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Corticosterone as a mediator of the tradeoff between survival and reproduction

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CORTICOSTERONE AS A MEDIATOR OF THE TRADEOFF BETWEEN SURVIVAL AND REPRODUCTION

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Organisms cannot simultaneously maximize their investment in survival and reproductive success, thus these traits are said to trade off against one another. The physiological mechanisms that help guide these investment decisions are not fully understood. Stress-induced corticosterone is a strong candidate mechanism, because it is thought to promote survival-oriented behavior and physiology at the expense of non-critical functions such as reproduction. In this dissertation, I studied multiple components of corticosterone physiology (baseline corticosterone and stress-induced corticosterone) in several species of passerine birds to address this question from a variety of angels. I first provide direct support for corticosterone playing a role in the reproduction-survival tradeoff. Stress-induced measures of corticosterone predicted greater survival and lower reproductive performance. Baseline corticosterone, however, appears to reflect quality, with greater baseline levels associated with greater survival and reproduction. I then explore the relationship between corticosterone and reproduction at a finer scale, using both correlative and experimental approaches. Individual variation in corticosterone was negatively associated with both brooding behavior and offspring feeding rate, but experimental manipulation of corticosterone in the latter study had no effect. And finally, I evaluated the relationship between environment and endogenous corticosterone levels, finding no support for any relationship between the two. Altogether these results show that corticosterone is closely tied to survival and reproduction and should be considered when evaluating mechanisms of investment in fitness.

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Chapter 1: Natural selection and glucocorticoid physiology

ABSTRACT

Studies of natural selection are important for understanding the adaptive evolution of traits. However, the vast majority of natural selection studies focus on morphological traits, so relatively little is known about how selection acts on physiological traits. In this study, we used a long-term dataset from a population of mountain white-crowned sparrows to estimate natural selection on glucocorticoid concentrations. Glucocorticoids are thought to be potent modulators of the tradeoff between survival and reproduction, and are thus a likely target of selection. We found positive reproductive selection on baseline glucocorticoids and negative reproductive selection on one measure of stress-induced glucocorticoids. We also found positive survival selection on measures of both baseline and stress-induced glucocorticoids. These results add to our knowledge of how selection operates on physiological traits and also provide an evolutionary and ecological perspective on several key open issues in the field of glucocorticoid physiology including. We address which elements of glucocorticoid physiology have the strongest associations with measures of fitness and how patterns of selection in this population relate to the current thinking on the fitness effects of glucocorticoids

INTRODUCTION

A fundamental goal of evolutionary biology is to understand how traits evolve and how organisms adapt to current conditions. Investigations of natural selection facilitate this effort, because natural selection is the main driver of adaptive evolution. Studies of selection in wild

populations have proliferated in recent decades and have documented various modes of selection (i.e. directional, stabilizing, and disruptive; (Endler 1986; Kingsolver et al. 2001; Kingsolver & Pfennig 2007)). The mode of selection can have important implications for the evolutionary trajectory of a population: directional selection may increase or decrease trait values, stabilizing selection may lead to canalization (Wagner et al. 1997), and disruptive selection may lead to speciation (Kirkpatrick & Ravigné 2002). However, most selection studies focus on either morphological traits or life history and phenology traits, so comparatively little is known about how selection acts on physiological and behavioral traits (Kingsolver et al. 2001). Physiological traits are critical components of organisms and may evolve differently than morphological traits for several reasons. Physiological systems often regulate multiple traits, which can constrain their evolution (Ketterson & Nolan 1999). Also, while morphological traits tend to be fixed during development, physiological traits often respond dynamically to current conditions and this plasticity may affect how they evolve (Kingsolver & Huey 1998). Physiologists increasingly incorporate evolutionary perspectives and techniques into their work (Feder et al. 2000) and the number of studies measuring selection on physiological traits is growing (Irschick et al. 2008). Despite this recent increase, more studies across a broader range of traits are needed to improve our understanding the modes of selection operating on this important class of traits.

Endocrine systems exert a powerful influence on an array of behavioral and physiological traits, and facilitate organisms' ability to interact appropriately with their environment (Husak et al. 2009). One such endocrine system is the glucocorticoid hormone axis, an ancient trait found across vertebrate taxa (Close et al. 2010). Glucocorticoids, a class of stress and metabolic hormones, are thought to comprise two separate traits, baseline levels and stress-induced

levels (Romero 2004). Baseline levels exhibit daily and seasonal variation (Romero 2002) and help regulate metabolism and activity (Sapolsky et al. 2000). Elevated (stress-induced) levels are secreted in response to all types of challenges including agonistic social interactions, encounters with predators, inclement weather, and food scarcity (Wingfield et al. 1998). Elevated concentrations of glucocorticoids promote survival-oriented physiology and behavior (e.g. glucose mobilization, escape behavior) at the expense of noncritical functions such as reproduction (Wingfield et al. 1998).

Based on these immediate physiological and behavioral effects, much of the endocrine stress literature asserts that glucocorticoids are related to fitness (e.g. survival and reproduction). Stress-induced glucocorticoids are thought to help mediate the tradeoff between survival and reproduction by redirecting resources towards survival (Wingfield & Sapolsky 2003). Additionally, Bonier et al (2009a) recently formalized the 'cort-fitness hypothesis'. The 'cort-fitness hypothesis' posits that endogenous glucocorticoids should correlate negatively with fitness, because environmental challenges should elevate glucocorticoids and reduce fitness. However, both supporting and contradictory evidence has been found (Bonier et al. 2009b). Despite the prevalence of the notion that glucocorticoid physiology is related to fitness and the appeal of its logic, relatively few studies actually measure survival or reproductive success in association with variation in glucocorticoid physiology. This is especially true for the acute glucocorticoid stress response (Breuner et al. 2008).

Studies of natural selection can also provide an ecological and evolutionary context to complement the mechanistic approach common in physiology (Feder et al. 2000). For example, the glucocorticoid response to stress is quantified in several ways, but physiologists do not

agree which elements within the glucocorticoid pathway are most biologically relevant. Elevated glucocorticoid secretion in response to standardized stressors (stress-induced levels) has variously been quantified as the maximal glucocorticoid concentration, integrated glucocorticoid concentration, and fold-increase of glucocorticoid concentration (Romero 2004). Additionally, glucocorticoid molecules are bound to a glycoprotein in the plasma, and it is not known if the total or unbound (free) concentration of glucocorticoids is more important (Romero 2002; Breuner & Orchinik 2002). Comparing the relationships between these elements of glucocorticoid physiology and fitness could give an indication of their biological relevance.

In this study we use a long-term data set from mountain white-crowned sparrows (*Zonotrichia leucophrys oriantha*) to investigate the relationships between measures of glucocorticoid physiology and fitness. We evaluate these relationships from both evolutionary and physiological perspectives. From the evolutionary perspective, we seek to understand if selection is operating on glucocorticoid physiology and, if so, what are the patterns of selection (i.e. mode and direction). From the physiological perspective, we seek to understand which elements of glucocorticoid physiology have the strongest associations with measures of fitness and how patterns of selection in this population relate to the current thinking on the fitness effects of glucocorticoids. Fitness was measured as reproductive success (number of offspring successfully fledged within a breeding season) and survival probability (estimated from capture-mark-recapture data).

METHODS

Study Area, Study Species, and Fieldwork

We studied a population of breeding mountain white-crowned sparrows at Tioga Pass Meadow (37.8°N 119.2°W; ~3,030 m) from 2001 to 2008. Tioga Pass Meadow is a subalpine meadow located just outside of the eastern entrance to Yosemite National Park. The study site has extensive patches of willow and scrub pine, which provide nesting substrate for the sparrows. Approximately 75 pairs breed at the study site each year. Males arrive at the breeding ground first and females arrive ~10 days later on average (Morton 2002). When the birds arrive, the breeding ground is covered with snow and they must wait for the snow to recede from nest sites before they initiate nesting (range of clutch initiation date: May 31st to June 13th). This results in a distinct arrival phase that precedes nesting (Wingfield et al. 2004).

Fieldwork consisted of two main tasks, trapping adult sparrows and finding and monitoring nests. Adults were captured using seed-baited potter traps at established locations throughout the study site. Time spent trapping varied by year. Researchers arrived in early May and began trapping as soon as the road to the study site was cleared of snow (range: May 3rd to May 15th). In the years 2001 to 2006, trapping effort continued through late July (range: July 26th to July 31st). In 2007 and 2008, trapping effort ceased on May 31st and June 6th respectively. Although trapping effort continued into the nesting period from 2001-2006, only blood samples drawn prior to clutch initiation were included in our study. A combination of systematic searching and parental cues were used to located as many nests as possible from 2001 - 2006. Nest searching began when adults were observed with nesting material and continued through late July. Once found, nests were checked every other day until fledging.

We used a standardized capture and restraint stress protocol to assess baseline and stress-induced corticosterone (the primary glucocorticoid in birds) levels (Wingfield 1984). An initial blood sample was taken from captured birds within 3 minutes of trap disturbance. Birds were then placed in cloth bags as part of the stress protocol and subsequent blood samples were drawn at 15 and 30 minutes post-capture. Blood samples were taken by puncturing the brachial vein with a 26-gauge needle and collecting ~ 40-60 µl of blood in a heparinized microcapillary tube. Blood samples were kept in a portable cooler with several icepacks. Within 6 hours, blood samples were centrifuged at 14,000 RPM for 10 min. After centrifugation, the plasma was removed. Plasma samples were stored at -20°C until they were assayed.

Hormone and Binding Protein Assays

Serial blood samples taken during the 30 minute stress protocol allow us to measure multiple aspects of glucocorticoid physiology (Fig 1). Baseline corticosterone was measured as the concentration of corticosterone in the initial blood sample. We tested for an effect of bleed time (interval between trap disturbance and completion of initial blood sample) on baseline corticosterone and found that samples taken within 3 minutes were unaffected by bleed time (linear model: $t_{614} = 1.006$, $p = 0.315$). When 4 minute samples were included in this analysis, baseline corticosterone was significantly positively related to bleed time (linear model: $t_{627} = 2.359$, $p = 0.019$). Thus, only samples taken within 3 minutes were included in the study.

Stress-induced corticosterone was quantified in several ways (Fig 1):

- Maximal corticosterone is the highest concentration of corticosterone measured over the sampling period.

- Integrated corticosterone is the total amount of corticosterone secreted over the sampling period.
- Fold increase in corticosterone is the maximal concentration divided by the baseline concentration.

These three measures offer insight into peak levels, total levels over time, and the increase in corticosterone that the target cell would experience, respectively.

Plasma corticosterone concentrations were measured using Enzyme Immunoassay (EIA) kits (cat # ADI-901-097, Enzo Life Sciences, Plymouth Meeting, PA) following the protocol laid out in Wada et al. (2007). Briefly, plasma was diluted 1:40 and 1% steroid displacement buffer was added to the plasma. Samples and standard curves were run in triplicate

Most glucocorticoid molecules in the blood are bound to a carrier protein called corticosteroid binding globulin (CBG). The glucocorticoid-CBG complex likely cannot pass through capillary walls, which would prevent access to intracellular receptors in target tissues. This is the basis of the 'free hormone hypothesis' which states that the concentration of unbound ('free') glucocorticoids is more biologically relevant than the total concentration of glucocorticoids (Mendel 1989). An alternative view of CBG is that it functions as a carrier molecule analogous to hemoglobin (Romero 2002).

Plasma corticosteroid binding globulin capacity was measured using a ligand-binding assay with tritiated corticosterone following an established protocol (Breuner et al. 2003). Breuner et al (2003) optimized the following assay parameters for mountain white-crowned sparrows: incubation time (2 h), incubation temperature (4°C), rinse volume (3x3 ml cold buffer), and

plasma dilution (1:900). All samples were run in triplicate. Assay tubes contained 50 µl of 1:300 diluted plasma, 50 µl [3H] corticosterone, and either 50 µl of 1 µM unlabelled corticosterone (non-specific binding) or 50 mM (pH 7.40) Tris assay buffer (total binding). Tubes were then incubated for 2 h at 4°C. After incubation, we separated bound and unbound (or free) radioligand using a rapid vacuum filtration harvester (Brandel, Gaithersburg, MD) over 1 µm binder-free glass mircofiber filters (GF/B, Whatman, Piscataway, NJ) soaked in 25 mM Tris buffer with 3% polyethylenimine for 1 hr. Filters were then rinsed 3 times with 3 ml of 25 mM Tris buffer (pH 7.40). Free hormone levels were estimated using an equation by Barsano and Baumann (1989):

$$Hfree = 0.5[Htotal - Bmax - \frac{1}{Ka} \pm \sqrt{\left(Bmax - Htotal + \frac{1}{Ka}\right)^2 + 4 * \left(\frac{Htotal}{Ka}\right)}$$

where Ka is 1/Kd (nmol/l), Kd is affinity of corticosterone for CBG, Bmax is total CBG capacity, and Htotal is total plasma hormone concentration. Affinity (Kd) of corticosterone for CBG was estimated as 3.68 ± 0.31 nM (mean ± SD) using pooled plasma in a separate equilibrium saturation binding assay (Breuner et al. 2003).

Survival Analysis

Survival probabilities were estimated using the 'recaptures only' model in Program MARK Version 6.0. Only resident individuals were included in the analysis (Pradel et al. 1997), where a resident was defined as an individual captured at least 4 days apart in the same year. Due to limitations on incorporating individually time-varying covariates in Program MARK, we used

average values for individuals with repeated measurements. Baseline corticosterone samples greater than 2 standard deviations above the mean were excluded from our analysis, because these individuals likely experienced a stressor prior to our sampling.

For some individuals we only had measures of baseline corticosterone. Therefore, we analyzed measures of baseline and stress-induced corticosterone with separate datasets to maximize sample sizes. For each dataset, we separately fit and compared the relative support for alternative models using an information theoretic approach (quasi Akaike information criterion, QAIC, (Burnham & Anderson 2010)). We first found the best general model (no individual covariates) for each dataset by comparing models containing effects of year and sex on survival and effects of year, sex, and trapping effort (number of trap-days) on resight probability. General models provided a standard for comparison for models with individual covariates. The individual covariates for our baseline dataset were baseline total and free corticosterone, mass, scaled-mass index (a measure of body condition, (Peig & Green 2009), and wing length. The individual covariates for our stress-induced dataset were maximal, integrated, and fold-increase for total and free corticosterone. For each corticosterone covariate, we fit separate models where survival probability varied as a linear function of the raw corticosterone value, a linear function of the natural log transformed corticosterone values, and quadratic function of the raw corticosterone values. We assessed whether the relationship between survival probability and our measures of corticosterone varied by year (interaction) with a subset of our data (2002-2006). There were relatively fewer capture events in the years 2001, 2007, 2008, which prevented our models for 2001-2008 from converging when separate parameters relating corticosterone to survival were estimated for each year.

Finally, we explored additional models wherein we truncated our dataset to exclude portions of corticosterone parameter space with sparse data, because we were concerned that rare high values may be driving the observed relationships. The shape of these functions and the support for the underlying model were similar to the untruncated data. Therefore we only present the more inclusive dataset.

We used parametric bootstrapping (1000 bootstraps) to test the goodness-of-fit of our general models (Cooch & White 2010) and to estimate a correction factor. Data from the bootstrap simulation adhere to the assumptions of the model. Thus we compared the observed deviances from our general models to the deviance from the bootstrapped models to assess how well our data meet the assumptions of the model. The observed model deviances from all of our general models were significant at the $\alpha = 0.1$ level, which suggests that our data were overdispersed (i.e. extra-binomial variation). A variance inflation factor ($\hat{c}$) can be applied to correct for departures from the assumptions of the binomial distribution (Burnham & Anderson 2010) and program MARK uses $\hat{c}$ to adjust the AICc using quasi-likelihoods (White & Burnham 1999). We estimated $\hat{c}$ by dividing the mean deviance from bootstrap simulations by the observed deviance from the general model and the mean dispersion parameter from the bootstrap simulation by the observed dispersion parameter from the general model (Cooch & White 2010). The higher of these values from each general model was used as our $\hat{c}$. Goodness-of-fit methods do not exist for models with individual covariates, so we used the $\hat{c}$ from the appropriate general model for our models with covariates (Cooch & White 2010).

Reproductive Success Analysis

The number of offspring successfully fledged was quantified as the number of offspring last seen in a nest before fledging minus any dead offspring found in the nests after fledging. We estimated linear (β) and quadratic (γ) selection gradients for our corticosterone measures and several morphological and energetic measures (wing length, mass, fat, and scaled-mass index) using regression analysis (Lande & Arnold 1983; Brodie et al. 1995). Briefly, we fit regressions using relative fitness (individual fitness / population mean fitness) and standardized values for each covariate ((individual value – population mean value) / population standard deviation). We analyzed each sex separately. We also compared fledging success between first time breeders and returning breeders, because returning breeders often have greater reproductive output (e.g. Nol & Smith 1987). In our population, returning breeders had greater reproductive success than first time breeders (t-test, $t_{40} = 3.18$, $p = 0.003$), so we analyzed these groups separately. All statistical analysis for reproductive success were done using 'R' version 2.11.1 for Windows (R Foundation for Statistical Computing, Vienna, Austria).

RESULTS

Survival

For the baseline corticosterone dataset, the best general model (no individual covariates) had a single parameter for survival probability and separate parameters for male and female resight probability. Resight probability was also a function of the number of trap days in that year. All models including baseline corticosterone terms had lower QAIC scores than the general model (Table 1). In the best model for the baseline corticosterone dataset (lowest QAIC scores), survival probability was a function of natural log transformed free corticosterone (Figure 2A). In

the best model including total baseline corticosterone (third lowest QAIC score overall), survival probability was a function of natural log transformed total corticosterone (Figure 2B). For the years 2002-2006, no model with a corticosterone-by-year interaction had lower QAIC scores than the general model (Table 2).

For the stress-induced corticosterone dataset, the best general model (no individual covariates) had a single parameter for survival probability and separate parameters for male and female resight probability. Only three models had lower QAIC scores than the general model (Table 3). In the best model, survival probability was a function of natural log transformed maximal free corticosterone (Figure 3A). In the other two models with lower QAIC scores than the null model, survival probability was a function of natural log transformed integrated free corticosterone and a linear function of free maximal corticosterone, respectively (Figure 3B, C). For the years 2002-2006, models with corticosterone-by-year interaction for natural log transformed free maximal corticosterone and natural log transformed free integrated corticosterone had lower QAIC scores than the general model (Table 4; Figure 4A-D).

Reproductive Success

All significant relationships between the individual covariates and reproductive success were found in female returning breeders. Returning females with longer wings had greater reproductive success (LM: $F_{1, 22} = 7.53$, $p = 0.012$; Table 5, Figure 5A). Also, returning females with greater baseline total corticosterone had greater reproductive success (LM: $F_{1, 25} = 9.03$, $p = 0.006$; Table 5, Figure 5E). Finally, returning females with a lower fold-increase for total corticosterone (a smaller proportional increase above baseline corticosterone) had greater

reproductive success (LM: $F_{1, 21} = 8.62$, $p = 0.008$, Table 5, Figure 5K). There were no other significant linear relationships for females or males. Linear selection gradients ranged from -0.18 to 0.14 (Table 5).

We also found two significant quadratic selection gradients among returning female breeders. Females with intermediate fat scores had greater reproductive success (LM: $t_{27} = 2.80$, $p = 0.009$; Table 6, Figure 5D). Also, females with intermediate fold increase in free corticosterone had lower reproductive success (LM: $t_{19} = 2.28$, $p = 0.034$; Table 6, Figure 5L). There were no other significant quadratic relationships for females or males. Quadratic selection gradients ranged from -0.11 to 0.15 (Table 6).

DISCUSSION

In this study, we set out to describe the relationships between fitness components and glucocorticoid physiology, evaluating this relationship from both evolutionary and physiological perspectives. Overall, we found significant relationships between corticosterone levels and reproductive success. Our results also support a relationship between corticosterone and survival probability.

Patterns of Selection

Patterns of selection varied by the measure of corticosterone and fitness. The absolute value of all of our linear selection gradients were ≤ 0.2 (Table 5). These values are well within the range found in Kingsolver et al's (2001) review, which described an exponential frequency

distribution of selection gradients with a mean of 0.22 and a median of 0.16. The magnitude of direction selection was the greatest on fold increase in total corticosterone (X ± SE = 0.18 ± 0.06), however its confidence interval overlapped with the magnitude of selection on wing length. Thus, directional selection on physiological and morphological systems appears to be similar in magnitude. The relative strength of selection on physiological versus morphological traits is a poorly document comparison, so this represents an important data point for our understanding of how natural selection operates on physiological systems.

The absolute values of our quadratic selection gradients were all ≤ 0.11 and only fat score and fold in increase in free corticosterone were significant relationships (Table 6). The relationship between fat score and relative fitness was convex, suggesting stabilizing selection. Curiously, the relationship between relative fitness and fold increase in free cort had a concave shape, which suggests disruptive selection. However, given the relatively small number of data points we are reluctant to place too much weight on this finding. Kingsolver et al's (2001) review found that most quadratic gradients fell between -0.1 and 0.1, with a mean absolute value of 0.10. Again our results are consistent with these findings.

