurring over an extended time frame, such as fecal GCs, may be better (i.e., more consistently) suited to this application in many species (Dantzer et al., 2014; Dickens and Romero, 2013 and Sheriff et al., 2010).

It is possible that preparatory or consequential increases in baseline GCs during reproduction may overshadow the ability of GCs to represent more gradual variation in the environment. For example, the preparatory role of baseline GCs in mediating investment in reproduction (Love et al., 2014) may be much more pronounced than the changes associated with finer-scale environmental variation. It is therefore possible that the non-breeding season may be better-suited to baseline GC measurements. However, accessing individuals during the overwintering season can be difficult in many species (e.g. migratory or hibernating species) and may therefore limit the applicability of baseline GC measures to some animal systems. There is therefore a need to determine which characteristics and contexts are most important to interpreting baseline GC levels as this will allow for the determination of which systems may be best suited to their use.

There is also a need for experimental approaches to better understand how GC levels can reflect changes in the environment, with particular attention to the changes most likely to be associated with climate change or other anthropogenic disturbances (e.g., agricultural intensification, urbanization, and resource extraction). Direct manipulation of environmental variables would be highly beneficial in determining these relationships. In addition, while outside the scope of this study, the most powerful experimental approaches will also include measures of fitness (productivity and survival) as this will allow the value of baseline GCs for predicting the demographic consequences that drive population viability to be determined. In this way it may also be possible to identify whether thresholds exist where baseline GCs become useful indicators of the environment and predictors of population change (Dantzer et al., 2014), allowing their application to be tailored to specific wildlife systems.

#### 4.4. Conclusions

We found that baseline GCs were not representative of the internal and external environment in tree swallows, regardless of reproductive stage, indicating that baseline GCs may be limited in their ability to reflect gradients in habitat quality or disturbance in some species. Overall, we currently lack a complete picture of if and how baseline GCs may fit into the conservation toolbox. Most importantly, growing evidence indicates that the application of GCs will be highly context-dependent and the method will need to be considered in light of its reliability, sensitivity, and ease of interpretation to determine when it will be a useful tool for conservation biologists and wildlife managers.

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