For reproductive output, we found contrasting directional selection in baseline (positive) and fold-increase in corticosterone (negative). While our data suggest a linear increase in reproduction with increasing baseline corticosterone, we expect that reproduction would begin to suffer at some higher value of baseline corticosterone (Wingfield & Sapolsky 2003). Previous studies of glucocorticoids and reproduction also found primarily directional selection (positive and negative) between glucocorticoids and reproduction (e.g. tree swallows, *Tachycineta bicolor,*Bonier et al. 2009a; Eastern fence lizard *Sceloporus undulatus*, John-Alder et al. 2009;

snow petrels, *Pagodroma nivea*, Goutte et al. 2010). Evidence has been found for quadratic (here, stabilizing) selection operating on a different endocrine trait, GnRH-induced testosterone levels. In male dark-eyed Juncos (*Junco hyemalis*), GnRH-induced testosterone levels showed primarily stabilizing selection for measures of reproductive output (McGlothlin et al. 2010). Other studies have found positive relationships between circulating testosterone and mating success, a less direct measure of reproductive output (e.g. Satin Bowerbird *Ptilonorhynchus violaceus*, Borgia & Wingfield 1991; black grouse, *Tetrao tetrix*, Alatalo et al. 1996).

The relationship between corticosterone and reproductive success was only present in females. In this species, females build the nest, provide all of the incubation, and perform the majority of offspring provisioning. Overall, females are more involved in the process of reproduction, which may explain why their glucocorticoid profiles are related to number of offspring fledged but males' glucocorticoid profiles are not. It is also worth noting that extra-pair paternity in this population is 30-56% (MacDougall-Shackleton et al. 2002), so males' fitness may be more dependent on their ability to attain sexual mates than their ability to successfully fledge offspring with their social mates. Thus the number of social offspring fledged may not be the appropriate response variable in which to look for a relationship between corticosterone and reproductive success in males.

For survival, we primarily found evidence for positive directional survival for both baseline and stress-induced corticosterone, although our data also provide some support for stabilizing selection acting on baseline corticosterone. For both baseline and stress-induced corticosterone, the vast majority of our samples come from the low end of the observed range

of values, which limits our ability to make inferences about survival at higher trait values. Thus, we can say with some certainty positive directional selection acts on the lower end of the range of values, but we cannot draw many conclusions about the shape of the selection at mid to high values. The most comparable studies found evidence of both stabilizing and negative directional survival selection for baseline corticosterone (cliff swallows, *Petrochelidon pyrrhonota,* Brown et al. 2005) and negative directional survival selection for stress-induced total corticosterone (European white storks, *Ciconia ciconia*, Blas et al. 2007). Positive directional survival selection on stress-induced corticosterone has been found in mammals (European wild rabbits, *Oryctolagus cuniculus*, Cabezas et al. 2007). Corticosterone also has been positively related to survival in reptiles (Common lizards, *Lacerta vivipara*, Cote et al. 2006; common side-blotched lizard, *Uta Stansburiana*, Comendant et al. 2003), but to our knowledge the shape of these relationships has not been described.

Finally, we found evidence that the shape of the relationship between some measures of stress-induced free glucocorticoids and survival varied across years. For the first four years of this analysis (2002-2005), the shape of the relationship was consistently positive and curvilinear over low values of corticosterone. In the last year of the analysis we found a slightly negative relationship over the entire range of corticosterone values. However, the confidence intervals were quite broad when we estimated a separate parameter for each year, so we are reluctant to draw firm conclusions about how selection changes through time.

Tradeoff between Survival and Reproduction

The two main components of fitness, survival and reproduction, are thought to tradeoff such that an increase in one is necessarily accompanied by a decrease in the other (Williams 1966). The existence of this tradeoff has been shown in a variety of systems (e.g. blue tits, *Cyanistes caeruleus*, Nur 1984), although this tradeoff can be obscured by differences in resources acquisition within species (van Noordwijk & de Jong 1986). Glucocorticoids have been put forward as a candidate mechanism mediating the tradeoff between survival and reproduction (Ricklefs & Wikelski 2002), because stress-induced glucocorticoids are thought to promote survival-oriented behavior and physiology at the expense of non-critical functions, such as reproduction (Wingfield et al. 1998). We assumed the existence of this tradeoff in our population and examined our results for evidence that stress-induced glucocorticoids mediate the relationship. Our results support this hypothesis, because higher stress-induced glucocorticoids are associated with reduced reproduction and increased survival. A stronger demonstration would entail manipulating trait values within an individual (or family) and showing that higher levels of stress-induced glucocorticoids are associated with greater survival and lower reproductive output. However, the technical challenges of such an experiment place it beyond the scope of this study.

CORT-Fitness Hypothesis

A pervasive theme in the glucocorticoid literature is the 'cort-fitness' hypothesis, which suggests that endogenous glucocorticoids should be negatively associated with fitness. Our results run contrary to 'cort-fitness hypothesis' as both survival and reproductive success were positively associated with our measures of baseline (endogenous) corticosterone. The foundation of the cort-fitness hypothesis is that environmental challenges elevate

glucocorticoids and impair fitness. Instead of reflecting environmental challenges, baseline corticosterone during the arrival phase reflects individual quality because both survival and reproductive output covary with baseline corticosterone (Fig 2, Fig 5E). Our data do not address the causality of this relationship. Individual quality could be the driver of this relationship if it is independently related to both fitness and corticosterone. Perhaps, high-quality individuals exert greater effort to acquire high-quality territories and high-quality mates during the arrival phase. Corticosterone increases in response to activity levels and energy expenditure (reviewed in Landys et al. 2006). If higher quality individuals invest more in these goals, we would expect glucocorticoids to be elevated and the observed positive association. Alternatively, higher levels of glucocorticoids may be driving this relationship. Glucocorticoids are known to mobilize energy (Sapolsky et al. 2000) and increase activity levels (Breuner et al. 1998). If this energy and activity is deployed in the service of resource acquisition, then individuals with higher baseline corticosterone may have higher survival or reproductive success as a result of the elevated glucocorticoids.

We also evaluated another hypothesis for the relationship between endogenous corticosterone and reproductive success. This hypothesis relates to the arrival pattern of the sparrows, seasonal changes in endogenous glucocorticoids, and age specific reproductive success. Previous work on this population found that older birds (returning breeders) tend to arrive earlier than first year breeders (Morton 2002). Also, corticosterone is generally upregulated during the breeding season in a sister subspecies (Romero & Wingfield 1999). Finally, we observed in our dataset that older individuals had greater reproductive output than first time breeders. Together, these facts raise the possibility that older individuals arrived earlier, were further along in their seasonal upregulation of the HPA axis and had higher reproductive

success. This set of circumstances would give the appearance of a relationship between corticosterone and reproductive success. However, we found no relationships between age and sample date, age and corticosterone, sample date and corticosterone, or sample date and reproductive success. This leads us to reject the hypothesis the relationship between corticosterone and reproductive success is an artifact of age or sampling date.

Biological Relevance of Different Glucocorticoid Measures

The glucocorticoid literature has not definitively identified which measures of glucocorticoids are most biologically relevant. One debate surrounds the relevance of free (unbound) versus total hormone concentrations (Romero 2002; Breuner & Orchinik 2002). Various studies have found support for importance of total hormone concentrations (e.g. European wild rabbits, Cabezas et al. 2007; tree swallows, Bonier et al. 2009a) and free hormone concentrations (e.g. white-crowned sparrows, Breuner et al. 2003; European starlings, *Sturnus vulgaris*, Love et al. 2004), and this remains an open question in the literature. Fitness is an integrated measure of biological performance, so we examine our results in light of this debate. For survival, we found roughly equal support for total and free baseline corticosterone. Models with stress-induced free corticosterone were better supported than stress-induced total corticosterone, suggesting that free corticosterone is more important stress-induced measure for survival in this system. For reproduction, we found that total baseline corticosterone is a better predictor of reproductive output than free baseline corticosterone, suggesting that total corticosterone is the more important baseline measure for reproduction in this system. Fold increase in total and free corticosterone were the only significant stress-induced predictors of reproductive output and they explained similar proportions of variance.

Endocrinologists also disagree on which measure of stress-induced glucocorticoids best reflects the glucocorticoid response to stress. Romero (2004) argues that the integrated response (total hormone secreted over sample period) is the most relevant measure glucocorticoid stress response and that the fold increase in glucocorticoids has little value, because baseline and stress-induced glucocorticoids exert their effect through different receptors. Breuner (Breuner 2010), however, argues that the fold-increase in corticosterone is relevant because baseline levels of glucocorticoids help set receptor numbers and influence stress-induced secretion via negative feedback and tonic inhibition. Again, we examine our fitness measures to assess the biological relevance of these stress-induced measures. For survival, we found that models with maximal and integrated (free) corticosterone were better supported than models with fold-increase. However, the opposite pattern was found in reproduction, where fold increase in total and free corticosterone were the only significant predictors of reproduction. An important caveat is that many the various measures of corticosterone are correlated with one another and the measure with the greatest effect size does not necessarily have the strongest functional link with fitness. Thus, our results do not provide a consistent answer as to which measures of glucocorticoid physiology are most important.

Overall, this study represents one of the few attempts to quantify how patterns of selection operate on a physiological trait. It is especially rare to have estimates of both survival and reproduction selection from a single population. This study contributes to our understanding of how natural selection acts on physiological systems and provides an ecological and evolutionary context for glucocorticoid physiology.

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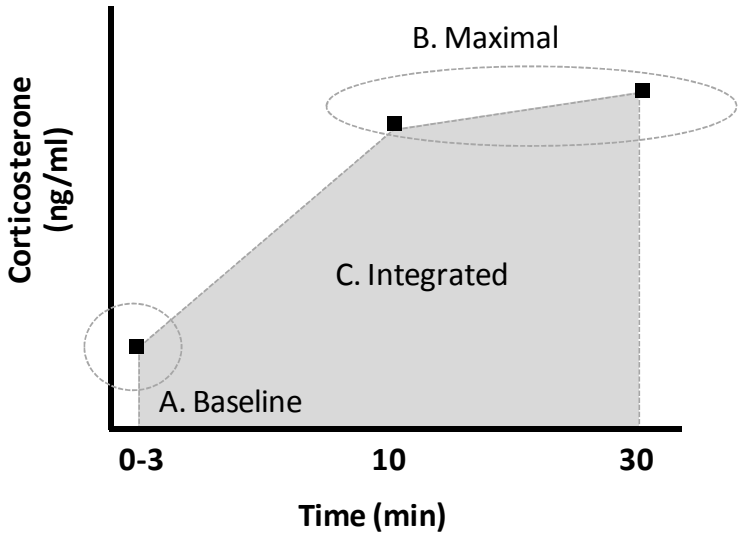
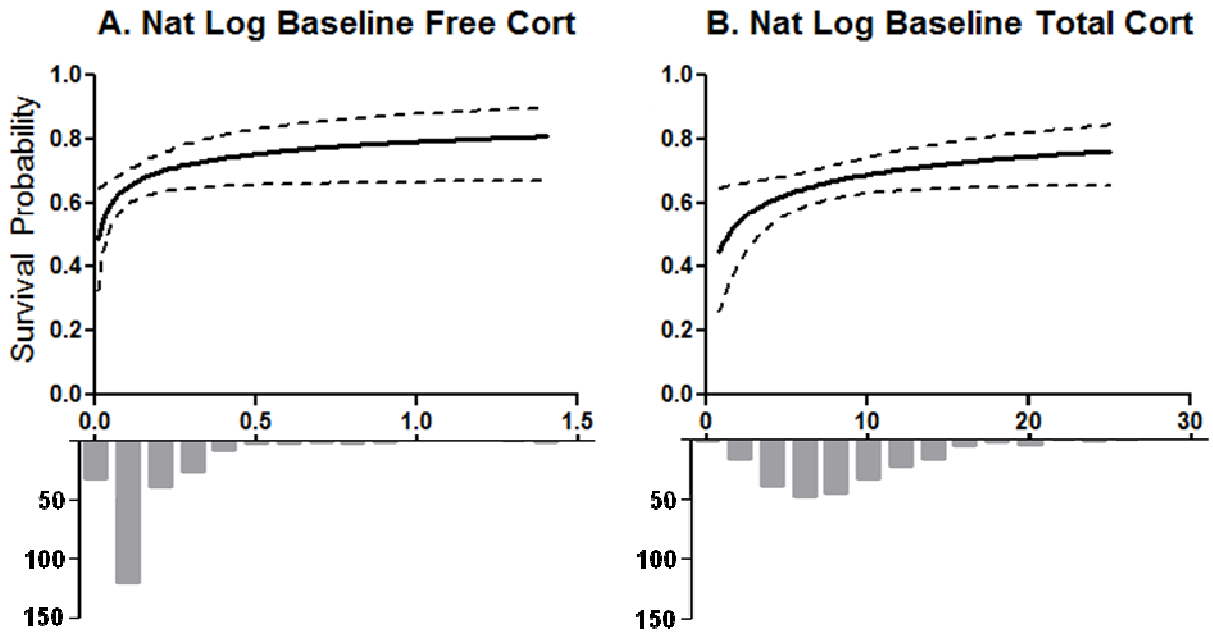


D. Fold Increase = Maximal / Baseline

Measure of Corticosterone (Cort)	How Measured	Reflects
A. Baseline	Cort concentration of 0 minute	Integration of recent activity & natural stressors
B. Maximal	Highest Cort concentration measured	Highest concentration of Cort that receptors experience during challenges (here experimental stressor)
C. Integrated	Area under curve created by sample points	Total quantity of Cort that receptors expereince during challenges (here experimental stressor)
D. Fold Increase	Maximal Cort / Baseline Cort	Proportional increase in Cort concentration that receptors experience during challenges (here experimental stressor) compared to the normal course of life (baseline concentrations) which set receptor levels.

Figure 1: Measures of corticosterone (Cort) from standardized capture and handling stress



protocol. A. Baseline Cort. B-D. Stress induced Cort.

Figure 2: Relationship between corticosterone and survival probability. A. Best model for baseline free corticosterone. B. Best model for baseline total corticosterone.

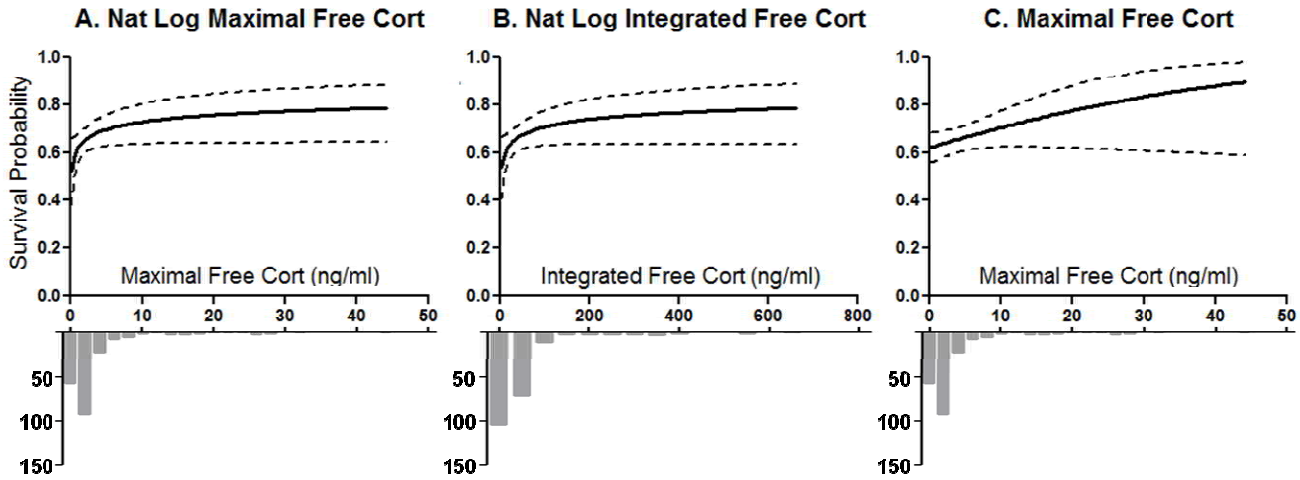


Figure 3: Relationship between corticosterone and survival probability for models with lower QAIC scores than general model. A. Survival as a function of natural log transformed free corticosterone. B.

Survival as a function of natural log transformed integrated corticosterone. C. Survival as a function of free corticosterone.

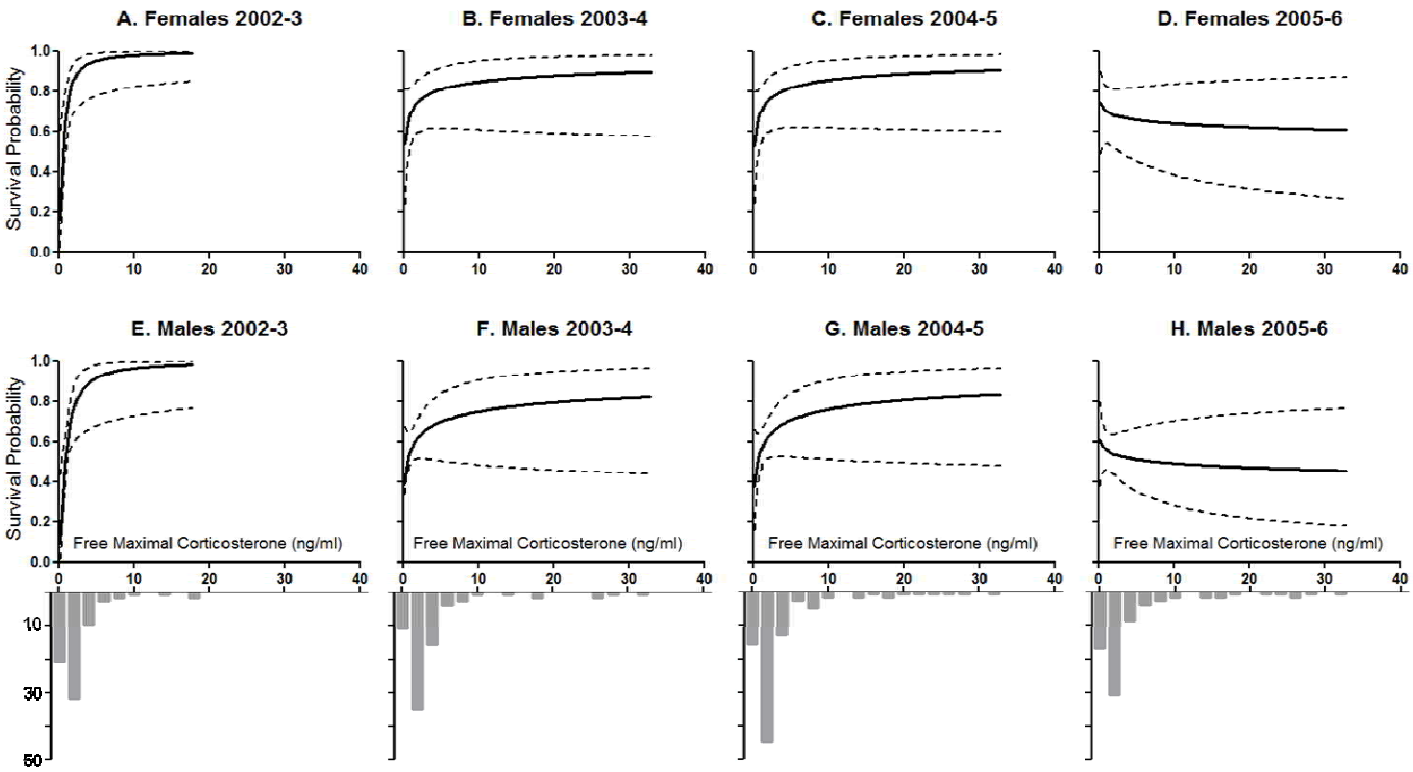
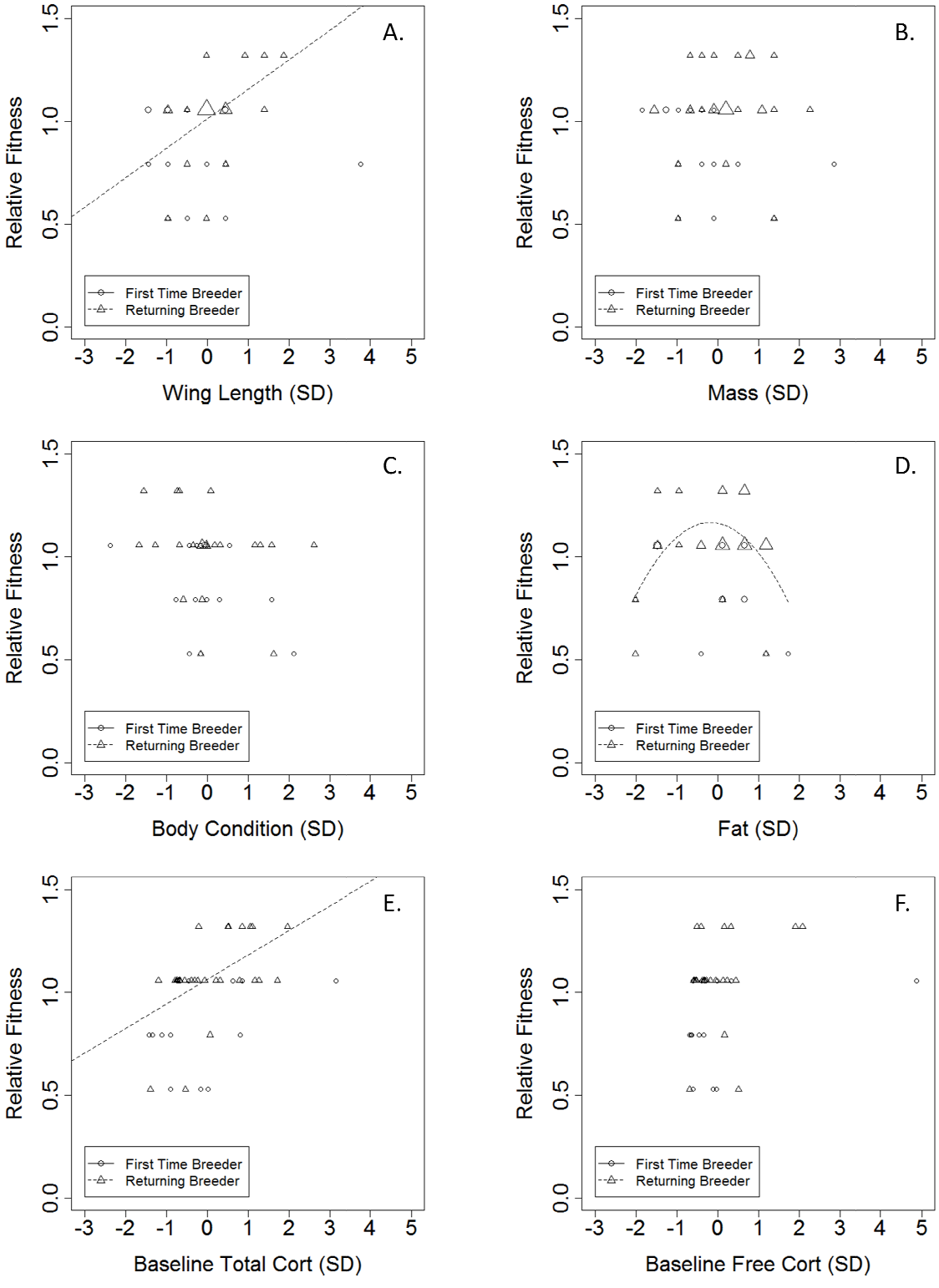


Figure 4: Relationship between corticosterone and survival probability where the survival probability is a function of sex and the interaction of year and natural log transformed maximal free corticosterone. A-D. Survival probabilities for females. E-H. Survival probabilities for males.


A.
Relative Fitness
Wing Length (SD)
First Time Breeder
Returning Breeder
B.
Mass (SD)
C.
Body Condition (SD)
D.
Fat (SD)
E.
Baseline Total Cort (SD)
F.
Baseline Free Cort (SD)

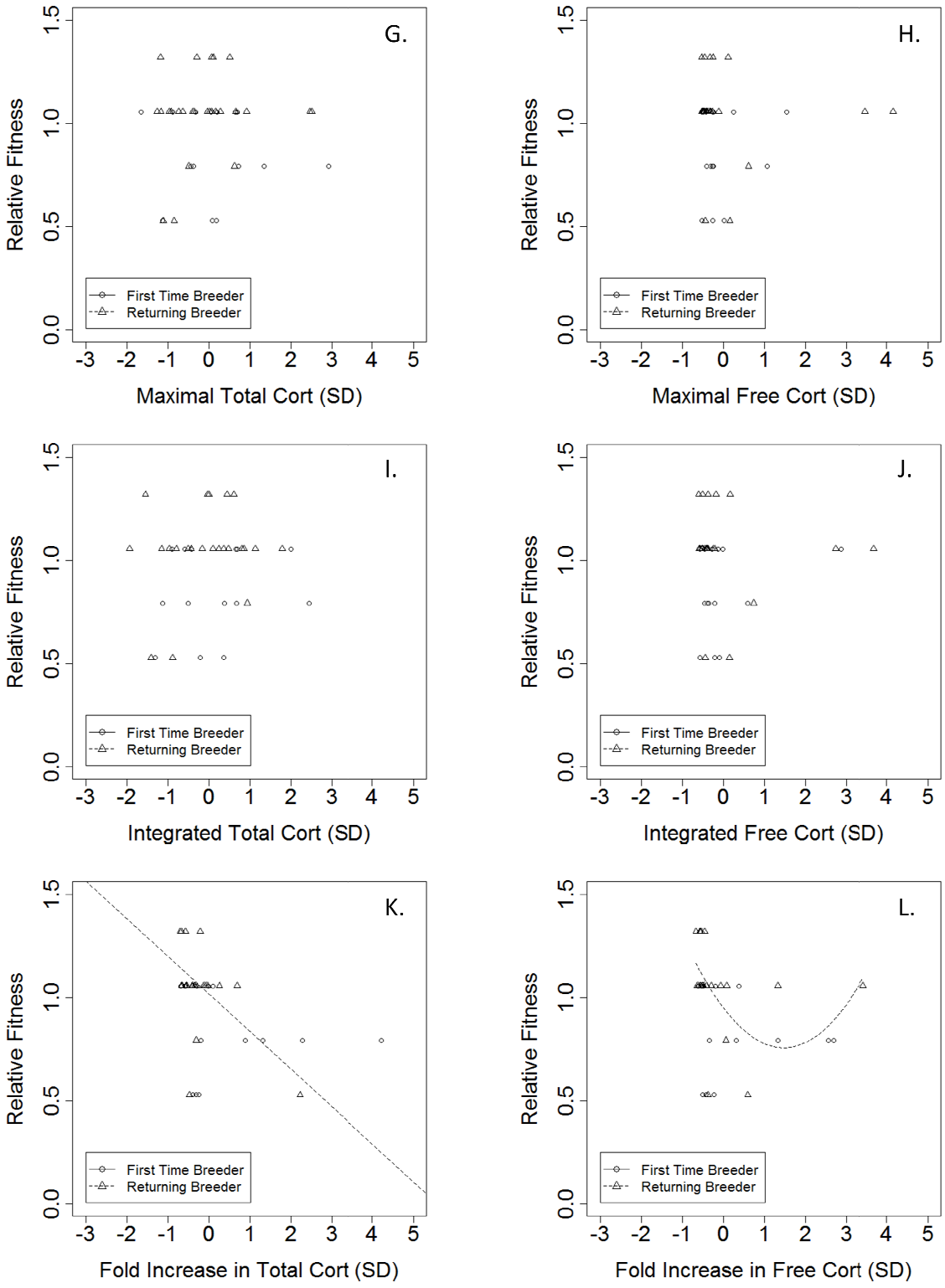


Figure 5: Relationship between relative fitness (individual reproductive output/population mean reproductive output) and standardized individual covariates ((individual value – population mean value) / population standard deviation). A-D. Morphological/energetic covariates. E-F. Baseline corticosterone

covariates. G-L. Stress-induced corticosterone covariates. Trend lines represent significant relationships ($P < 0.05$).

Table 1: Summary of baseline corticosterone survival models

Model Survival (ϕ)	Capture (p)	QAICc	Delta QAICc	QAICc Weight	Model Likelihood	# Par	QDeviance
Ln (Free)	Sex + Effort	632.151	0	0.224	1	5	622.032
Free + Free^2	Sex + Effort	632.726	0.575	0.168	0.750	6	620.558
Ln (Total)	Sex + Effort	632.803	0.652	0.162	0.722	5	622.683
Total	Sex + Effort	633.701	1.549	0.103	0.461	5	623.581
Total + Total^2	Sex + Effort	633.796	1.644	0.099	0.439	6	621.628
Free	Sex + Effort	634.266	2.115	0.078	0.347	5	624.147
Null	**Sex + Effort**	**635.469**	**3.318**	**0.043**	**0.190**	**4**	**627.390**
Mass	Sex + Effort	636.952	4.801	0.020	0.091	5	626.832
Scaled Mass Index	Sex + Effort	637.135	4.984	0.019	0.083	5	627.016
Wing	Sex + Effort	637.430	1.961	0.084	0.375	5	627.311

Survival was a function of total baseline corticosterone (Total), free baseline corticosterone (Free), mass, body condition (Scaled Mass Index), wing length (Wing), or no individual covariates (Null, in bold). Resight probability was a function of sex and trapping effort (Effort, number of trap days in each year).

Table 2: Summary of baseline corticosterone by year survival models

Model Survival (ϕ)	Capture (p)	QAICc	Delta QAICc	QAICc Weight	Model Likelihood	# Par	QDeviance
Sex + Ln (Total)	Sex	309.321	0	0.265	1	5	299.143
Sex + Ln (Free)	Sex	309.635	0.314	0.226	0.8546	5	299.457
Sex + Free	Sex	309.833	0.512	0.205	0.7742	5	299.655
Null	**Sex**	**312.906**	**3.585**	**0.044**	**0.1665**	**4**	**304.788**
Sex + SMI	Sex	312.932	3.611	0.044	0.1644	5	302.754
Sex + Wing	Sex	313.173	3.852	0.039	0.1457	5	302.995
Sex + Free * Yr	Sex	313.426	4.105	0.034	0.1284	8	296.995
Sex + Ln (Free)* Yr	Sex	313.465	4.144	0.033	0.1259	8	297.034
Sex + Total * Yr	Sex	313.475	4.154	0.033	0.1253	8	297.044
Sex + Ln (Total) * Yr	Sex	313.529	4.209	0.032	0.1219	8	297.098
Sex + Mass	Sex	314.959	5.638	0.016	0.0597	5	304.781
Sex + SMI * Yr	Sex	316.491	7.170	0.007	0.0277	8	304.781
Yr	Sex	316.692	7.371	0.007	0.0251	6	304.442
Sex + Free * Yr	Sex	317.413	8.092	0.005	0.0175	12	292.467
Sex + Free + $Free^2$ * Yr	Sex	317.483	8.162	0.004	0.0169	12	292.538
Sex + Total * Yr	Sex	318.115	8.794	0.003	0.0123	12	293.169
Sex + Mass * Yr	Sex	318.650	9.329	0.002	0.0094	8	302.219

Survival was a function of total baseline corticosterone (Total), free baseline corticosterone (Free), year (Yr), sex (Sex), mass, body condition (Scaled Mass Index), or no individual covariates (Null, in bold). Resight probability was a function of sex.

Table 3: Summary of stress-induced corticosterone survival models

Model Survival (ϕ)	Capture (p)	QAICc	Delta QAICc	QAICc Weight	Model Likelihood	# Par	QDeviance
Ln (Free Max)	Sex + Effort	586.376	0	0.179	1	5	576.242
Ln (Free Integr)	Sex + Effort	586.743	0.3668	0.149	0.8325	5	576.609
Free Max	Sex + Effort	587.149	0.7729	0.122	0.6795	5	577.015
Null	**Sex + Effort**	588.417	2.0414	0.064	0.3604	4	580.328
Free Integr	Sex + Effort	588.427	2.0507	0.064	0.3587	5	578.293
Free Max + Free Max^2	Sex + Effort	588.608	2.2321	0.059	0.3276	5	578.474
Total Fold Incr + Total Fold^2	Sex + Effort	589.441	3.0651	0.039	0.216	5	579.307
Ln (Free Fold Increase)	Sex + Effort	589.741	3.3654	0.033	0.1858	5	579.608
Free Fold Incr	Sex + Effort	589.799	3.4227	0.032	0.1806	5	579.665
Free Integr + Free Integr^2	Sex + Effort	589.867	3.4913	0.031	0.1745	5	579.734
Total Integr + Total Integr^2	Sex + Effort	590.154	3.7782	0.027	0.1512	5	580.020
Ln (Total Fold Incr)	Sex + Effort	590.212	3.8358	0.026	0.1469	5	580.078
Total Integr	Sex + Effort	590.221	3.8448	0.026	0.1463	5	580.087
Ln (Total Integr)	Sex + Effort	590.227	3.8509	0.026	0.1458	5	580.093
Total Max + Total Max^2	Sex + Effort	590.288	3.9125	0.025	0.1414	5	580.155
Ln (Total Max)	Sex + Effort	590.338	3.9626	0.025	0.1379	5	580.205
Total Max	Sex + Effort	590.343	3.9671	0.025	0.1376	5	580.209
Free Fold Incr	Sex + Effort	590.375	3.9996	0.024	0.1354	5	580.242
Free Incr + Free Fold Incr^2	Sex + Effort	590.452	4.0761	0.023	0.1303	5	580.318

Survival was a function of maximal total corticosterone (Total Max), integrated total corticosterone (Total Integr), fold increase in total corticosterone (Total Fold Incr), maximal free corticosterone (Free Max), integrated free corticosterone (Free Integr), fold increase in free corticosterone (Total Free Incr), mass, body condition (Scaled Mass Index), or no individual covariates (Null, in bold). Resight probability was a function of sex and trapping effort (Effort, number of trap days in each year).

Table 4: Summary of stress-induced corticosterone survival models

Model Survival (ϕ)	Capture (p)	QAICc	Delta QAICc	QAICc Weight	Model Likelihood	# Par	QDeviance
Sex + Ln (Free Max) * Yr	sex	369.153	0	0.751	1	8	352.692
Sex + Yr + Ln (Free Integr) * Yr	sex	371.924	2.771	0.188	0.250	11	349.070
Sex	**sex**	**375.994**	**6.841**	**0.025**	**0.033**	**4**	**367.868**
Sex + Ln (Total Integr) * Yr	sex	376.494	7.341	0.019	0.026	8	360.033
Sex + Ln (Total Max) * Yr	sex	378.044	8.890	0.009	0.012	8	361.582
Sex + Yr + Ln (Free Fold) * Yr	sex	378.580	9.427	0.007	0.009	11	355.725
Sex + Ln (Total Fold) * Yr	sex	380.961	11.808	0.002	0.003	8	364.499

Survival was a function of maximal total corticosterone (Total Max), integrated total corticosterone (Total Integr), fold increase in total corticosterone (Total Fold Incr), maximal free corticosterone (Free Max), integrated free corticosterone (Free Integr), fold increase in free corticosterone (Total Free Incr), Yr (year), sex (Sex), mass, body condition (Scaled Mass Index), or no individual covariates (Null, in bold). Resight probability was a function of sex.

Table 5: Linear (β) selection gradients measure relationships between corticosterone or energetic resources and fitness

Trait	β	SE	F	df	P
Wing Length					
Age = 1	-0.026	0.044	0.359	1, 13	0.559
Age > 1	**0.143**	**0.052**	**7.525**	**1, 22**	**0.012**
Mass					
Age = 1	-0.078	0.045	2.997	1, 13	0.107
Age > 1	0.024	0.043	0.324	1, 28	0.574
Body Condition (SMI)					
Age = 1	-0.078	0.054	2.101	1, 13	0.171
Age > 1	-0.056	0.041	1.835	1, 22	0.189
Fat					
Age = 1	-0.081	0.049	2.782	1, 13	0.119
Age > 1	0.032	0.039	0.067	1, 28	0.421
Baseline Total Cort					
Age = 1	0.053	0.047	1.231	1, 13	0.287
Age > 1	**0.119**	**0.040**	**9.032**	**1, 25**	**0.006**
Baseline Free Cort					
Age = 1	0.043	0.041	1.102	1, 13	0.313
Age > 1	0.086	0.062	1.931	1, 22	0.179
Maximal Total Cort					
Age = 1	-0.016	0.053	0.085	1, 13	0.775
Age > 1	0.032	0.041	0.608	1, 25	0.443
Max Free Cort					
Age = 1	0.047	0.097	0.238	1, 13	0.634
Age > 1	-0.012	0.037	0.109	1, 22	0.744
Integrated Total Cort					
Age = 1	0.036	0.054	0.438	1, 12	0.520
Age > 1	0.042	0.048	0.768	1, 21	0.391
Integrated Free Cort					
Age = 1	0.063	0.067	0.871	1, 12	0.369
Age > 1	-0.017	0.044	0.153	1, 20	0.700
Fold Increase in Total Cort					
Age = 1	-0.031	0.042	0.534	1, 13	0.478
Age > 1	**-0.182**	**0.062**	**8.618**	**1, 21**	**0.008**
Fold Increase in Free Cort					
Age = 1	-0.033	0.052	0.407	1, 13	0.535
Age > 1	-0.049	0.051	0.917	1, 20	0.350

Bold text represents significant relationship ($P < 0.05$)

Table 6: Quadratic (γ) selection gradients measure relationships between corticosterone or energetic resources and fitness

Trait	γ	SE	t	df	P
Wing Length					
Age = 1	0.010	0.024	0.407	12	0.692
Age > 1	0.012	0.055	0.221	21	0.827
Mass					
Age = 1	0.031	0.030	1.053	12	0.313
Age > 1	-0.035	0.036	0.977	27	0.337
Body Condition (SMI)					
Age = 1	-0.013	0.031	0.422	12	0.680
Age > 1	0.020	0.031	0.661	21	0.516
Fat					
Age = 1	-0.064	0.045	1.410	12	0.184
Age > 1	**-0.107**	**0.038**	**2.800**	**27**	**0.009**
Baseline Total Cort					
Age = 1	0.007	0.032	0.205	12	0.841
Age > 1	-0.055	0.044	1.261	24	0.219
Baseline Free Cort					
Age = 1	-0.013	0.041	0.318	12	0.756
Age > 1	0.056	0.074	0.758	21	0.457
Maximal Total Cort					
Age = 1	-0.001	0.034	0.027	12	0.979
Age > 1	-0.030	0.031	0.978	24	0.338
Max Free Cort					
Age = 1	0.152	0.194	0.784	12	0.448
Age > 1	0.055	0.042	1.303	21	0.207
Integrated Total Cort					
Age = 1	-0.016	0.048	0.339	11	0.741
Age > 1	-0.030	0.046	0.642	20	0.528
Integrated Free Cort					
Age = 1	0.029	0.083	0.353	11	0.731
Age > 1	0.051	0.046	1.106	19	0.283
Fold Increase in Total Cort					
Age = 1	0.017	0.031	0.556	12	0.589
Age > 1	-0.040	0.066	0.606	20	0.551
Fold Increase in Free Cort					
Age = 1	0.017	0.069	0.252	12	0.805
Age > 1	**0.089**	**0.039**	**2.280**	**19**	**0.034**

Bold text represents significant relationship ($P < 0.05$)

Chapter 2: An integrative look at physiological condition

ABSTRACT

Physiological condition compromises multiple components of an individual's physiology (e.g. energy balance, immune function, endocrine system) and has important consequence for survival and reproduction. These various components draw from a common pool of resources and therefore tradeoff against one another. However, most studies focus on a single physiological system. Thus we argue that a more holistic approach will yield a better understanding of physiological condition. In this study, we investigated a number of different measures from a range of physiological systems in a breeding population of gray-headed juncos (*Junco hyemalis caniceps*). We used linear mixed effects models to assess which measure or suite of measures best predict individuals' ability to perform an important reproductive task. Our best models of condition included multiple measures: corticosterone titer, presence of blood parasites and fat score. Incorporating multiple components of condition, spanning several physiological systems improved the predictive power of our models, which highlights the value of this multisystem approach.

INTRODUCTION

Physiology condition comprises multiple components of an individual's physiology and has important consequences for fitness (McNamara & Houston 1996). It reflects previous success in performing vital tasks such as foraging, competing with conspecifics, and coping with environmental challenges (Jakob et al. 1996). It also represents the current pool of resources available for investment in survival and reproduction, thereby driving individual differences in fitness (van Noordwijk & de Jong 1986). For these

reasons, biologists have had a longstanding interest in measuring physiological condition, with measures dating back to the early nineteenth century (Jelliffe & Jelliffe 1979). Despite this longstanding interest, we still lack a consensus as to the best measure.

A vast literature surrounds physiological condition and a variety of condition indices have been developed (reviewed in Brown 1996, Stevenson & Woods 2006). Different types of condition indices attempt to capture different underlying attributes of an organism's health or well-being. One of the most prevalent measures is energetic stores. Researchers typically use morphological measures to assess an individual's mass relative to its body size, where greater mass for a given body size indicates greater energy stores and thus greater resources to allocate to survival and reproduction (reviewed in Peig & Green 2010). Other common approaches include assessing immune system parameters (e.g. Møller & Petrie 2002), parasite loads (e.g. Hakkarainen et al. 2007), hormone titers (e.g. Moore et al. 2000), and blood metabolites (e.g. Jenni-Eiermann & Jenni 1997). Each of these measures has been individually related to some measure of performance which supports its relevance as a measure of condition. However, no single measure of physiological condition consistently predicts individual performance.

The link between physiological condition and individual performance has both theoretical and empirical support. McNamara and Houston (1996) used a modeling approach to show that physiological condition should dictate the level of investment in reproduction and that these initial differences in reproductive investment can have multigenerational effects. Other theoretical work suggests that individuals with access to large energetic resources, in particular, should have both high survival and high reproductive output, because they have ample resources to devote to each of these tasks (van Noordwijk & de Jong 1986).

Empirical evidence also supports the link between physiological condition and individual performance across a range of taxa and physiological systems. In a population of black-browed albatrosses (*Thalassarchemelanophrys*), baseline corticosterone levels were negatively associated with male reproductive success over a 5-year period (Angelier et al. 2010). Female great tits (*Parus major*) infected by the blood parasite *Trypanosoma* had smaller egg volumes, lower hatching success, smaller nestlings, and nestlings in worse condition (Dufva 1996). The likelihood of getting pregnant increased with body condition (length-mass residuals) in South African fur seals (*Acrtocephalus pusillusi,* Guinet et al. 1998). Again, these studies focus on a single or few measures of physiological condition and may not reflect the well being of the individual as a whole.

A holistic approach may provide a more accurate picture of well-being or new insight (Stevenson & Woods 2006). Physiological systems draw from a common pool of resources, and therefore interact with and trade off against one another (Lochmiller & Deerenberg 2000). Thus, a multivariate approach may outperform any single measure of condition. Wilson and Nussey (2010) make a similar argument when discussing individual quality, which they define as "an axis of among-individual heterogeneity that is positively correlated with fitness". They argue that different traits putatively reflecting quality may be positively correlated with fitness and weakly correlated with one another. In such circumstances, a multivariate approach will capture more information about individual quality.

Any measure of physiological condition must be validated. From a physiological perspective, validation entails relating a condition index (e.g. fat score) to a direct measure of the underlying

physiological system (e.g. percentage of body mass made up of lipids). However, from an ecological or evolutionary perspective validation requires using the condition index to predict fitness-related traits. Reproduction is a primary component of fitness. In most mammals and birds, parental care contributes to reproductive performance because it is critical to the development of young and can have profound effects on offspring quality (Clutton-Brock 1991). Therefore, we used measures of parental care to validate our integrative condition index.

In this study we used a population of breeding gray-headed juncos (*Junco hyemalis caniceps*) to develop an integrative measure of physiological condition. We assessed multiple components of condition across several physiological systems and evaluated the ability of those metrics to predict variation in parental care. Our measures of condition were scaled mass index (an unbiased measure of mass relative to body size), fat score, hematocrit, presence of blood parasites, corticosterone titers, and an immune parameter (hemagglutination, measures of innate immunity) (Table 1). Our measure of performance was female brooding behavior.

METHODS:

Study Area and Study Species

We studied a population of breeding gray-headed juncos in a series of high elevation (2600 m) snowmelt drainages along the Mogollon Rim in central Arizona from May-June 2007 (see Martin 2007 for details of study area). Juncos are small passerines (ca. 24 g) that build open-cup nest on the ground or in low shrubs. Females incubate eggs (clutch size: 3 to 5 eggs) and brood nestling. As altricial birds, nestling juncos are reliant on maternal brooding to maintain their body

temperature until they develop the capacity to thermoregulate (ca. 7 days post hatching, Cheng 2008).

Sampling

Females were captured in mist nets set up in their preferred flight path near the nest. Captured birds were subject to a standardized capture and restraint stress protocol to measure their baseline and stress-induced corticosterone (the primary glucocorticoid in birds) (Wingfield 1994). An initial blood sample was taken from birds as soon as possible after they hit the net and time to blood sample did not affect corticosterone level ($t_{34} = 0.55$, $p=0.586$). Birds were then placed in cloth bags as part of the stress protocol and subsequent blood samples were drawn at 10 and 30 minutes post-capture. Blood samples were taken by puncturing the brachial vein with a 26-gauge needle and collecting ~ 40-60 µl of blood in a heparinized microcapillary tube. Several drops of blood were also used to make a blood smear. Blood samples were kept in a portable cooler with several icepacks. Within 6 hours, blood samples were centrifuged at 14,000 RPM for 10 min. After centrifugation, the plasma was removed and stored at -20°C until it was assayed. Prior to releasing the birds, we measured several morphological traits. Morphological measurements included mass (nearest 0.25 g), tarsus length (nearest 0.1 mm), flattened and straightened wing length (0.5 mm), and subcutaneous fat deposits in furcular and abdominal cavities on a scale of 0 (no fat) to 5 (fattest) (Wingfield & Farner 1978). Scores were summed across the two cavities for a final range of 0 to 10.

Blood parasites

Blood smears were fixed with methanol and stained for 50 minutes with Giesma stain. Smears were then inspected under oil immersion at 1000x magnification for the presence of *Haemoproteus*, an intracellular parasite (Foreyt 2002). Each smear was examined for 10 minutes or until an infected red blood cell was seen.

Immune Function

Constitutive innate humoral immunity was assessed using a hemolysis-hemagglutination assay, following a protocol adapted from Matson et al (2005). This assay measures natural antibodies and complement-mediated lysis. Briefly, we serially diluted plasma with 0.01 M PBS (Sigma #P3813, St Louis, MO) down the long axis (12 rows) of U-bottomed 96-well plates. After serial dilutions, the first column had pure plasma and the last column had pure PBS. The concentration for each intervening column was half the concentration of the preceding column. We then added 25 µl of 1% rabbit blood cell suspension in PBS (defibrinated whole rabbit blood; Colorado Serum Co. Ref CS1072; Denver, CO) to each well. We sealed the plates with parafilm and incubated at 37 degrees for 90 min. After incubation, the plates were tilted at a 45° angle and incubated at room temperature for 20 minutes. After the 20 min incubation, plates were inspected for agglutination. After another 70 minutes, plates were inspected again for lyisis. Agglutination and lysis were scored as the lowest dilution which showed agglutination or lysis, respectively.

Hormones

Serial blood samples taken during the 30 minute stress protocol allow us to measure multiple aspects of corticosterone physiology. Baseline corticosterone was measured as the concentration of corticosterone

in the initial blood sample. Stress-induced corticosterone was quantified in two ways: maximal corticosterone and integrated corticosterone. Maximal corticosterone was defined as the highest concentration of corticosterone measured over the sampling period. Integrated corticosterone was measured as the total amount of corticosterone secreted over the sampling period. These analyses offer insight into peak levels and total levels over time respectively.

We measured plasma corticosterone concentrations using Enzyme Immunoassay (EIA) kits (catalogue number ADI-901-097, Enzo Life Sciences, Plymouth Meeting, PA, U.S.A.) following the protocol laid out in Wada et al. (2007), with one notable exception. Instead of using steroid displacement buffer, we ran EIAs following an extraction using anhydrous ether. Recoveries after extraction averaged 89% (range 81–94%). Recoveries were estimated by adding a known amount of tritiated corticosterone to each sample. After the ether extraction of steroid hormones (including corticosterone), the percentage of tritiated corticosterone remaining each sample indicates the percentage of total hormone recovered. Thus, each sample can be corrected for the corticosterone lost in the extraction process and the final assayed concentration was adjusted accordingly. Additionally, the plasma was diluted 1:45 (instead of 1:40), based on the optimization protocol laid out in Wada et al. (2007). Samples and standard curves were run in triplicate. All samples from the same individual were run on the same plate, but individuals were randomized across plates, and samples were randomized within each plate. Mean ± SD detection limits were 0.33±0.15 ng/ml (detectability = percentage bound of total binding minus two standard deviations; i.e. corticosterone values that were significantly different from blank wells). All samples were above the detection limit. Interplate and intraplate variations were 19.1% and 9.3%, respectively. Interplate variation is measured as the coefficient of variation of the external standard across plates; intraplate variation is the plate average of the within-sample coefficient of variation.

Behavioral Analysis

Brooding behavior was quantified from videotapes (See Martin et al. 2011 for details). Briefly, nests were videotaped during the nestling period (nestlings age range 1-10 days old), starting within a half hour of sunrise and lasted for approximately 6 hours. Video cameras were placed approximately 5 m from the nests. The start and finish times for each brooding event were recorded and the total time on the nest was summed for the six-hours of videotape. We then calculated the proportion of time spent brooding by dividing amount of time the female spent on the nest by the total duration of the videotape.

Statistical Analysis

Scaled mass index was calculated following Peig & Green (2009). This approach accounts for the allometric relationship between length and mass and is thought to be a better indicator of relative energy reserves than ordinary least squares residuals. Scaled mass index is calculated as:

$$\widehat{M}_i = M_i[\frac{L_0}{L_i}]^{b_{SMA}}$$

where M_i and L_i are each individual's respective mass and linear body measurements (here, head–bill length); L_0 is an arbitrary value of L (here, the sample mean); and b_{SMA} is the scaling exponent estimated by the standardized major axis (SMA) regression of ln (M) on ln (L); and $\widehat{M}_i$ is the predicted body mass for individual *i,* where the linear body size is scaled to L_0 (Peig & Green 2009).

We conduct several preliminary analyses to ensure we used the most appropriate data set. As nestlings acquire the ability to thermoregulate, females reduce then cease brooding. Therefore, we first plotted nestling age against percentage of time spent brooding to determine when brooding behavior ceased. We excluded all videos taken after this threshold. We then checked for collinearity among our independent variables. Measures of corticosterone were correlated with one another, so we created two sets of models: one for baseline corticosterone and one for the measure of stress-induced corticosterone that best correlated with brooding. Brooding data were arcsine transformed for all analyses. The remaining predictor variables in our models were independent (all correlation coefficients < 0.5).

For each measure of corticosterone, we fit a linear mixed effects model with brooding behavior as the dependent variable and nestling age, fat score, scaled mass index, hematocrit, corticosterone, agglutination, and parasite infection as independent variables. Some nests were videotaped multiple times, so we included female ID as a random effect. We then used a bidirectional stepwise Akaike Information Criterion (AIC) selection process to determine the most parsimonious model (stepAIC function in R package MASS; Venables & Ripley 2002). The model selection algorithm started with the full model and evaluated the change in AIC associated with removing each term from the model. The algorithm then removed the term whose removal resulted in the biggest decline in AIC. All subsequent iterations also investigated the effect on AIC of adding back each of the previously removed terms. This process was repeated until no further alterations to the model improved the AIC score. For some individuals, we had incomplete data. Therefore, we performed the model selection process on the dataset with complete cases, and then fit a final model with those predictor variables to all records with those parameters to maximize our sample size.

We assessed the explanatory power of our predictor variables by calculating the R^2 for fixed effects only (Liu et al. 2008). Nestling age has a known powerful effect on brooding behavior independent of maternal physiological condition, so we report the additional explanatory power of our physiological condition variables after nestling age effects were removed. We also explored a number of other measures of female performance (number of eggs laid, number of eggs hatched, hatching success, number offspring fledged, and fledging success). However, a lack of variation in these performance measures prevented our models from converging. Finally, we investigated whether ambient temperature had an effect on brooding behavior to determine if it should be included as a covariate. There was no effect of ambient temperature on brooding behavior either as a single covariate (LME: $t_{26} = 0.627$, $p = 0.536$) or after controlling for nestling age (LME: $t_{25} = 1.22$, $p = 0.233$). Therefore we did not include it in our final analyses. All statistical analyses were done using R v.2.11.1 for Windows (R Foundation for Statistical Computing, Vienna, Austria).

RESULTS

Brooding ceased after day 7 of the nestling period (Fig. 1). These results are consistent with other work done on this population of juncos, which showed that endothermy developed at 7.15 days of age (Cheng 2008). Therefore we only included behavioral samples taken on or before nestling day 7 in our models. In the top baseline corticosterone model, we found a negative relationship between brooding and nestling age, presence of parasites and corticosterone, and a positive relationship between brooding and female fat score (Table 2, Fig 2). We found similar results in our integrated corticosterone dataset. Brooding was negatively associated with nestling age, presence of parasites and corticosterone, and positively associated with female fat score (Table 3, Fig 2).

By itself, nestling age had an R^2 of 0.58 (Fig 1, 3, 4). In both sets of models, corticosterone added the most explanatory power (25% of the remaining variation for baseline corticosterone and 22% of the remaining variation for integrated corticosterone). Adding parasite presence or fat score resulted in smaller gains. When all of our condition measures were included, 40% and 36% of the remaining variation in brooding behavior is explained by models for baseline and integrated corticosterone datasets, respectively (Fig 2, 3).

DISCUSSION

In this study we sought to determine which measure or suite of measures of physiological condition best predicted brooding behavior, a critical component of parental care in altricial birds. The best model for brooding behavior included nestling age, fat score, presence of parasites, and either baseline or integrated corticosterone. Each of these three measures of physiological condition represents a different underlying physiological system. All of these relationships were in the expected direction. The other factors investigated in our study (hematocrit, scaled mass index, and hemagglutination) did not improve the fit our model for brooding behavior.

Corticosterone explained more remaining variation in brooding behavior than our other condition measures (22-25% vs. 14% and 4%, Fig 3, 4). Another study linking multiple condition measures to individual performance also identified the importance of corticosterone. In famine-stressed Galapagos marine iguanas (*Amblyrhynchus cristatus*), stress-induced corticosterone was a better predictor of survival than a morphologically-based body condition index (Romero & Wikelski 2001). Furthermore, they found that adding body condition to their corticosterone model explained approximately half of the remaining variation (R^2 improved from 0.87 to 0.94) in survival. Our measures of corticosterone were

negatively associated with brooding behavior, which is the expected direction for this relationship. Classically, stress-induced corticosterone is thought to promote survival-oriented behavior and physiology at the expense of noncritical functions such as reproduction or parental care (Wingfield et al. 1998). This relationship has been supported in some studies (e.g. Silverin 1986; Jessop 2001), but not others (Angelier et al. 2007). Combined with these literature studies, our results suggest that measures of corticosterone are an important predictor of reproductive performance, and should be included in an integrated measure of condition.

Parasite presence was also negatively associated with brooding behavior. The improvement in R^2 was smaller for parasites than corticosterone; however parasite presence is still in the best models. The negative relationship between parasite presence and brooding behavior is consistent with numerous previous studies showing a negative effect of parasite infection on reproductive performance. For example, experimental suppression of blood parasites with antibiotics improved fledging success in blue tits (*Cyanistes caeruleus,* Merino et al. 2000). Other studies have shown that the immune response to infection itself is energetically costly. Female pied flycatchers injected with non-pathogenic antigens showed reduced offspring feeding and fledged fewer offspring (Ilmonen et al. 2000). Thus, parasite infection may reduce the resources available for brooding either through direct pathogenic effects or by triggering costly immune response (reviewed in Sheldon & Verhulst 1996; Lochmiller & Deerenberg 2000). Our data do not distinguish between these two alternatives. However, these results show the relevance of parasite infection in predicting parental performance.

Finally, we found only limited evidence that energetic stores were important in predicting brooding behavior. Fat score showed a trend for a positive association with brooding behavior. Fat score has been shown to be a good indicator of lipid reserves (Krementz & Pendleton 1990) and increased energy stores

have been associated with better parental performance (e.g. Spencer & Bryant 2002). However, we were unable to find any studies linking natural variation in avian fat scores to differences in parental performance. Furthermore, including fat score in our models only explained an additional 2-8% of the remaining variation. Therefore, fat score has limited utility in our integrative condition index. Our other measure of energetic reserves, scaled mass index, was not included in our best models. Surprisingly, then, neither measure of energetic stores was a good predictor of brooding behavior. Time spent brooding cannot be spent foraging and brooding itself is metabolically costly (Bryant & Westerterp 1983), so we expected that females with greater energetic stores would brood more because they could afford it energetically. Alternatively, females that chose to brood more may have used more of their energetic reserves.

Altogether, our results suggest that looking across multiple physiological systems produces the best prediction of parental performance. These results make sense in light of the fact that physiological systems interact with and trade off against one another (Lochmiller & Deerenberg 2000). Our study indicates the strength of an integrated approach; however this type of validation should be performed for each species of interest. Future studies would be strengthened by measuring multiple components of performance or fitness to generate a fuller picture. Consistent results across multiple components of fitness would confirm the relevance of the integrative condition index. We incorporated multiple measures of fitness into our analysis, but a lack of variation in many of these fitness measures prevented us from fitting models with many predictor variables. The most appropriate measures of performance will likely depend on the natural history and life history strategy of the study species.

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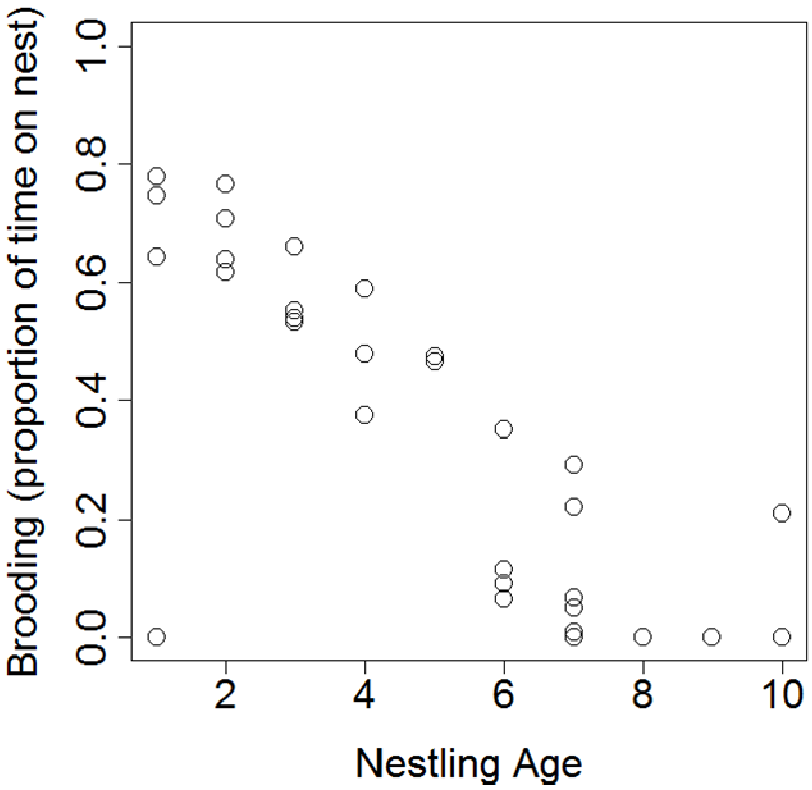

Figure 1: Brooding behavior versus nestling age

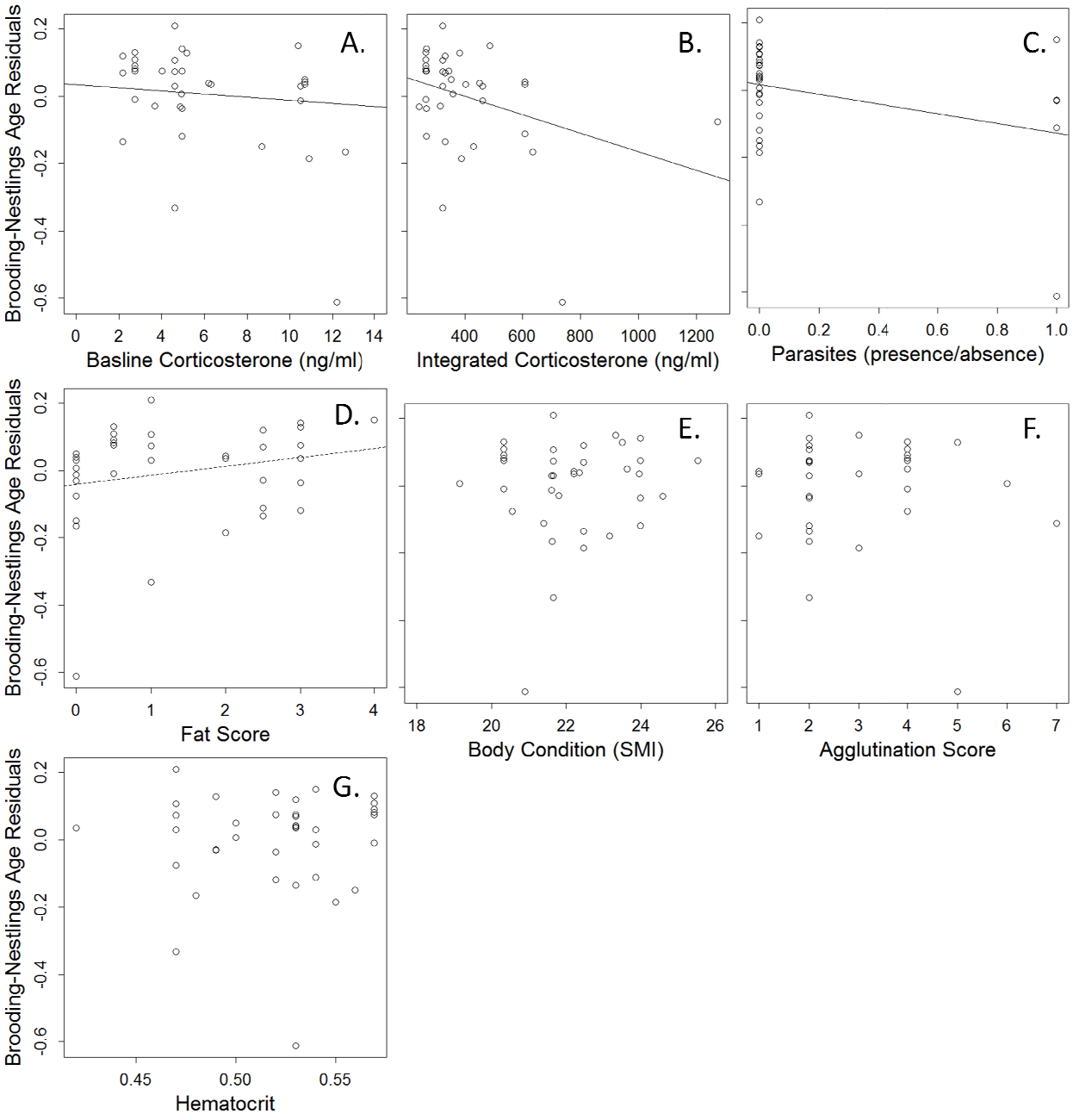

Figure 2: Physiological condition measures versus the residuals of brooding and nestling age.

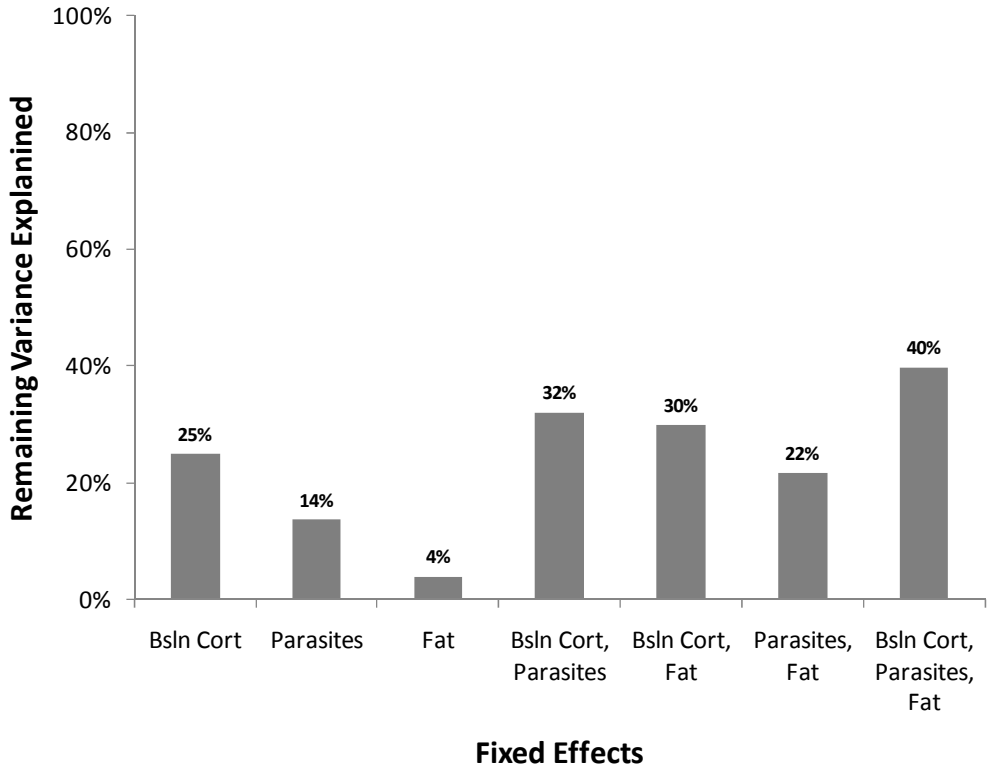

Figure 3: Proportion of remaining variance explained by models from baseline corticosterone dataset, after accounting for nestling age. Bsln Cort = baseline corticosterone.

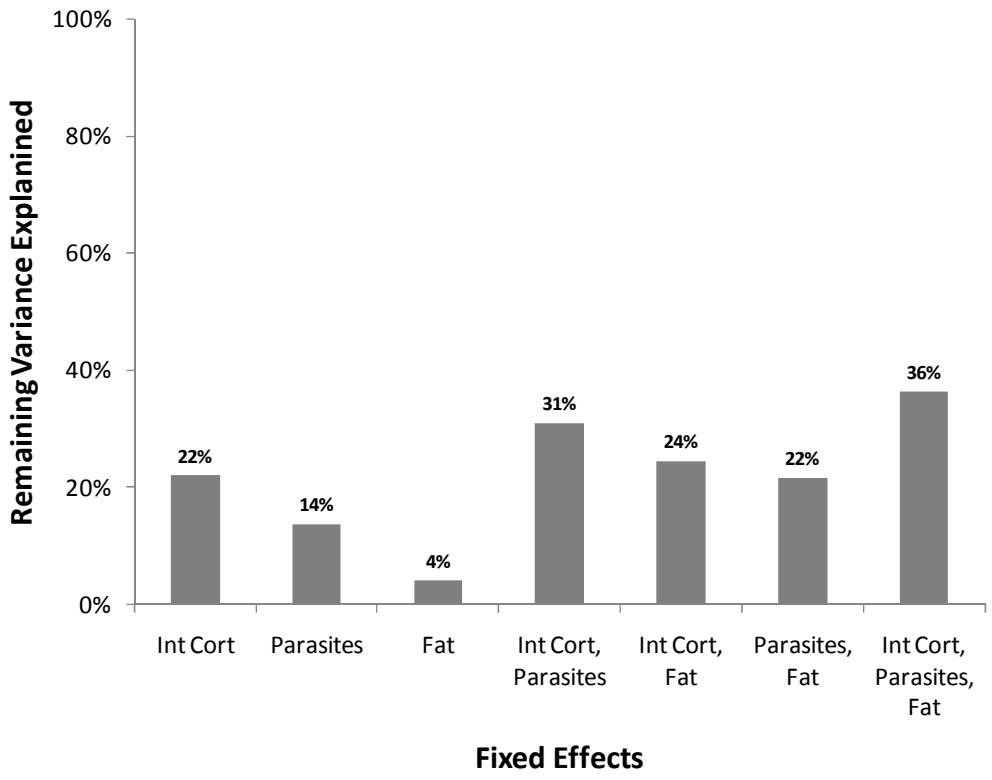

Figure 4: Proportion of remaining variance explained by models from stress-induced corticosterone dataset, after accounting for nestling age. Int Cort = integrated (stress-induced) corticosterone

Table 1: Summary of condition measures

	Mean	SD	Min	Max	n
Fat Score	1.34	1.23	0	4	38
Scale Mass Index	25.52	1.44	19.15	25.52	38
Hematocrit	0.52	0.04	0.42	0.57	38
Baseline Cort	6.10	3.36	2.17	12.60	36
Integrated Cort	376.30	126.20	242.41	736.15	36
Agglutination Score	2.91	1.49	1	7	33
Parasites	0.14[†]	0.36	0	1	35

† 5 of 35 individuals were infected with blood parasites

Table 2: Top model for baseline corticosterone dataset

	Value	Std.Error	DF	t-value	p-value	
(Intercept)	1.235	0.153	17	8.052	0.000	***
Nestling Age	-0.107	0.015	13	-7.350	0.000	***
Fat Score	0.068	0.032	13	2.121	0.054	+
Baseline Total Cort	-0.131	0.062	13	-2.126	0.053	+
Parasites	-0.230	0.107	17	-2.148	0.046	*

n = 35 observations from 19 females

Table 3: Top model for stress-induced corticosterone dataset

	Value	Std.Error	DF	t-value	p-value
(Intercept)	2.521	0.698	17	3.611	0.002
Nestling Age	-0.110	0.014	13	-7.724	0.000
Fat Score	0.062	0.033	13	1.870	0.084
Integrated Cort	-0.253	0.113	13	-2.228	0.044
Parasites	-0.242	0.108	17	-2.235	0.039

n = 35 observations from 19 females

Chapter 3: Glucocorticoids, individual quality and reproductive investment in a passerine bird

ABSTRACT

Measures of individual quality, especially energetic resources, have long been linked to investment in reproduction. However, the physiological mechanisms underlying this relationship are not well understood. In this study, we examined glucocorticoids as a potential mediator linking individual quality to investment in reproduction, because glucocorticoids have been associated with measures of both energetic resources and reproduction. We manipulated energetic resources (feather-clipping handicap) and glucocorticoids (corticosterone-soaked dermal patch) in female tree swallows*, Tachycineta bicolor,* and measured reproductive investment (offspring provisioning rate). Feather clipping was performed approximately 10 days before the first behavioral trial, while corticosterone patches were applied the day before the second behavioral trial. Prior to corticosterone manipulation, handicapped females provisioned their offspring at a lower rate and had lower levels of endogenous free corticosterone than control females. Also prior to the corticosterone manipulation, there was a trend for a negative association between endogenous corticosterone and offspring provisioning rate. There was no effect of the corticosterone manipulation on offspring provisioning rates, but there is some uncertainty regarding the efficacy of the manipulation. Overall, these results do not support the hypothesis that glucocorticoids are the primary physiological mechanism linking quality and reproduction, as measured by offspring provisioning behavior. Instead, individuals facing a sustained reduction in quality lower their circulating levels of free corticosterone perhaps to mitigate the negative effects of their handicap to themselves and their offspring.

INTRODUCTION

All organisms face resource limitations that prevent maximal investment in multiple goals. For example, resources devoted to current reproduction cannot be spent on self-maintenance or future reproduction (Stearns 1989). Thus, there is strong selective pressure to optimize resource allocation, and the optimal allocation for each individual depends on its access to resources (McNamara & Houston 1996). Individual variation in quality (here, defined as access to energetic resources) arises from variation in attributes such as territory quality, foraging ability, or social dominance. High-quality individuals tend to invest more in reproduction than low-quality individuals. This correlation between quality and reproduction makes sense intuitively because high-quality individuals will devote more resources towards reproduction in absolute terms even if they allocate the same proportion of resources to reproduction as low-quality individuals (van Noordwijk & de Jong 1986).

Numerous studies across a broad range of taxa have found positive correlations between various measures of energetic resources and reproductive investment. For example, among birds, greater adult mass or body condition index has been associated with larger clutch size and more time allocated to incubation (white storks, *Ciconia ciconia*: Sasvari & Hegyi 2001) and greater nestling growth rates (Atlantic puffins, *Fratercula arctica*: Erikstad et al. 1997). Foraging ability predicts clutch size in kestrels, *Falco tinnunculus* (Daan et al. 1990), and offspring provisioning rate in blue tits, *Cyanistes caeruleus* (Senar et al. 2002). Among mammals, heavier females have greater total milk energy output than lighter females (grey seals, *Halichoerus grypus*: Lang et al. 2009), and dominant females have higher fecundity than their subordinates (red deer, *Cervus elaphus*: Clutton-Brock et al. 1986). Among invertebrates, females fed higher-quality diets have higher fecundity and greater egg hatching success than females fed low-quality diets (*Bicyclus anynana* butterfly: Geister et al. 2008).

Hormones rapidly modulate physiology and behavior in response to internal, environmental and social cues (Ricklefs & Wikelski 2002), which makes them excellent candidates to explore as mechanisms linking individual energetic resources and reproductive investment. Indeed, much is known about the hormones involved in reproduction and parental care. In vertebrates, prolactin, oxytocin, oestrogens, androgens and progesterone tend to promote reproduction (Nelson 2005), while stress-induced levels of glucocorticoids typically inhibit reproduction (Wingfield & Sapolsky 2003). The endocrine systems that mediate reproduction in general are well described, but we understand relatively little about how hormones respond to or interact with individual quality to determine the effort and resources devoted to reproduction.

Glucocorticoids are a particularly promising class of hormones to consider as a link between individual quality and reproduction, because glucocorticoid levels often covary with energetic resources and reproduction. Many studies have found negative associations between various measures of energy stores and circulating levels of glucocorticoids. For example, subcutaneous fat stores (Müller et al. 2006), mass corrected for size (body condition; Jenni-Eiermann et al. 2008) and environmental food availability (Kitaysky et al. 1999; Jenni-Eiermann et al. 2008) are often negatively associated with circulating glucocorticoids (but see Scheuerlein et al. 2001; Walsberg 2003; Cyr & Romero 2007). Furthermore, stress-induced glucocorticoids suppress multiple components of reproductive physiology and behavior (Wingfield & Sapolsky 2003; but see Bókony et al. 2009 for a positive relationship between baseline glucocorticoids and reproduction). In mammals, elevated glucocorticoids are associated with smaller litters and offspring size (faecal cortisol metabolites; Sheriff et al. 2009). In reptiles, glucocorticoid implants can decrease territory size and delay egg laying (morph-dependent effects: Denardo & Sinervo 1994; Svennson et al. 2002). In birds, elevated glucocorticoids are associated with increased nest abandonment (endogenous free baseline levels; Love et al. 2004) and reduced nest

attendance and offspring provisioning (experimentally elevated levels: Silverin 1986; Angelier et al. 2009).

However, other avian studies have found evidence of a more complicated relationship between glucocorticoids and reproduction. A study of female tree swallows found a negative association between clutch mass and baseline corticosterone during incubation, but a positive association between clutch mass and baseline corticosterone during nestling provisioning (Bonier et al. 2009). They also found a concomitant positive association between brood growth and baseline corticosterone. The authors suggested that females with heavier broods and greater nestling growth show higher levels of corticosterone because corticosterone helps mobilize energy that the females use for reproduction.

These studies suggest a role for glucocorticoids as mediators of the relationship between individual quality and reproduction, but several outstanding issues remain. First, many of the studies are based on correlations between traits rather than experiments, so causation cannot be assessed. Furthermore, the studies that did manipulate glucocorticoid concentration typically elevated them outside the normal physiological range, which confounds the interpretation of results. Finally, to our knowledge, the proposed relationship between energetic resources, glucocorticoids and reproduction has never been tested as an integrated pathway, wherein both individual quality and glucocorticoids are manipulated and some component of reproduction is measured.

In this study, we used female tree swallows, *Tachycineta bicolor*, to test the hypothesis that glucocorticoids function as a physiological link between individual quality and reproduction. Specifically, we hypothesized that an individual's quality influences its glucocorticoid levels, which in turn affect investment in reproduction. Individual quality was manipulated by clipping three primary flight feathers

per wing. This treatment has been shown to reduce parental body condition and offspring provisioning rates in tree swallows (Winkler & Allen 1995). Glucocorticoids were manipulated using corticosterone-soaked dermal patches to mimic elevations of glucocorticoids within the natural range of variation (Wada and Breuner 2008). Finally, offspring provisioning rate was chosen as the measure of reproduction. In most mammals and birds, parental care is critical to the development of young and can have profound effects on offspring quality (Clutton-Brock 1991). Furthermore, parents can adjust offspring provisioning rate instantaneously in response to changing conditions or a change in their energetic resources. Thus, changes in energetic resources or the underlying physiological mechanisms should result in changes in offspring provisioning rate. Altogether, this approach should enable us to assess glucocorticoids as a potential mediator in the relationship between energetic resources and one critical component of reproduction.

METHODS

Study Area and Species

Use of animals for this research was approved by the Institutional Animal Care and Use Committees at the University of Montana (protocol number 031-08CBDBS-061908) and Cornell University (protocol number 2001-0051). Breeding female tree swallows were studied during 26 May–17 June 2009 and 7–13 June 2010, on the Cornell Teaching and Research Center near Harford, NY, U.S.A. (~42°26'N, 76°14'W; 400 m elevation), approximately 20 km west of the central Cornell University campus in Ithaca. Tree swallows are obligate secondary cavity nesters that readily use artificial nestboxes. Birds in this population nest in standard Golondrinas nestboxes (13 x 13 x 25 cm; http://golondrinas.cornell.edu) placed on fence posts in a hillside cow pasture. There were a total of 129 nestboxes at the study site and

each box was at least 20 m from its nearest neighbour. Old material from previous nesting attempts was removed from nestboxes before the breeding season.

Boxes were checked every 2 days starting in early May to determine the clutch initiation date of each nest. Monitoring continued every other day until clutches were complete. Clutch sizes range from three to eight in this population (mean ± SD = 5.2±1.1 for study birds in 2009; 5.4±0.9 in 2010). Once clutches were complete, nests were left undisturbed until late in the incubation stage.

Quality Treatment

Female quality was manipulated by clipping every third primary flight feather. Tree swallows are aerial insectivores, which makes them well suited for a handicap study. This technique has been shown previously to reduce body condition, offspring provisioning rate and return rate the following year in female tree swallows (Winkler & Allen 1995). Females were caught at the end of the incubation stage (incubation day 13 ± 1 day) and were randomly assigned either a handicap or control handling treatment. Females assigned the handicap treatment had their third, sixth and ninth primary feathers clipped just below the coverts on each wing (Fig. 1a). Control females were handled in the same manner as the handicapped females, but no feathers were clipped in the sham treatment.

Corticosterone Treatment

Corticosterone was manipulated using dermal patches containing crystalline corticosterone dissolved in peanut oil (as per Wada and Breuner 2008). We added 20 µl of 13.75 µg/µl corticosterone solution to the corticosterone dermal patches (dosing determined from estimates of body size and blood level

achieved in nestling Nuttall's white-crowned sparrows, *Zonotrichia leucophrys nuttalli*, in Wada and Breuner 2008). An equal volume of plain peanut oil was added to the control dermal patches. Dermal patches consisted of three layers. The layer touching the skin was a 4 x 8 mm rectangle cut from the gauzy portion (including the plastic backing) of a Band-Aid (Johnson & Johnson, New Brunswick, NJ, U.S.A.). The middle layer was an 8 x 12 mm rectangle of vinyl electrical tape. The outer layer was a 14 x 18 mm rectangle of Nexcare Tegaderm Transparent Dressing (3M, St Paul, MN, U.S.A.). Patches were placed on the back between the spinal and humeral feather tracts approximately in line with the wing (Fig. 1b). Dermal patches are less invasive than surgically implanted silastic tubing, and produce lower, more transient (and perhaps realistic) elevations in corticosterone than silastic implants. Patches were constructed the evening before application, and the corticosterone solution or peanut oil was added less than 4 h before application. Dermal patches did not appear to affect flying ability (S.H. Patterson, personal observation). Females were randomly assigned to the corticosterone treatment or the control treatment. Thirteen of 38 patches (34%) fell off of females between the application of the patch and the final capture. Results were similar in direction and significance when the females that lost patches were excluded from the analysis.

In 2010, we conducted a follow-up study to verify the efficacy of the dermal patches and to determine the associated time course of corticosterone elevation. We captured females, collected a baseline blood sample and applied a corticosterone patch. Each female was caught again 4, 16 or 20 h following the initial capture. Females were randomly assigned to recapture groups and each was bled only once after the corticosterone patch was applied. Seven of 25 patches (28%) fell off of females between the application of the patch and the final capture. Results were similar in direction and significance when the females that lost patches were excluded from the analysis, so we present results of analyses that included all females.

Sample Design

We used a two-by-two factorial design with individual quality and corticosterone treatments as factors. This resulted in four treatment groups: clipped–corticosterone patch (N = 9), clipped–control patch (N = 8), control handled–corticosterone patch (N = 12) and control handled–control patch (N = 9). Females were grouped into quartets based on hatch date and clutch size, because both factors may affect offspring provisioning rates. Within these quartets, females were randomly assigned to a treatment group. Group sizes were not equal because some nests were dropped from the study due to nest abandonment or inability to recapture females.

Females were captured using 'wigwag' nestbox traps (http://golondrinas.cornell.edu/) at the end of the incubation stage (incubation day 13 ± 1 day). Briefly, 'wigwag' traps consist of a wooden slat attached on a pivot to the front of the nestbox, a simple pulley system, and a length of fishing line. The fishing line ran through the pulley system and was attached to the wooden slat on one end and held by the researcher on the other end. Pulling on the fishing line caused the wooden slat to rotate and cover the entrance hole, thereby trapping the bird. Researchers trapping birds waited in a crouched or seated position at least 35 m from the nestbox.

Capturing females allowed researchers to collect a blood sample, measure morphological traits, apply a field mark (see below) and administer a handicap or sham manipulation. Morphological measurements included mass (nearest 0.25 g), head–bill length (maximal distance between the tip of the bill and the back of the head; nearest 0.1 mm) and flattened and straightened wing length (0.5 mm) (Winkler & Allen 1996). To distinguish between parents in videos, females were marked on the throat with a red

permanent marker and on the back of the head with Wite-Out (Bic USA, Shelton, CT, U.S.A.) correction fluid. We videotaped the nestbox 10±3 days later (observation day 1; nestling day 7 ± 1 day) in the morning to measure offspring provisioning rate (see Behavioral Analysis below). There was no effect of interval between initial and subsequent captures on either offspring provisioning rate or corticosterone level. After the videotaping was completed, we captured females to collect a blood sample, measure mass and administer a corticosterone-soaked or control dermal patch. On the following day (observation day 2), we videotaped the nestboxes again, collected a final blood sample and removed the dermal patch.

Blood Sampling

Blood samples were taken by puncturing the brachial vein with a 26-gauge needle and collecting approximately 75 µl of blood in a heparinized microcapillary tube. All but one sample was taken within 3 min of capture, and the remaining sample was taken within 4 min. Capture time was considered to be the time at which the researcher engaged the nestbox trap. We evaluated the effect of the time between capture and blood sampling on corticosterone level. To avoid complications associated with the corticosterone treatment, we restricted this analysis to samples from observation day 1, which included the 4 min sample, and found no effect of time on corticosterone. Blood samples were kept in a portable cooler with several icepacks. Within 8 h, blood samples were centrifuged at 14 000 revolutions/min for 10 min. After centrifugation, the plasma was removed. Plasma samples were stored at -20 °C until they were assayed.

Fitness Measures

To assess fitness, we investigated reproductive success and survival. We measured reproductive success in two ways: number of offspring fledged and percentage of brood fledged. Nests were left alone following the second day of behavioral observations until the 15th day of the nestling period. Starting on the 15th or 16th day of the nestling period, we checked nests for fledging every other day. We quantified the number of offspring fledged as the number of live nestlings last directly counted in the nest minus the number found dead in the box after fledging. We calculated the percentage of brood fledged by dividing the number of offspring fledged by the number of chicks that successfully hatched. Survival was estimated as adult return rate.

Insect Sampling

We sampled daily insect abundance with an aerial suction sampler at a sampling height of about 1.5 m (McCarty & Winkler 1999). The insect sampler was located centrally within the study site. The components of the sampler were encased in a section of 14-inch (36 cm) stove piping. Just inside the top of the stove piping was a 27 cm diameter x 28 cm fibreglass mesh funnel that shunted insects into a bottle of 70% ethanol. Below this was a motor-driven fan (30.5 cm diameter, 78 W, 1650 revolutions/min) to vacuum insects out of the air. The insect sampler was run from 0600 to 1200 hours each day, which included the entire period of behavioral observations. At the end of each day, the insects inside of the ethanol bottle were counted and archived.

Hormone and Binding Protein Assays

We measured plasma corticosterone concentrations using Enzyme Immunoassay (EIA) kits (catalogue number ADI-901-097, Enzo Life Sciences, Plymouth Meeting, PA, U.S.A.) following the protocol laid out

in Wada et al. (2007), with one notable exception. Instead of using steroid displacement buffer, we ran EIAs following an extraction using anhydrous ether. Recoveries after extraction averaged 84% (range 72–100%) for 2009 and 75% (range 63–85%) for 2010. Recoveries were estimated by adding a known amount of tritiated corticosterone to each sample. After the ether extraction of steroid hormones (including corticosterone), the percentage of tritiated corticosterone remaining each sample indicates the percentage of total hormone recovered. Thus, each sample can be corrected for the corticosterone lost in the extraction process and the final assayed concentration was adjusted accordingly. Additionally, the plasma was diluted 1:20 (instead of 1:40), based on the optimization protocol laid out in Wada et al. (2007). Samples and standard curves were run in triplicate. All samples from the same individual were run on the same plate, but individuals were randomized across plates, and samples were randomized within each plate. Mean ± SD detection limits were 0.75±0.19 ng/ml (detectability = percentage bound of total binding minus two standard deviations; i.e. corticosterone values that were significantly different from blank wells). All samples were above the detection limit. Interplate and intraplate variations were 10.0% and 7.87%, respectively, in 2009, and 6.38% and 5.30%, respectively, in 2010. Interplate variation is measured as the coefficient of variation of the external standard across plates; intraplate variation is the plate average of the within-sample coefficient of variation.

Corticosteroid binding globulin (CBG) is a protein that binds to corticosterone in the blood. The CBG–corticosterone complex is too large to pass through capillary walls and, according to the free hormone hypothesis, only corticosterone that is not bound to CBG (free corticosterone) is biologically active (Mendel 1989). An alternate hypothesis of the role of CBG is that it functions as a carrier molecule analogous to hemoglobin, and does not limit access of corticosterone to target tissues (Romero 2002). At present, it is not known which CBG hypothesis is correct or, by extension, which measure of glucocorticoids (total or free) is more biologically relevant. Therefore, in the present study, we report

both total and free corticosterone concentrations. We measured plasma corticosteroid binding globulin capacity using a ligand-binding assay with tritiated corticosterone following the protocol laid out by Breuner et al. (2003).

The following assay parameters were optimized for tree swallows: incubation time (2 h), incubation temperature (4 °C), rinse volume (3 ml of cold buffer) and plasma dilution (1:250). All samples were run in triplicate. Assay tubes contained 50 µl of 1:84 diluted plasma, 50 µl [3H] of corticosterone, and either 50 µl of 1 µM unlabelled corticosterone (nonspecific binding) or 50 mM (pH 7.40) Tris assay buffer (total binding). Tubes were then incubated for 2 h at 4 °C. After incubation, we separated bound and unbound (or free) radioligand using a rapid vacuum filtration harvester (Brandel, Gaithersburg, MD, U.S.A.) over 1 µm binder-free glass mircofibre filters (GF/B, Whatman, Piscataway, NJ, U.S.A.) soaked in 25 mM Tris buffer with 3% polyethylenimine for 1 h. Filters were then rinsed three times with 3 ml of 25 mM Tris buffer (pH 7.40). Interfilter variation for the point sample assay was 10.6% and 75.3% for 2009 and 2010, respectively. Free hormone levels were estimated using an equation by Barsano & Baumann (1989):

$$Hfree = 0.5[Htotal - Bmax - \frac{1}{Ka} \pm \sqrt{\left(Bmax - Htotal + \frac{1}{Ka}\right)^2 + 4 * \left(\frac{Htotal}{Ka}\right)}$$

where Ka is 1/Kd (nmol/litre), Kd is the affinity of corticosterone for CBG, Bmax is total CBG capacity, and Htotal is total plasma hormone concentration. We estimated the mean ± SD affinity (Kd) of corticosterone for CBG as 7.25±1.15 nM using pooled plasma in a separate equilibrium saturation binding assay.

Behavioral Analysis

Offspring provisioning rates were quantified from videotapes. Offspring provisioning rate was defined as the number of provisioning trips by the parent divided by the total time of the video sample. Video cameras were set up at least 20 m from the nestbox. Videotapes started at 0800 hours ± 2 h and ran for approximately 90 min. For some provisioning visits, it was not possible to identify the sex of the parent. These unknown visits were allocated to each sex based on the proportion of known provisioning visits for each sex. A reduced analysis was also run wherein nests were eliminated (*N*=7) if the number of unknown parent visits made up more than 10% of the total provisioning visits. In this reduced analysis, the direction, magnitude and significance of effects on female provisioning rate were similar. Given the similarity of the reduced analysis to the complete analysis, all nests were included in the results presented here.

Statistical Analysis

Body condition was estimated as scaled mass index (Peig & Green 2009). This approach accounts for the allometric relationship between length and mass and is thought to be a better indicator of relative energy reserves than ordinary least squares residuals. Scaled mass index is calculated as:

$$\widehat{M}_i = M_i[\frac{L_0}{L_i}]^{b_{SMA}}$$

where M_i and L_i are each individual's respective mass and linear body measurements (here, head–bill length); L_0 is an arbitrary value of L (here, the sample mean); and b_{SMA} is the scaling exponent estimated by the standardized major axis (SMA) regression of ln (M) on ln (L); and $\widehat{M}_i$ is the predicted body mass for individual *i,* where the linear body size is scaled to L_0 (Peig & Green 2009).

We used *t* tests and linear models (LM) to assess the effects of treatment groups (i.e. handicap and dermal patch) on circulating levels of glucocorticoids and offspring provisioning rate. Corticosterone, CBG and free corticosterone were normalized by natural log transformation in all analyses. Our initial (full) models for offspring provisioning rate included our treatments, measured levels of corticosterone and other factors we believed likely to influence offspring provisioning rates or corticosterone. These factors were insect abundance from the day of the behavioral observation, brood size and body condition. We also used a bidirectional stepwise Akaike Information Criterion (AIC) selection process to determine the most parsimonious model (stepAIC function in R package MASS; Venables & Ripley 2002). The model selection algorithm started with the full model and evaluated the change in AIC associated with removing each term from the model. It removed the term whose removal resulted in the biggest decline in AIC. All subsequent iterations also investigated the effect on AIC of adding back each of the previously removed terms. This process was repeated until no further alterations to the model improved the AIC score. Results from both the full models and the most parsimonious models are reported. We emphasize the results from full models because corticosterone has context-dependent effects and often interacts subtly with environmental and physiological factors. We tested for collinearity among the predictor variables in our models and found them to be independent (all correlation coefficients < 0.4). Separate models were constructed for day 1 and day 2 of behavioral observations. Day 1 models did not include the dermal patch treatment because patches had not been applied at that point.

We also conducted an alternative analysis for day 1, wherein we excluded the handicap treatment from our analysis. The rationale for this approach is that the handicap treatment should alter energetic resources. Including the handicap treatment in our model may prevent us from detecting an effect of

body condition (scaled mass index). There were no significant relationships in a model excluding the handicap treatment, so these non-significant results were are not reported.

Linear models with a clipping-by-patch interaction term were also investigated for observation day 2. We conducted likelihood ratio tests to compare models with and without the interaction term. *P* values were nonsignificant in all cases ($P > 0.9$), and thus, only the main effects models are presented below. We explored similar linear models to investigate whether our treatments or endogenous corticosterone were related to two measures of reproductive success (number of offspring fledged and percentage of brood fledged). We assessed the potential cost of clipping on adult survival (estimated by return rates) using a one-tailed Fisher's exact test. All statistical analyses were done using R v.2.11.1 for Windows (R Foundation for Statistical Computing, Vienna, Austria).

RESULTS

Observation Day 1: Pre-corticosterone Treatment

On the first day of behavioral observations, clipped females had a lower body condition index than control females (mean ± SE scaled mass index: 19.6±0.30 g versus 20.5±0.26 g; two-tailed Student's *t* test: $t_{35} = 2.25$, $P = 0.031$). Clipped females also fed nestlings at a lower rate than control females (two-tailed Student's *t* test: $t_{36} = 2.05$, $P = 0.048$; Fig. 2a). When males were included, there was no difference in total offspring provisioning rate between nests with clipped females and nests with control females (two-tailed Student's *t* test: $t_{36} = 0.08$, $P = 0.935$; Fig. 2c). However, there was no significant difference in male provisioning rates (two-tailed Student's *t* test: $t_{36} = 1.22$, $P = 0.229$; Fig. 2b). We also investigated linear models for female offspring provisioning rate. The full linear model found a negative relationship

between feather clipping and offspring provisioning rate (LM: t_{31} = 2.53, P = 0.017; Table 1), and a weak negative association between endogenous corticosterone and offspring provisioning rate (LM: t_{31} = 1.72, P = 0.095; Table 1). The best model from the stepwise model selection process (2.20 AIC points lower than full model) included only the feather-clipping treatment and corticosterone. Again, the direction of the relationship was negative for both terms (LM: clip: t_{35} = 2.48, P = 0.018; corticosterone: t_{35} = 1.67, P = 0.104). A partial correlation analysis yielded similar results ($r^2_{clipping.feeding|cort}$ = 0.1491, p = 0.0167; $r^2_{cort.feeding|clipping}$ = 0.0737, p = 0.0991).

Total corticosterone tended to be lower in clipped females than in control females, but the difference was not significant (mean ± SE = 5.4±0.5 ng/ml versus 7.0±0.7 ng/ml; LM: t_{34} = 1.93, P = 0.062), and there was no relationship between body condition and total corticosterone (LM: t_{34} = 1.25, P = 0.221). CBG levels were not affected by clipping treatment (mean ± SE = 50.7±6.7 mM versus 36.7±3.2 mM; LM: t_{20} = 1.32, P = 0.198), but there was a weak positive association between body condition and CBG (LM: t_{20} = 1.73, P = 0.099). Free corticosterone was lower in clipped females than in control females (mean ± SE = 2.8±0.5 ng/ml versus 5.6±1.0 ng/ml; LM: t_{20} = 3.07, P = 0.006), and there was a weak negative association between body condition score and free corticosterone (LM: t_{20} = 1.85, P = 0.077). The CBG and free corticosterone analyses were done on a reduced data set (N = 23; clipped = 13, control = 10) due to an error in the harvesting portion of the binding globulin assay.

Observation Day 2: about 18 h Post-corticosterone Treatment

In the day 2 full linear model, clipping treatment was the only significant (negative) predictor of females' provisioning rates (LM: clipped: t_{31} = 2.55, P = 0.016; patch: t_{31} = 0.19, P = 0.852; corticosterone: t_{31} = 0.36, P = 0.723; Fig. 3). The best model from the stepwise model selection process (6.47 AIC points lower

than full model) only included feather clipping, which again had a negative effect on offspring provisioning rate (LM: $t_{36} = 3.33$, $P = 0.002$).

About 22 h after application (mean ± SD = 22.3±2.4 h), total corticosterone levels did not differ between birds receiving corticosterone patches and those receiving control patches (LM: clipped: $t_{35} = 0.06$, $P = 0.953$; patch: $t_{35} = 1.13$, $P = 0.265$; Table 2). Free corticosterone levels were also not significantly different (LM: clipped: $t_{21} = 0.53$, $P = 0.604$; patch: $t_{21} = 1.55$, $P = 0.137$; Table 2).

2010 Hormones and Binding Proteins

In 2010, there were three treatment groups, which varied in the amount of time between the application of the corticosterone patch and the follow-up corticosterone sampling (4, 16 and 20 h). All groups had similar baseline (0 h) corticosterone, CBG and free corticosterone (LMs: all P values >0.7). Baseline corticosterone values were higher in 2010 than they were in 2009 (two-tailed Student's t test: $t_{60} = 6.51$, $P < 0.001$; Fig. 4). Total corticosterone was significantly elevated at 4, 16 and 20 h following application of corticosterone compared to the baseline sample (LM: 4 h: $t_{42} = 3.02$, $P = 0.004$; 16 h: $t_{42} = 4.30$, $P < 0.001$; 20 h: $t_{42} = 3.20$, $P = 0.003$; Fig. 4).

Fitness Measures

There was no effect of treatment or day 1 endogenous corticosterone on the number of offspring fledged (LM: clipped: $t_{34} = 0.94$, $P = 0.356$; patch: $t_{34} = 0.60$, $P = 0.556$; corticosterone: $t_{34} = 0.91$, $P = 0.370$) or the percentage of brood fledged (LM: clipped: $t_{34} = 0.81$, $P = 0.425$; patch: $t_{34} = 0.25$, $P = 0.801$; corticosterone: $t_{34} = 0.74$, $P = 0.466$). In addition, there was no effect of clipping treatment on female

return rates (14 of 24 clipped females and 15 of 24 control females returned in 2010; one-tailed Fisher's exact test: $P = 0.500$).

DISCUSSION

Our study investigated the roles of individual quality and glucocorticoids in regulating investment in reproduction. We confirmed that impairing individual quality by clipping flight feathers can have a negative effect on offspring provisioning rate. In contrast, our corticosterone manipulation did not alter offspring provisioning rate. Furthermore, we found no relationship between either of our treatments and our measures of fitness (total offspring fledged, percentage of brood fledged and return rate). The feather-clipping treatment was the only significant predictor for offspring provisioning rate on observation day 1. No relationships were found between provisioning rate and brood size, insect abundance or body condition, all factors that seemed likely to affect provisioning. Overall, the data do not support our hypothesis that corticosterone is a primary physiological mechanism linking individual quality and reproductive effort. However, there was a weak negative association between endogenous corticosterone and provisioning rate in our full model, which is consistent with the idea that corticosterone is involved in reproductive investment decisions. Additionally, it is important to consider the severity and duration of our condition manipulation as well as possible confounding factors when interpreting these results.

Fitness Measures

There was no relationship between either of our treatments and our fitness measures, despite the negative effect of feather clipping on female provisioning rates. The absence of a relationship between

feather clipping and reproductive success is probably the result of male provisioning behavior. When male provisioning visits were included, there was no difference in the frequency of provisioning visits at nests with clipped females and nests with control females. Thus, males appeared to compensate for the decline in female provisioning rate and prevented a reduction in reproductive success at nests with clipped females. There was also no effect of clipping treatment on return rates, a result that differs from the negative effect found in a previous feather-clipping study of tree swallows at a sister site about 20 km away (Winkler & Allen 1995). This suggests that the effects of feather clipping on return rates vary by site or by year. Further investigation with a larger sample size looking across sites and years would be required to understand how feather clipping affects survival.

Observation Day 1: Endogenous Corticosterone

On the first day of behavioral observations (pre-corticosterone treatment), clipping had a negative effect on offspring provisioning rate and body condition. These results are consistent with our hypothesized pathway. However, contrary to our original hypothesis, the clipping treatment and the accompanying reduction in body condition resulted in significantly lower free corticosterone levels and tended to lower total corticosterone levels. While the results of this study appear not to support the first link in our proposed pathway from quality to corticosterone, the absence of our predicted relationship may be due to the duration and severity of our clipping treatment.

This unexpected negative effect of our handicap on endogenous corticosterone may reflect the difference between chronic and acute stress. Short-term challenges (e.g. food deprivation; Astheimer et al. 1992) typically elevate corticosterone, but longer-term insults (the 7–13 days between feather clipping and corticosterone sample) probably represent chronic stressors, which can alter corticosterone

levels in diverse ways. Studies have found both decreases (European starlings, *Sturnus vulgaris*: Cyr & Romero 2007) and increases (black-legged kittiwakes, *Rissa tridactyla*: Kitaysky at al. 1999; song sparrows, *Melospiza melodia*: Clinchy et al. 2004) in baseline corticosterone associated with chronic stress. Hence, there is precedent for a decrease in circulating corticosterone in handicapped birds if we draw the reasonable conclusion that our handicap was a chronic stressor. Nevertheless, to our knowledge, this represents only the second documented instance of a species downregulating baseline corticosterone in response to chronic stress. Also note that we imposed a physical stressor in our study, whereas the starling study used a variety of psychological stressors (Cyr & Romero 2007).

There are at least two nonexclusive ultimate explanations for why chronically stressed parents may downregulate corticosterone. First, they may do so as a strategy to shield their offspring from excessive loss of parental care associated with elevated corticosterone. Other researchers have suggested that it may be beneficial to suppress the glucocorticoid response to acute stress in certain circumstances (e.g. limited possibility of future reproduction: Wingfield & Sapolsky 2003; a given brood represents a large proportion of expected lifetime reproductive output (brood value): Bókony et al. 2009). The same cost–benefit analysis that favours a lower-magnitude elevation of corticosterone in response to acute stress may favour lowering circulating levels of corticosterone in response to chronic stress, as seen in European starlings (Cyr & Romero 2007). Evidence for the negative effects of baseline corticosterone on parental performance comes from our study (a weak negative association between baseline corticosterone and offspring provisioning rate) and the literature (association between higher free corticosterone levels and complete cessation of parental care; i.e. nest abandonment; Love et al. 2004). As a relatively short-lived species (average life span = 2.7 years; Butler 1988) with relatively large clutch sizes (up to 8 eggs), tree swallows may benefit from suppressing their circulating levels of glucocorticoids in response to chronic stress and favouring reproduction over self-maintenance.

Second, handicapped parents may be trying to avoid the physiological costs associated with sustained elevated corticosterone, which include severe protein loss, neuronal cell death and reduced immune function (Wingfield et al. 1998). The negative effects of sustained elevated glucocorticoids are well established in both the comparative and biomedical literature (McEwan & Wingfield 2003). Overall, the relationship between quality and corticosterone may be different for sustained challenges (chronic stress) than it is for transient challenges (acute stress). Our feather-clipping treatment was probably a chronic stressor, so we cannot reject the possibility that corticosterone mediates the relationship between quality and investment in reproduction over either a shorter timescale of challenges or a narrower range in quality.

Observation Day: Exogenous Corticosterone

Exogenous corticosterone treatment did not affect offspring provisioning rate; however, we are uncertain of our corticosterone patches' efficacy. In the clip-by-corticosterone treatment experiment (2009), we were unable to assess corticosterone levels until approximately 2.5 h after the behavioral trials were completed. Therefore, the lack of a difference in corticosterone between control and corticosterone-treated birds may represent either ineffective corticosterone patches or a return to baseline following an elevation (total hormone levels were not significantly different; Fig. 4, grey bars). In 2010, we tested the efficacy of the corticosterone patch, measuring levels after 4, 16 and 20 h (Fig. 4, black bars). Circulating corticosterone remained elevated after 16 and 20 h of corticosterone patch treatment. These two time points bracket the interval between the application of the dermal patches and the start of behavioral trials from 2009 (mean ± SD = 18.0±1.8 h). Thus, our 2009 behavioral trials occurred within the window of elevated corticosterone, if the patches performed the same in 2009 as

they did in 2010. Overall, the 2010 data indicate that corticosterone dermal patches have the capacity to elevate corticosterone in a manner similar to elevations from naturally occurring acute stressors.

If we assume that the corticosterone treatment succeeded in elevating corticosterone in 2009, then the day 2 data do not support the hypothesis that glucocorticoids are a primary mediator of the relationship between individual quality and offspring provisioning rate. Neither the corticosterone treatment (Fig. 3), nor measured levels of corticosterone were associated with differences in offspring provisioning rate, which argues against corticosterone's involvement in regulating offspring provisioning rates. However, it is again important to consider the context the birds were experiencing. On observation day 2, the females were being captured and handled for the second day in a row, which may have influenced their behavior and corticosterone physiology. It is possible that corticosterone mediates the relationship between individual quality and reproduction over the natural range of quality, but that our manipulation may have pushed females outside of this natural range. The effects of many hormones are context specific (Orchinik 1998), and, by introducing an artificial context (clipped feathers), we may have altered the effect of corticosterone.

It is also possible that corticosterone affects offspring provisioning indirectly by altering other factors, such as prolactin. Prolactin is a hormone tightly associated with parental care in birds. In a study of black-legged kittiwakes, Angelier et al. (2009) found that a 2-day elevation of corticosterone suppressed prolactin for the duration of the study (8 days), while corticosterone returned to normal within 2 days of treatment. Thus, our condition treatment may have elevated corticosterone over a brief period, which, in turn, could have had an extended effect on prolactin. However, if corticosterone affects provisioning rate via prolactin (or some other mechanism), the corticosterone dermal patches should have reduced offspring provisioning rate, which we did not observe.

Finally, it is worth evaluating whether circulating corticosterone levels represent stress-induced levels or not. We could only find one study that describes baseline and handling-induced corticosterone levels in adult tree swallows (Franceschini et al. 2008). In that study, handling-induced corticosterone levels were similar in magnitude to our 2010 patch-induced levels. However, baseline levels in the Franceschini study were an order of magnitude lower than ours, making the two data sets difficult to compare. A third study (Bonier et al. 2009) found baseline levels in between our values and those of Franceschini et al. (2008).

Future work investigating corticosterone's effects across multiple measures of reproduction at different points of the breeding cycle (e.g. Bonier et al. 2009) would enhance the picture developed here. Also, future studies may consider using a range of quality manipulations imposed for different times to gain a more nuanced understanding of how quality interacts with corticosterone and reproduction.

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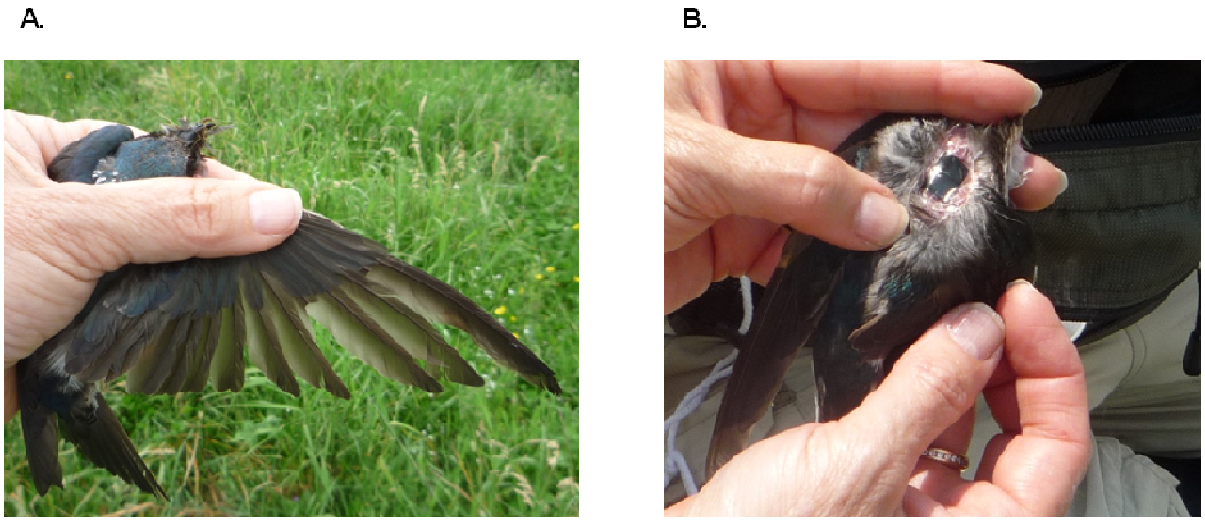

Figure 1: Experimental treatments. (A) Females receiving the handicap treatment had the 3rd, 6th, and 9th primary feathers clipped below the coverts. (B) Dermal patches were placed between the spinal and humeral feather tracks in line with the wing.

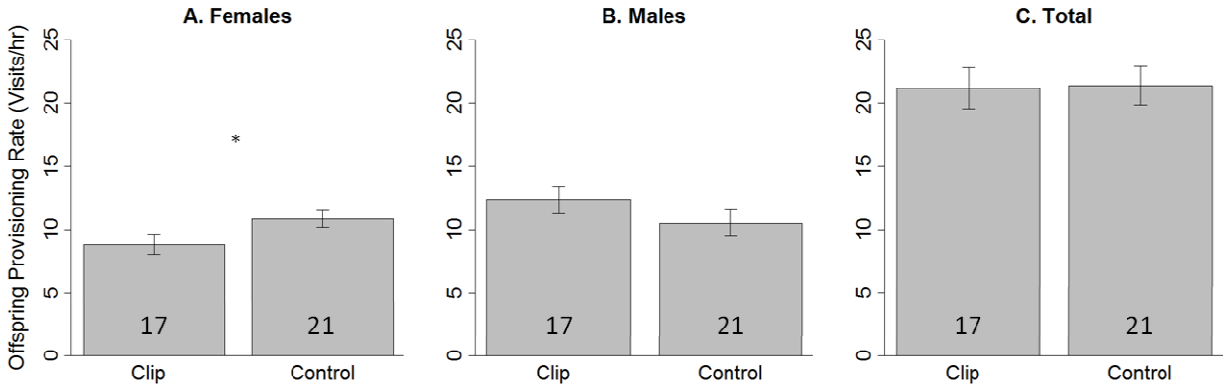

Figure 2: Day 1 offspring provisioning rates of (A) clipped females (B) males at nests with clipped versus control control females and (C) female and male combined total. Sample sizes shown within bars. *$P <$ 0.05

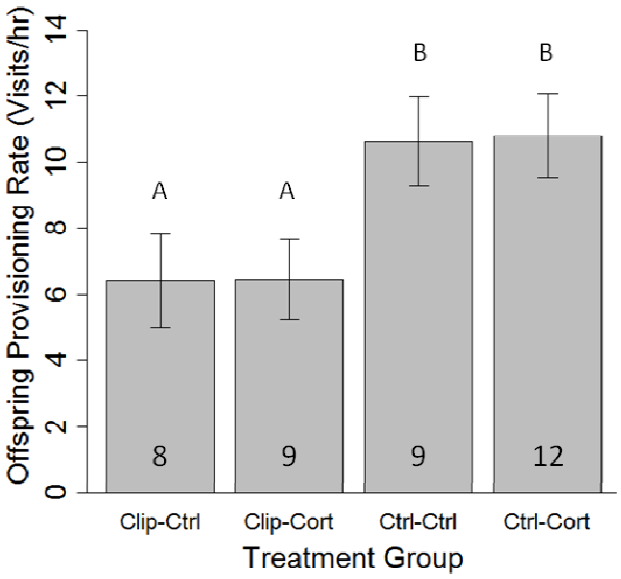

Figure 3: Day 2 offspring provisioning rates of females in each treatment group: clipped-control patch (Clip-Ctrl), clipped-corticosterone patch (Clip-Cort), sham handled-control patch (Ctrl-Ctrl), sham handled-corticosterone patch. Different letters above bars represent significant differences ($P < 0.05$) between treatment groups. Sample sizes are given within bars.

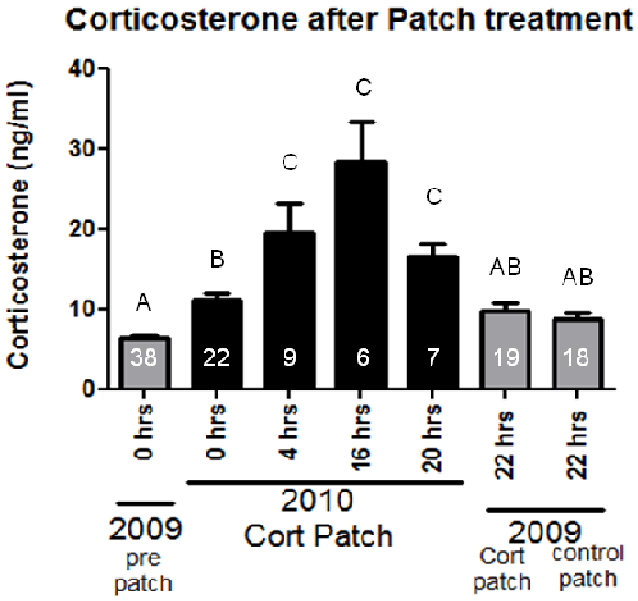

Figure 4: Mean + SEM corticosterone level by year, treatment and time after application of patch. Grey bars: 2009 data; black bars 2010 data (mean + SE). Different letters above bars represent significant differences ($P < 0.05$) in Tukey's post hoc comparison. Sample sizes given within bars.

Table 1

Offspring provisioning rate (visits/h)

	Estimate (Beta)	SE	*t*	*P*
(Intercept)	11.794	10.190	1.157	0.256
Insects	0.029	0.025	0.256	0.256
Corticosterone	-2.118	1.229	1.723	0.095†
Body condition	-0.149	0.447	0.334	0.741
Brood size	0.246	0.407	0.605	0.550
Clip treatment	2.742	1.086	2.526	0.017*

Statistics are taken from linear model of provisioning behavior on observation day 1 (pre-corticosterone treatment). All *P* values are two tailed. Sample size = 37 nests (one control female was omitted because no mass was recorded for her).

†$P < 0.1$; *$P < 0.05$.

Table 2

Effects of feather-clipping handicap (Clip) and corticosterone patch treatment (Cort) on corticosterone levels in female tree swallows

	Clip–Control	Clip–Cort	Control–Control	Control–Cort	P_{clip}	P_{patch}
Total corticosterone (ng/ml)	9.2±1.5 (8)	11.6±3.3 (9)	8.2±1.1 (9)	10.5±1.2 (12)	0.953	0.265
CBG (mM)	58.6±6.9 (6)	48.1±8.1 (7)	42.7±8.0 (5)	42.1±7.1 (6)	0.178	0.398
Free corticosterone (ng/ml)	3.6±0.5 (6)	10.0±4.1 (7)	6.1±2.4 (5)	8.9±3.1 (6)	0.604	0.137

Statistics are taken from separate linear models relating treatments to total corticosterone, corticosterone binding globulin (CBG) and free corticosterone from observation day 2. All *P* values are two tailed. Sample sizes are given in parentheses.

Chapter 4: Effects of weather and energetic stores on glucocorticoid levels

ABSTRACT

Environments are variable and organisms should adjust their physiology in response to current or expected conditions. The endocrine system modulates behavioral and physiological responses to the environment and therefore plays an important role in this process. Glucocorticoids, in particular, help regulate energy balance and should therefore be related to energetic stores and energetically demanding environmental conditions. However, findings from previous studies on this topic are mixed. In this study, we used a long-term dataset from a population of mountain white-crowned sparrows (*Zonotricia leucophrys*) to investigate the relationships between glucocorticoids, energetic stores, and weather. We used a multimodel information theoretic approach and found that none of our environmental or energetic variables were associated with measures of glucocorticoids. However, baseline corticosterone was positively associated with stress-induced corticosterone. Overall, these results suggest that the sparrows in our study population were not challenged by the observed range of environmental conditions or by low internal energy reserves. Given the large sample size (n=249 to n=316) used here relative to other studies, our results sound a note of caution against the assumption that glucocorticoid secretion is infinitely plastic and modulates responses to fine scale differences in the internal and external environment.

INTRODUCTION

No environment is perfectly stable. Therefore, organisms should tailor their physiology and behavior to respond to prevailing or anticipated conditions. Hormones act as internal signals transducing environmental and physiological information into biological responses (Ricklefs & Wikelski 2002). Glucocorticoids, in particular, should be considered because they are often associated with environmental (e.g. food availability, inclement weather) and energetic conditions (e.g. fat stores, body condition), and are known to affect a broad range of behaviors and physiological systems (Wingfield & Kitaysky 2002). Most of the work investigating the relationship between glucocorticoids and the environment focuses on severe environmental challenges such as El Niño-induced food shortages (e.g. Galapagos marine iguanas, *Amblyrhynchus cristatus,* (Romero & Wikelski 2001) or winter storms (e.g. dark-eyed Juncos, *Junco hyemalis hyemalis*, (Rogers et al. 1993). However, environmental variation also exists on a finer scale and less is known about how these more subtle environmental changes may be related to glucocorticoid physiology (but see Frigerio et al. 2004). Furthermore, the internal environment (i.e. energetic stores) should also affect glucocorticoid levels because energetic stores influence organisms' ability to cope with environmental challenges (McEwen & Wingfield 2003).

While severe weather events have been linked to elevated glucocorticoids in multiple studies, we know less about how milder weather affects glucocorticoid levels. Some studies show associations between glucocorticoids, ambient temperature and barometric pressure (e.g., fecal metabolites in graylag geese, *Anser anser,* (Frigerio et al. 2004). Other studies have found inconsistent results. In a study of three species of passerines, Romero et al (2000) found that weather factors (e.g. wind speed, temperature, precipitation, and humidity) did a poor job explaining variation in baseline and stress-induced corticosterone during the breeding season.

However, during molt, weather explained 19 to 88% of the variation of corticosterone depending on the species and measure of corticosterone (Romero et al. 2000). A major conclusion from this study is that the relationship between corticosterone and environmental factors depends on life history stage.

Glucocorticoid levels can respond to the internal environment as well as the external environment. Specifically, internal energy stores are thought to influence glucocorticoid secretion. Greater energetic reserves may allow organisms to endure a challenge without altering their physiology or behavior, thus obviating the need to elevate glucocorticoid levels (Breuner 2010). In all likelihood, the internal and external environments interact to determine glucocorticoid levels, because glucocorticoids act to balance environmentally determined energy demand against internal energy stores (McEwen & Wingfield 2003). Numerous studies across a broad range of taxa have found negative associations between measures of body condition and glucocorticoid levels (e.g. dusky flycatchers, *Empidonax oberholseri*,(Pereyra & Wingfield 2003); Galapagos marine Iguanas, (Romero & Wikelski 2001); red-spotted garter snakes, *Thamnophis sirtalis concinnus*, (Moore et al. 2000); wild rabbits, *Oryctolagus cuniculus*, (Cabezas et al. 2007). While prevalent, the negative association between measures of body condition and glucocorticoids is not universally observed (Breuner 2010) and many studies documenting the relationship have small sample sizes.

In this study, we use a long-term data set from mountain white-crowned sparrows (*Zonotrichia leucophrys oriantha*) to examine the relationship environmental and physiological conditions and several measures of glucocorticoids from the arrival phase of the breeding season. Our environmental measures were temperature, change in barometric pressure, and snow cover.

Our physiological measures were body condition and fat score. We also included age and date as covariates in our analysis.

METHODS

Study Area, Study Species, and Fieldwork

We studied a population of mountain white-crowned sparrows at Tioga Pass Meadow (37.8°N 119.2°W; ~3,030 m) of the breeding season from 2002 to 2006. Tioga Pass Meadow is a subalpine meadow located just outside of the eastern entrance to Yosemite National Park. When the birds arrive following the spring migration, they initially spend time foraging at lower elevation before ascending to the study site. Thus, they arrive at the study site in relatively good condition. Males arrive at the breeding ground first and females arrive approximately 10 days later on average (Morton 2002). When the birds arrive, the breeding ground is covered with snow and they must wait for the snow to recede before they can initiate nesting (range of clutch initiation date: June 3rd to June 13th). This results in a distinct arrival phase that precedes nesting. All samples were taken during the arrival phase. Adults were captured during using seed-baited potter traps at established locations throughout the study site. The duration of the trapping period varied by year: researchers arrived in early May and began trapping as soon as the road to the study site was cleared of snow (range: May 4th to May 15th) and trapping effort continued until the first clutch was initiated.

Adults were subject to a standardized capture and restraint stress protocol to measure their baseline and stress-induced corticosterone (the primary glucocorticoid in birds) levels (Wingfield

1994). An initial blood sample was taken from captured birds immediately following their removal from the trap. Birds were then placed in cloth bags as part of the stress protocol and subsequent blood samples were drawn at 15 and 30 minutes post-capture. Blood samples were taken by puncturing the brachial vein with a 26-gauge needle and collecting ~ 40-60 µl of blood in a heparinized microcapillary tube. Blood samples were kept in a portable cooler with several icepacks. Within 6 hours, blood samples were centrifuged at 14,000 RPM for 10 min. After centrifugation, the plasma was removed and stored at -20°C until it was assayed. Prior to releasing the birds, we measured several morphological traits, including mass (nearest 0.5 g), tarsus length (nearest 0.1 mm), and unflattened wing length (0.5 mm). We also scored subcutaneous fat deposits in furcular and abdominal cavities on a scale of 0 (no fat) to 5 (fattest). Scores were summed across the two cavities for a final range of 0 to 10.

Weather

We measured temperature, barometric pressure, and new snowfall as our weather variables. Temperature was recorded hourly using a HOBO temperature logger (Onset, Bourne, MA) situated under a stand of trees near the center of the study site. Barometric pressure was recorded hourly at Tuolumne Meadows (~10km west-southwest of Tioga Pass Meadow). Snow fall was recorded as personal observations during field work.

Hormone and Binding Protein Assays

Serial blood samples taken during the 30 minute stress protocol allow us to measure multiple aspects of glucocorticoid physiology. Baseline corticosterone was measured as the

concentration of corticosterone in the initial blood sample. We tested for an effect of bleed time (interval between trap disturbance and completion of initial blood sample) on baseline corticosterone and found that samples taken within 3 minutes were unaffected by bleed time (linear model: $t_{614} = 1.006$, $p = 0.315$). When 4 minute samples were included in this analysis, baseline corticosterone was significantly positively related to bleed time (linear model: $t_{627} = 2.359$, $p = 0.019$). Thus, only samples taken within 3 minutes were included in the study. Stress-induced corticosterone was measured in two ways: maximal corticosterone and integrated corticosterone. Maximal corticosterone was defined as the highest concentration of corticosterone measured for an individual over the sampling period. Integrated corticosterone was measured as the total amount of corticosterone secreted over the sampling period.

Plasma corticosterone concentrations were measured using Enzyme Immunoassay (EIA) kits (cat # ADI-901-097, Enzo Life Sciences, Plymouth Meeting, PA) following the protocol laid out in Wada et al. (2007). Briefly, 1% steroid displacement buffer was added to raw plasma, which was then diluted 1:40 for the assay. Samples and standard curves were run in triplicate.

Most glucocorticoid molecules in the blood are bound to a carrier protein called corticosteroid binding globulin (CBG). The glucocorticoid-CBG complex likely cannot pass through capillary walls, which would prevent access to intracellular receptors in target tissues. This is the basis of the 'free hormone hypothesis' which states that the concentration of unbound ('free') glucocorticoids is more biologically relevant than the total concentration of glucocorticoids (Mendel 1989). An alternative view of CBG is that it functions as a carrier molecule analogous to hemoglobin (Romero 2002). The biological relevance of total versus free hormone

concentrations remains an open question, so both total and free hormone concentrations were measured and are reported in this study.

Plasma corticosteroid binding globulin capacity was measured using a ligand-binding assay with tritiated corticosterone following an established protocol (Breuner et al. 2003). Breuner et al (2003) optimized the following assay parameters for mountain white-crowned sparrows: incubation time (2 h), incubation temperature (4°C), rinse volume (3 x 3 ml cold buffer), and plasma dilution (1:900). All samples were run in triplicate. Assay tubes contained 50 µl of 1:300 diluted plasma, 50 µl [3H] corticosterone, and either 50 µl of 1 µM unlabelled corticosterone (non-specific binding) or 50 mM (pH 7.40) Tris assay buffer (total binding). Tubes were then incubated for 2 h at 4°C. After incubation, we separated bound and unbound (or free) radioligand using a rapid vacuum filtration harvester (Brandel, Gaithersburg, MD) over 1 µm binder-free glass mircofiber filters (GF/B, Whatman, Piscataway, NJ) soaked in 25 mM Tris buffer with 3% polyethylenimine for 1 hr. Filters were then rinsed 3 times with 3 ml of 25 mM Tris buffer (pH 7.40). Free hormone levels were estimated using an equation by Barsano and Baumann (1989):

$$Hfree = 0.5[Htotal - Bmax - \frac{1}{Ka} \pm \sqrt{\left(Bmax - Htotal + \frac{1}{Ka}\right)^2 + 4 * \left(\frac{Htotal}{Ka}\right)}$$

where Ka is 1/Kd (nmol/l), Kd is affinity of corticosterone for CBG, Bmax is total CBG capacity, and Htotal is total plasma hormone concentration. Affinity (Kd) of corticosterone for CBG was estimated as 3.68 ± 0.31 nM (mean ± SD) using pooled plasma in a separate equilibrium saturation binding assay (Breuner et al. 2006).

Statistical Analysis

A combination of linear models and linear mixed-effects models were used to relate our measures of corticosterone to our environmental and energetic predictor variables, as well as several covariates. There were six measures of corticosterone (total baseline, free baseline, total maximal, free maximal, total integrated, and free integrated) and each measure was the dependent variable in a separated set of models. All measures of corticosterone were natural log transformed for all analyses. The sets of models for baseline total and free corticosterone investigated seven energetic and environmental factors: age, phenological date, new snowfall, change in barometric pressure, previous night's low temperature, fat score, and body condition. The four set sets of models for stress-induced corticosterone included all of the predictor variables of the baseline models, as well as total or free baseline corticosterone. Year and individual ID were included as random factors in our linear mixed-effects models.

Several factors used in our models bear further explanation. Phenological date was the number of days before the first egg of the season was laid. New snowfall was a binary variable and birds were considered to have experienced new snowfall if snow was recorded in the 48 hours preceding sampling. Change in barometric pressure was quantified as the ordinary least squares regression (OLS) slope of the hourly barometric pressure values from the 12 hours preceding capture. Lastly, body condition was estimated as scaled mass index (Peig & Green 2009). This approach accounts for the allometric relationship between length and mass and is thought to be a better indicator of relative energy reserves than OLS residuals. Scaled mass index is calculated as:

$$\widehat{M}_i = M_i[\frac{L_0}{L_i}]^{b_{SMA}}$$

where M_i and L_i are each individual's respective mass and linear body measurements (here, unflattened wing length); L_0 is an arbitrary value of L (here, the sample mean); and b_{SMA} is the scaling exponent estimated by the standardized major axis (SMA) regression of ln (M) on ln (L); and $\widehat{M}_i$ is the predicted body mass for individual *i* where the linear body size is scaled to L_0 (Peig & Green 2009).

For each measure of corticosterone, we fit all possible permutations of our fixed and random effects because all factors had the potential to affect corticosterone levels and there were no obvious biological rational to exclude particular combinations (Stephens et al. 2007). We took a weighted averaged of the parameter estimates for each predictor variable using the Aikaike model weights (Burnham & Anderson 2002). A 95% confidence interval was constructed by multiplying the unconditional variance estimator for each predictor variable by 1.96. Unconditional variance estimators were calculated as

$$\widehat{var}\left(\bar{\hat{\theta}}\right) = \left[\sum_{i=1}^{R} w_i \sqrt{\widehat{var}\left(\hat{\theta}_i | g_i\right) + \left(\hat{\theta} - \bar{\hat{\theta}}\right)^2}\right]^2$$

where $\bar{\hat{\theta}}$ is the model-averaged estimate, w_i is the Akaike weight, $\hat{\theta}$ is the parameter estimate, and g_i is a given model (Burnham & Anderson 2002). We also calculated the relative importance weight of each predictor variable by summing the Akaike weights for all models in which the

predictor variable was included (Burnham & Anderson 2002). All statistical analysis were done using 'R' version 2.11.1 for Windows (R Foundation for Statistical Computing, Vienna, Austria).

RESULTS

Baseline Corticosterone

For baseline total and free corticosterone, the 95% confidence intervals for all environmental and physiological predictor variables included zero (Table 1, Fig. 1A-B). Also, both random factors (year and individual) had relative importance weights of ≤0.02. For total baseline corticosterone, phenological date and body condition had relative importance weights of nearly 1 or 1 (the maximum value). The remaining fixed factors had relative importance weights ranging from 0.29 to 0.67 (Table 1). For free baseline corticosterone, phenological date and body condition had relative importance weights that approached one (0.97 and 0.94 respectively; Table 1). Fat score, low temperature, and new snowfall had a somewhat lower relative importance weights (range: 0.78-0.89). The remaining terms had relative importance weights (age = 0.34, change in barometric pressure = 0.29; Table 1).

Stress-Induced Corticosterone

For stress-induced total corticosterone, all 95% confidence intervals for our predictor variables included zero (Table 1, Fig. 1C-D). For both measures of stress-induced total corticosterone, fat score, low temperature and total baseline corticosterone had relative importance weights of nearly 1 or 1. All other fixed factors had intermediate relative importance weights (range: 0.28 –

0.75). Also, both random factors had relative importance weights of zero for both measures of total stress-induced corticosterone (Table 1).

Maximal and integrated free corticosterone were positively associated with baseline free corticosterone (Fig. 2) and the 95% confidence interval for free baseline corticosterone did not overlap zero (Table 1, Fig. 1E-F). All other predictor variables for models of stress-induced free corticosterone had 95% confidence intervals that overlapped zero (Table 1, Fig 1E-F). Baseline free corticosterone had a relative importance weight of 1 and date had relative importance weight of ~0.9 for both measures of stress-induced free corticosterone. All other fixed factors had intermediate relative importance weights (range: 0.26 to 0.59). Also, both random factors had relative importance weights of nearly zero (≤0.01) for both measures of stress-induced free corticosterone (Table 1).

DISCUSSION

In this study, we investigated how weather-related variables and measures of energetic stores affect corticosterone profiles. We found no evidence that any measure of weather or energetic stores was associated with any measure of corticosterone. We did, however, find evidence for a positive association between baseline free corticosterone and both measures of stress-induced free corticosterone. Given the large sample sizes (n=249 to n=316) used in our study relative to other studies, our results sound a note of caution against the assumption that glucocorticoid secretion is infinitely plastic and modulates responses to fine scale differences in the internal and external environment.

Weather

Each weather-related variable (barometric pressure, temperature, and new snowfall) has previously been related to corticosterone (e.g. barometric pressure, (Frigerio et al. 2004); temperature, (Lobato et al. 2008)et al 2008; new snowfall, (Rogers et al. 1993)) and we have a biological rationale for the existence of each relationship. Changes in barometric pressure can presage a coming storm, which is often energetically challenging. Corticosterone stimulates feeding behavior and mobilizes energy stores (Sapolsky et al. 2000). Therefore, elevating corticosterone in response to changes in barometric pressure could help optimize energetic state in preparation for an impending storm. Lower nighttime temperatures require birds to spend more energy to maintain their body temperature. Thus, birds may face a greater energy deficit following colder nights and corticosterone may be elevated to stimulate foraging behavior and the mobilization of energy stores. Finally, new snowfall can affect the availability of food, and corticosterone could be adjusted to optimize foraging or energy mobilization in response to food availability (Wingfield & Romero 1998).

Despite these plausible biological rationales, we found no associations between any measures of weather and any measures of corticosterone. Some environmental factors had high relative importance weights, however their 95% confidence intervals all overlap zero. This suggests that the factors with high relative importance weights were most informative of a collection of poor predictors. At least two possible non-exclusive hypotheses could explain the absence an effect of weather on corticosterone. First, the conditions experienced during our study may simply have been too mild to cause an elevation in glucocorticoids. Most studies linking weather to corticosterone have focused on severe weather events (Smith et al. 1994), while our study

encompassed milder forms of weather (daily minimum temperature range: -12C to 7C). Second, the relationship between glucocorticoids and weather may be life history stage specific. Previous studies have found an individual's life history stage influences the relationship between weather and corticosterone (Wingfield et al. 1983; Romero et al. 2000). In a study of adult Puget Sound white-crowned sparrows, Wingfield et al (1983) found that storms during the nestling phase elevated corticosterone levels, but storms before the onset of nesting did not. Our study took place prior to the onset of nesting, and, similar to Wingfield et al (1983), we found no effect of weather on corticosterone.

Energetic Stores

We found no evidence that energetic stores affected corticosterone, a result that runs counter to much of the literature. While not a universal finding, negative associations between glucocorticoids and energetic stores have been shown in a number of other studies, including a previous study in this population (Breuner & Hahn 2003). However, that result was based on fewer than 20 individuals and two individuals appear to drive the relationship (Breuner & Hahn 2003). Indeed, many of the previous studies have relatively small sample sizes, so a combination of sampling error and publication bias may inflate the level of support for the relationship between glucocorticoids and energetic stores. Thus, the relationship between energetic stores and glucocorticoids may be weaker or more idiosyncratic than is currently appreciated. Other previous work suggests the negative relationship between energetic stores and glucocorticoids only occurs below a certain energetic threshold. In a study of Galapagos marine iguanas, Romero and Wikelski (2001) found a negative relationship between glucocorticoids and body condition below a certain body condition threshold, but no relationship above that threshold.

Birds in this population forage at low elevation prior to arriving that the study site, so it is likely they are not below the critical threshold, should such a threshold exists in this species.

Other Predictor Variables

The one positive association observed occurred between baseline and stress-induced measures of free corticosterone. Total corticosterone showed a similar pattern. In a phylogenetic comparative study across 64 species of birds, Bokony et al (2009) found that baseline corticosterone was a significant predictor of stress-induced corticosterone. This relationship is likely due to the effect of baseline levels on negative feedback and tonic inhibition which regulate stress-induced glucocorticoid levels (Breuner 2010).

We found no effect of age on corticosterone levels. Theory predicts that aged individuals should suppress glucocorticoid secretion to maintain the current reproductive effort, because they have relatively few reproductive opportunities remaining (Wingfield & Sapolsky 2003). This is especially applicable to long-lived species where the future schedule of reproductive opportunities is relatively predictable. Results from long-lived species are mixed with some studies finding a decline in stress-induced corticosterone with age (e.g. common terns, *Sterna hirundo,* Heidinger et al. 2006), while other studies found no relationship (e.g. snow petrels, *Pagodroma nivea*, (Angelier et al. 2007). Our study focused on a short-lived species with relatively stochastic mortality. Unpredictable mortality means future reproduction is less likely to be a function of age, which reduces the selective pressure for age-specific suppression of corticosterone.

Finally, we did not find an association between phenological date and corticosterone. Glucocorticoids are often modulated on a seasonal basis (Romero 2002), with glucocorticoids peaking during breading in birds. Studies of Gambel's white-crowned sparrows, a sister subspecies, found that corticosterone was higher in the breeding season than pre-breeding (Romero & Wingfield 1999). The absence of a relationship in our study may result from the fact that all samples were taken within a single life history stage.

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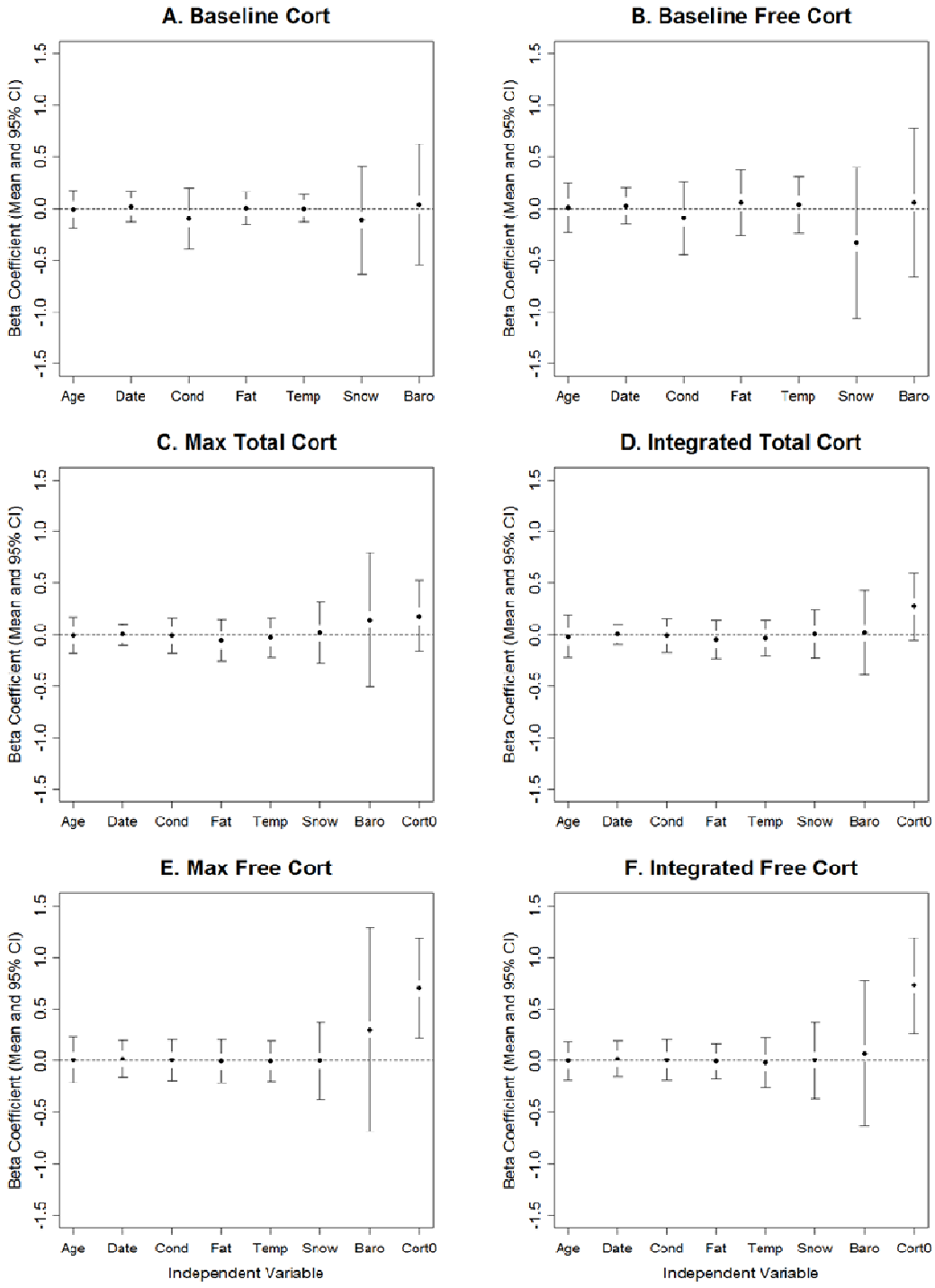

Figure 1: Model-averaged beta coefficient (generalized linear model slope parameter) for predictor variables. Age is from the year of the sample. Date is number of days before the first egg of the season was laid. Cond is body condition as estimated by scaled mass index. Fat is the sum of the furcular and abdonminal fat scores. Temp is the previous night's low temperature.s Snow is the presence of new snow in the previous 48 hours. Baro is the slope of a regression of hourly barometric pressure from the

preceding 12 hours. Cort0 is an individual's baseline corticosterone (total and free baseline corticosterone were used in models with total and free stress-induced corticosterone, respectively).

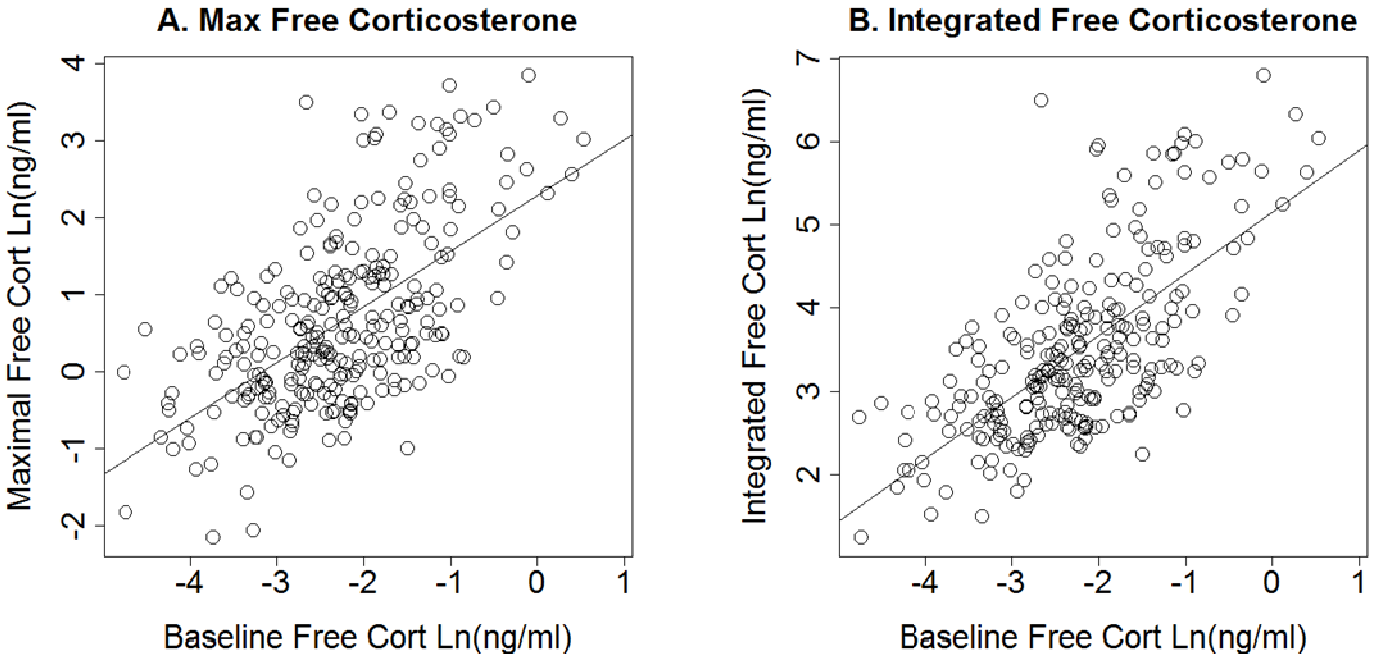

Figure 2: Relationship between free baseline corticosterone and free stress-induced corticosterone. A. Maximal free corticosterone ($t_{268} = 12.45$, $p<0.001$, $R^2 = 0.366$). B. Integrated free corticosterone ($t_{247} = 14.31$, $p<0.001$, $R^2 = 0.453$).

Table 1: Summary of model-averaged relationships between predictor variables and measures of corticosterone

Response Variable	Age	Date	Body Cond.	Fat	Low Temp	Snow	Chg Baro Pressure	Baseline CORT	Year	Band
Total Baseline CORT (n = 316)										
Variable Relative Importance Wt	0.30	0.98	1.00	0.31	0.30	0.67	0.29	-	0.00	0.00
Beta coefficent (GLM slope parameter)	-0.01	0.02	-0.10	0.00	0.00	-0.11	0.04	-	-	-
95% Confidence Interval	(-0.19, 0.18)	(-0.13, 0.17)	(-0.39, 0.2)	(-0.16, 0.16)	(-0.13, 0.14)	(-0.63, 0.41)	(-0.55, 0.63)	-	-	-
Free Baseline CORT (n = 308)										
Variable Relative Importance Wt	0.34	0.97	0.94	0.86	0.78	0.89	0.29	-	0.01	0.02
Beta coefficent (GLM slope parameter)	0.01	0.03	-0.09	0.06	0.04	-0.33	0.06	-	-	-
95% Confidence Interval	(-0.23, 0.25)	(-0.15, 0.21)	(-0.45, 0.26)	(-0.25, 0.37)	(-0.23, 0.31)	(-1.07, 0.4)	(-0.66, 0.78)	-	-	-
Total Max CORT (n = 274)										
Variable Relative Importance Wt	0.46	0.61	0.48	1.00	0.98	0.35	0.56	1.00	0.00	0.00
Beta coefficent (GLM slope parameter)	-0.01	0.00	-0.01	-0.05	-0.03	0.02	0.14	0.18	-	-
95% Confidence Interval	(-0.19, 0.17)	(-0.1, 0.1)	(-0.18, 0.16)	(-0.25, 0.15)	(-0.22, 0.16)	(-0.28, 0.31)	(-0.51, 0.79)	(-0.17, 0.52)	-	-
Free Max CORT (n = 270)										
Variable Relative Importance Wt	0.30	0.89	0.29	0.35	0.34	0.27	0.52	1.00	0.00	0.00
Beta coefficent (GLM slope parameter)	0.01	0.02	0.00	-0.01	-0.01	0.00	0.30	0.70	-	-
95% Confidence Interval	(-0.21, 0.23)	(-0.16, 0.2)	(-0.19, 0.2)	(-0.21, 0.2)	(-0.2, 0.19)	(-0.38, 0.37)	(-0.68, 1.29)	**(0.22, 1.18)**	-	-
Total Integrated CORT (n = 253)										
Variable Relative Importance Wt	0.75	0.60	0.52	1.00	1.00	0.28	0.28	1.00	0.00	0.00
Beta coefficent (GLM slope parameter)	-0.02	0.00	-0.01	-0.04	-0.04	0.01	0.02	0.27	-	-
95% Confidence Interval	(-0.23, 0.19)	(-0.09, 0.1)	(-0.18, 0.16)	(-0.23, 0.15)	(-0.21, 0.14)	(-0.23, 0.25)	(-0.39, 0.43)	(-0.05, 0.6)	-	-
Free Integrated CORT (n = 249)										
Variable Relative Importance Wt	0.26	0.91	0.30	0.29	0.59	0.27	0.31	1.00	0.01	0.01
Beta coefficent (GLM slope parameter)	0.00	0.02	0.01	0.00	-0.02	0.00	0.07	0.73	-	-
95% Confidence Interval	(-0.19, 0.19)	(-0.15, 0.19)	(-0.19, 0.2)	(-0.17, 0.17)	(-0.26, 0.22)	(-0.37, 0.38)	(-0.63, 0.78)	**(0.27, 1.19)**	-	-

Separate analyses were run for each corticosterone response variable. Age is the individual's age in the year of the sample. Date is number of days before the first egg of the season was laid. Cond is body condition as estimated by scaled mass index. Fat is the sum of the furcular and abdonminal fat scores. Low Temp is the previous night's low temperature. Snow is the presence of new snow in the previous 48 hours. Baro is the slope of a regression of hourly barometric pressure from the preceding 12 hours. Cort0 is an individual's baseline corticosterone (total and free baseline corticosterone were used in models with total and free stress-induced corticosterone, respectively).